BIOTHERAPY OF MALIGNANT TUMOURS

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PREFACE TO ENGLISH EDITION

WE should like to preface our book with a few words regarding its purpose, the questions it answers and how it came to be written, since this will enable the reader to understand our intentions more clearly and to evaluate our results more correctly.

This book does not deal with all the searchings, mistakes and confusions arising in the course of our search for factors of microbial origin capable of inducing the regression of malignant tumours. As soon as experiments had shown that such a course was possible, and that the use of products obtained from microbes may give a positive effect not only experimentally but also clinically, we termed this method of treatment *the biotherapy of malignant tumours.*

The basic and most important distinction between biotherapy and chemotherapy is the appropriate application of some or other components of microbial or other cells which have been formed in them in the process of a prolonged development under particular conditions of existence, in the process of their complex and peculiar relationships with the medium and with surrounding organisms. While in chemotherapy the investigator creates suitably designed chemical compounds, in biotherapy the investigator observes, finds and makes use of the biological principles laid down by nature itself in the great diversity of relationships existing between the various cells of different beings. Therefore, while chemotherapy has its fundamental origins in chemistry, biotherapy finds its sources primarily in general biology, microbiology and cytology. It is therefore no coincidence that the book to which we would draw the reader's attention is the result of the combined efforts of a clinical microbiologist and a cytologist.

Among the many representatives of the microbe kingdom which were the objects of our study or were learned of from the literature, we gave particular attention to *Schizotrypanum (Trypanosma) cruzi.* This also was by no means accidental, since it was impossible to overlook our findings on the very marked antagonistic effects of infections caused by *Schizotrypanum* on spontaneous and transplantable tumours of laboratory animals. Experiments with experimentally induced infection in human

patients in the terminal stages of malignant diseases (Gaillard, Brumpt, Martinez, 1950) showed that in this case also infection with *Schizotrypanum cruzi* causes suppression of the growth of tumours and in some cases their diminution. But since the course of the infection is short, and the number of developing trypanosomes is not great, in the human patient the phenomenon of antagonism between the infection and malignant growth is expressed less clearly than in laboratory animals. Prof. Jean Coudert (Faculté de Medecine, Lyons, France) has informed us that endemic and chronic trypanosome infection (Chagas' disease) can give a remarkable protection against cancer. Thus, for example, according to the Cancer Centre in Sao-Paulo (Brazil), among tens of thousands of cancer patients only two gave a positive Machado reaction (typical of chronic cases and patients recovered from trypanosome infection), whereas among the remaining population the number suffering from this infection varies from 10 to 20 per cent.

The main task confronting us was, on the basis of the antagonism between the course of trypanosomiasis and the growth of tumours noted experimentally and in nature, to find a way to obtain the active principle from *Schizotrypanum cruzi*, to devise methods for its application, if only for a few kinds of malignant tumours in man, and finally, using histopathological, cytological, cytochemical and biochemical techniques, to reveal to however slight an extent the mechanism of action of the antiblastomatous preparation later known as the antibiotic "Cruzin".

The more we found out about its properties and mechanism of action, the more clearly we realized that the substance obtained by us from one species of pathogenic protozoan cannot be exclusive and that there must be a number of other substances which may be obtained for various biotherapeutic purposes from the cells of different species of the extensive phylum Protozoa *in this respect completely uninvestigated.*

Our work began in 1929, and our early observations were subsequently published in 1931, 1936, 1937, 1938, 1939 and 1940. The war interrupted our work. Only in 1944–45 did the opportunity occur to continue our long-begun experimental investigations and to set about the first clinical trials. In carrying them out we remembered the need to consider the *long-term results* of the use of an extract from *Schizotrypanum* after a 5–10 year period, and this circumstance was bound to delay the appearance of our book.

Our purpose, or rather our desire, was to strive to extend, however little, the tragically limited powers of the doctor in treating the cancer patient. Naturally, we could not have carried out our clinical observations

on the use of Cruzin had it not been for the very real help we received from a number of experienced specialists, among whom a particular amount of work, attention and experience was provided by Prof. V. M. Sviatukhin and also Dr. G. S. Yumashev.

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INTRODUCTION

OUR research on the biotherapy of malignant tumours has been based on the working hypothesis that even the strongest cells must have their weak, easily injurable points—our job as researchers is to find them. This seemed to us to be a feasible task, since cancer cells, because of their modified physiological, biochemical and immunobiological characteristics, react atypically to a number of factors.

Antineoplastic substances of microbial origin (the progress made in experimental chemotherapy of tumours does not concern us here) have been discovered by various workers, including the authors, in a number of bacteria, bacilli, viruses, fungi and finally protozoa (*Trypanosoma cruzi*). With appropriate treatment it proved possible to isolate from *T. cruzi* a substance which is *harmless to normal tissue but selectively active against human malignant tumours.*

A comparison of our experimental and clinical observations with modern ideas on the chemical structure of micro-organisms and the wealth and functional diversity of the factors contained in their cytoplasm has convinced us that the facts which we shall present are not the results of chance. The properties of microbes which have served to maintain their species through the course of evolution are so varied and well differentiated that they open up wide new fields in the search for microbial cell fractions, similar to the antineoplastic fraction of *T. cruzi* but having still greater activity against malignant tissues. Although antineoplastic substances, as shown experimentally, occur fairly frequently in the protoplasmic elements of various micro-organisms, only a few of them may be used in the treatment of human cancer, because of their toxicity or low activity or for other reasons. The experience gained from numerous experiments shows the complex problems which must be overcome before a positive clinical effect can be obtained.

During the last ten years investigators in many countries have joined the search for cancer antibiotics. So far a number of new antibiotics have been tried with positive results, mainly experimentally, and some antibiotics have even been used to obtain a greater or lesser degree of regression of malignant tumours in man. We believe that some useful purpose will

be achieved by setting out at this stage the results of our clinical and experimental application of the trypanosome antibiotic. The first part of this book deals therefore with the various searches for cancer antibiotics now being carried out by us and by other authors, while the subsequent parts describe the principles of the process of regression of malignant tumours under the influence of the trypanosome preparation, clinically and experimentally.

We consider the theoretical importance of the principles described to be that they demonstrate the possibilities of purposeful and controlled interference in the development of human malignant tumours; their practical importance is that they provide a basis for the development of a new line in cancer treatment—treatment with preparations of bacterial origin.

This discussion of the results of biotherapy of malignant tumours is not only an evaluation of the reliability of one or another experiment or observation. Everything described in this book is experimentally reproducible and clinically repeatable. The discussion of biotherapy is of fundamental importance in principle: exogenous factors, in this case of microbial origin, can regulate and suppress malignant growth processes; this means that cancer may be treated by specific therapeutic measures, a cure being achieved more rapidly the earlier in the process of treatment the physiological defensive powers of the body become involved.

The final test of the theoretical and practical importance of our premises and conclusions lay in our original research. In a relatively short period (3–4 years) this had to embrace a large number of problems in microbiology, protozoology, cytology, immunity, through to complex biochemistry and, finally, clinical trials of antineoplastic preparations. Before extensive experimental and clinical work was possible there was the problem of finding large supplies of the raw materials required for the preparation of the trypanosome substance—not an easy problem, but one which is now in the first stages of solution. Obviously, this study of the biotherapy of cancer on such a large scale demanded the attention of a considerable number of specialists in various fields, whose names are mentioned with gratitude in the appropriate parts of this book.

The variety of investigations and observations carried out should throw some light on the following cardinal questions:

Is it possible purposefully to cause regression of malignant tumours of man and animals with the aid of factors of microbial origin?

What principles are involved in this process?

What is the practical value of cancer antibiotics in cancer therapy?

What are the prospects for the production of new anti-cancer preparations of microbial origin, apart from the trypanosome preparation which was first investigated?

Finally, what can these investigations offer in the further development of the theory of biotherapy of malignant tumours, and what can they offer now in the treatment of patients with cancer?

While working on the various aspects of the biotherapy of malignant tumours we were reminded more than once of the words of Claude Bernard: "Above all material and to some extent objective difficulties, the obstacles facing experimental medicine are those arising from poor technique, slovenly mental habits and false ideas". Indeed, the greatest difficulties and complexities of our clinical and laboratory investigations of the biotherapy of malignant tumours have lain not so much in overcoming the experimental problems, with much labour and sacrifice, but in the need for a simultaneous revision of certain accepted and firmly established conceptions of the nature, properties and development of malignant tumours and the principles of their treatment. We had, willingly or unwillingly, to criticize and overcome a number of theoretical standpoints in the general study of tumours, otherwise there would have been no hope of understanding the outcome of our observations or of making some progress, however slight, among the diverse, often contradictory, and still more often unsuccessful experiments.

We would like to stress particularly that we could never have comprehended the process of tumour regression had we not become convinced that Mechnikov's outstanding theory—the study of the defensive role of macrophages in its classical form—is also fully applicable in oncology, a field in which it has been either underestimated or completely ignored.

MICROBIAL NOMENCLATURE

SOME of the terms used in the text differ from those now commonly accepted. Such terms are listed below in the order in which they appear in the text, together with the accepted versions.

B. prodigiosus	Serratia marcescens
B. coli	Escherichia coli
Shigella paradysenteriae Flexneri	Shigella flexneri
B. disenteriae Flexner A.	Shigella flexneri Type 1
Streptoc. ovalis	Streptococcus faecalis
Streptothrix felis	Streptomyces felis
Oidium albicans	Candida albicans
Actinomyces erythromogenes	Actinomyces erythrochromogenes
Klebsiella pulmonum	Klebsiella pneumoniae
Bact. typhi	Salmonella typhosa
Bac. histolyticus	Clostridium histolyticum

Part I

THE ACTION OF VARIOUS BACTERIAL PRODUCTS ON MALIGNANT TUMOURS, EXPERIMENTALLY AND CLINICALLY

ALMOST 25 years ago, a group of investigators in Moscow began work on a new line in cancer treatment—biotherapy—using certain products of microbial cells, i.e. the substances which much later came to be termed antibiotics.

In recent years the idea of biotherapy has become increasingly prominent among scientists working in the field of experimental cancer therapy. Many of them would agree with the words of the well-known Italian oncologist Rondoni (1948): "We are now starting to recognise the existence of certain microbes, the products of which may have a selective activity against malignant tumours". Indeed, a considerable amount of literature has appeared during the last few years—evidence that many authors in different countries are engaged in the search for anti-cancer preparations of bacterial origin. For example, the far from complete review by Reilly (1953) entitled "Microbiology and Cancer Therapy" mentions 146 recently published investigations in the field of biotherapy dealing with the search for cancer antibiotics. Work of this nature has been going on for a relatively long period and in various directions. Each successful search can only serve as a starting point for more important research. This part of the book gives a review of such work, chiefly that carried out in the last decade.

1. THE PRESENT POSITION IN THE TREATMENT OF MALIGNANT TUMOURS BY COLEY'S METHOD

In 1896, the journal *Sovremennaia Meditsina (Modern Medicine)* published a long article by Dr. Eiger introducing to Russian medical circles *the first experiments* in the treatment of cancer patients by inoculating them with erysipelas and, mainly, with Coley's toxins. In the con-

clusion to this article Eiger wrote: "*We therefore conclude that toxin therapy deserves further investigation. This form of treatment should be used mainly against sarcomata, and only against those which prove inoperable*". Half a century has passed since the publication of that article. This line of research is still being followed, with great difficulty, and it presents some scientific problems which are still far from being solved. In the development of this work Coley (1891–1936) is outstanding for his perseverance and adherence to principle.

* * *

The treatment of malignant tumours with bacterial toxins is based on the observation that nearly all types of neoplasm regress *in isolated cases* under the influence of acute bacterial infections, particularly erysipelas. The literature contains instances where this effect was so marked as to bring about a clinical cure. Starting in 1891, Coley attempted to induce, in the aims of therapy, an erysipelas inflammation in 10 patients with inoperable tumours. Having encountered many difficulties, he started to try out cultures of streptococci sterilized by heat or filtration, but these experiments produced no positive results. In 1892, having learned from the literaure that *Bacillus prodigiosus (Serratia marcescens)* or its toxins increase the virulence of other bacteria, he included this organism in the preparation which came to be known as "Coley's mixed toxins". It is appropriate to mention here that as early as 1886 Gamaleia first noted the effects of *B. prodigiosus* on a cancer patient. He prepared killed cultures, which were injected into the inoperable patient. There was considerable improvement in the patient's condition due to the effects of *B. prodigiosus*.

Microbiologists prepared for Coley a number of different modifications of the so-called "mixed toxins" which were also used clinically. Coley always favoured *local injection*, believing the effects to be more rapid and definite than after subcutaneous injection remote from the tumour site. The highest number of successful results with various tumour types were obtained during the period from 1906 to 1912, when the potent and stable products prepared by Tracy were used. It was only after Coley's death in 1936 that other workers showed that for maximum effectiveness the toxins must reach the tumour in the blood stream before they are neutralized by the tissues. This explains why intratumoural and intravenous injections cause more rapid breakdown of tumours. However, Coley's method, although giving a number of successes, was associated with many difficulties and outright failures, and physicians gradually lost interest in "mixed toxins".

Coley's method has been examined in detail by Nauts, Swift and B. Coley (1946). These authors established that from the time the method was first used in 1894 at least fifteen different "Coley's toxins" preparations were employed, only three of which were more active than the others. The technique of using the preparations varied extensively with regard to the means of injection, dosage, frequency and duration of treatment. The only preparation available in the U.S.A. in 1921 was *extremely* weak, and therefore "*the use of this method, even at this late stage, was in most cases much less effective than in previous years*". Therefore—these authors suggest—"most doctors of this generation *have not seen the remarkable results obtained in the beginning*". Nauts, Swift and B. Coley summarized observations on 484 cancer patients from 1892, almost half of these observations being Coley's own. They also include 65 cases in which intercurrent infection, mainly with erysipelas, had been of importance in causing the regression of different types of malignant tumours. In more than 88 per cent of the cases the diagnoses were based on reliable microscopical as well as clinical and radiological indications.

We should stress that the greatest number of positive results achieved by Coley's method *did not occur during the last 20 years*. This gives the impression that the method was never perfected, but for some unknown reasons (or for the good reason that a strict critical standard developed in the evaluation of results and in the diagnosis of the condition) became degraded. We believe that—like all unknowns—this process deserves analysis.

When one considers the reasons for the failure of Coley's "mixed toxins" one must, of course, recognize the factors mentioned by Nauts, Swift and B. Coley—failure to produce standardized and active preparations for general use, the imperfection of the methods of using the "mixed toxins", the lack of consideration of the effects of accompanying methods of treatment, etc.—all this is true and important, but these do not appear to be deciding factors. The cardinal moment in the fate of "mixed toxins" was the *complete absence of any appropriate experimental oncological research during a period so long in the development of oncology as that from 1907 to 1930*. This gave rise to a situation whereby Coley and a number of his followers were trying to solve the question of the importance of mixed toxins in the treatment of malignant tumours by *oncological empiricism* alone, completely divorced from experimental investigations, and, no less important, on the basis of inadequate microbiological studies of the properties of the erysipelas organism and *B. prodigiosus*.

All these weak points in the clinical and experimental study of Coley's mixed toxins were by no means balanced by the unsatisfactory progress made in the preparation of standardized preparations from bacterial cells, although this task was taken on by such large organizations as the Lister Institute in England, the pharmaceutical firm of Parke, Davis and Co. in the U.S.A. and other laboratories, who in 40 years of almost continual experiments could not produce a standardized, stable and sufficiently effective while minimally toxic biological preparation of the "mixed toxins" type.

In order to decide whether there is *any truth at all* in the numerous papers devoted to the role of streptococcal infection and streptococcal toxins in the treatment of cancer, Mikhailova, at our suggestion, undertook a special investigation. Her experiments were on two lines:

(1) A study of the effects of a streptococcal exotoxin on the Crocker sarcoma;

(2) A study of the effects of streptococcal lysates on the same tumour. Twenty-four different strains of streptococus were used. These were obtained from the Tarasevich Control Institute.*

The following conclusions may be drawn from Mikhailova's experiments:

(1) The toxin from the scarlet fever streptococcus *does not affect* the development of the Crocker sarcoma.

(2) Lysates from a mixture of different streptococcal strains prepared by Grassa's method have an inhibitory influence on the development of the Crocker sarcoma.

(3) Lysates prepared from a mixture of different streptococcal strains by prolonged growth in broth culture have an inhibitory influence on the development of the Crocker sarcoma.

(4) In comparing the effects of streptococcal lysates prepared by Grassa's method with those of lysates prepared by prolonged growth in nutrient media, it may be concluded that lysates prepared by the latter method *are more active* than lysates prepared by Grassa's method.

(5) The activity of the preparation is obviously not caused by the summated effects on the Crocker sarcoma of different streptococcal strains, which taken individually have no inhibitory effect. The effects of the preparation on the development of the Crocker sarcoma depend on the activity *of the individual strains of streptococci.*

Editor's note: The institute referred to is the L. A. Tarasevich Central State Scientific Control Institute of Sera and Vaccines (U.S.P.H.S. Directory of Medical and Biological Research Institutes).

(6) The inhibitory effects of the lysate on the Crocker sarcoma do *not* depend *on the action of nonspecific proteins* but *on a specifically active agent* contained in the lysate, since a number of streptococcal lysates (strains 4544, 4542 and some others) have no active effect on the Crocker sarcoma.

In three different experiments on the effects of streptococcal lysates the ratios of the average tumour weights in experimental and control animals were: in the controls, 3·8; 3·8; 1·6; experimental, 1·5; 2·1; 1·3.

This investigation also shows conclusively that all the arguments and doubts regarding the activity of streptococcal toxins, at least experimentally, have been caused by insufficiently differentiating analysis of the properties of the very complex streptococcal group. By all appearances, only isolated strains of streptococci may in fact contain substances producing some slight inhibitory effect on the development of this transplantable mouse sarcoma. This also goes to show that the isolated and by no means frequent observations of Soviet and foreign physicians on the inhibitory effects of erysipelas inflammation on the development of malignant tumours in man may find their experimental basis in Mikhailova's work.

2. A POLYSACCHARIDE FROM *B. PRODIGIOSUS (SERRATIA MARCESCENS)* AND THE PRODUCTS OF CERTAIN OTHER GRAM-NEGATIVE BACTERIA

Interest in the study of bacterial toxins in experimental oncology has been revived by the work of Gratia and Linz (1931), who showed that filtrates of an *E. coli* culture cause haemorrhagic necrosis in guinea-pig tumours without preliminary sensitizing injections. Duran-Reynals (1933) has rightly pointed out that these experiments, besides their general significance, are of particular interest in two respects—a) no local tissue reactivity is usually shown by mice, and apparently special conditions in the tumour produce a sensitivity of its vessels to the toxins; b) the state of tumour reactivity is constant both in sensitive and in insensitive animals, and no preparatory injections are necessary as in the production of the typical Schwartzman reaction in the rabbit and guinea-pig. The vascular reactions described are seen only in malignant and rapidly growing tumours. Duran-Reynals considers that these phenomena are conditioned by two factors: (a) an intrinsic factor, related to the sensitivity of the tumour itself, and (b) an extrinsic factor, related to the activity and mode of injection of the toxin. These facts show that newly-formed vessels in

malignant tumours are extremely sensitive to bacterial toxins, making the tumour vulnerable, and this property may be exploited in attaining regression of the tumour.

Andervont (1939) showed by similar experiments that a transplanted tumour only regresses under the influence of such injections when considerable haemorrhage occurs in it—the regression is directly proportional to the extent of the haemorrhage produced. If only the centre of a tumour is affected, growth at its periphery is unchecked.

The next stage in the study of the effects of bacterial products is linked with the work of Shear and co-workers (1943; 1944), who isolated from *B. prodigiosus* active fractions rich in polysaccharides (the best preparations gave a negative reaction for protein). Shear experimented with mouse tumours induced by carcinogenic substances. Nearly all the tumours studied were sarcomata. In these experiments the mice were each injected with 0·4 ml of a preparation with a highly active polysaccharide fraction. The haemorrhagic effect obtained could be clearly delineated. Only a few tumours proved refractory. In most cases there was clearly marked suppression of tumour growth and in many cases complete regression. The tumours could, however, renew their growth and eventually kill the mouse. The changes in the tumour occurred, as a rule, within a few hours after the injection. After repeated injections the tumour became oedematous. Shear's microscopical findings confirmed those of other investigators — haemorrhages are not seen in normal tissues but only in the tumours. The cytological changes occurring in the cells in such cases were the subject of a special investigation by Diller (1947). Subsequently Shear, in association with Brues, tested a highly active polysaccharide preparation obtained by them in the treatment of patients with malignant tumours. They were inoperable tumours with widespread metastases which had not responded to radiation therapy. The polysaccharide injections were given daily for 5 days. All the patients died after various periods. There were no detectable changes in the tumours.

Shear and his co-workers had to admit that clinical observations did not support the suggestion that a polysaccharide from *B. prodigiosus* could be recommended for the treatment of malignant tumours. This negative result did not however prevent further work on the polysaccharide fraction from *B. prodigiosus*, as shown by the report by these authors given at the International Cancer Congress in 1947.

It can be seen from recent scientific publications that these first unsuccessful results in the treatment of patients have not deterred the investigators. Diller (1947) mentions 16 patients treated with polysaccharide

from *B. prodigiosus*. More detailed results are given by Oakey (1947), who describes the reactions of nine patients with malignant tumours to injections of prodigiosus polysaccharide. The types of tumour included lymphosarcoma, spindle-cell sarcoma, metastisizing Ewing tumour, fibrosarcoma, haemangioblastoma, multiple malignant melanoma and one case of leukaemia. None of the tumours was completely destroyed, although most of them showed changes in size and consistency.

Hence, these investigations into the biotherapy of malignant tumours can by no means be considered completed.

The encouraging progress made in the experimental use of active fractions from *B. prodigiosus* has not always been reflected in clinical trials. Shear and his co-workers, taking into account all their past experience and the results of new clinical experiments, have selected two principle lines for further study: (1) a search for *B. prodigiosus* strains in which the toxic fraction is minimal and the antitumour fraction most marked; (2) a search for methods enabling the prevention of the rapidly developing process of immunization on injection of prodigiosus polysaccharide.

Certain factors may influence the effects induced by prodigiosus polysaccharide. For example, ascorbic acid inhibits the haemorrhagic effect caused by bacterial polysaccharides in transplanted tumours (Andervont and Schimkin, 1936). The absence of ascorbic acid from the diet of experimental animals increases the haemorrhagic effect by about 5 times in comparison with the tumours in control animals (Eddy *et al.*, 1952). Finally, there is a definite link between blood-pressure and necrotic phenomena in tumours under the influence of bacterial toxins (Algire *et al.*, 1952). Attempts have been made to reduce the toxicity of the polysaccharide by iodination (Sack and Seligman, 1947, 1948) and also by the introduction of radioactive iodine into its molecule (Seligman *et al.*, 1948). Clinical trials with these preparations gave no encouraging results. Thus, the study of prodigiosus polysaccharide still calls for a great deal of united work by microbiologists and biochemists. Only after the completion of this work can clinical trials again be attempted. The work of the investigator is not limited, as we shall see, to the study of toxins from *B. prodigiosus*. The factor causing haemorrhage in tumours is not only contained in cultures of *B. prodigiosus*. Ikawa *et al.* (1954) isolated from *E. coli* a haemorrhagic factor consisting of a polysaccharide complex containing peptides and phospholipids. After treatment with trichloracetic acid they were able to show that the peptide fraction is inactive and that the lipo-polysaccharide fraction possesses the antitumour activity.

The work of Zahl and his co-workers (1942–1945) is among that running parallel with the study of prodigiosus polysaccharide. After studying 100 strains of over 30 species of bacteria they showed that micro-organisms producing a "haemorrhagic substance" have the following general characteristics: (1) they are gram-negative; (2) they contain a complete endotoxin complex (complete antigen); (3) they have similar pathogenic effects, consisting of vascular damage, disturbances of carbohydrate metabolism and irritation of the intestinal mucous membrane. Zahl suggests that the substance producing the haemorrhagic effect in tumours is identical with the polysaccharide complex of the endotoxin antigen. If mice are previously made immune (in experiments with the endotoxin of *Shigella paradysenteriae Flexner* and *Salmonella typhi-murium*) tumours in these animals become extremely resistant to the effects of the corresponding endotoxin and no haemorrhage occurs in them. Huthner and Zahl (1943) concluded that toxins causing tumour haemorrhage and associated with the O-antigen complex are characteristic of a number of gram-negative bacteria. However, not only gram-negative bacteria may be a source of antineoplastic substances, as shown by experiments with diphtheria toxin.

3. DIPHTHERIA TOXIN

Diphtheria toxin, when injected in certain doses, not only inhibits the development of transplantable carcinomata in rats and mice but also gives a complete cure in a considerable number of experimental animals (Roskin and Romanova, 1938; Roskin and Khaikina, 1946; Klyuyeva and Roskin, 1946). The toxin is effective against spontaneous mouse tumours. As a result of treatment of 132 spontaneous tumours, 79 tumours showed considerable decrease in size, 31 disappeared completely, 13 ceased to grow and only 9 of the 132 continued to enlarge. It was also found that in the treated mice, as distinct from the controls, metastases were generally absent — diphtheria toxin acts on both the primary tumour and its metastases. As is known, mice are refractory to diphtheria toxin, but neoplastic modifications in mouse cells render them sensitive to it. It may be thought that the mechanism of the effects of the *B. prodigiosus* toxin and diphtheria toxin are identical or at least similar. That this is not so is clearly shown by the following comparison:

Effects of B. prodigiosus *toxin on cancer in mice*	*Effects of diphtheria toxin on cancer in mice*
(1) Effective doses are toxic	(1) Effective doses not toxic
(2) Injection causes state resembling shock	(2) Injection causes no apparent reaction
(3) Effect decreases with repeated doses	(3) Effect increases with repeated doses
(4) Large haemorrhages produced in tumour	(4) Haemorrhages are usually absent
(5) Necrosis of cancer cells occurs as result of breakdown of tumour's vascular network, i.e. as secondary phenomenon	(5) "Liquefaction" of large areas of malignant tissue is observed
(6) Effect is absent in immune animals	(6) Immune state of the animal does not affect action on tumour

Thus, the nature of the effects of these toxins on malignant tumours is completely different, and two different phenomena are observed in the tumours.

These differences may be associated with the different chemical natures of *B. prodigiosus* toxin and diphtheria toxin: the former is a polysaccharide and the latter a protein. These experiments have left open the question of the response of malignant tumours to diphtheria toxin in an animal having no species immunity to diphtheria infection. What would be the effects of this toxin on a malignant tumour in an animal having no natural, physiological defence against it, as well as being affected by cancer? Would it be possible in such a case to induce artificially a state in which only the tumour is injurable and the normal tissues are protected against the damaging effects of the toxin? This problem has been studied by Klyuyeva and Gintsburg (1949) in the treatment of the Brown–Pearce tumour in rabbits (highly susceptible to diphtheria infection) which had previosly been immunized. Their experiments showed that diphtheria toxin retards the growth of the Brown–Pearce tumour. The effects of the toxin are most marked when optimum doses are used. Excessive or insufficient doses either have no effect or only act in occasional cases (the optimum dose is 150–200 MLD per course of injections). Our knowledge of the nature of malignant tumours is so limited that it is hard to analyse the remarkable fact that in a body immune to diphtheria toxin the latter can reach the tumour and destroy it without damaging normal tissues. At first sight it would appear logical to expect that in the hyperimmune body the toxin would become fixed by specific antitoxin and thus, being neutralized, would lose its ability to damage cancer cells just as it has lost its activity against the normal tissues of the body. There is no doubt that the toxin is in fact fixed by the antitoxin, because otherwise the animals would die, showing the typical signs of diphtheria intoxication. However, the rabbits show no such signs. Why then are the cancer cells destroyed? It may be suggested that the immunological reaction arising in the normal tissues does not take place in the tumour because

of the loss of reactivity and immunological inertness of cancer cells. However, this assumption still leaves the question of how the toxin enters the tumour through the immune barrier formed by the specific antitoxin it meets on the way. It may only be supposed that fixation by the antitoxin is not associated with the loss of the toxin's antineoplastic properties. This theory is also tenable in that the "toxin-antitoxin" link is not chemical but occurs by adsorption, when the properties of the toxin with regard to the tumour are unaffected. But how does one explain the inverse relationship between the amount of toxin injected and its inhibitory effects? Observations show that the inhibitory effects of diphtheria toxin on malignant tumours are in the form of a biological reaction arising and developing not in isolation but involving the physiological mechanisms of the body. Experiments described earlier (Klyuyeva and Roskin, 1946) have convinced us that blocking the reticulo-endothelial system and extirpation of the spleen inhibits the carcinolytic effects of a biological tumour-retarding substance and halts the progress of tumour regression. These experiments show that an excess of toxin breaks through the immune barrier and suppresses the defence system. Under conditions of reduced immunobiological activity of the body the influence of diphtheria toxin does not become apparent.

4. LYSATES AND FILTRATES OF CERTAIN OTHER BACTERIA

Among work carried out in the last decade is an investigation by Vollmar and Knoll (1944), who in their search for antineoplastic substances of microbial origin used an extensive collection of bacterial strains. Filtrates and lysates of a large number of bacteria were added directly to cultures of malignant tissues *in vitro*, or else small pieces of tumour tissue were placed for 24–48 hours in the bacterial lysates, the growth of these malignant tissues then being observed under conditions of culture *in vitro*. Particular activity against cultures of malignant tissues was shown by lysates of the following 5 species: *B. subtilis, E. coli, Shig. paradysenteriae Flexner A, Strep. Sg.* 104 and *Strep. ovalis*. It should be noted here that all these experiments were not easily reproducible by other workers because the properties described as belonging to certain species related *only to the strains mentioned above, having their own laboratory number.* The investigators were faced with the question of the nature of the substances active against cancer cells. They therefore carried out experiments involving boiling the lysates and filtrates for 15 minutes. The preparations *did not lose* their activity against tumour cells after this treatment, in fact

in some cases it was even increased, except for the *B. subtilis* lysate, where the activity was decreased. It should be added that treatment of the coli lysate with X- and ultraviolet rays did not destroy its activity.

The coli lysate resisted the action of sodium hydroxide (5:1) and normal hydrochloric acid (24 hours in the thermostat followed by neutralization). Treatment with pepsin or trypsin also failed to reduce the activity of the coli lysate. All these experiments by Vollmar and Knoll show that the substances active against cancer cells are not bacterial cell enzymes, since they resist boiling. We must therefore consider different component fractions of the bacterial cell, which have so far not been accurately determined. Ultrafiltration experiments show that the active substance is of high molecular weight or is linked to a high-molecular-weight carrier. It should be added that the question of the effects of these microbial preparations on experimental tumours was hardly mentioned in the work of Vollmar and Knoll, although everyday experience teaches us that the antineoplastic properties of one or another antibiotic cannot be decided merely by experiments with cultures of malignant tissue.

Among more recent investigations we would mention that of Freiman (1952), who showed that the injection of certain lysates of *Staph. aureus* directly into the Crocker sarcoma or near it causes necrosis, breakdown and resolution of a significant number of tumours. However, lysates of various staphylococcal strains possess some degree of necrotic activity. The polysaccharide fraction of staphylococci, as distinct from the protein fraction, causes necrosis or growth inhibition in mouse tumours, particularly marked on intravenous injection. Freiman also notes that the polysaccharide fraction of staphylococci shows marked tropism to tumour tissues. These findings are not in agreement with those of Grabchenko and Podil'chak (1952). The divergence is probably explained by the use of different bacterial strains and experimental techniques. This also applies to the negative results obtained by Zayeva (1953) in the treatment of mouse tumours with certain bacterial toxins.

5. THE EFFECTS OF BACTERIAL PREPARATION 221 ON TRANSPLANTABLE AND SPONTANEOUS MOUSE TUMOURS

In the course of our search for new biotherapeutic antineoplastic preparations of microbial origin we were able to obtain from *B. subtilis* the so-called preparation 221. First experiments carried out in collaboration with Milovanova on the Crocker sarcoma showed the considerable effectiveness of this preparation when used in the normal screening routine

for antitumour preparations of microbial origin: the tumour is implanted, and injections of the preparation are started after 24 hours. These experiments led us to test the preparation on spontaneous mouse tumours, for which we used 21 mice with very large tumours. The results of the use of preparation 221 against spontaneous tumours were: tumour enlargement in 6 mice, cessation of growth in 6 and decrease in size in 8 mice.

Nos. of mice	Original size of tumour (cm)	Final size of tumour (cm)	Duration of treatment (days)
3	2.0×1.6	1.5×1.5	28
8	1.9×1.9	pea-sized (sic)	25
9	a) 1.5×1.4	1.0×1.8	17
	b) pea-sized	tumour disappeared	—
12	1.8×1.2	1.2×1.0	14
14	3.0×1.9	2.0×1.4	26
19	1.1×0.9	0.7×0.7	8
24	1.6×1.4	1.1×0.7	19
5	2.3×1.6	1.4×1.4	31

Tumours decreasing in size under the influence of preparation 221 were studied histologically. The action of this preparation was typified by the formation of extensive cavities with sero-sanguineous contents. The breakdown occurring in the tumour is accompanied by changes in the cancer cells, nuclear pyknosis, a fall in the number of mitoses or a complete absence of mitosis. In summing up the histological observations we were bound to note that the microscopical appearance of tumours treated with preparation 221 is essentially different from that described by us for tumours submitted to the effects of the trypanosome preparation.

These experiments may usefully be considered in association with Gregory's observations (1950), which showed that destruction of the "cancer virus" may occur under the influence of an antibiotic from *B. subtilis*. Gregory observed this process in cancer cells under the electron microscope *in vitro*. By chemical and electrophoretic methods he obtained from extracts of the bacterium a crystalline material which he called "magnesium tracinate". The crystals were administered to eight patients with advanced cancer, when clinical improvement was observed.

6. THE INFLUENCE OF BACTERIOPHAGE ON MALIGNANT TUMOURS

In recent years investigations on cancer antibiotics have involved not only various species of bacteria but also bacteriophage and other viruses.

In experiments with coli-phage, Bloch (1940) noted that the phage is preserved in mouse carcinoma and fowl sarcoma tissues for longer than in normal tissues, some of the tumours losing their powers of further transplantability. However Koch (1943) could not support this finding. At the same time, Vollmar and Knoll (1944), studying the effects of coliphage on mouse tumours, concluded that it has an inhibitory effect on cancer tissue. At our suggestion, Mikhailova carried out an investigation of the effects of dysentery phage (obtained from the Mechnikov Institute) on Sarcoma 180; for 10 days, starting from the day of implantation of the tumour, dysentery phage was injected in doses of 0·2 ml into mice in a region remote from the site of the sarcoma implant. These experiments enabled her to draw the following conclusions: (1) under the influence of dysentery phage there is inhibition of the development of sarcomata, but no cases were seen where tumour growth was halted; (2) heat-inactivated phage has no inhibitory effect on the development of Sarcoma 180. It should be added that these conclusions cannot be confined to the simple statement that there is a noticeable inhibitory effect on the tumour. A large number of substances of varying chemical nature are known to have some inhibitory effect on experimental tumours. If, however, we take the view that phage is a living substance, the whole question becomes far more significant, since in this case we are touching upon one of the most fundamental problems of biology—that of biological antagonists—irrespective of how we ourselves wish to view the matter: as a problem of antagonism between phage and the cancer cell or of antagonism between the hypothetical virus of the cancer cell and phage. In spite of the great theoretical and possibly also clinical significance of the study of phage in oncology, this work has only just been begun.

7. THE INFLUENCE OF VIRUSES ON MALIGNANT TUMOURS

Levaditi and Nicolau (1922) first showed that certain viruses (yellow fever, fowl pox) may become selectively concentrated in tumours. Other workers have established that filterable viruses may infect malignant tumours and exist in them for considerable periods. These circumstances lead us to believe that malignant cells are unable to develop an immunity to a number of filterable viruses. The presence of these viruses in the tumour tissue has no noticeable effect on the growth of the tumour and the malignant cells undergo no apparent change in their life-processes. However, Andrews (1940) states that the multiplication of cancer cells may be somewhat suppressed by the presence of virus in the tumour. In 1949 Shen, studying

in Moscow the problem of symbiosis between viruses and malignant tumours, established that the Scottish encephalitis virus actively multiplies in mouse sarcoma cells and in doing this produces a somewhat lower degree of proliferation of the tumour on subsequent transplantation into normal mice. The American encephalitis virus multiplies in mouse sarcomata and carcinomata but does not affect subsequent development of the tumours.

Shen observed that under the influence of a virus there were signs of early necrosis in sarcomata and carcinomata; sometimes vacuolation occurred, with a decrease in the staining properties of the tumour cells. However, as well as the degenerating cells many well-preserved cancer cells remain, many of them in a state of active division. Shen adds that the presence of a virus in a tumour does not usually induce inflammatory phenomena.

Turner and Mulliken (1947), having studied in detail the effects of pox virus on Sarcoma 180, drew two main conclusions; firstly, the pox virus may have a marked affinity for this tumour and may reach a high concentration in it, living in it for an unusually long period without causing any noticeable harm to the host; secondly, tumours containing large amounts of virus grow more slowly than usual after transplantation. With regard to the multiplication of viruses within neoplastic cells, the ability of viruses to become concentrated in rapidly multiplying normal tissues, adult and embryonic, is well known.

It should also be mentioned that the viruses of influenza and herpes (Moore, 1949) have little influence on the growth and development of experimental tumours, although they may in fact become selectively concentrated and exist in malignant tissues. Results differing completely from those mentioned above were obtained in experiments by Roskin and Banag (1951), when mice with transplanted Sarcoma 180 were infected with the Russian Far East encephalitis virus. On the day following implantation the mice received intraperitoneally a previously titrated sublethal dose of Russian Far East encephalitis virus. These experiments enabled the following to be established:

(1) The Russian Far East encephalitis virus becomes selectively concentrated in malignant tumours.

(2) Under the influence of this virus there is marked inhibition of the growth of the tumours, or they disappear completely; for example, in one experiment 1 of 15 transplanted sarcomata showed marked increase in size, 7 were of hardly measurable proportions, the size of a small seed (their weight at the end of the experiment did not exceed 0.02–0.05 g)

and in 7 mice the tumours disappeared, whereas normal growth occurred in control animals.

(3) The effect of Russian Far East encephalitis virus on the tumour depends upon the dose administered to the experimental animal: only massive (sublethal) doses have any clear antineoplastic effects.

(4) Under the influence of the virus profound histophysiological and cytological changes occur in the tumour cells.

During the course of this investigation we became acquainted with the work of Moore (1949), who after a long search for viruses having an antagonistic effect on tumours drew conclusions similar to our own: the Russian Far East encephalitis virus selectively destroys malignant cells. In Moore's experiments, however, only lethal doses of the virus were active, sublethal doses having no effect at all on the growth of Sarcoma 180, whereas we were able to obtain clearly marked inhibition of its growth by injecting sublethal doses of the virus. Moore also established that virus injected subcutaneously in mice first appears (at high concentrations) in the tumour; 5 days after injection the virus is found in equal concentration in the tumour and the brain. In a number of papers (1949, 1950, 1951, 1952) she has described in detail the effects of Russian Far East encephalitis on many types of transplantable tumours. Not only Sarcoma 180 is destroyed by the virus. Of 13 tumours studied, 6 types were completely destroyed: (1) fibrosarcoma MC 1, induced by methylcholanthrene; (2) mammary adenocarcinoma EO 771; (3) Sarcoma G241; (4) carcinoma 1025; (5) Ridgway osteogenic sarcoma; (6) neuroblastoma C 1300. The virus had a weak effect on a lymphosarcoma and two strains of leukaemia, and no effect at all on the other tumours (Harding–Passey melanoma, sarcoma Ma 387, squamous-cell carcinoma and the Wagner sarcoma). The first five tumours mentioned differed in that some of them needed greater amounts of virus to produce tumour destruction. Moore states that the reasons for these different effects in different tumours are still unknown; it may only be supposed that the cells of susceptible tumours contain a substance essential for virus multiplication, and that destruction of the cancer cells occurs when the virus begins to "compete" with the cells for this hypothetical substance. Since this particular virus is neurotropic, it may be expected that the substance is also found in nerve cells. Moore also established that continuous passage of the virus from tumour to tumour increases its ability to destroy Sarcoma 180 (from 20 to 100 per cent of inoculated tumours). On the other hand, brain passage leads to a gradual loss of the oncolytic properties of the virus. On prolonged passage (80–90 passages) through osteogenic sarcomata the virus started to

cause complete tumour lysis, whereas without previous passages the virus had hardly any effect on the tumours. Moore's work has been confirmed and developed by a number of investigators. Koprowski and Norton (1950) showed that certain other neurotropic viruses possess similar properties: the St. Louis encephalitis virus, Japanese encephalitis virus, Venezuelan encephalitis virus and West Nile encephalitis virus. The last two viruses are active against lymphosarcomata, osteogenic sarcomata and epithelioma EO 771. Buckley, Buckley and Snipes (1951), studying the effects of the St. Louis encephalitis virus on Sarcoma 180, established that this virus can enter the tumour and multiply in it, but only for a limited period. On injecting the virus into mice with 4-day tumours there was some lowering of the growth rate of the tumours and a noticeable prolongation of the survival-time of the tumour-bearing mice. Under the influence of the virus increased signs of pyknosis were visible in the tumour cells, with a fall in the number of mitoses and increased necrotic areas. Tumours infected with the virus failed to grow after transplantation in 5 of 10 cases, i.e. the virus had a marked effect on the viability of Sarcoma 180 tissue. Sharpless, Davies and Cox (1950) found that the viruses of Russian and West Nile encephalitis can cause regression not only of Sarcoma 180 but also of malignant lymphoid tumours of chickens, without any apparent harm to these animals. Other authors (Southam, Bronstein and Webber, 1951) have established that the West Nile virus can produce temporary inhibition of leukaemia in mice, although the life of the animals is not prolonged. The work carried out on the effects of viruses on transplantable tumours also includes attempts to use viruses in the treatment of malignant neoplasms of the human patient. Bierman and his co-workers (1950) observed a positive effect of the feline agranulocytosis virus on the course of leukaemias in humans. Higgins and Pack (1951), injecting rabies virus with therapeutic intent into 30 patients with melanomata, observed a decrease in size and consolidation of dermal metastases in 6 cases, while in the authors' opinion the development of further metastases was arrested. Southam and Moore (1952) carried out observations on a large number of cancer patients to whom neurotropic viruses had been administered with therapeutic aims. No prolonged effect was achieved in a single case, but there was temporary regression of tumours in 34 patients injected with the Egypt encephalitis virus. Toolan and Moore (1952) claim that Egypt 101 virus has a destructive effect on epidermoid carcinomata in the human patient. Finally, Southam and Moore (1954) injected intramuscularly West Nile virus therapeutically into 84 patients with various malignant neoplasms. The following observations were made:

Intensity of viral infection	Tumour tropism of virus	Tumour lysis
+ in 36 0 in 21 ± in 12	absent in 6 positive in 36 selective in 14 (only in tumour)	negative in 56 cases doubtful in 10 cases positive in 6 cases

These results give no indication of survival time or of any real or lasting changes in the course of the conditions. On the other hand, Birth (1955) mentions in his review article an experiment involving inoculation of virus into 128 patients with various malignant neoplasms, when marked improvement was seen in occasional cases. Virus therapy—writes Birth—is still in the stage of overcoming the initial difficulties, but much progress may be expected in the next few years. The American author Sebin, in his paper given in Moscow (1956), also mentioned this question, and expressed the opinion that it may become necessary to inject in turn not one but two or more different viruses in the aims of cancer therapy. Taking all this into account, it would be wrong to consider this field of cancer biotherapy to be in any way closed. The most interesting work on the influence of viruses on human and animal tumours is only just beginning.

8. FILTRATES OF FUNGAL CULTURES AND THE PRODUCTS OF YEAST CELLS

Among work in this field that devoted to *Aspergillus fumigatus* is particularly worthy of attention. Kidd (1947) showed that fragments of the Brown-Pearce rabbit carcinoma were rendered nonviable after immersion in a crude culture filtrate. In Meyer's experiments (1947–1948) fragments of a transplantable mouse tumour were treated by immersion in a filtrate from a culture of *Aspergillus flavus*. The filtrate was effective in retarding the growth of the cancer cells, this effect being shown on the 3rd day of cultivation, reaching a maximum on the 7th day and then disappearing spontaneously by the end of the 8th day. This antineoplastic effect is ascribed to a gliotoxin, which is also present in filtrates from cultures of *Gliocladium* and according to Kidd (1947), is capable of inhibiting the development of the Gardner mouse lymphosarcoma.

A study of the influence of metabolic products from cultures of various strains of *Penicillium, Aspergillus, Actinomycetes and Basidiomycetes* on the development of carcinomata, sarcomata and melanomata in mice was

the subject of an investigation by Stock, Sugiura and Rhoads (1949). It was shown that culture filtrates of *Penicillium notatum in vitro* act not only on carcinomatous tissues but also have a clear inhibitory effect on the growth of the transplantable Sarcoma 180, although they have only a weak effect on spontaneous mammary tumours of mice. In distinction from bacterial polysaccharides (for example *B. prodigiosus*), filtrates from such cultures do not cause haemorrhages in the tumours.

Reilly and Stock (1951) isolated from culture filtrates of *Aspergillus fumigatus* a factor, protein in nature, having a marked inhibitory effect on Sarcoma 180 of mice, but this substance was extremely toxic. Further purification of the active principle produced no noticeable decrease in its toxicity for mice. It was established by electrophoretic methods that the substance is related to a group of proteins with highly basic properties (Petermann, Hamilton and Reilly, 1952). While discussing *A. fumigatus*, we must mention earlier experiments (McLeod and Ravenel, 1938) on treatment using extracts of *A. niger* and *Saccharomyces cerevisiae*: decrease in tumour size was seen in a number of cases of advanced cancer in human patients. Pieces of tumour taken at biopsy showed degeneration and necrosis of the tumour tissue. However, this work includes no detailed description of the clinical and laboratory observations, or a description of the means of preparation of the extracts used for treatment.

The difficulties in solving the problems arising here (not only with regard to mould products but also all antineoplastic antibiotics) are conditioned by a whole series of reasons. One of them may be illustrated by an example taken from Reilly's paper (1953): of seven isolated strains of *A. fumigatus*, five contained cancer-inhibitory substances in culture filtrates while two strains were completely inactive. Another reason is that the nature of their substrate and conditions of cultivation may have a great deal of influence on microbial metabolism and on their ability to form specific substances. This very real circumstance explains the contradictions which constantly arise in the assessment and checking of the results of any work in the field of antiblastic antibiotics.

* * *

Among other work in this section of the biotherapy of experimental tumours one should include that of DeAngelis (1949), who suggested the use in clinical oncology of a commercial preparation "mycetin" for the treatment of malignant tumours in man, and also for preoperative and postoperative treatment or treatment in combination with radiation therapy,

etc. "Mycetin" was obtained from filtrates of adult cultures of the fungus *Streptothrix felis*.

It should be mentioned that the minimum lethal dose of "mycetin" is very close to the therapeutic dose. For this or other reasons there has been no further development of this experimental work, not to mention clinical observations.

* * *

Attempts to use various species of yeasts or their metabolic products for the treatment of malignant tumours have been going on for some time (see Petrov, 1947). Earlier work in this direction included that by Nevorozhkin (1935), who showed that when a dense suspension of the yeast *Saccharomyces cerevisiae* No. XII (Berlin race) was injected directly into the Ehrlich adenocarcinoma of mice tumour regression occurred in a number of cases.

More recently, various papers (for example Protti, 1948–1950) have indicated the oncolytic effects of living enzymes from different saccharomycetes. Castelli and Gaggini (1949) observed that on contact *in vitro* between the cells of a mouse adenocarcinoma and a number of species of blastomycetes there is lysis of a considerable proportion of the cancer cells. Among yeasts, the authors noted particularly *Oidium albicans*. On intraperitoneal injection of this yeast into mice with adenocarcinomata tumour development was arrested and tumour necrosis took place in some cases. Castelli and Gaggini consider that (a) *O. albicans* has a lytic effect on the cells of mouse adenocarcinomata, (b) this yeast does not affect normal cells, (c) its lytic activity is enhanced by passage through mice — *O. albicans* is capable of suppressing mitosis of tumour cells when injected into cancer-affected mice.

9. THE EFFECTS ON MALIGNANT TUMOURS OF ANTIBIOTICS PRODUCED BY THE PHARMACEUTICAL INDUSTRY

The widespread application of penicillin in general therapy could not but lead to corresponding work in experimental oncology. Cornman (1944) observed that crude penicillin has a marked lethal effect on mouse tumour cells in tissue culture without damaging normal cells. Beard (1944) established that crude penicillin not only acts on malignant cells in tissue culture but also causes regression of tumours in about 20 per cent of cases.

Dobrovolskaya-Zavadskaya (1946) also confirmed these observations on penicillin in experiments with mouse tumours and noted the appearance

of an intense hyperaemia in the malignant tissues. This effect was only temporary, and if the antibiotic therapy was continued (sic) the tumour began to grow again. In one patient with disseminated mammary adenocarcinoma she observed the same effects on penicillin treatment as had occurred in mice. The activity of crude penicillin with respect to certain tumours may be connected with the fact, as shown by Burk, Hesselbach and Fischer (1947), that amorphous penicillin brought about marked inhibition of respiration in mouse tumours and normal tissues, whereas crystalline penicillin was only one-tenth as active. However, crude penicillin is not effective against all transplantable tumours: Bennison (1949) could observe no activity of impure penicillin on mammary tumours in mice caused by the milk factor. If purified penicillin is inactive against tumours, we are left with the question of which accompanying substance possesses the antineoplastic activity. A number of antibiotics have recently been used in experimental oncology.

Antibiotic	Tumour	Result of experiment	Author
Clavacin (patulin)	Sarcoma 180	Sarcoma cells become nonviable when tumour fragments immersed in the antibiotic	Stock, Sugiura and Rhoads (1949)
Aureomycin	Rat carcinoma	Small doses stimulate, large doses inhibit carcinoma growth	Sokoloff and Eddy (1951)
Actinomycin and citrinin	Sarcoma 180	Weak suppressing effect on sarcoma growth	Stock (1950)
Terramycin, aureomycin and neomycin	Rouse sarcoma in tissue culture	Inhibition of growth	Chinn (1952)
Actinomycin, actidione, illudin M, illudin S, terramycin	Sarcoma 180	Weak inhibition of tumour growth	Reilly, Stock, Buckley and Clarke (1953)
Actinomycin C	Rat carcinoma	Weak inhibition of tumour growth	Hackmann (1952)
Azaserine	Sarcoma 180	Inhibition of tumour growth	Stock, Reilly, Buckley, Clarke and Rhoads (1954)

Culture filtrates of 1068 different soil microorganisms have also been investigated. Using 5059 filtrates, 878 organisms (82 per cent) gave negative

results, a weak inhibitory effect was shown by 174 (16.2 per cent) and 16 (1.5 per cent) produced a moderate inhibitory effect, i.e. the average diameter of the treated tumours was $\frac{1}{4}$–$\frac{1}{2}$ that of the control tumours (Sarcoma 180) (Reilly and Stock, 1954–1955).

Aureomycin was used by Bateman, Klopp and Barbario (1952) as an adjuvant in the treatment of inoperable tumours by X-rays; these authors claim that in five cases the tumour was reduced to a size suitable for surgical interference. An experiment on 17 patients involved intraperitoneal injections of nitrogen mustard and aureomycin; Bateman and co-workers (1953) state that four inoperable patients could then be classified as operable. Finally, Bernard and Kessler (1953) observed clinical improvement in a number of cancer patients treated with aureomycin (and terramycin) in combination with ACTH.

A number of investigators have drawn attention to the antineoplastic properties of *stylomycin*—6-dimethylamino-9-(3′-paramethoxy-L-phenylalaninamino-3′-deoxy-β-D-ribofuranosyl)-purine. Troy *et al.* (1953, 1954), studying the effects of stylomycin on nine different experimental tumuors, showed that it has a marked effect on glioblastomata and mammary adenocarcinomata of C3H mice. The antitumour activity of this antibiotic is related to the aminonucleoside part of its molecule (Bennet, Haliday *et al.*, 1954, 1955). Amino-acid analogues of stylomycin were tested on mammary adenocarcinomata in C3H mice. Of 21 stylomycin derivatives, 5 compounds had an inhibitory effect in 50 per cent of the cases, 6 compounds in 25–80 per cent and the rest were ineffective. The activity of the derivatives was affected by the optical configuration of the amino-acid and also by the amino group substituent, the position of the amino-acid and the nature of the amino-acid itself. According to Wright, Dolgopol *et al.* (1955) prolonged administration of this antibiotic to rats causes loss of weight and liver and kidney affections. Stylomycin has been used in 51 patients with inoperable tumours of the oral cavity, oesophagus, stomach, intestine, lungs and mammary gland. In 15 patients there was temporary decrease in tumour size, but this was not accompanied by improvement in the patients' general condition. Little is known of the mode of action of stylomycin on cancer cells. Investigators have concentrated on the antagonism between stylomycin (and closely related compounds) with biologically important purine compounds, and also the disturbance of nucleic acid metabolism by this antibiotic (Khokhlov and Vikhrov, 1956).

In recent years much attention has been given to cancer antibiotics isolated from certain species of actinomycetes. This work has been conducted simultaneously by groups of Japanese, German and American investiga-

tors. Most of the experiments and clinical observations have been carried out with azaserine, sarcomycin and actinomycin.

A recently published report by Stock, Reilly, Buckley, Clarke and Rhoads (1954) states that the antibiotic *azaserine*, obtained from culture filtrates of a species of streptomycete, (Parke, Davis and Co., 1926) has an inhibitory effect on the growth of the transplantable tumour Sarcoma 180. Azaserine (0-diazoacetyl-L-serine), in Cardinali's opinion (1955), is a serine antimetabolite—this group of substances is very important, since it is related to nucleic acid metabolism. A similar preparation produced by Fusari *et al.* (1954) has given some promising experimental results. Skipper *et al.* (1954), analysing the mode of action of azaserine, showed that it inhibits the inclusion of ^{14}C into nucleic acids, but unfortunately this occurs in both normal and cancer-affected mice.

Azaserine has a marked inhibitory effect on mouse adenocarcinomata, lymphosarcomata and leukaemia of mice, and also on the Flexner-Jobling tumour in rats, provided that treatment is commenced within 24 hours after implantation of the tumour. In these experiments azaserine was usually injected intraperitoneally in doses of 2–10 mg/kg daily for 7 days. However, under similar conditions no tumour-retarding activity could be demonstrated against the Brown-Pearce carcinoma of rabbits, glioma 26, sarcoma T-241 and a number of other tumours of laboratory animals. It is interesting to note that when treatment was commenced later, for example 7 days after implantation, marked inhibition of tumour growth was only seen in the Crocker sarcoma, while other tumour types grew just as rapidly as in the control animals (Stock, Reilly *et al.*, 1954; Clarke, 1955; Sugiura, 1955). One important disadvantage of azaserine is its toxicity: therapeutically active doses cause liver changes, disturbance of bone marrow function and lesions in the intestinal mucosa.

Clinically, on administration of azaserine in doses of 8–10 mg/kg per day (intravenously or *per os*) there was no significant improvement in the condition of cancer patients; 5–20 days after the start of the treatment many of them suffered from nausea, vomiting, symptoms of liver disturbances and leucopenia (Ellison *et al.*, 1954). Another cancer antibiotic, the so-called DON (6-diazo-5-oxo-1-norleucine), isolated from cultures of an actinomycete (the exact species has not yet been determined), is very similar to azaserine in its therapeutic activity and toxic properties. DON has given promising results in experiments on various transplantable tumours. In clinical trials involving 37 patients with various malignant tumours temporary improvement was seen in 4 patients (1 case of lymphoma, 1 melanoma and 2 carcinomata) (see Khokhlov and Vikhrov, 1956, 1957).

Yumesawa and co-workers (1953, 1954) isolated an antibiotic *sarcomycin*, having marked antineoplastic properties, from agar cultures of a strain resembling *Actinomyces erythromogenes*. The most promising experimental results were in mice with the Ehrlich carcinoma, using intraperitoneal injections of 2.5 mg per mouse daily (from the second day after implantation) for 18 days. After recalculating their results to correspond with our own index of effectiveness sarcomycin was found to have an activity index of 2.4, i.e. the average tumour weight in the experimental group was 2.4 times lower than in control animals.

After the administration of 250 mg/kg sarcomycin 5 hours after implantation of 5 different ascitic tumours into rats and mice there was marked prolongation of the lives of the treated animals, varying with the different types of tumour (Oboshi *et al.*, 1955). The same authors consider that sarcomycin is not a specific mitotic poison nor an antimetabolite, but has a specific destructive effect on cancer cells both during division and in interphase.

Takeuchi and co-workers (1955) determined the effects of sarcomycin on the Ehrlich carcinoma; on transferring their results to our activity index this becomes 2.6, when treatment is started not later than 24–72 hours after implantation of the tumour. The administration of sarcomycin prolonged the lives of treated mice by 62–65 per cent, whereas experiments with the Yoshida sarcoma and Hirosaki sarcoma gave no positive results. Experiments with cancer cells in tissue culture showed that the addition of glutathione significantly decreases the toxicity of sarcomycin while only slightly decreasing its activity against cancer cells.

Sarcomycin has not yet been isolated in pure crystalline form. According to the literature (Hooper *et al.*, 1955) the active antitumour component of sarcomycin is 2-methylene-3-oxocyclopentane-carboxylic acid. Sarcomycin has been used clinically and has produced objective and subjective improvement in 7 of 11 children with malignant tumours. Especially noteworthy was the arrest of tumour growth in cases of sympathogonioma and microsarcoma and clinical improvement in leukaemia. However, more significant changes were not seen, and the authors consider sarcomycin to be of use only as an adjuvant to surgery and X-ray therapy (Fujii *et al.*, 1955). Sarcomycin was also used in 2 cases of cancer of the *cervix uteri*. One patient was given 52 g over 60 days, the other 117.5 g over 128 days. However, no real clinical results could be claimed (Momose and Kobayashi, 1955).

Among the few positive clinical results given by sarcomycin is that obtained in a patient with a malignant tumour of the *cervix uteri* (chorion-

epithelioma). Two months after operation radioscopy revealed metastases in both lungs. Sarcomycin treatment was instituted and continued for 12 days. The blood-tinged discharge ceased, and the patient gained weight. After a second course of therapy, during which the patient received 15 g sarcomycin, there was further improvement in her condition. Radiographs showed diminution of the lung metastases. Two months after the end of the antibiotic treatment radiographs again revealed a shadow in the right cardiac lobe. After a third course of sarcomycin therapy (32 g) the lung shadow disappeared and the patient gained 4 kg in weight. Together with this report we may include the work of Magill and co-workers (1956), who state that sarcomycin in doses of 26–210 g given intravenously produced no significant changes in the condition of 26 patients with various forms of disseminated cancer.

A number of experimental and clinical observations have been made on the antibiotic *carcinophyllin,* isolated from cultures of *Streptomyces sahachiroi.* This antibiotic, which has not yet been isolated in a chemically pure form, has a marked effect, even after a single injection, on the Yoshida sarcoma, Ehrlich adenocarcinoma and ascitic hepatoma. Much interest has been aroused by experiments on the Yoshida sarcoma, which on implantation into the peritoneal cavity of mice causes death in 6–10 days. In this instance the administration of carcinophyllin at a dose of 960 units per day (not later than the second day after implantation) significantly depresses tumour growth, and treated animals lived for more than 40 days. The administration of carcinophyllin later, after 4–6 days, had no noticeable effects on the tumour. In clinical trials carcinophyllin was given intravenously in 5% glucose, starting with doses of 2000 units, gradually increasing to 10,000 units daily. Each patient received an average of 200–300,000 units over the course. Subjective improvement occurred in 3 of 28 advanced cases and objective improvement in 8. Marked diminution of the tumour was seen in 2 cases of reticulosarcoma. All these changes were of a temporary character. The most clear-cut result was obtained in the treatment of a patient with skin cancer, in whom an ulcer became cicatrized after all other means of treatment had failed. Carcinophyllin produced clinical improvement in a case of Hodgkin's disease. This antibiotic is toxic: the patients showed a marked leucopenia (Hata *et al.,* 1954; Shimada *et al.,* 1955).

Much more work, with more positive results, has been carried out with *actinomycin C.* This antibiotic was isolated in 1949 by Brockmann, who also determined its approximate chemical formula. Actinomycin C is apparently very similar to the actinomycin A isolated earlier by Waksman. Actinomycin C itself probably comprises three substances, known as C_1, C_2 and C_3. (Hackmann, 1955). It has marked lymphotropic properties. It has a particular cytostatic effect on lymphoid tissue, producing abnormal mitoses. This has given rise to its use in malignant lymphogranulomatosis and leukoses. Actinomycin may be obtained from various actinomycete species (*Streptomyces antibioticus, parvus, chrysomalus,* and others) and it is produced under several names — A, B, C_1, C_2, C_3, J_1, J_2 and X.

Clinically, the most interesting of these is actinomycin C. It is active against gram-positive bacteria. The cytostatic effects of the antibiotic are particularly well shown as nuclear disturbances in spleen cells. Actinomycin has an anti-allergic action, as confirmed by its beneficial effects in bronchial asthma (Businco, 1955). Hackmann (1949–1950) first established the inhibitory effect of actinomycin on tumours of rats and mice. Crystalline actinomycin C, according to Field and co-workers, is obtainable from culture filtrates of *S. chrysomalus,* and has an inhibitory effect on Sarcoma 180. On administration of 100 mg/kg actinomycin C the average tumour size was 46 per cent of that in controls, and on administration of 50 mg/kg per day it was 70 per cent. Actinomycin C produces considerable disorganization of the tumour tissue. When mice with the RC carcinoma were given 80 mg/kg actinomycin daily the average tumour size was 52 per cent of that in the controls, and 74 per cent when the dose was 50 mg/kg per day. Actinomycin prolongs the life of mice inoculated with leukaemia L 4946. Doses of more than 50 mg/kg daily cause serious liver changes in mice. On transferring the results obtained by these workers to our own system, the activity index of actinomycin is shown to be not greater than 2. This must be considered in relation to the effectiveness of other cancer antibiotics.

Schulte (1952) used actinomycin C (in some cases in combination with X-ray therapy) in 150 cancer patients. It had little effect on carcinomata and a more marked action in 50 patients with lymphogranulomatosis. The patients each received 50–250 μg daily for several weeks. They gained weight, and their clinical condition improved. Superficial tumours decreased rapidly in size, but mediastinal tumours appeared to decrease only slightly. Schulte rates the anticancer activity of actinomycin C very highly, but it should be noted that his observations have covered only a short period, lasting no more than $1^1/_2$ years.

Starting in November 1953, Croizat and Lacoste (1955) treated 44 patients: 23 with lymphogranulomatosis, 6 with lymphosarcoma, 10 with reticulosarcoma, one with a lung neoplasm, and some others. Most of

these patients had been treated previously (radiotherapy, nitrogen mustard). Intravenous injections of 300 μg were given to women, 400 μg to men and 200 μg to a child aged 4 years. The results were in general much poorer than those achieved by combined nitrogen mustard and radiotherapy. The authors considered, however, that the combination of actinomycin and radiotherapy enables the dose of radiant energy to be decreased.

Doer and Stein (1954) established that after treatment of Hodgkin's disease with actinomycin C (9000 gamma) there was extensive fibrous cicatrization in affected nodes.

Gregory and co-workers (1956) observed a significant therapeutic effect when actinomycin D was injected into mice with the ascitic form of Sarcoma 180 and lymphosarcoma.

Further findings regarding actinomycin C are summarized in the following table.

Disease	Results of treatment	Authors
6 cases of Hodgkin's disease and 1 of reticulum cell sarcoma	In 2 cases, noticeable regression of lymph nodes and diminution of spleen Stomatitis and thrombocytopenia.	Trounce *et. al.* (1955)
1 case of hypernephroma	Regression and disappearance of lung metastases and improvement in general condition; treatment continued for 9 months.	Schmidt and Watrin (1954)
12 cases of Hodgkin's disease, lung cancer and a number of cases of reticulum cell sarcoma	Weak and transient effect on Hodgkin's disease, no effect on lung cancer, but significant therapeutic effect on reticulum cell sarcoma, the duration of which is still difficult to determine	Ravina and Pestel (1954)
30 cases, including 24 of Hodgkin's disease	Temporary improvement. Authors suggest combination of antibiotic with radiotherapy.	Huguenin, Truhaut and Bourdin (1954)
15 cases of Hodgkin's disease	No effect	Bertrand-Fontaine, Mallarme and Schneider (1954)
16 cases of Hodgkin's disease, 6 of lymphosarcoma, 4 of reticulosarcoma, 1 epithelioma	Partial and mainly transient improvement	Croizat (1954)
2 cases of agranulocytosis	No effect, in spite of high doses of antibiotic used	Jambon, Bertran and Carli (1955)
7 cases of various types of malignant neoplasm	Permanent improvement only in 1 patient (laryngeal epithelioma), transient improvement in another; no effect in remainder	Tapie (1955)
Reticulum cell sarcoma	Negative result	Gilbert and Thommen (1955)
3 cases of Hodgkin's disease, 1 of lung cancer	Clear but temporary improvement in 1 case of Hodgkin's disease; slight diminution of primary nodule in lung cancer, without noticeable influence on general course of the condition	Gernez-Rieux and Gondemand (1954)

Such are the main results, very interesting but still far from complete, of work on the use in experimental and clinical oncology of antibiotics produced by the pharmaceutical industry.

In summing up the work on cancer antibiotics and on actinomycin C in particular we are bound to admit that this work shows great promise, but it is still in the very first stages of experimental investigation and clinical trial.

We must now consider the last section—a summary of the work on a cancer antibiotic obtained not from plant cells (to which all the previous substances relate) but from animal cells, namely a preparation* from a species of trypanosome *(Trypanosoma cruzi)*.

10. A CANCER ANTIBIOTIC FROM *TRYPANOSOMA CRUZI*

Almost 25 years ago a group of Moscow biologists, followed by clinical workers, started work on the study of an antiblastic preparation derived from *Trypanosoma cruzi*. With considerable interruptions, this investigation has continued since then and is still being carried out.

The material set out in our earlier reports has been studied experimentally by a number of workers in various countries — we shall deal with this in more detail later. In 1946, the considerable volume of experimental results and the first, very limited, clinical observations were described in our book *The Biotherapy of Malignant Tumours*. Further investi-

* Waksman, in his article "Antibiotics" (*Biol. Rev.* 23: No. 4, 1948) includes the trypanosome preparation among the antibiotics.

gations have shown that the trypanosome preparation is of an antibiotic nature.

The use of the trypanosome antibiotic in the treatment of cancer finds its rational basis in the antagonism which exists between trypanosome infection and the development of malignant tumours. "Confirmation of the Russian work may be found in Chile"—wrote Barry Commoner (1946)—"where a study has been made of cancer among persons suffering from the chronic form of trypanosome infection. Preliminary results show that cancer is rare in those parts of the country where trypanosome infection is common". The influence of trypanosomiasis on animals and humans affected by cancer has been studied by Hauschka, Saxe and Blair (1946), who confirmed our claim that "trypanosome infection considerably retarded the growth of three different types of tumours in animals and had a relatively weak effect on a fourth type of tumour". In another paper, Hauschka and Blair report that under the influence of trypanosome infection growth of the tumours was *almost completely inhibited,* but if the trypanosome is destroyed by the action of a specific drug the previously inhibited tumour growth becomes restored to its usual rate and the animals die of cancer. It may thus be considered that the antagonistic influence of trypanosomiasis on tumour development has been adequately confirmed.

Particular mention should be given to an experiment on the treatment of hopeless cases of human cancer by inoculation of *T. cruzi,* as carried out in 1950 by the three prominent French scientists Gaillard, Brumpt and Martinez, who undertook this work at the request of leading workers at a number of Paris hospitals. Seven prominent specialists from these hospitals* took part in the evaluation of the results, and also controlled the clinical observations. The authors came to the conclusion that in hopeless cases of far advanced cancer trypanosome infection brought about definite beneficial results: diminution of tumours, disappearance of malignant proliferations and scars and improved general condition. In one case of an enormous epithelioma there was clinical improvement and considerable diminution of the tumour. Pain rapidly ceased in cases of cancer of the parotid gland and tongue. In a number of other cases there was a decrease in size of the tumour mass and disappearance of nodules and scars. In reporting this work on the effects of trypanosome infection on human malignant tumours it must be noted that the French investigators used two different strains of *T. cruzi,* which differed in their effectiveness, the strain isolated by Brumpt giving the better results.

* The Bichat, Hôtel-Dieu, Salpetrière and Broussais clinics and the Paris Cancer Institute.—*Ed.*

Talice and co-workers (1954), like the French investigators, inoculated *T. cruzi* into 4 patients with inoperable tumours and observed temporary improvement in them.

A second group of investigations was devoted to attempts to obtain a trypanosome preparation. Unsuccessful attempts to do this were made by Engel (1944) and Hauschka and co-workers (1947–1948). Similar failures were recorded by Belkina *et al.* (1949) and Lob (1950). The reasons for the failures experienced by these authors probably lie in the properties of the *T. cruzi* strains with which they worked. *T. cruzi* races with differing properties (and showing different organotropisms: myotropic, neurotropic strains, etc.) were described in the literature long ago by a number of authoritative specialists. This question has been dealt with experimentally by Brand *et al.* (1949). Their paper starts with the sentence: "Klyuyeva and Roskin have reported that extracts from *Trypanosoma cruzi* and even infection with this parasite may cure certain types of malignant tumours. Other investigators such as Engel and Hauschka have been unable to confirm this experimentally, and it has been suggested that the use of different strains may have influenced their results. In view of this, it seemed desirable to find out whether in fact different strains of *T. cruzi* have different properties".

As a result of careful experiments the above mentioned authors give a positive answer to this question—*different strains of T. cruzi do* have different properties. Somewhat later, Hauschka *et al.* (1950) also worked on the question of different strains of *T. cruzi.* Cultures of 7 different strains, isolated at various times in varying geographical localities, showed considerable variations among themselves in their reactions to natural agglutinins in normal human sera. This means that different strains of *T. cruzi* have different protein carbohydrate complexes.

In the Soviet Union the question of *T. cruzi* strains has been investigated by Levinson, studying two Chilean strains—22 and 28. A characteristic of these strains is their ability to cause a chronic process (infected mice live 2–3 months), the infection showing remissions during its course, with few trypanosomes in the blood. Levinson's findings show that strains 22 and 28 differ in their inhibitory effects on Sarcoma 180, although they both cause approximately similar diseases in mice. Strain 22 undoubtedly has some antineoplastic effect, while this property is absent from strain 28. Malisoff (1947) has analysed in detail the difficulties involved in the preparation of the trypanosome substance.

In his paper read at the IVth International Cancer Congress, Malisoff described the positive results of treating experimental tumours with "a trypanosome extract having a destructive effect on certain malignant tumours". In his conclusion, Malisoff stated: "It would appear that by means of active trypanosome preparations it is possible to obtain 100 per cent regression of tumours without harm to the rest of the body"; and "Our experiments show that the trypanosome preparation is selectively active against malignant tumours". He stated that a very careful technique is required for the preparation of the trypanosome endotoxin, since *T. cruzi* is easily affected by temperature, the nutrient medium and other factors. "Also, variations in a strain may lead to the production of completely inactive extracts, as occurred with Hauschka and his colleagues". "In spite of all the efforts made to prepare trypanosome extracts of standard potency, the greatest difficulties in this field still lie in obtaining an exact account of the trypanosome itself. There may also be unnoticed deviations in the seeding techniques, slight variations in the growth and harvesting of the cultures, in the chemical purification and in the conditions of lysis, all of which go to determine the activity of the preparation".

Summing up his experiments on the treatment of experimental tumours, Malisoff claims "It would appear that 10 or more daily injections of the active preparation can produce 100 per cent regression of tumours without damage to any other organs.... When tumour regression takes place under the influence of the preparation, this effect occurs rapidly and in direct association with the injections". Malisoff's overall conclusion was: "Our experiments show that the endotoxin from *T. cruzi* has a selective lysing effect on malignant mouse tumours, namely spontaneous mammary carcinomata and Sarcoma 180. The endotoxin is not toxic for normal tissues both under therapeutic conditions and in concentrations 4 times as great". It should also be noted that the American author Spain and his co-workers (1948), having checked our work, obtained a positive result in the treatment of spontaneous malignant mouse tumours, but only in those measuring less than 1 cm. These authors write: "We established a degree of tumour inhibition sufficient to warrant further careful study".

The best results were obtained in 1950 by the French scientists Coudert and Judin, at the French National Institute of Health. Their report, the outcome of a 3-year study, starts "For 50 years many investigators of all countries have studied the effects of the various substances able to induce, accelerate or propagate the growth of malignant tumours of laboratory animals. In a new line of experimental clinical research—the biotherapy of malignant tumours.... the investigators were Moscow scientists. As early

as 1932, they claimed that *T. cruzi* selectively parasitizes and destroys tumour cells. Klyuyeva and Roskin have extracted from *T. cruzi* a substance possessing similar properties". Further, Coudert and Justin write: "Control experiments have been carried out in several countries: in France they started with Rudali's thesis (Paris, 1941), which gives a rather general description of the experiments. Much more significant experimental work was done in the U.S.A.: Malisoff (1947); Spain *et al.* (1948); Hauschka and Blair (1948). The first of these authors drew some hopeful conclusions, the others reported varying results, without, however, completely denying the effect".

As a result of their experiments, Coudert and Judin concluded:

(1) In those experiments where treatment with the trypanosome substance was instituted early, i.e. between 7 and 10 days after implanting the tumour, *outstanding* results were obtained in 60 per cent of the cases (the tumours disappeared and none of the animals died). *Good* results were obtained in 30 per cent and *indifferent* results in 10 per cent of the cases. The authors consider that the trypanosome lysate has a definite effect on tumour cells, this effect being more marked the earlier in the course of tumour development treatment with the lysate is commenced.

(2) *T. cruzi* contains an active substance having a selective effect on malignant tissues. Injections made into normal tissues do not damage them at all.

(3) "The *T. cruzi* lysate apparently acts as a specific antibiotic as a result of the particular susceptibility of malignant cells, independently of their origin—this has been confirmed by recent clinical observations".

Both infection with *T. cruzi* and the cancer antibiotic obtained from this trypanosome have a broad spectrum of activity, i.e. they have a positive effect on various types of malignant tumours, as shown by the findings mentioned below. An inhibitory or "cancerolytic" effect of *T. cruzi* infection has been shown for the following types of tumours: (1) the Ehrlich mouse adenocarcinoma (Roskin and Romanova, 1931, 1935); (2) the Jobling rat carcinoma (Roskin and Romanova, 1935); (3) spontaneous mouse carcinoma (Klyuyeva and Roskin, 1946); (4) mouse Sarcoma 180 (Klyuyeva and Bobritskaia, 1946; Levinson, 1947); (5) the Brown–Pearce rabbit carcinoma (Yumasheva, 1949); (6) mouse squamous-cell carcinoma 119 (Malisoff, 1947); (7) mouse sarcoma 37 (Hauschka, 1947); (8) spontaneous mouse mammary adenocarcinoma—a relatively weak effect (Hauschka, 1947); (9) certain types of malignant tumours of man, in observations on incurable patients (Gaillard *et al.*, 1950). An inhibitory or cancerolytic effect of trypanosome substance prepared by various methods has been demonstrated in the following types of tumours: (1) the Ehrlich

mouse adenocarcinoma (Roskin and Romanova, 1935); (2) the Jobling rat carcinoma (Roskin and Romanova, 1935); (3) Sarcoma 180 (Klyuyeva and Bobritskaia, 1946); (4) mouse carcinoma 63 (Klyuyeva and Bobritskaia, 1946); (5) the Brown-Pearce rabbit carcinoma (Klyuyeva and Gintsburg, 1947–49); (6) spontaneous mouse mammary tumours (Klyuyeva and Gintsburg, 1949; Klyuyeva and Milovanovaia); (7) spontaneous mouse mammary carcinoma (Malisoff, 1947); (8) mouse Sarcoma 180 (Malisoff, 1947); (9) spontaneous mouse carcinoma, with retardation of tumours measuring under 1 cm (Spain *et al.*, 1948); (10) rat sarcoma (Coudert and Jutin, 1950). No worker in the field of experimental cancer therapy can afford to underestimate the significance of these findings. The question naturally arises as to the presence of antiblastic substances in other Protozoa. In their search for cancer antibiotics, our colleagues have therefore studied members of various groups of the Phylum Protozoa: Amoebida *(Entamoeba moschkowskii);* Infusoriae *(Paramaecium caudatum);* Haemosporidiae *(Plasmodium gallinaceum);* Trypanosomidae *(T. equiperdum, gambiense, lewisi, kohl-jakimow);* Leishmania (*L. tropica* — 2 strains). The results of these studies will be described later, but in this connection it may be mentioned that malarial infection caused by *Plasmodium bergei* can prolong the life of leukaemic mice (Nadel and Greenberg, 1953).

The extensive accumulation of material regarding the activity of a cancer antibiotic from *T. cruzi,* both against transplantable and spontaneous tumours of laboratory animals and in preliminary clinical observations, has caused us to carry out wider clinical trials of this preparation, and to work on new methods of culturing the trypanosome, the development of more active strains, and finally to discover the mode of action of this cancer antibiotic on malignant tumours of man and laboratory animals. Subsequent chapters deal with this work. Before this, however, we must consider two questions arising directly from the foregoing, and of no little theoretical importance, namely: (1) certain general aspects of the problem of antibiotics of protozoan origin, (2) the influence of certain infections on malignant tumours.

Shortly before publication of our book, we became acquainted with an article by the French worker Coudert (1956). On the basis of our work and his own experiments Coudert, in collaboration with a number of doctors, made observations on the use of a trypanosome preparation in patients with far advanced forms of cancer. The positive results of these clinical trials enabled the Mérieux Institute (Lyons) to produce in 1957 a trypanosome preparation "Trypanosa" for use in general practice for the treatment of cancer.

Part II

PROTOZOAN STIMULATORS AND INHIBITORS IN RELATION TO ANTIBIOTICS OF PROTOZOAN ORIGIN

Our observations on the inhibitory effects of protoplasmic components of *Trypanosoma cruzi* on malignant tumour cells and the presence of similar but much less effective substances in the cells of some other protozoan blood parasites have led us to consider the more general biological problem, so far hardly dealt with by investigators, of stimulator and inhibitor substances produced by protozoans. This problem is directly related to the one which particularly interests us: can protozoans serve as a source of antibiotic substances?

It has long been known that the trichocysts of a number of Infusoria, the suctorial tentacles of the Suctoria and the pseudopodia of Amoebae secrete substances capable of immobilizing rapidly-swimming small creatures. As well as these poisons, secreted specifically for defence or food capture, certain free-living and also parasitic protozoa secrete substances poisonous for various bacteria, protozoans and also the tissues of higher animals. A phenomenon of a different character has also been discovered in Protozoa.

Robertson (1924) was apparently one of the first to draw attention to the fact that in equal volumes of nutrient medium two paramaecia multiply more rapidly than one. This phenomenon was termed *allelocatalysis.* The paramaecia are apparently able to produce not only substances of the allelocatalyst type but also inhibitor substances: published reports indicate that some races of paramaecia can secrete substances which retard or even inhibit the development of paramaecia of another race, as has been shown by Sonneborn's studies (1945).

The phenomenon of allelocatalysis has a wider distribution and is not limited only to paramaecia. Merbarger (1943), observing the multiplication-rate of *Colpidium striatum* in watch-glasses (containing varying amounts of 2% proteose-peptone fluid), established that as the number

of Infusoria in the watch-glass increased, the multiplication rate rose to a maximum and then decreased. It should be noted that this decrease in old medium was not due to shortage of food. This acceleration and retardation depends, in Merbarger's opinion, on the concentration of a substance secreted by *Colpidium* which is able, according to its concentration, either to stimulate or inhibit development of the culture. This substance is destroyed by heating at 100°C for 3 hours.

Mast and Pace (1942) reached similar conclusions in their study of the multiplication of an unpigmented flagellate *(Chilomonas paramaecium)*. These Mastigophora apparently secrete a thermolabile multiplication-stimulating substance. If this substance is present in the cultural fluid in high concentrations it inhibits multiplication of the Mastigophora.

Particular interest is presented by experiments showing the existence of antibiotic factors in various species of Protozoa. The literature includes a reference to the soil infusorium *Colpoda saprophila*, which can inhibit the development of pathogenic fungi such as *Fusarium* or the mould *Penicillium expansus*. No less interesting is the observation that *Colpoda saprophila* is also able to inhibit the pathogenic effects of the bacterium *Bacillus proidae*. These findings induced Professor Brodskii (1942) to test the activity of soil Infusoria against the very dangerous parasite of many agricultural plants *Verticillium dahliae* Klebahn (the cause of so-called "wilt"), which affects cotton, potatoes, sunflowers, soya and many other cultivated plants.

For his experiments, Brodskii chose two plants—the cotton plant and the tomato—and the same genus of Infusoria *Colpoda*, various species of which abound in soils planted with Lucerne (each 1 g soil contains 30–60 thousand individuals). Numerous observations carried out over 10 days in microaquaria and in small flasks (10 ml) showed that in the presence of living Infusoria fungal spores do not germinate and form hyphae. The same effect was shown in experiments on the growth of fungal spores in a fluid (hay infusion) containing *Colpoda* killed by heating at 60°C. It is noteworthy that the findings regarding the ability of Infusoria to produce a fungicidal substance were obtained by observations on the growth of *V. dahliae* spores in culture filtrates of *Colpoda:* in the great majority of cases the fungal spores would not grow in these filtrates. Experiments with bacteriologically almost sterile Infusoria cultures gave a similar result. In those cases where the Infusoria were in the encysted state the fungicidal effect was still shown, but it was much weaker.

All these experiments confirm that the protoplasm of *Colpoda* contains a substance which inhibits the vital processes of *V. dahliae* spores, and

that this substance may be secreted by the Infusoria into the surrounding medium.

Experiments on the fungicidal effects of *Colpoda* on *Verticillium* when these were in direct contact with a test-plant (mainly tomato) also gave clearly positive results. Phytopathological examination of plants germinating in a medium containing large numbers of Infusoria and Pseudosclerosia showed clearly the absence of both fungi and signs of disease. Hence, contact of the Infusoria *Colpoda* with the Pseudosclerosia *Verticillium* deprived this organism of its sporulatory ability and protected the plants from the penetration of this infection.

In the same field of research into new antibiotics of protozoan origin the work of Gardin (1943) is of some interest; this author, observing cultures of *Oikomonas termo* grown with various bacteria, established that these Mastigophora are able to flocculate five different species of bacteria.

Other examples of antibiotic substances of protozoan origin are those obtained by McKee et al. (1947) from a number of flagellates. McKee studied the effects of lysates from the free living flagellates *Astasia klebsi, Chilomonas paramaecium, Euglena gracilis* and *Tetrahymena gelei*, all easily culturable in fluid media. To obtain lysates from these cultures, the flagellates were precipitated in the cold, suspended in one-tenth the volume of the clear supernatant fluid and then repeatedly frozen and thawed. It was shown that the lysates from the infusorium *Tetrahymena gelei* so obtained were active against *Mycobacterium tuberculosis* (1 : 300–1 : 800) and *Mycobacterium phlei* (1 : 200–1 : 670), but had no observable effect on cultures of *Staphylococcus aureus, Escherichia coli, Klebsiella pulmonum* or *Photobacterium ficheri*. Experiments showed that acetone, methyl alcohol and ether extract from the *Tetrahymena* lysates contained an active antibacterial substance of a lipoid nature. It consists of 75 per cent saturated and unsaturated free fatty acids and 15 per cent neutral fat and neutral steroid-like compounds.

McKee carried out experiments involving the treatment of mice infected with tuberculosis with the lipoid fraction of *Tetrahymena*, but obtained negative results, which the author ascribed to combination of the fatty acids with proteins or phospholipids in the body. It should be added that in *in vitro* experiments the activity of the *Tetrahymena* preparations was markedly decreased by the addition of blood or serum. Filtration through asbestos filters also destroyed completely the activity of *Tetrahymena* lysates.

Finally, we would mention the work of Koch (1943), who on infecting tissue cultures of human foetuses and chick embryos with *Trichomonas*

vaginalis established that this organism shows a positive chemotropism to explanted tissues. The trichomonads first attacked epithelial cells, then connective tissue fibroblasts. The ultimate death of the tissue culture cells is not caused by mechanical damage but by toxic products secreted by the flagellates. Filtrates of old *T. vaginalis* cultures also damaged mammalian and fowl cells.

Very recently, Chen (1955) obtained an antibiotic from *Paramaecium* (paramaecin 34) having a selective activity against other species of this genus.

All these facts go to illuminate the little-studied question of antibiotics of protozoan origin (to which the trypanosome preparation relates) and thus provide a basis for extending the search for antibiotic substances. However, they still leave open the question raised at the beginning of this chapter: are allelocatalytic substances present in *T. cruzi* and are they of any importance in its cultivation? With a great deal of probability we may give a positive answer to this question, which is of such significance in the production of the trypanosome substance. Although the literature contains few references to this subject, two relatively recent papers by Subbarow and Little (1945) and Sampath and Little (1949) state that to obtain a culture of *T. cruzi* in the medium they suggest, a very large number of trypanosomes must be seeded.

This shows that one of the conditions for the successful development of a culture is the introduction of a certain seeding dose, the lower limit of which is still very high and justly surprises microbiologists used to entirely different principles. This peculiar phenomenon may be explained if we accept the hypothesis of the presence of allelocatalytic, i.e. growth-stimulating substances produced by the trypanosomes. A significant degree of culture growth is only possible when these substances reach a certain level in the nutrient medium. This hypothesis also explains the latent period, longer than for many other micro-organisms, which every trypanosome culture undergoes and during which there is no increase in the number of trypanosomes. If this is so, we must conclude that we are dealing here with at least two factors—one stimulatory and one inhibitory.

The stimulatory substance can affect not only cells of the same species, i.e. it is not only allelocatalytic, but is capable of affecting cells of an entirely different species. This is confirmed by experiments described in the literature involving simultaneous cultivation of *Entamoeba histolytica* and *Trypanosoma cruzi:* the addition of the latter markedly stimulates development of the amoeba culture.

MacIlwain (1942) and many other investigators have shown it to be

probable that the growth-paralysing factor is chemically similar to substances essential for cell growth, and because of this similarity it becomes involved in cell metabolism, disrupting the enzymes necessary for normal intracellular metabolism. The aim of the investigator in the work we have undertaken is (1) to make a general study of the conditions of existence under which *T. cruzi* cells come to contain an inhibitor substance which may be used as an antiblastic factor, due to its properties of selective concentration by malignant cells and its ability to disturb their metabolism; (2) to remove, during preparation of the cancer antibiotic, other substances inhibiting or adversely affecting the action of the antiblastic factor.

Part III

INFECTION AND CANCER IN RELATION TO CANCER ANTIBIOTICS

OUR past and present experiments on the biotherapy of malignant tumours, a number of new and old studies by Soviet and foreign workers, a considerable amount of research work on the influence of various microbes on cancer, some of it completely asystematic and random, but particularly prominent in recent years, when the success of antibiotics had become obvious—all these investigations bring up the general problem of the interrelationship between certain infections and malignant tumours.

In order to make something of this complicated and, as far as we can tell, still unsolved problem, we should like first of all to consider the effects of some of the more fully studied infections on the development of malignant tumours. The literature includes several positive examples of cases where the inhibitory effect of infection on malignant tumours has been clearly apparent, and we shall deal with these in some detail.

The first example is an observation by Braunstein (1929) relating to the influence of malaria on malignant tumours in man. The basis of this experiment lay in the claims of certain authors regarding a visible or suspected antagonism between malaria and malignant tumours. These observations show particularly the role of malaria as a disease of long duration.

Braunstein treated 6 patients with advanced cancer by malaria injections. Almost all the patients showed enlargement of the spleen and a simultaneous reaction in the tumour after inoculation of the malaria organism, before the onset of a malarial attack. Superficial tumours showed hyperaemia and swelling, accompanied by pain. Patients with cancer of the liver and rectum complained of pain and a feeling of pressure in the region of the tumour. After the onset of the febrile stage the local symptoms increased, and there was extensive breakdown of the tumour tissues. After the end of the malarial attacks *a temporary decrease in the size of the tumours could be discerned.*

Braunstein considered his experiments as an attempt to use nonspecific "irritation" therapy, the main factor of which was stimulation of the activity of the spleen and the reticulo-endothelial system as a whole. The effects on malignant tumours were ascribed to a hypothetical antiblastic humoral factor produced by the reticulo-endothelial system and spleen. If we do not choose to agree with this explanation, it is not because we do not believe it possible that humoral factors produced by the reticulo-endothelial cell system and spleen may play a part in the body's anticancer defences.

Moreover, both a considerable volume of literature devoted to the role of the reticulo-endothelial system in cancer and our own experiments, published earlier, on the stimulation of the spleen by ultra-violet rays or experiments on blocking the reticulo-endothelial system or splenectomy after implantation of tumours have convinced us of the important part played by this system in the fight against malignant neoplasms. All the same, there is no convincing proof that the anticancer defences of the body may be related to the action of so-called nonspecific humoral factors, as was held a quarter of a century ago and is still held, through inertia, in some quarters today.

In the light of many new facts, it is now hard to assess the accuracy of Braunstein's theories on the mode of action of malaria on human tumours.

We can see the complexity of the stages of even temporary tumour regression, the characteristic and fundamental cellular and tissue changes taking place and the importance of the timely intervention of the body's defence mechanisms in this already initiated process. The loss of any of the links in this complex chain of cellular and humoral factors in tumour regression can halt the process or even cause the undoing of the temporary effect achieved. For this reason it is not so easy to judge which were the main factors involved in the definite, although transient, positive effects of malaria infection on cancer. We cannot ascribe this positive effect of malaria simply to improved function of the reticulo-endothelial system. We have seen that under the influence of certain microbial factors there have been alterations in the cancer cells themselves, with changes in their cytoplasm and nuclei and a sharp fall in their mitotic index. There were also changes in the stroma, with significant modifications of the composition and activity of connective tissue cells, and all the cells which are known to be of some importance in the body's defence system began to penetrate and become active among the malignant tissue, where there was a simultaneous increase in the various processes of cellular degeneration and necro-

sis. And if today we are unable to comment on the reaction of the nervous system of tumours to microbial factors, we do know—on the evidence of many investigations—about the reaction of tumour vessels to the group of microbial factors which cause destruction of their capillary networks and thus bring about profound disturbances of both the nutrition and the metabolism of the malignant cells. Finally, it has been shown that certain microbial factors disrupt definite stages in the division of malignant cells.

We have so far only mentioned the category of microbial factors which act *directly* on various tumour elements. There is no doubt, however, that equal importance must be ascribed to microbial factors acting on tumours not directly but *indirectly*, through the medium of the body's diverse physiological systems, involved directly or indirectly in the infectious process. There is also no doubt that we must consider these processes as having a direct or indirect influence on the tumour.

When all this is considered, it is clear that any simplified answer to the question of the effects of malaria on cancer is impossible, as we have seen the diversity of the body's means of defence against malignant cells, and how these natural defences may be restored, directed and strengthened in the cancer-affected body.

We have intentionally chosen the effects of malarial infection on cancer as our first example, since it brings out the whole complexity of this problem, particularly important to us in that *Plasmodium*, like *Trypanosoma cruzi*, is a member of the Protozoa. The real solution to the question of the significance of malarial infection must be sought experimentally. We must mention here our attempts to create an experimental model for the solution of this problem, when we started to study the effects of fowl malaria on the development of the Rous sarcoma. As already stated, this infection has no effect on the fowl sarcoma. This result was for us a direct indication of the *complexity of the relationship between a particular type of infection, a particular type of tumour and the conditions under which the relationship exists*. The fowl sarcoma experiments were also instructive in that another infection—tuberculosis—definitely has some inhibitory influence on the development of such tumours.

In this connection Teutschlander's observations (1931) on the influence of tuberculous infection on the fowl sarcoma are particularly interesting. His experiments showed that of 80 birds inoculated with the sarcoma, tumours developed in 53, and of the remaining 27 birds tumours did not develop in 8, there was tumour regression in 16 and in 3 the tumours took badly. Tuberculosis of the lungs or intestine was present in 23 of these 27 birds. In subsequent experiments sarcoma-affected birds were intentionally infected with tuberculosis. In this case it was shown that tuberculosis infection causes regression of the Rous sarcoma.

Teutschlander considers his observations to provide experimental proof that "complication" by tuberculosis, at least in the early development of the malignant neoplasm, has a marked inhibitory influence on it. That tumour regression or resolution does in fact result from the tuberculosis is supported by the fact that spontaneous regression of the Rous sarcoma is very rare in birds unaffected by tuberculosis. The regression in tuberculous birds was directly related to the development of infection, *the birds maintaining their condition*, whereas control birds died much sooner.

Teutschlander writes: "It is therefore possible to achieve, by means of intercurrent infection, regression and resolution of developing tumours, as long as the tuberculosis has time to exert its effects. Since none of the phenomena associated with tuberculosis occurs in other infectious diseases such as diphtheria, fowl pox (natural and experimental) and suppurative peritonitis, I assume that tubercle bacilli have the special property of producing a nonspecific immunity against the Rous sarcoma". There is no doubt that Teutschlander's observations are of interest, but there is some doubt as to the accuracy of his claims that the inhibitory effects of tuberculous infection are entirely due to so-called "nonspecific immunity". Teutschlander himself states that no other infections (diphtheria, fowl pox, suppurative peritonitis) *had any effects even resembling that produced by tuberculosis.*

We believe that two working hypotheses may be put forward. The first is that the fowl sarcoma is known to be caused by a filterable virus and it may therefore be supposed that tubercle toxin inhibits the Rous sarcoma virus. The second hypothesis is that tubercle toxin specifically affects the sarcoma cells. These two suggestions are more acceptable than Teutschlander's idea of "nonspecific immunity", particularly as the latter has had no confirmation and is in contradiction to what he himself observed in other infections which did not have any effect.

Teutschlander's observations are ideologically related to numerous clinical and *post mortem* observations, but the conclusions are various and often contradictory: one school claims that tuberculosis has a cancer-inhibitory effect, while another school refutes this. Heddaeus (1935), who made a particular study of this subject, believes that the combination of tuberculosis and cancer is only possible under definite conditions, which include cases where latent tuberculosis is activated by cancer cachexia. When active tuberculosis and cancer arise at different times, the rapid development of tuberculosis suppresses the process of tumour growth. For this

reason active tuberculosis and a developing tumour are rarely seen simultaneously. Heddaeus ascribes this to activation of the reticuloendothelial system by tuberculosis.

It should be added that many investigators (Pearl, 1928, 1929; Sturm, 1928; Ruhe, 1929; MacIntosh, 1930, and others) have written in support of the existence of an antagonism between tuberculous infection and malignant tumours. Numerous histopathological, statistical and clinical observations induced some authors to make use of an available product of tubercle bacilli—tuberculin. Attempts to treat cancer by tuberculin injections did not on the whole give good results, although many observations were made. Experiments on the effects of tuberculin on experimental tumours in animals also gave varied results in the hands of different investigators. Karnel', Brantsburg and Khanenia (1947) concluded that bovine tuberculin injected subcutaneously has some inhibitory effect on the growth of rat sarcoma 16 and the Crocker sarcoma in mice. These and similar experiments gave no clear-cut results. It may be thought that until a systematic study is made of the effects of *different protoplasmic fractions* from tubercle bacilli of the various types and until the significance of the amount of the active agent injected is studied, etc., we shall not know whether the effects of tuberculous infection on tumours are direct or incidental. The effect of tuberculosis on animal tumours is selective—this is shown by the *negative* results of Kon and Cole with rabbit carcinoma; *in this case there is no antagonism between the carcinoma and tuberculous infection.*

From examples of the influence of infection on cancer taken from the literature we shall now turn to experiments carried out in our own laboratory.

The influence of these infections on the Brown–Pearce rabbit carcinoma has been studied in detail by Yumashev (1953). The first study involved *diphtheria infection*. This forms a continuation of our work on the effects of diphtheria toxin on spontaneous mouse tumours and on the rabbit tumour. In Yumashev's experiments rabbits were subjected to subcutaneous immunization with diphtheria toxoid, after which the Brown–Pearce tumour was implanted intratesticularly. After 5–8 days, during the time of development of the tumour in the testis, a live culture of diphtheria bacilli was injected subcutaneously, and again 12 days after the start of the experiment.

The experiments with diphtheria infection enabled the following conclusions to be drawn:

(1) Subcutaneous injection of a live diphtheria culture after previous immunization causes regression of the rabbit carcinoma.

(2) Live cultures of diphtheria bacteria prevent the development of metastases in carcinoma-affected rabbits.

(3) A control group of rabbits received only diphtheria toxoid, which had no effect on tumour development or metastasis formation. This result fully supports our earlier observations on the complete ineffectiveness of toxoid against mouse adenocarcinomata.

The experiments with live diphtheria cultures are interesting in that the effect produced by this infection *can be reproduced by injection of the corresponding dose of diphtheria toxin.* It follows from this that regression of the carcinoma depends on a specific effect of diphtheria toxin, which may be injected direct or may be formed in the bodies of previously immunized rabbits as a result of the multiplication and metabolism of diphtheria bacteria. It is already well known that the toxin is the main factor in the pathogenic action of the diphtheria organism, though the infectious process produced by this organism is still very complex and involves the function of various systems in the body. We repeat, however, that *it is not the infectious process, with its complex mechanisms, which brings about suppression of metastasis formation and regression of the primary tumour, but diphtheria toxin, or one of its chemical components.*

The second example of the influence of infection on the Brown–Pearce carcinoma of rabbits is that of *E. coli* infection. Preliminary observations have shown that 2 billion cells injected intraperitoneally produce disease in rabbits within 8–10 days. In the experiment, the Brown-Pearce tumour was inoculated into the testes of 30 rabbits. Some of the rabbits received intraperitoneal injections of *E. coli*, the others serving as controls. The results of these experiments showed that:

(1) in 40 per cent of the experimental group, testicular tumours formed but there were no metastases;

(2) in 15 per cent there were neither metastases nor any testicular enlargement;

(3) in 45 per cent with testicular tumours there were metastases in the internal organs, though widespread metastases were seen in only 25 per cent;

(4) in 60 per cent of the experimental group the survival period was over 70 days from the time of implantation of the carcinoma, whereas most of the control rabbits died much sooner, showing extreme emaciation.

Thus, live *E. coli* cultures injected intraperitoneally retard the development of the Brown–Pearce tumour in rabbits, with a complete absence of metastases in 55 per cent. Our experiments, still incomplete, have revealed

that the action of *E. coli* is related to a definite chemical fraction, but that its effects on *spontaneous* mouse tumours are relatively weak.

The third example is the effect of experimental typhoid infection on the Brown–Pearce rabbit carcinoma. Rabbits with tumour implants in the testicle were given intraperitoneal injections of a *Salm. typhosa* culture in quantities of 1·2 billion bacterial cells. This experiment gave negative results. Live *S. typhosa* cultures injected intraperitoneally into carcinoma-bearing rabbits *had no noticeable effect either on the development of the primary testicular tumour or on metastasis formation.* Only in isolated cases was there some slight decrease in the number of metastases.

* * *

We have carried out numerous experiments showing various degrees of tumour regression in animals infected by a variety of micro-organisms—*E. coli,* salmonellae, *Trypanosoma* species, streptococci and others. In analysing this phenemenon, the first question to arise is that of the role of pyrogenic factors in microbial cells — is tumour regression brought about by the high temperature usually associated with an infectious process? This question must be answered in the negative. The following observations provide the basis for such an answer:

(1) While studying the effects of *E. coli* on spontaneous carcinomata of white mice we attempted to obtain separate effects by injecting different fractions — those possessing pyrogenic properties and those not possessing them. Tumour regression occurred *only* in animals which had received the apyrogenic fraction, while injection of the fraction with pyrogenic properties had no influence on the spontaneous carcinomata.

(2) The same conclusions may be drawn from observations on the course of the Brown–Pearce tumour in animals suffering from trypanosomiasis. After inoculating *T. cruzi* into rabbits, the animals showed a high temperature for the first few days after infection. Then the temperature fell to normal and did not rise again for a considerable time—sometimes for 2 or 3 months. The trypanosomiasis took a mild form in the rabbits. Rabbits having a normal temperature for the whole course of the disease still showed frequent and marked tumour regression, as stated earlier. This does not enable us to ascribe regression of the Brown-Pearce tumour in rabbits suffering from trypanosomiasis to the influence of pyrogenic factors manifesting their effects in the early days of the infection.

(3) In a series of experiments, animals inoculated with the Brown–Pearce tumour then received intraperitoneal injections of foreign blood to which

they reacted with a rise in temperature. However, the pyrogenic effect of foreign protein in this case had no influence on tumour growth in any of the experiments.

(4) In several experiments we were able to produce tumour regression by injecting living or killed typhoid organisms. In both cases the animals received, as well as the other fractions of the typhoid organism, a pyrogenic substance which is always present in the intact cell of this bacterium. We could have concluded from this that these substances are responsible for the regression of malignant tumours. However, experiments showed that the fraction of this typhoid strain containing the pyrogenic factors is incapable of producing such regression.

(5) Numerous experiments have shown that one trypanosome strain yields a preparation with no pyrogenic properties but still containing an antiblastic factor.

Hence the combined observations made in various experiments allow us to exclude the effects of the pyrexia developing during an infectious process as a factor inhibitory to malignant growth. As an independent factor, high temperature *does not lead* to tumour destruction, and the pyrogenic fraction of microbial cells does not bear any antineoplastic agent.

In this account of the relationship between the development of an infectious process and the development of a malignant neoplasm, one more moment is left to be dealt with—the effects of the spreading factor contained in microbial cells. *Trypanosoma cruzi,* like other parasites, contains a spreading factor. This was established by Gintsburg in our laboratory. As with other pathogenic microbes, hyaluronidase itself has no toxic activity, does not destroy the cells and tissues of the infected body, and even in malignant tumours hyaluronidase can act only on the intercellular ground substance—hyaluronic acid. Malignant cells remain unharmed, as do the cells of normal tissues when the intercellular cement substance is affected by hyaluronidase. All this shows that hyaluronidase can only play an auxiliary role in the process of tumour regression.

The deciding role belongs to an antiblastic factor attached to one of the chemical fractions of the microbial cell, independently of the enzyme hyaluronidase. If the reverse were true, tumour regression would occur on injection of any of the numerous products of microbial origin obtainable from cultures of hyaluronidase-containing organisms. In fact, this process is only produced by certain microbial species, of various systematic classifications.

Experiments have shown that:

(1) A destructive effect on tumours may be connected with a microbial cell fraction which is harmless to normal tissues and organs. The antiblastic fraction from *T. cruzi* serves as an example of this.

(2) A cancerolytic effect may be connected with products of microbial origin which, while not harming all somatic cells, affect only highly specialized tissue (such as nervous tissue), where they cause *functional* and organic disturbances. Yet these substances, which appear very remote in their functional specificity from an antiblastic function, have a cancerolytic effect on malignant tumours, as we have seen in earlier experiments on the effects of tetanus toxin on adenocarcinomata of white mice.

(3) At the same time, products of microbial metabolism highly toxic to normal tissues may even have no harmful effects on malignant cells, as occurs with *Clostridium histolyticum*, which has a powerful lytic action on normal tissue but does not affect malignant tissue.

It must therefore be accepted that the carrier of the antiblastic factor in microbial cells is a definite chemical fraction, which is by no means necessarily also a bearer of toxic, lytic or necrotizing properties with respect to normal cells. This fraction acts according to the general rule of specificity governing biological phenomena.

Turning now to *T. cruzi* infection, one very important fact must be considered: although it has a very wide spectrum of antineoplastic activity against tumours in man and many species of animals, trypanosome infection produces inhibitory effects of *varying* degrees. This was shown in experiments by Fradkina *et al.*, who established a varying index of inhibitory activity of *T. cruzi* strains on the Crocker sarcoma (from 2.88 to 15). The same phenomenon had earlier been seen in our laboratory by Levinson, who showed in a number of experiments that *T. cruzi* infection inhibits the growth of the Crocker sarcoma by 2, 4, 5 or 6 times in comparison with the controls. Fradkina and her co-workers used cultures produced on synthetic culture media. This may explain the variable antineoplastic properties of their strains. Levinson's experiments involved the infection of sarcoma-bearing mice with mouse blood containing the trypanosomes. The question arises: upon what does the varying degree of activity of *T. cruzi* infection depend, given similar experimental conditions?

It is not difficult to show that the tumour regression caused by this infection depends on the presence in the trypanosome cell of an antineoplastic fraction, which may be extracted and purified from other, non-antineoplastic fractions of the trypanosome cell protoplasm. But now we must decide what might be the cause of varying activity by one and

the same antineoplastic factor, in this case injected as a live trypanosome culture. It should be remembered that the tumour regression occurring in such a case is a very complex process, involving the physiological mechanisms of the body, pathogenic factors from the neoplasm and the antineoplastic factor from *T. cruzi*. There is no doubt that each of these factors may introduce an element of variability into the regression process. Which of them, however, is most active in this respect?

Observations extending over several years indicate that trypanosome strains cultured in normal mice maintain their species characteristics for many years, including their antineoplastic properties. Strains of the Crocker sarcoma, with strict observation of the rules of transplantation, also give relatively uniform results in each different series of transplantations. We must assume that the main variable factor in the experiments described was the experimental animals. The significance of the properties or state of the body in the process of interaction between tumours and *T. cruzi* infection is shown by Hauschka's experiments (1947) establishing the influence of the sex of the animal on the course of the infection; in one mouse strain the average number of trypanosomes in the blood was 3250 for bucks and 1400 for does; in another strain the corresponding figures were 1022 and 502.

Consequently, trypanosome infection must also develop differently in sarcoma-affected mice, according to their hormonal and metabolic characteristics. As a direct result of this, the reaction produced by the antineoplastic fraction of *T. cruzi* must also be expressed in different ways—different dynamics and different intensities.

The fundamental significance of these facts is that they give rise to the question: cannot the physiological characteristics related to metabolism have their own effect not only on live cultures of *T. cruzi* but also on the protoplasm of the trypanosome, and what is the significance of the inhibition of trypanosome growth in female mice? It may be that the physiological mechanisms of the infected body can destroy certain of the trypanosome components, thus inhibiting the multiplication and neutralizing the effects of the parasite. What conclusion may be drawn from these experiments, analysing them with respect to cancer biotherapy by means of the trypanosome preparation? In any case, the experiments mentioned above create the idea that in the human body the reaction to the products of *T. cruzi* cells used in cancer therapy may vary slightly according to the body's hormonal or physiological characteristics. It is also plausible that under certain physiological conditions there may

be partial or complete destruction of the trypanosome fraction bearing antineoplastic activity.

However, the most important and decisive fact to be emphasized here is the *stability* of the antineoplastic factor from *T. cruzi,* as a result of which its specific activity is repeated in principle, irrespective of the physiological differences ascribable to species, race or sex, varying in degree according to these circumstances. Our clinical observations have shown that in some cases the defensive functions of the body are highly developed, whereas in others, because of some physiological peculiarity, they are undeveloped. The effects produced by one antineoplastic preparation will vary according to the correlation between three primary factors:

(1) the active microbial regression factor;

(2) the character and properties of the malignant tumour;

(3) the state of the cancer-affected body.

None of these can play a subordinate or minor role in tumour regression. They are of equal significance, and their co-reaction can bring about regression of a malignant tumour to an extent sufficient to lead to clinical recovery. This irreversible rule of regression relates to all cases of the action of antineoplastic preparations, irrespective of which microbes serve as their source.

When one considers as a whole all that has been learned from experiments on the effects of different infections on cancer, one may for a moment form the completely false impression that almost any microbe injected into the body has an inhibitory action on tumours. This idea is, however, very far from the truth. It should be remembered that a very large number of microbes have been proved to be inactive—we have not mentioned all the experiments concerned, as they lie outside our sphere. Most of it is learned, however, by everyday experience. Every investigator has found an extensive flora in ulcerated tumours of animals, every clinician has seen infected tumours, but they could see no regressive influence of bacterial contamination of tumours, either of the rectum or lung. However, although the number of inactive microbes is very large, there are still a few infections which have a noticeable and in some cases indisputable effect. *It would be an inexcusable error to assume that the regression factors associated with the metabolism of various microbes are identical, while their modes of action differ.* There is, as we have seen, no basis for such a suggestion. It might sooner be suggested that we have here a situation similar to that of the antibiotics active against different groups of bacteria. It is now known that the antibiotics are a chemically unrelated group. However, one antibiotic may be active against various species of bacteria, and, more significantly, antibiotics of differing chemical structure may act against the same bacterial species, *by affecting different physiological systems in them and producing the end result by different means.* The same thing obviously applies to antineoplastic substances of microbial origin. We have tried to show differences in the effects on cancer cells by comparing the trypanosome preparation with the *B. prodigiosus* polysaccharide. A similar difference would appear to exist between the modes of action of diphtheria toxin and prodigiosus polysaccharide. If we take it as proven that there is a whole series of biochemically differing antineoplastic substances of microbial origin which in proper doses and in the appropriate tumours may bring about either single stages or the whole multistage process of regression of malignant tumours, we then have the question: how can we reconcile this position with the orthodox assertions of the *autonomy,* high biological activity and aggressiveness of cancer cells? There can only be one answer to this question: cancer cells are extremely susceptible to a number of external factors — much more susceptible than any normal cells. It follows that the aggressiveness of malignant tissues is inevitably associated with increased sensitivity to a whole series of external factors, of which we have studied factors of microbial origin.

On comparing all the facts given in this and the previous chapters, the following conclusions may be drawn:

(1) In a number of cases, in both man and animals, an antagonism can be seen to exist between certain infections and the development of malignant tumours.

(2) The mechanism of the inhibitory effects of these infections on cancer is much more complex than was thought after first analysis.

(3) The inhibitory effects of these infections on cancer are connected with the fact that the organisms causing the infections form substances influencing, to a greater or lesser extent and more or less selectively, the metabolism of malignant cells, damaging various components in them.

(4) Microbes producing antineoplastic substances are much more varied and numerous than microbes causing infectious diseases. If the causal organism of any infection contains an antineoplastic fraction, the infectious disease produced by it may in the cancer-affected body be accompanied by inhibition of the malignant process. But an antineoplastic fraction may be contained in non-pathogenic microbes, or the microbe may be pathogenic only for one species of animal, while its antineoplastic fraction may be capable of producing tumour regression in another species.

* * *

The many facts and theories mentioned in this part of the book are an essential prerequisite to a consideration of the following observations on the effects of the cancer antibiotic from *T. cruzi* on malignant tumours, primarily on cancerous diseases in the human patient.

Part IV

REGRESSION OF HUMAN MALIGNANT TUMOURS AND THE PRINCIPLES OF THE ACTION OF THE TRYPANOSOME SUBSTANCE

EVER since various experiments first showed the antineoplastic properties of *T. cruzi* cultures, and special investigations showed the safety of the use of a preparation from these cultures in human patients, clinical observations have been carried out with the primary object of establishing the principles of the course of cancer under conditions of systematic injections of the trypanosome preparation. The clinical observations were supervized by Prof. Limberg, Prof. Sviatukhin and Prof. Nisnevich, and carried out by clinical workers Andreev, Yumashev, Dreitser, Rutkovskaya, Chegis and Marantidi. The case-histories prepared by these clinicians served as the material for an analysis of the principles of regression of human malignant tumours under the influence of the cancer antibiotic from *T. cruzi*. In a large number of cases we were in consultation with Profs. Egorov, Faerman, Aleksandrov and Ratner. Pathomorphological diagnoses were provided by Profs. Talalaev, Pozhariskii and Rapoport.

The main subjects for the clinical use of the preparation were cancer of the lip and mammary cancer.

Cancer of the lip was chosen as an open form of affection accessible for visual observation, which allowed systematic control of the effects of the antibiotic both at the tumour lesion and in the patient's body as a whole. Mammary cancer was chosen as a closed form of the condition, which was more accessible than other forms for observation clinically and by repeated biopsies.

During the three years when the culture preparation was tested clinically (1948–1951), observations were made on 24 patients with cancer of the lip. In 5 of the 24 patients the preparation gave no effect, while varying effects were observed in 19 patients, 11 of whom were in stage I of the disease, 7 in stage II and 1 in stage III.

Of the 73 patients with breast cancer, 33 showed negative results, while varying effects were observed in 40 patients, 13 of whom were in stage I of the disease, 23 in stage II and 4 in stage III. It should be noted particularly that in cancer of the breast relatively prolonged effects were obtained from the use of the preparation only after the clinicians started to resort to excision of the primary tumour nodule to the extent of the macroscopically affected tissue, carrying out this doubly palliative interference during an uninterrupted course of intramuscular injections of the trypanosome preparation.

The group of patients reacting to injections of the preparation by lasting signs of tumour regression and improved general condition were re-examined by a committee from the Scientific Council of the Ministry of Health of the U.S.S.R. in 1955, after 5 or more years had elapsed from the end of the treatment. These observations will be described, along with others involving shorter periods.

1. REGRESSION OF CANCER OF THE LIP

OBSERVATION NO. 1

Patient G., male, aged 48 years. Clinical diagnosis: *carcinoma of the lower lip,* histologically a *squamous-cell carcinoma.*

In the winter of 1946 a wart had appeared on the lower lip which the patient had cauterized with acid; an ulcer developed at the site. In the spring of 1947 the affected area was removed surgically. Six months later, in January 1948, a scab developed on the site of the scar; this repeatedly appeared and fell off, leaving an ulcer.

SYMPTOMS OF THE CONDITION (12 MAY 1948)

On the left of the lower lip, in the red portion, there was a firm, fibrous tumour measuring 1·0×0·5 cm. On palpation its periphery was recognisably more dense. The whole growth did not protrude beyond the level of the surrounding mucosa. It was the same colour as the surrounding mucosa, but with a dull surface. The neoplasm had a narrow circumvallate zone. On the right this crevasse was 0·2 cm wide and 0·3 cm deep.

The red border of the lip was covered by areas of hyperkeratosis. The left commissure of the lips bore a scar—the remains of the operative interference carried out a year previously in the spring of 1947 because of the ulcer following cauterization.

The submandibular lymphatic nodes were not palpatable.

On 14 May 1948 a biopsy was performed, when a quarter of one area of hyperkeratosis was excised under local infiltration anaesthesia. Pathomorphological findings: "early squamous-cell carcinoma".

On 14 May 1948 the patient was put on to a course of injections of a dried preparation obtained from *T. cruzi* cultures grown on synthetic culture media (modification VI).

INJECTIONS OF THE PREPARATION AND COURSE OF THE DISEASE

The preparation was injected intramuscularly in the buttocks, once daily. The following individual doses were given:

Injection	Dose in arbitrary units	Date
1	200	14 April
2	400	15 April
3	400	17 April
4	600	18 April
5	800	19 April
6–37	1000–1800	20 April–29 June
38–75	2000–2500	30 June–27 Aug.
76–88	3000–4000	28 Aug.–11 Sept.

After 62 injections (100,800 units) the firm growth palpated earlier had disappeared. The scar—the remains of the biopsy carried out on 14 May 1948—was in good condition. The surface of the red border of the lower lip bore an area of hyperkeratosis covering about 2 cm, with a width of 0·2–0·3 cm. The crevasse on the right extended for 0·2 cm and had become superficial. Soft lymphatic nodes could be felt in the submandibular region, the one on the left measuring 1·25×0·5 cm and the three on the right each measuring 1·0×0·5 cm.

The injections were stopped after 22 September 1948 (*sic*). There were no visible signs of the condition. The first course consisted in all of 88 injections involving 171,170 units of the preparation.

A clinical examination on 20 October 1948 revealed no changes in the patient's condition, nor any evidence of recurrence or metastases. The submandibular lymphatic nodes were soft in consistency, measuring 1×0·5 cm on the right and 1·7×0·5 cm on the left.

SECOND SERIES OF INJECTIONS AND COURSE OF THE DISEASE

After a 75-day interval, on 27 November 1948 a second course of injections of the same preparation was started as a prophylactic measure. The first injection was of 1000 units, the others of 2000 units daily.

After eleven injections, on the advice of a committee from the Academy of Medical Sciences, the patient underwent wedge resection of the biopsy scar and the submandibolar lymphatic nodes as a precautionary measure.

The excised portion of the lower lip had a base measuring 2 cm, sides of 2 cm and a depth of 1·75 cm. The lymphatic nodes measured 1.0×0·5 cm and 0·5×0·5 cm. Outwardly they did not appear cancerous.

On histological examination no evidence of a primary or metastatic tumour process was found in any of the excised portions.

The second course finished on 17 January 1949; it consisted in all of 42 injections involving 93,000 units of the preparation—from 2000 to 3000 units per injection. Clinical checks carried out on 17 January, 17 February, 25 March and 1 August 1949 and in April 1955 showed the absence of any signs of recurrence or metastases. The period of observation was 7 years (Plates 1, 2, 3 and 4*).

OBSERVATION NO. 2

Patient P., male, aged 40 years. Clinical diagnosis: *cancer of the lower lip*. Histologically, a *keratinizing squamous-cell carcinoma*.

In December 1947 the patient felt a tumour on the lower lip with his tongue. Because it irritated him, he punctured it with his teeth. Several days later a persistent ulcer formed at the puncture site. He did not adopt any particular treatment, except to apply streptomycin to the ulcer occasionally.

The patient was admitted to our clinic for biotherapy on 8 May 1948.

SYMPTOMS OF THE CONDITION

At the time of admittance on 8 May 1948 the middle part of patient P.'s lower lip had an ulcerated surface covered by a thin scab, about 1 cm in diameter. The edge of the ulcer protruded as a rim 0·2-0·3 cm wide. The whole tumour had a firmer consistency on palpation than the remainder of the lip. The posterior half of the peripheral rim was of a very firm, almost cartilagenous consistency. Mobile, oval lymphatic nodes measuring 1·5×1·0 cm on the right and 1·0×0·5 cm on the left could be palpated in the submandibular region. No lymphatic nodes could be felt in the mental region.

* Note: figures in the text are denoted by Roman numerals (I, II, III, etc.), while Plates on separate pages are given Arabic numbers (1, 2, 3, etc.).

On 14 May 1948 a biopsy was carried out, consisting of the excision under local anaesthesia of a segment one quarter the size of the tumour.

Histological investigation revealed a picture of "keratinizing squamous-cell carcinoma with epithelial pearl formation, large numbers of mitoses in the epithelial cells, extensive inflammatory infiltration of the connective tissue core and superficial ulceration".

A course of injections of the preparation (modification VI) was started 14 May 1948.

INJECTIONS AND COURSE OF THE DISEASE

The patient received daily intramuscular injections of the preparation in the buttocks (except for the first, which was given as a paratumoural injection). During the course of the injections the preparation was given in individual doses of from 200 to 3000 units:

Injection	Dose in units	Date
1	200	14 May
2	400	15 May
3	400	17 May
4	600	18 May
5	800	19 May
6–47	1000–2000	20 May–15 July
48–63	2000–3000	16 July–10 Aug.

After 13 injections (12,140 units) the area corresponding to the biopsy site consisted of a superficial erosion 1 cm long and 0·3 cm wide, covered by a serous exudate forming a scab. To the right of this erosion was an affected area 0·8×0·3 cm, of firm consistency.

After 21 injections (22,400 units) a firm area 0·25–0·3 cm in diameter remained in the middle portion of the lip. The ulcerated surface had disappeared.

After 31 injections (38,400 units), only a central thickened area corresponding to the biopsy scar was found, during a consultation examination.

After 52 injections (82,500 units) palpation of the whole thickness of the lip revealed a firm area without definite edges.

The patient received a total of 63 injections, involving 107,500 units.

On 10 August 1948 the patient voluntarily terminated the injections.

30 December 1948. Lower lip normal in colour. An insignificant small scar—the remains of the biopsy—could be seen in the centre of its mucosal surface. The lip was soft on palpation, with slight cicatricial thickening

round the scar. A lymphatic node measuring 1.25×0.75 cm could be palpated in the right submandibular region, and a node 0.75×0.5 cm on the left. No nodes could be palpated in the mental region. No evidence of recurrence or metastases was seen.

15 April 1949. No signs of recurrence or metastases.

Examination of the patient in April 1951 and on 20 March 1955 showed the absence of signs of recurrence or metastases.

The observation period was 6 years and 10 months.

Figure I and Plates 5, 6 and 7 show the state of the affected lip before and after the course of injections and also a diagram of the tumour regression during the injections.

BLOOD ANALYSES

Date	11 May '48	21 May '48	2 June '48	11 June '48
Haemoglobin	70	71	70	70
Red cells	4,280,000	4,400,000	4,220,000	4,340,000
Colour index	0.8	0.8	0.8	0.8
Leucocytes	7200	7400	6800	7000
E.S.R.	3	15	19	28
Eosinophils	2	3	3	6
Juvenile cells	2	5	5	4
Segmented cells	71	68	66	65
Lymphocytes	22	20	22	21
Monocytes	3	4	5	4

	22 June '48	11 July '48	21 July '48
Haemoglobin	73	75	75
Red cells	4,460,000	4,500,000	4,500,000
Colour index	0·8	0·83	0·83
Leucocytes	9400	8800	6100
E.S.R.	33	20	21
Eosinophils	4	5	2
Juvenile cells	6	2	2
Segmented cells	66	64	59
Lymphocytes	20	27	34
Monocytes	4	2	4

In analysing the observations on patient P., the following points should be considered:

(1) 6 years 10 months has elapsed since the start of the disease in this patient.

(2) The patient was admitted to hospital 5 months after he first noticed the condition. Histological examination revealed a *keratinizing squamous-cell carcinoma.*

(3) During the course of the disease—from 14 May to 10 August 1948—the patient received injections of modification VI of a preparation obtained from *T. cruzi* cultures grown on a synthetic culture medium. The patient received *no other treatment.*

(4) During the last 6½ years the patient has been *in good health*; the lip previously affected by the carcinomatous growth has a normal external appearance and is soft on palpation. There are no signs of recurrence or metastases.

FURTHER COURSE OF THE CONDITION

15 September 1948. Scar at biopsy site softening, with no thickened areas.

21 September 1948. No thickening evident in the middle of the scar on examination. Lymphatic nodes not palpatable.

6 October 1948. Lower lip of soft consistency. No signs of recurrence or metastases.

OBSERVATION NO. 2. PATIENT P. CARCINOMA OF LOWER LIP

Consideration of these facts compels us to accept that in this case a clinical cure was obtained as the result of the use of the modification VI preparation.

Attention is drawn to the fact that the injections were accompanied by a sometimes more, sometimes less marked temperature reaction. This was transient, but sometimes intense, with rigors and in some cases pains in the joints, also of a temporary nature (Fig. II).

The general reaction of the body to injections of the preparation in patient P., except for the temperature reaction described, was not accompanied by any pathological symptoms. The patient's weight increased slightly during treatment. Haematological examinations were made 7 times at intervals of 10–20 days, and showed no variations from the normal (Fig. III).

Patient P. has been perfectly fit for 6½ years, without noticing any abnormalities in this condition.

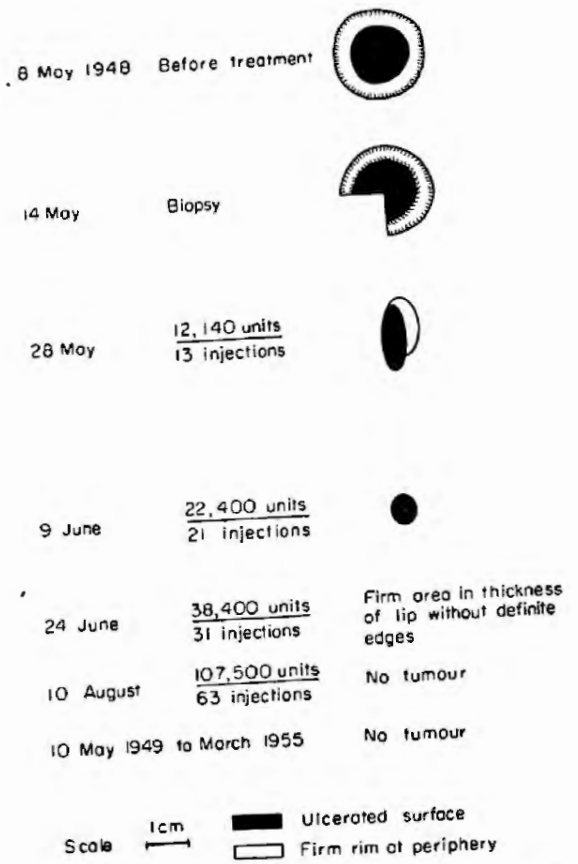


FIG. I. Diagram of tumour regression in Patient P.

OBSERVATION NO. 3

Patient R., male, aged 44 years. Clinical diagnosis: *carcinoma of lower lip*, histologically *a keratinizing squamous-cell carcinoma.*

During the two previous years the patient had punctured his lower lip on more than one occasion. An ulcer, and then a tubercle, formed at the site of the puncture.

On 23 May 1949 the patient was admitted to the clinic for biotherapy.

SYMPTOMS OF THE CONDITION (23 MAY 1949)

On the right of the lower lip there was a tumour measuring 1.5×1.0 cm. At its centre was a dry scab measuring 0.8×0.5 cm; on palpation the

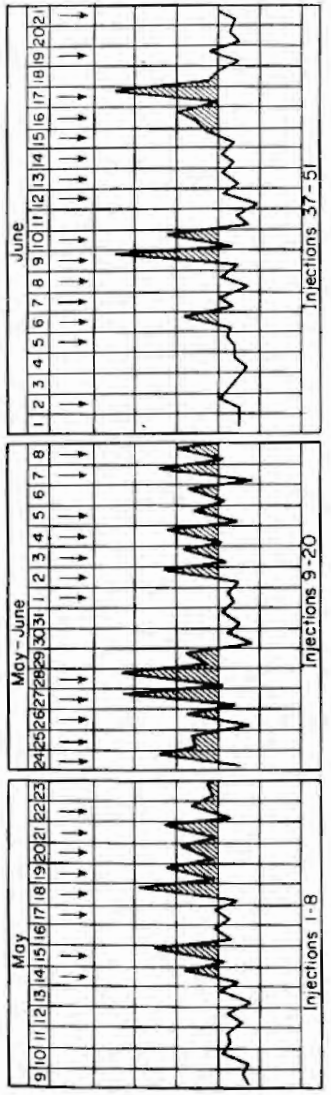


FIG. II. Temperature chart of patient P. during course of injections of the preparation.

growth was firm in consistency and could be felt to a depth of 1 cm in the thickness of the lip. Its posterior hemisphere was in the form of a rim of cartilagenous consistency. The right submandibular lymphatic node measured 2×1.2 cm, the left was soft and measured 0.75×0.5 cm.

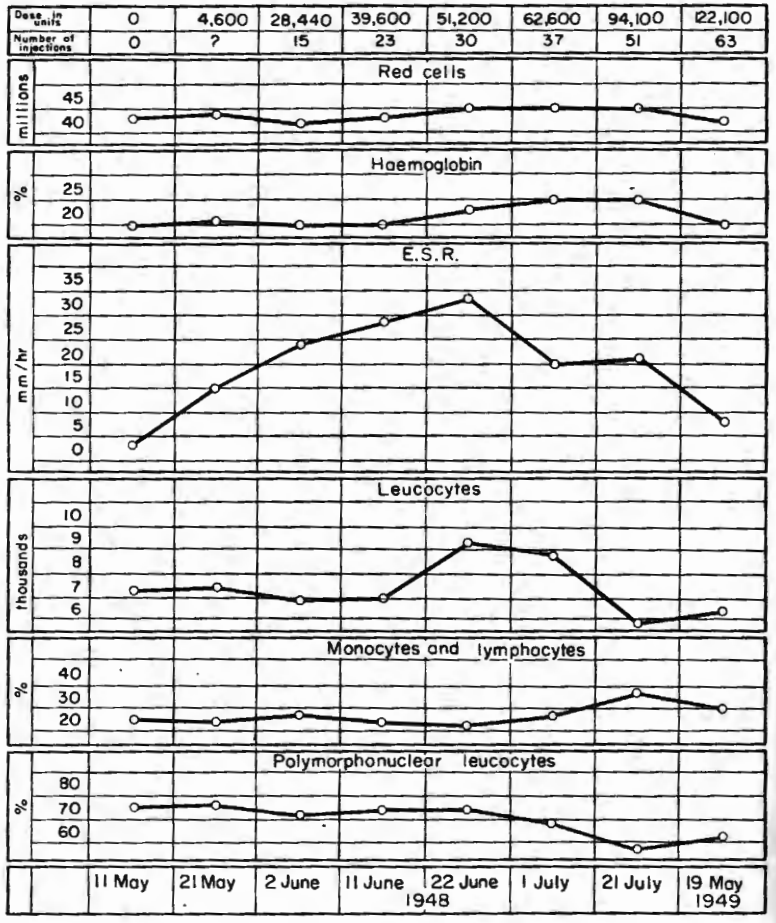


FIG. III. Diagram of blood changes in patient P. during course of injections of the preparation.

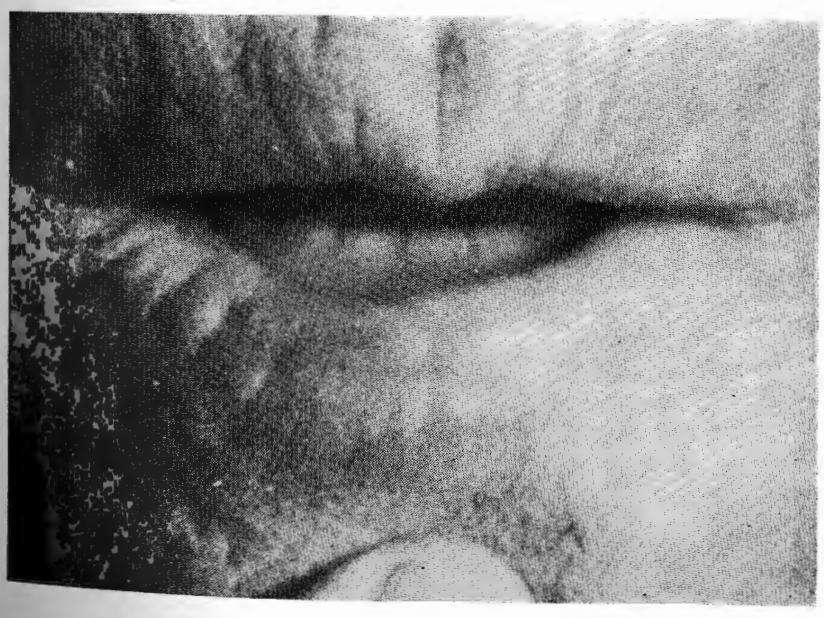
28 May 1949—*biopsy*. Excision of a quarter of the affected area.

Histological findings: "A picture suspicious of early squamous-cell carcinoma".

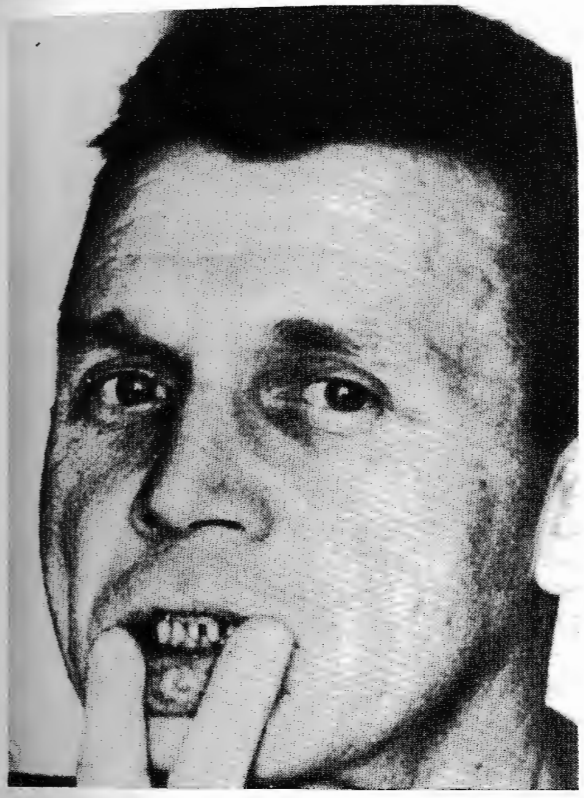
PLATE 1. Patient G., Observation No. 1. Squamous-cell carcinoma of the lower lip. 14 May 1948. Affected lower lip before injections were started. The tumour is of fibrous density, measuring 1.0×0.5 cm, and bordered by a firm ridge. On the right is a fissure 0.2 cm across and 0.3 cm deep. Areas of hyperkeratosis cover the red border of the lip.

PLATE 2. Patient G., 14 May 1948. Histological structure of one of the areas of hyperkeratosis taken at a biopsy before the start of the course of injections. Extremely dense and abundant infiltration of the connective tissue core by round cells plus a few polymorphonuclear leucocytes. Among the infiltrate lie abnormal cords and nests of squamous epithelial cells, penetrating the tissue in various directions. The central portions of the epithelial complexes show keratinization with pearl formation. The periphery shows a marked acanthosis, with moderate hyper- and parakeratosis, and a loose infiltration of the core by various cells, including many plasma cells.

Squamous cell carcinoma. Low magnification.



PLATES 3 and 4. Patient G., 14 March 1951. 2 years and 10 months after the start of the injections. There are no signs of the malignant affection. Control histological examinations showed the absence of malignant tumour elements either in the scar remaining from the diagnobiopsy or in regional lymphatic nodes. This position did not change in subsequent years. The last examination was in April 1955: signs of recurrence or metastases were absent. Period of observation, 7 years.



PLATES 5 and 6. Patient P. Observation No. 2. Keratinizing squamous-cell carcinoma of the lower lip. 8 May 1948. Affected portion of the lip before start of a course of injections of the preparation. A malignant tumour about 1 cm in diameter, surrounded by a firm projecting ridge 0.2–0.3 cm across. The surface is ulcerated and covered by a scab. In the submandibular region are two lymphatic nodes—on the right, 1.5×1.0 cm and on the left 1.0×0.5 cm. Histological structure of the tumour before treatment. Keratinizing squamous-cell carcinoma. One quarter of the ulcersted tumour was excised for biopsy.

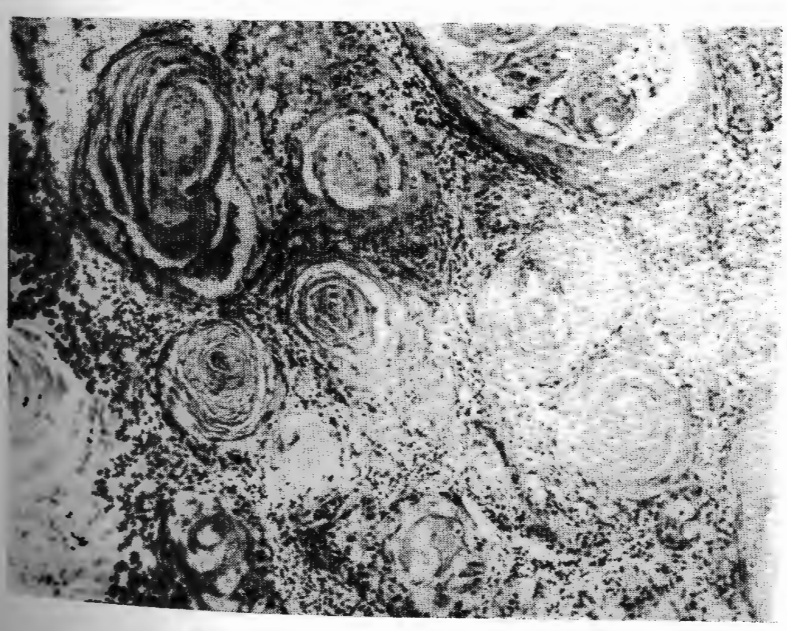


PLATE 7. Patient P., 1 October 1948. After the course of injections of the preparation. The course had lasted from 14 May to 10 August 1948. The patient received 63 injections—107,500 units of the preparation. No tumour is present. This position remained unchanged during subsequent years. The last examination was in March 1955: there were no signs of recurrence or metastases. Period of observation, 6 years 10 months.

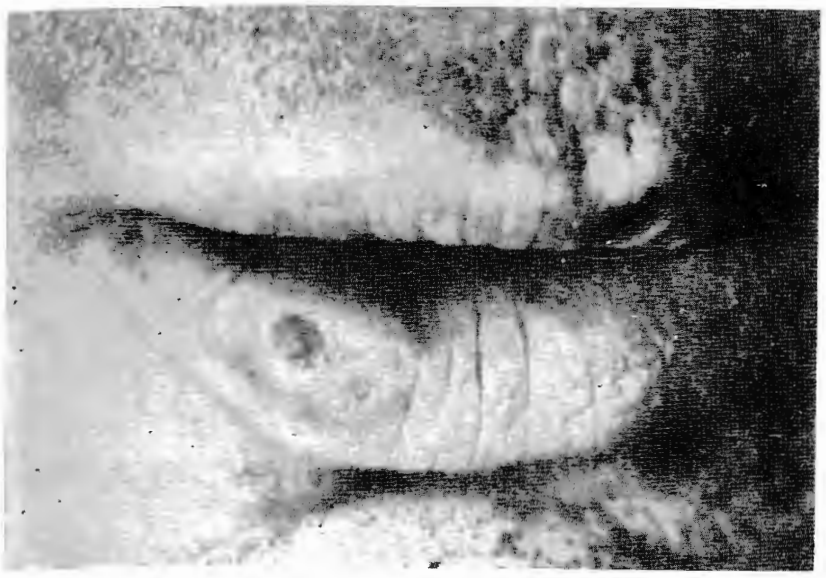


PLATE 8. Patient R., Observation No. 3. Keratinizing squamous-cell carcinoma. 24 May 1949. Affected portion of lower lip before the start of injections of the preparation. The tumour measures 1.5×1.0 cm; it can be palpated to a depth of 1 cm, at its centre is a dry scab 0.8×0.5 cm, and a lymphatic node 2.0×1.2 cm is present in the right submandibular region.

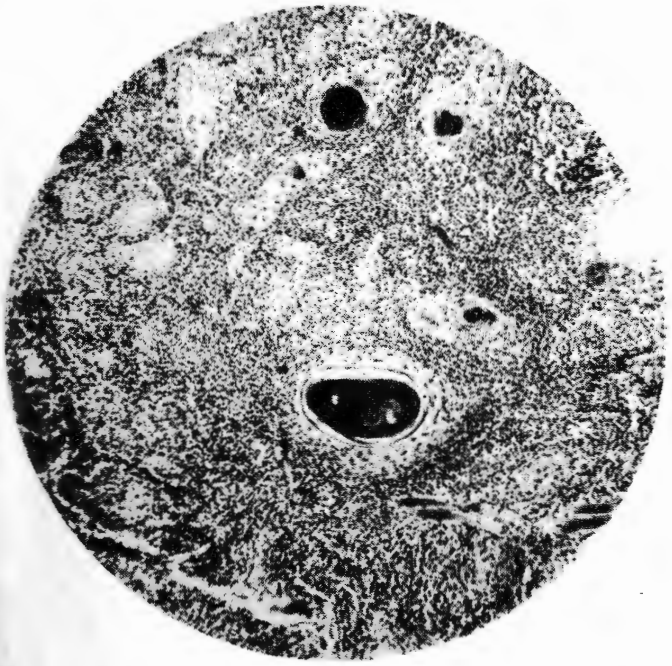


PLATE 9. Patient, R., 4 June 1949. Histological structure of the tumour before the start of treatment. Keratinizing squamous-cell carcinoma. Low magnification.

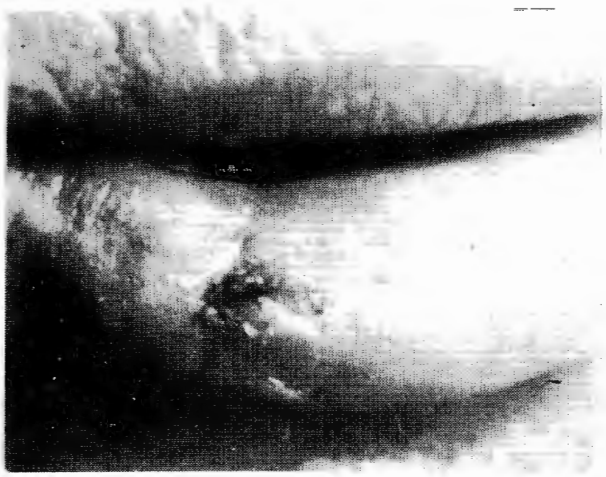


PLATE 10. Patient R. 18 days from the start of treatment, after 15 injections (15 June 1949). The size of the affected area remains the same (1.5×1.0 cm)

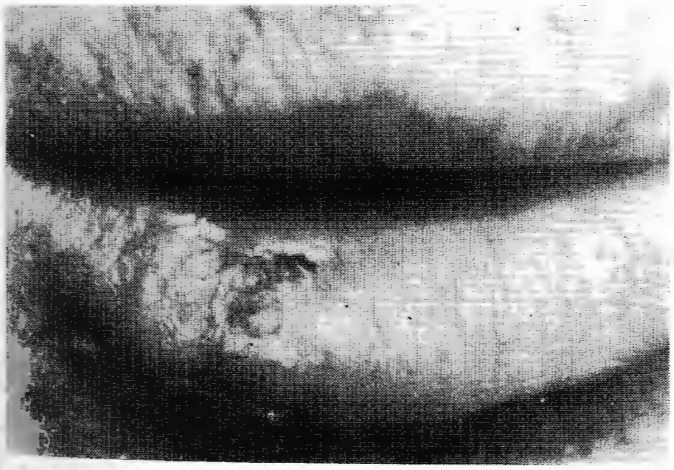
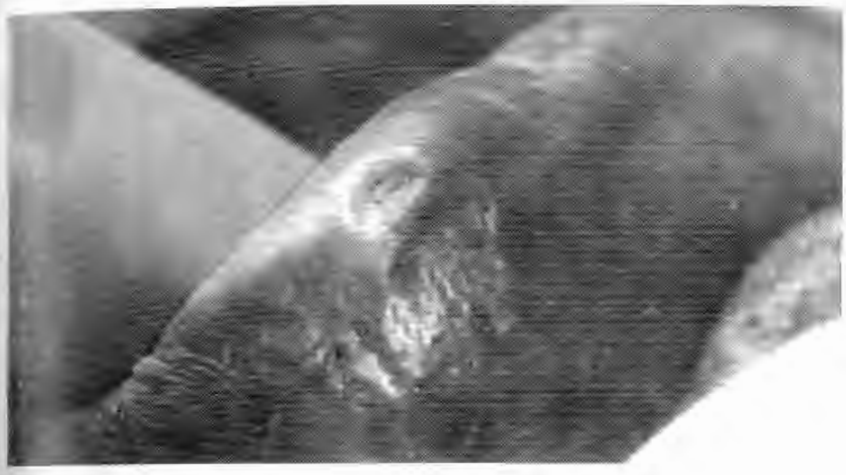


PLATE 11. Patient R., 24 days from the start of the course of injections, after 23 injections (24 June 1949). The size of the affected area remains the same—1.5×1.0 cm with 2 surface scabs, one 0.2×0.3, the other 0.5×0.3 cm.



PLATES 12 and 13. Patient R., 1 September 1949. 3 months from the start of treatment, after 67 injections. No tumour is present. At the site of the former affection is a depression covered by normal mucous membrane. Photo 12 was taken on the operating table before a control biopsy.

PLATE 14. Patient R., 1 September 1949. On the operating table. Control wedge resection of the formerly affected portion of the lip.

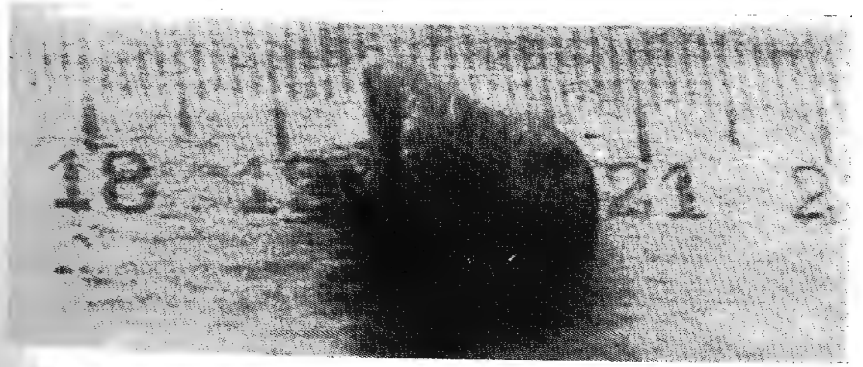


PLATE 15. Patient R., Segment of lip excised during control investigation on 1 September 1949.

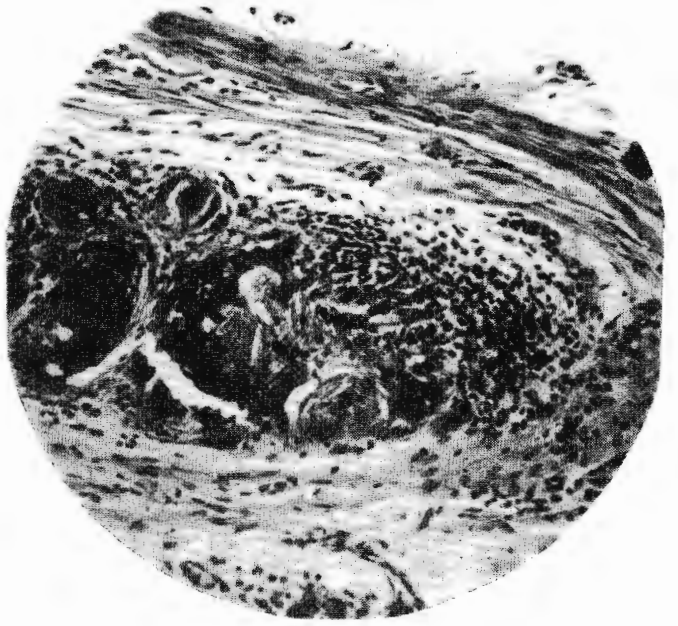
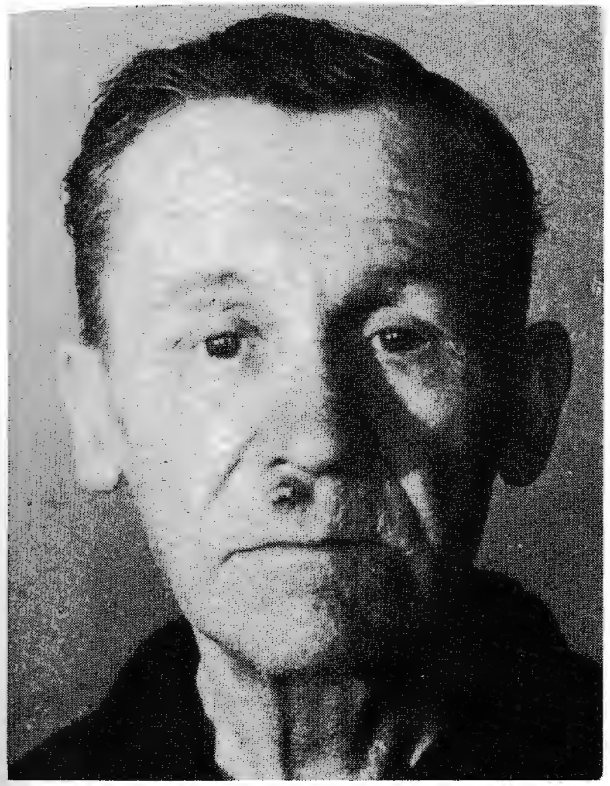
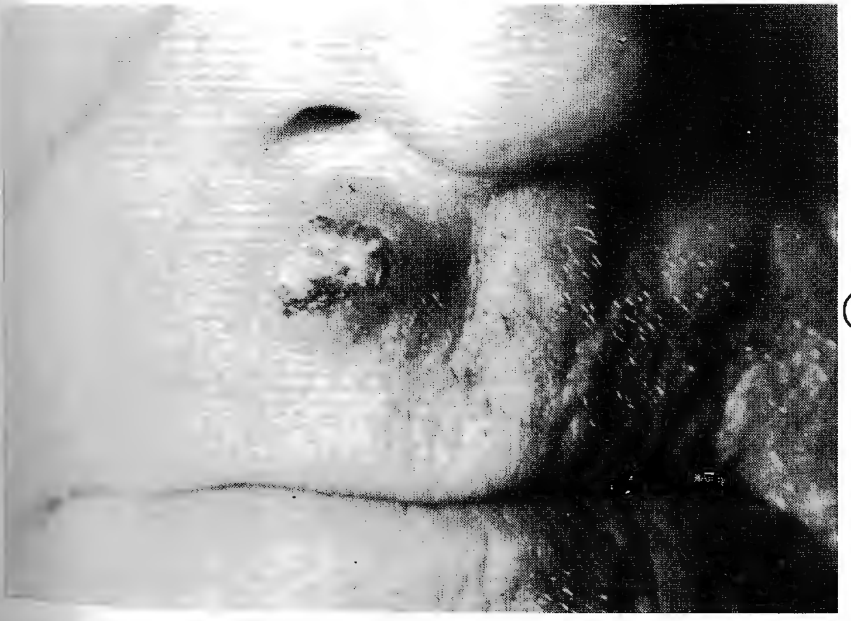


PLATE 16. Patient R., 1 September 1949. Control histological investigation of the formerly affected portion of the lip after 67 injections of the preparation. No elements of a malignant tumour are present.

The last examination of this patient was on 10 March 1955: the lip was in a satisfactory state, with no signs of recurrence or metastases, and no lymphatic nodes could be palpated. Period of observation, 5 years 10 months.



(*a*)



(*b*)

PLATE 17, *a*, *b*. Patient L., Observation No. 4. Keratizing squamous-cell carcinoma. 12 April 1949. The tumour measures 1.5×1.25 cm, with a height of 1 cm. Affected portion of upper lip before a course of injections of the preparation. The upper part is covered by a scab. In the right submandibular region are two firm lymphatic nodes measuring 1.5×0.75cm and 0.8×0.5cm.

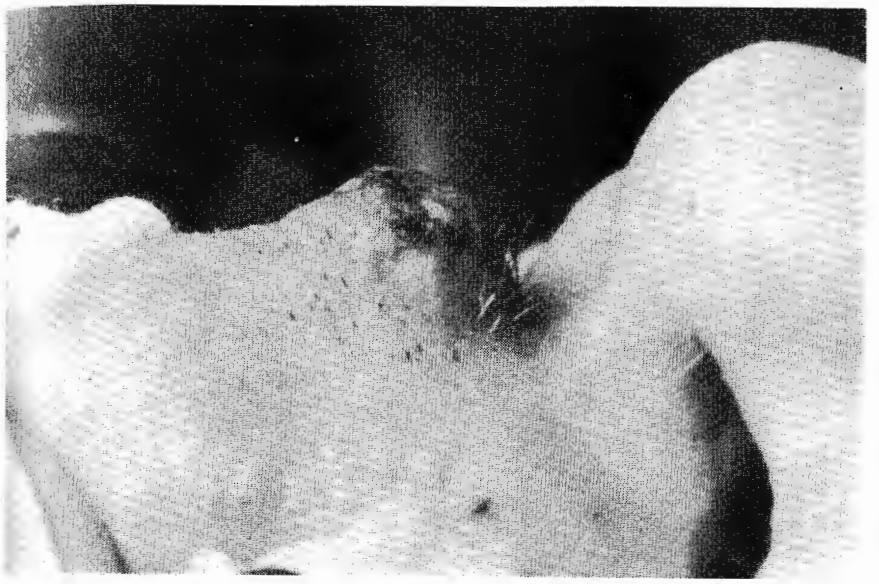


PLATE 18. Patient L., 19 April 1949. Before biopsy. Photograph taken on the operating table.

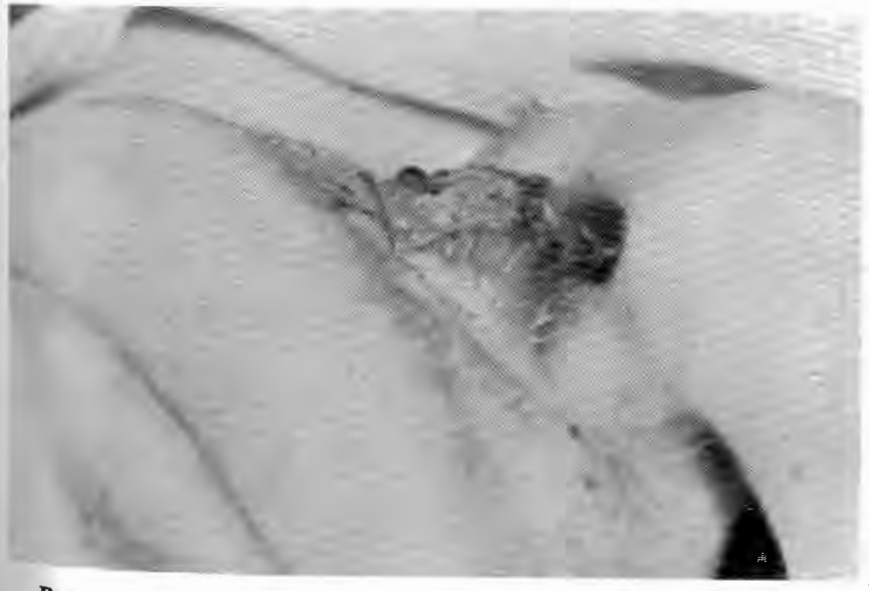


PLATE 19. Patient L., 19 April 1949. During biopsy. Photograph taken on the operating table.

PLATE 20. Patient L., 19 April 1949. Tumour segment measuring 0.5 cm excised for biopsy.

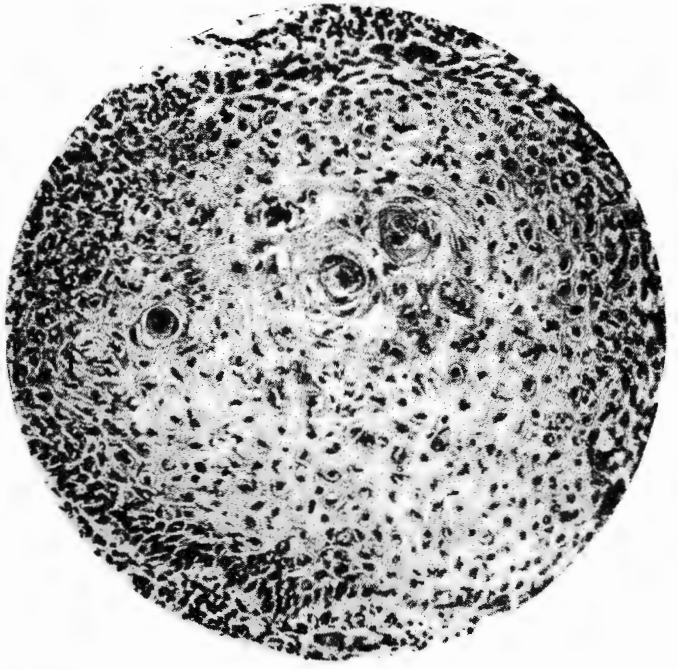


PLATE 21. Patient L., April 1949. Histological structure of the tumour before treatment with the preparation: keratinizing squamous-cell carcinoma, with carcinomatous pearls.

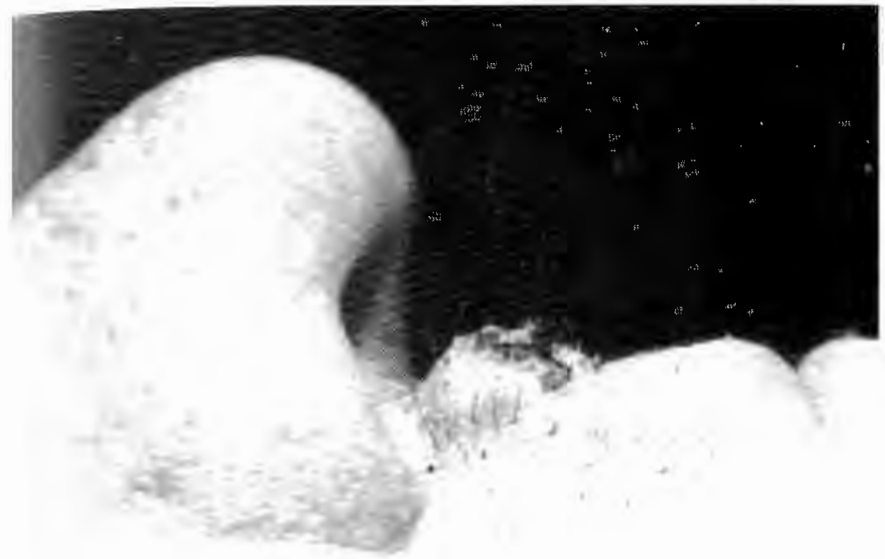


PLATE 22. Patient L., 9 May 1949. 3 weeks after the biopsy and from the start of the course of injections. The affected portion remaining after biopsy.

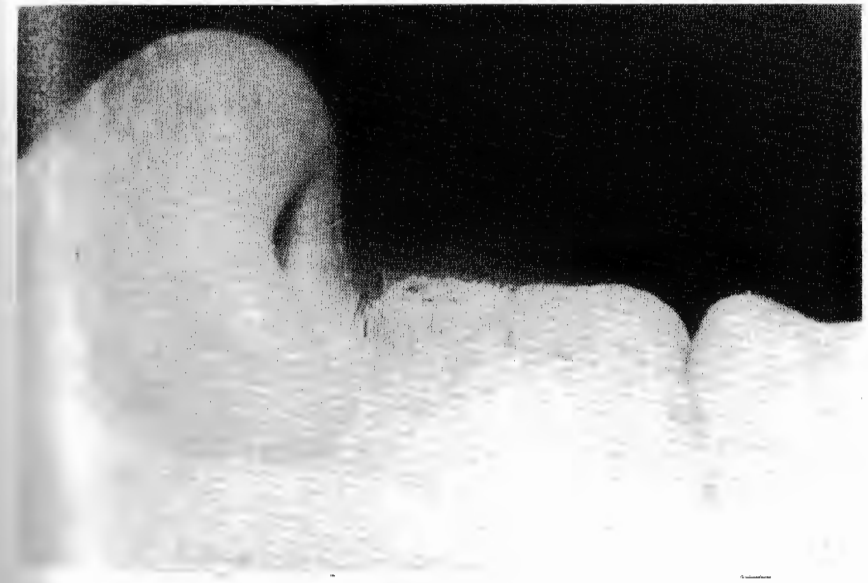


PLATE 23. Patient L., 4 June 1949. 1½ months from the start of the course of injections. 9000 units injected. The scab has fallen off. The neoplasm has become flatter: it projects 0.2 cm above the level of the normal tissue instead of the previous 1 cm.

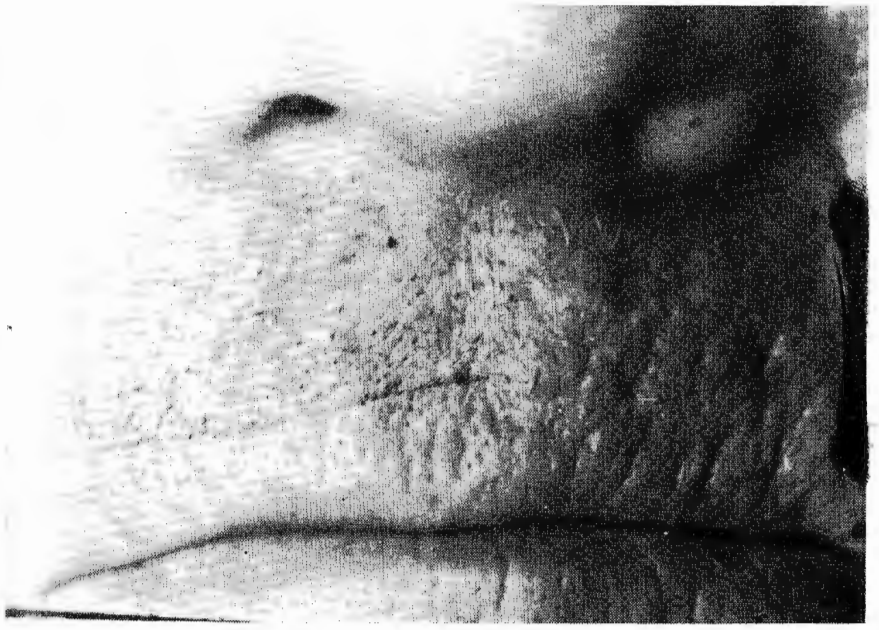


PLATE 24. Patient L., 1 July 1949. $2\frac{1}{2}$ months after the start of the course of injections. 12,150 units injected. No tumour is present. At its site is a puckered depression 0.2 cm below the level of the normal tissue. State of the lymphatic nodes: on the right—of the two nodes, one measuring 1.2×0.75 cm remains, on the left—a soft node.

PLATE 25. Patient L., 6 months after the start of treatment. No tumour is present. Clinical recovery.

In view of the insufficiently conclusive histological picture a second *biopsy* was carried out 7 days later, 4 June 1949, with excision of a segment of the ulcerated surface from the medial aspect representing one quarter of its area. A photograph (Plate 8) shows the state of the affected area before the start of the injections.

Histological examination of material taken at the *second biopsy* revealed a picture of keratinizing squamous-cell carcinoma with extensive histiocyte infiltration of the connective tissue core (Plate 9).

A course of injections of modification VI of the trypanosome preparation was started on 29 May 1949.

INJECTIONS OF THE PREPARATION AND COURSE OF THE DISEASE

The preparation (modification VI) was administered to the patient intramuscularly once daily in the doses shown below:

Injection	Dose in units	Date
1	50	29 May 1949
2–4	100 each	30 May–1 June
5	150	2 June
6–19	200 each	3–18 June
20	250	19 June
21–23	200 each	20–23 June
24–67	200–300 each	24 June–31 Aug. 1949

A total of 15,015 units was given in 67 injections.

On 25 June, after 24 injections, the affected area measured 1.5×1.0 cm, and on palpation merged without distinct borders into the surrounding tissues; it was of a firmer consistency than the latter. Its surface bore two scabs. One of them measured 0.2×0.3 cm, and on removal left an area covered by a thin layer of epithelium. The second scab measured 0.5×0.3 cm. Oval, rather consolidated lymphatic nodes measuring 1.2×0.75 cm could be palpated on both sides of the submandibular region. After 30 injections (5,650 units) the tumour had decreased markedly in volume and become flatter. After 2 July the patient started to receive his injections as an out-patient, allowing frequent intervals, so that during the next two months he received only 30 injections instead of 60 (Plates 10 and 11).

On 27 July, after 48 injections a sunken area had formed at the former tumour site; it was soft to the touch, 1 cm in diameter and 0.2 cm deep.

By 1 September, after 67 injections involving 15,015 units of the preparation, normal mucosa had grown over the former tumour site. The

ulcerated surface had become completely covered by epithelium. The submandibular lymphatic nodes were soft (Plates 12 and 13).

On 1 September the patient underwent a control wedge resection to allow histological examination of the area corresponding to the previously ulcerated surface together with the biopsy scar and part of the surrounding mucosa (Plates 14 and 15).

Here is a report on histological examination No. 267, carried out 2 September 1949." The specimen examined was a portion of the lower lip consisting of the biopsy scar and the site of the formerly ulcerated surface.

The examination revealed a marked acanthosis with moderate hyperkeratosis. The connective tissue core showed a picture of chronic inflammatory granulation, the various cellular elements of which (chiefly round plasma cells) had infiltrated into the dermis in large numbers. In some areas scar tissue was forming. Various parts of the excised portion contained large foreign body granulomata, with many giant cells surrounding an amorphous mass" (Plate 16).

After this control biopsy the patient was given a supplementary course of 10 injections, after which, on 14 September 1949, he voluntarily terminated the treatment, having received a total of 78 injections in 110 days.

Analysis of the observations on the course of the carcinomatous process in patient R. shows that injections of the trypanosome preparation (modification VI) caused regression of a keratinizing squamous-cell carcinoma of the lower lip.

The regression was marked by the following clinical signs: epithelialization of the ulcerated surface, softening of the previously firm tumour mass, and the disappearance of firm regional lymphatic nodes.

The clinical regression was supported by histological findings: the picture typical of a malignant neoplasm disappeared and its place was taken by the elements of an inflammatory reaction and scar tissue.

The combination of all these findings enables us to conclude that injections of the trypanosome preparation caused in patient R. a *complete* process of regression, as a result of which there was *clinical recovery*.

Examinations on 18 September 1949 and 20 March 1955: lip in normal condition, no signs of recurrence, lymphatic nodes not palpatable.

Period of observation—5 years 10 months.

OBSERVATION NO. 4

Patient L., male, aged 64 years. Clinical diagnosis: *carcinoma of the upper lip*. Histologically: *keratinizing squamous-cell carcinoma.*

At the beginning of February 1949 a wart appeared on the right of the patient's upper lip. It gradually increased in size and a scab formed at its centre.

The patient did not attempt any treatment.

On 8 April 1949 he was admitted to our clinic for biotherapy.

SYMPTOMS OF THE CONDITION

On admittance to the clinic on 8 April 1949 patient L. had on his upper lip to the right of the mid-line a tumour mass measuring 1.5×1.25 cm, 1 cm high. The upper part of the tumour was covered by a scab.

In the right submandibular region there were two firm lymphatic nodes, one measuring 1.5×0.75 cm and the other 0.8×0.5 cm. On the left were two nodes of soft consistency measuring 0.6×0.4 cm and 0.5×0.3 cm (Plate 17 *a*, *b*).

A biopsy was performed on 19 April, involving excision of a portion of the tumour 0.5 cm in diameter (Plates 18–21). A course of injections of the trypanosome preparation (modification VI) was beg un on 19 April

INJECTIONS OF THE PREPARATION AND COURSE OF THE CONDITION

The injections were made intramuscularly in the buttocks, using daily doses of modification VI of the preparation. The individual doses were as follows:

Injection	Dose in units	Date
1–2	100 each	19–20 April 1949
3–60	200 each	21 April–29 June 1949

Reaction to the injections was expressed as a rise in temperature, usually to 37·5–37·8°C and in a few cases to 38°C.

The scab was sloughed after 31 injections (6000 units). The tumour became flatter, extending only 0·2 cm beyond the level of the surrounding normal tissue instead of the previous 1.0 cm. The tumour core, formerly firm, grew softer.

After 60 injections, involving 12,150 units, the tumour had disappeared. In its place there remained a puckering associated with the biopsy scar. The surrounding normal tissue appeared to project beyond the puckered region by 0.2 cm, due to the fact that the central part of the former tumour was sunken. The previously affected area was somewhat firmer on palpation than the surrounding tissue.

The state of the lymphatic nodes was: on the right, one of the two nodes remained, somewhat consolidated and measuring 1.2×0.75 cm, while on the left were two nodes, one 0.75×0.5 cm and the other 0.6×0.6 cm, soft in consistency as before.

Plates 22–24 (*a*, *b*) show the process of tumour regression in patient L. during course of the injections of trypanosome substance.

Clinical observations on patient L. showed a picture of complete regression, brought about by the trypanosome preparation.

Microscopical examination was still required to exclude the presence of malignant cells, which could have existed among the normal tissue. A control biopsy was eventually carried out at the end of the course of injections on 12 September 1949. Here is the report on the histological investigation:

"Report No. 257, dated 13 September 1949.

The examination was of the scar remaining after a tumour of the upper lip. The epithelial covering and Malpighian layer were uneven. In some places there were small papillary outgrowths, and in others thin sunken cords of squamous epithelial cells. The deeper layers of the dermis contained abnormal cords of atrophic epithelial cells, some of them with central keratinization and peripheral granulation. The connective tissue core of the biopsy sample was infiltrated by friable strands of a lympho-histiocyte infiltrate containing many plasma cells. One of the preparations showed in the deeper layers of the dermis a clearly defined nodule, the central part of which consisted of keratinized scales surrounded by a layer of giant cells, and the periphery of several layers of lympho-histiocyte elements. This nodule was obviously the remains of an epithelial pearl which had undergone giant cell resorption. No evidence of malignancy was seen in any of the preparations".

Histological investigation therefore supported the clinical picture of regression of the malignant tumour in patient L. (Plate 25).

Examination on 15 February 1950: no recurrence or metastases.

OBSERVATION NO. 5

Patient K., male, aged 52 years. Clinical diagnosis: *carcinoma of the lower lip*. Histologically—*early squamous-cell carcinoma*.

In 1921, after a bruise, a dark spot formed on the lower lip; it gave no pain, did not interfere with speech or eating, did not enlarge and did not alter its external appearance. In May 1947 this spot began to enlarge

and occupy the whole thickness of the lower lip; it grew firmer and changed colour from blue-red to white.

The patient sought treatment on 30 July 1947, complaining that the tumour on his lip interfered with eating and speaking.

SYMPTOMS OF THE LIP AFFECTION ON ADMITTANCE TO CLINIC, 30 JULY 1947

On the left of the lower lip was a tumour measuring 1.2×0.8 cm, protruding 0.1–0.2 cm above the level of the mucosa, fairly firm in consistency, with a somewhat macerated surface dull white in colour or lilac in places. On pressure it took on a mother-of-pearl appearance. The whole growth was clearly defined and irregularly oval in shape. Its limits could be clearly palpated, merging into the posterior surface without definite borders. Some parts of the lip showed areas of hyperkeratosis, the largest being 0.1–0.2 cm in diameter (Plate 26).

Fairly firm lymphatic nodes, oval in shape and measuring 1.0×0.5 cm, could be palpated on both sides in the submandibular region. Lymphatic nodes measuring 0.1×0.2 cm could be felt indistinctly in the mental region.

During 1947 and 1948 patient K. received two courses of injections, with a 7-month interval.

FIRST SERIES OF INJECTIONS AND COURSE OF THE CONDITION

The patient received 57 injections of modification VI of the preparation between 5 August and 11 September 1947. The preparation was given intramuscularly in daily doses, except on rest days. After the sixth day 200 units daily were also injected paratumourally. The patient received the following intramuscular doses:

1st	injection	200 units
2nd	"	400 units
3rd	"	600 units
4–10th	"	800 units each
11–33rd	"	1000 units each
33–57th	"	1600–2000 units each

The 57 injections involved a total of 73,700 units.

A histological examination was made on 5 August by three pathologists: one specialist concluded that the biopsy material showed a picture of chronic inflammation of the Malpighian layer, the second concluded it to be "a malignant neoplasm" and the third "an epithelioma".

The first signs of improvement were evident after 12 injections (4000 units). The tumour grew flatter, and hardly projected beyond the level

of the mucosa. Of the areas of hyperkeratosis, one, measuring 0.2×0.3 cm, remained.

After 22 injections a second biopsy was made, involving the excision a tumour fragment 0.3×0.2 cm. Histological diagnosis: "hypertrophic angioma".

After the administration of 73,300 units (57 injections) the former tumour site was occupied by two areas 0.3 and 0.2 cm in diameter, separated by a narrow scar—the remains of the earlier biopsy. They were rather more firm on palpation than the surrounding tissue. Externally this part of the lip was indistinguishable from the rest. The tumour, which had earlier shown clinical sings of malignancy, had vanished. The hyperkeratosis had vanished. The lymphatic nodes remained unchanged (Plate 27).

By this time the patient had gained 7 kg in weight. Because of the clinical improvement in the tumour process and the divergence between the histological findings and the clinical picture, the first course of biotherapy was terminated on 11 September 1947.

FURTHER COURSE OF THE CONDITION

The patient remained under observation as an out-patient. Seven months later, at the end of April 1948, a tumour appeared *again* on the site of the former tumour, resembling externally a papilloma with hyperkeratosis.

The tumour was firm (of fibrous consistency), painless on palpation, protruded a little above the level of the red portion of the lip, and on pressure stood out clearly as a white growth like a corn.

Lymphatic nodes could be palpated on both sides of the submandibular region, oval in shape, soft in consistency, mobile and measuring 1.0×0.75 cm on the right and 1.0×0.6 cm on the left.

Histological examination on 14 May revealed an early squamous-cell carcinoma (Plates 28 and 29). The patient was given another course of biotherapy.

SECOND SERIES OF INJECTIONS OF THE PREPARATION AND COURSE OF THE DISEASE IN 1948

1st	injection	— 14 May 1948	—	200 units—biopsy
2nd	,,	— 15 May	,,	— 400 units
		— 16 May	,,	— No injection
3rd	,,	— 17 May	,,	— 400 units
4th	,,	— 18 May	,,	— 600 units
5th	,,	— 19 May	,,	— 800 units
6th	,,	— 20 May	,,	— 1000 units

7th	injection	— 21 May	,,	— 1200 units
		— 22–23May		— No injection
8th	,,	— 24 May	,,	— 1400 units
9th	,,	— 25 May	,,	— 500 units

The injections were interrupted as the patient had to go away. They were recommenced on 7 June:

10th	injection	— 7 June 1948	— 1500 units
11–41st	,,	— 8 June—14 July	— 1200–2100 units
42–88th	,,	— 15 July–4 Sept.	—2000–3000 units

From 14 May to 4 September, 88 injections were given, involving 273,220 units.

On 7 June, 19 days after the biopsy, the affected area of the lip consisted of an elevation, whitish in colour, fibrous in density and 0.3×0.4 cm in size, situated in the region of the red border of the lip, 1 cm from the left commissure.

After 56 injections (112,220 units) there was a marked improvement in the process.

After 88 injections, when 184,720 units of the preparation had been given, it was decided to carry out a *control biopsy* to establish whether the tumour had suffered structural changes. By this time the affected area appeared as a regularly circular papule 0.2 cm in diameter, somewhat pigmented, greywhite in colour and firm on palpation. Several soft, mobile lymphatic nodes could be palpated in the submandibular region; on 10 August the patient underwent wedge resection of the *whole* of the visibly affected portion of the lip.

Pathohistological findings: "Histological examination showed considerable thickening of the epithelial layer with hyper- and parakeratosis and extensive subepithelial infiltration. *There were no signs whatever of malignant growth*" (Plate 30). The course of biotherapy was continued until 4 September 1948.

Patient K. remained under observation until April 1951. The previously affected area of the lower lip was consistently soft on palpation, as was all the remaining surface of the red border of the lower lip. Soft or somewhat firm mobile lymphatic nodes could be palpated from time to time in the submandibular region, disappearing meanwhile.

The last examination on 2 April 1951 showed the absence of any signs of recurrence or metastases. The scar was soft.

Period of observation—3 years 8 months (Plates 31 and 32).

To assess the role of biotherapeutic interference in the development of the disease in patient K., the following facts must be considered.

The condition arose 3 years 7 months previously, in May 1947. During this time the patient had undergone the following:

(1) two courses of injections of the trypanosome substance

(2) four biopsies.

The first phase of the disease was characterized by a period of progressive tumour growth. It lasted for three months from the onset of the condition (May, June and July 1947). The patient received no treatment during this period. The second phase (August and September)—the period of injections of the preparation—was characterized by cessation of growth and subsequent regression of the tumour, which decreased in size, softened and grew flatter. At this time, as histological investigation shed some doubt on the malignant nature of patient K.'s condition, the course of biotherapy was terminated in spite of the clinical diagnosis and the patient discharged with residual signs, showing marked improvement, but without having undergone a complete course of treatment. This was followed by a third phase—the period of about 7 months from October 1947 to April 1948—a state of maintained clinical quiescence, with a flattened structure measuring 0.5×0.3 cm remaining at the site of the former tumour.

Seven months later in April 1948, the patient again approached the clinic because of renewed tumour growth at the same site. This time histological examination confirmed the clinical diagnosis of carcinoma and the course of injections of the preparation was re-instituted.

The progressive growth was replaced by tumour regression, later confirmed histologically.

Finally, the last phase—clinical recovery—commenced in August 1948 and lasted until observations were discontinued in April 1951.

What, in this case, is our *estimate of the therapeutic significance* of the preparation?

Patient K. did not receive any other form of treatment during the whole course of his disease. The repeated biopsies carried out for diagnostic purposes cannot be regarded as therapeutic interference. The whole practice of modern oncology indicates that a biopsy does not halt the growth of a malignant tumour and does not bring about tumour regression. If it has any effect on the development of a malignant neoplasm it is in fact in quite the opposite sense, i.e. it exacerbates its growth. Consequently, in this and in many similar cases it would be at best implausible to ascribe the cessation of growth, regression and finally clinical recovery to the repeated biopsies. If we believed this, we would have to accept the therapeutic effectiveness of such a peculiar and impracticable operative interference as the removal of a malignant tumour piece by piece over

$1\frac{1}{2}$ years, the excision of a piece of the tumour *each time* bringing about cessation of growth and regression of the neoplasm, finally leading to clinical recovery of the patient. This sort of explanation is in direct contradiction to our concrete knowledge of the harmful role of partial operative interferences and of the extreme dangers of nonradical surgical treatment.

"When any kind of radical excision of malignant tumours is known to be impossible, it is better to refrain from operating altogether than to knowingly remove only part of the tumour..." "It is better to take this course, because *nonradical operations not infrequently exacerbate the condition, leading to uncontrollably rapid growth and metastasis formation by the tumour** (this is particularly dangerous in sarcomata and pigmented tumours)" (N. N. Petrov (1947), Vol. 1, Pt. 1, p. 49).

The following should be emphasized: in the preparations of the biopsy material, as shown by the findings of the pathologist Prof. Rapoport and as can be seen microscopically, the border of the biopsy sample passed *within the malignant tissue*. The preparations show its transversely cut strands. Thus, only *part* of the malignant tumour was excised at biopsy, the rest remaining untouched in the body.

The second procedure carried out in patient K. was a course of biotherapy consisting of injections of the trypanosome preparation. Two coures were given, covering two years—1947 and 1948. Both the first and second courses were accompanied first by cessation of the growth of the tumour and then by its flattening and softening.

Termination of the biotherapy was associated at first with stabilization of the process, but later with renewed tumour growth, which was again arrested on re-institution of the injections. Thus, in this case the phenomenon of *controllable regression* of the tumour was seen. Clinically apparent regression of the tumour was accompanied by changes in its morphological structure: the elements of malignant neoplasia disappeared and were replaced by normal tissue.

This parallelism between tumour development and injection of the preparation—progressive growth in the absence of injections and regression during administration of the preparation—forces us to recognize the *direct dependency* of patient K.'s clinical recovery upon the course of biotherapy, as the only therapeutic factor giving a positive result in this case of a squamous-cell carcinoma, a condition which was further complicated by repeated biopsies.

*Authors' italics

The observations on patient K. draw our attention to the following: *repeated* administration of the preparation after an interval of several months gave a *positive cancerolytic result*, whereby tumour regression took place during the *second* course of injections, just as it had during the *first* course.

The observations also enabled us to decide *whether the antineoplastic substance in the trypanosome preparation possesses any immunizing powers.*

In patient K. a *positive action* by the preparation *was achieved twice* within 2 years, with an interval of 7 months between the two courses of injections.

The conditions of administration of the preparation were such that in the presence of any immunogenic factor the injections would inevitably have produced an immunity in the body. During the first course in 1947 the patient received intramuscular injections of the preparation daily for 2 months. The total number of injections was 57, followed by an interval of 7 months. Then the injections were renewed, giving a positive effect. During the first course the patient had received an adequate dose of the preparation parenterally, i.e. by the route most effective in producing antigenic stimulation of the body.

If we take an immunological parallel, the second course of injections, also parenteral, given 7 months later, would be a revaccination, when, as is well known, antibodies are formed to a high titre even in refractory bodies which are distinguished by their inability to produce antibodies in response to a primary cycle of immunization.

Hence, in patient K., conditions were exceptionally favourable for the formation of immunity to the trypanosome substance.

The presence or absence of an immunity could be decided firstly by demonstrating antibodies acting specifically on the trypanosome antigen *in vitro*, and secondly by observing (and this is the more important) the effects of the trypanosome antigen on the tumour after subsequent injections.

The findings described earlier show clearly that the second course of injections of the trypanosome extract was active with regard to the tumour. The picture of progressive remission is incompatible with and excludes any idea of an immunizing action by the preparation.

Thus, under conditions which were optimum for the production of immunity in the body, *no immunity developed in the tumour*. In this particular case this was demonstrated by the fact that tumour regression was induced *twice* in one year in one patient. This absence of immunity in the tumour tissues is in peculiar juxtaposition to the general state of immunity of the body. It should be borne in mind that modification VI of the preparation as used for the treatment of patient K. contained an antigenic agent, possessed immunizing properties and on injection into the body led to the accumulation of specific antibodies. For all this, the circulating antibodies were obviously incapable of inactivating the cancerolytic factor, which for this reason continued to act on the tumour even in a body containing specific anti-trypanosome antibodies.

It should be noted that paratumoural injection has no apparent advantages over the intramuscular route, since the former is no more effective and moreover has a number of drawbacks: it limits the possibilities of raising the volume of the doses, and is accompanied by pain and oedema at the injection site.

OBSERVATION NO. 6

Patient A., female, aged 60 years. Clinical diagnosis: *cancer of the lower lip*. Histological diagnosis: *keratinizing squamous-cell carcinoma.*

Examination on admittance to the clinic on 2 August 1947 revealed: the right half of the lower lip was slightly thicker than the left, and the edge of the red portion bore two ulcers — one measuring 1.7×0.4 cm, oval in form, the other smaller, with firm edges (Plate 33).

On 11 August 1947 the patient started a course of injections of the preparation in doses increasing from 50 to 1000 and 2000 units a day. Some of the preparation was given paratumourally (all round the tumour) and some intramuscularly. On 25 August, i.e. 14 days later, a *biopsy* was carried out — part of the ulcer (Plate 34) was excised under local anaesthesia.

After 30 injections the ulcer, part of which had been removed during the biopsy, had healed over with a smooth scar covered by fresh delicate epithelium of normal appearance. The other ulcer was also covered by normal epithelium over two thirds of its surface. An ulcerated surface 3 mm in diameter remained, covered by a very thin scab. The patient gained 2 kg in weight.

After 62 injections (48,000 units) there remained only an insignificant papule, 0.5 cm in diameter, white in colour, with a fairly firm peripheral ring and a central scab.

Subsequently during the process of the injections the scab was sloughed several times, leaving an ulcerated surface over which the scab reformed. The remaining affected area grew gradually smaller, but did not heal like the neighbouring surface.

After 129 injections (184,960 units) the scab had grown still smaller and become more delicate.

After 142 injections, including 28,000 units given paratumourally and 181,070 units intramuscularly, the situation showed little change: the previously affected surface was almost completely covered with normal epithelium, but a small portion still bore a dry scab, and no change could be observed in this fragment.

At this stage, the injections were terminated. An examination 3 weeks later showed the same picture: a scab, 0.3 mm in diameter, with a firm rim on one side. During the next month the consolidated area dispersed and the whole lip softened. A small scab 0.2–0.3 cm across remained.

In this observation attention is drawn by the fact that the regression process continued after the cessation of injections, as was also seen in the case of patient L. (observation No. 9). In the absence of other findings we can only note that both these cases differed from the others in that some of the injections were made immediately round the tumour lesion—paratumourally—as well as intramuscularly. It cannot be exlcuded that in both cases tissue oedema obscured the picture of regression, which became obvious as the local reaction subsided. This is, of course, only a suggestion, and further studies are required.

A clinical examination of the patient on 27 December 1950 showed the absence of any signs of the condition. The biopsy scar was soft and smooth. No lymphatic nodes could be palpated in the mental or submandibular regions (Plate 35).

Period of observation: 3 years 4 months.

OBSERVATION NO. 7

Patient V., male, aged 48 years. Clinical diagnosis: *cancer of the lower lip*. Histological diagnosis: *keratinizing squamous-cell carcinoma.*

On admittance, the right half of the lower lip bore an ulcer measuring 1.3×1.5 cm. Its central portion was covered by a dark scab, and it was bordered by an irregular firm rim. The ulcer was not painful. An enlarged, firm lymphatic node, 2×1.5 cm, mobile and painless, could be palpated in the submandibular region. The cervical nodes were not palpatable (Plate 36).

A *biopsy* was performed on 8 March 1948, involving excision of half of the lower lip tumour under local anaesthesia (Plates 37a and b).

Injections of the preparation were commenced on 9 March 1948; 100–200 units were given daily for the first 5 days, 600–800 units on days 6 to 10 and 1000–2000 and 4000 units on days 11 to 81 — a total of 111,000 units in 81 injections.

After 20 injections the tumour remaining after the biopsy measured 0.7×0.3 cm. Epithelium of normal appearance was forming at the edges of the ulcer. The ulcer was irregularly shaped, since in some parts of it there were strands of proliferating epithelium running in from the periphery to the centre.

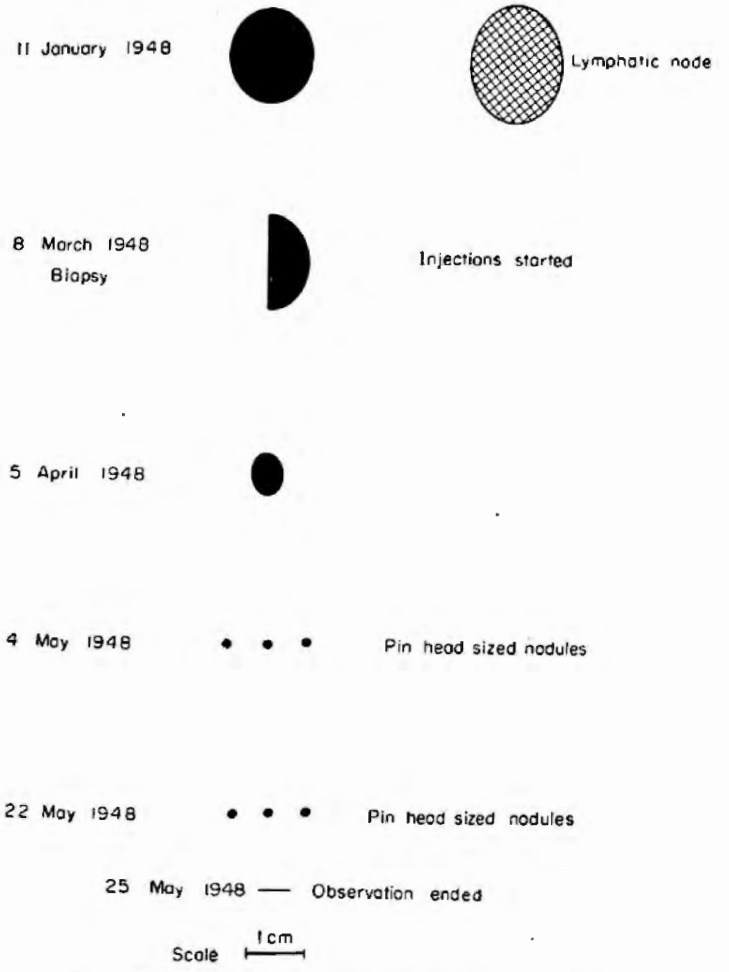


FIG. IV. Diagram of tumour regression in patient V.

After 44 injections (59,860 units) the site of the former tumour was occupied by three papules the size of a pin-head. Zones of pale epithelium had formed at its centre, spreading radially. The ulcer had healed over (Fig. IV).

After 57 injections a firm rim-like thickening 0·2 cm across appeared on the lateral edge of the affected area on the mucosal side. Its nature remained obscure, since three weeks later, on June 1948, the patient left for reasons beyond our control.

Hence, although regression of a squamous-cell carcinoma in patient V. had reached a high degree, regression of the tumour was still not completed when the observations ceased. Nevertheless, the clinical picture gave reliable evidence that injections of the preparation had produced a cancerolytic reaction resulting in regression of the tumour (Plate 38). The biopsy carried out during the course of injections was not associated with any provocation of malignant growth and did not affect the regression process.

* * *

One of the basic and guiding principles in the production of a cancerolytic reaction is the maintenance of continuous saturation of the body with the preparation containing the regression factor. If the administration of such a product gives rise to the regression process, then the termination of injections causes this process to stop. At first the tumour remains, as it were, stabilized, and the process appears permanent in nature, but soon growth of the neoplasm returns to the rate prevailing before the onset of the injections. This phenomenon can be explained if we remember the effects of antibiotic substances on bacterial populations. Even without resorting to this analogy, it can easily be imagined that malignant cells will not multiply or a tumour grow only when this growth is opposed by a factor inhibiting the viability of the tumour cells. If this factor is removed, and some of the cells remain in the body undamaged, their growth is inevitable until some active effect halts it.

Observation No. 8 illustrates this position.

OBSERVATION NO. 8

Patient F., male, aged 35 years. Clinical diagnosis: *cancer of the lower lip*. Histological diagnosis: *keratinizing squamous-cell carcinoma*.

In August 1948 a fissure, and later a papule, appeared on the lower lip; the patient punctured it several times with his teeth, after which an ulcer, covered by a scab, developed on this portion of the lip. It increased in size. The patient attempted no treatment.

Patient F. was admitted to our clinic for biotherapy 28 December 1948.

SYMPTOMS ON ADMITTANCE

On the lower lip, on the right of the red portion, there was a tumour measuring 1.7×1.7 cm, of fibrous consistency, protruding 0.5 cm above the level of the normal mucosa. The neoplasm was surrounded by a firm, raised rim. On palpation the tumour could be felt to a depth of 1–1.2 cm. In the right submandibular region was an indurated lymphatic node of a cancerous nature measuring 2×1 cm, and on the left there were two enlarged, soft nodes, 1×0.5 cm and 1×0.6 cm. A firm nodule, apparently lying on the bone, measuring 0.5×0.3 cm, and a soft node measuring 1×0.6 cm could be palpated in the mental region (Plates 39*a* and *b*).

On 6 January 1949 the patient underwent a biopsy, consisting of wedge resection (Plates 40 and 41) of part of the tumour ulcer. A segment 0.5×0.5×0.3 cm was excised. Histological examination showed "keratinizing squamous-cell carcinoma, with marked infiltration of the stroma with round cells and small histiocytes" (report No. 126).

FIRST SERIES OF INJECTIONS AND COURSE OF THE CONDITION

Patient F. was given two courses of injections of the preparation. The first course extended from 7 January to 7 March—59 days, during which time 47 injections were made. One injection was given daily. Thus, the patient had 12 rest days in the 59-day course. The injections were of modification VI of the preparation, given intramuscularly in the following doses:

Injections	Dose in units	Date
1	300	7 Jan.
2	700	8 Jan.
3	500	9 Jan.
4–6	600 each	10–14 Jan.
7–47	1000 each	15 Jan.—7 March

Signs of tumour regression began to appear during the course of the injections. After 19 injections, when 16,300 units of the preparation had been given, the tumour measurements had decreased to 1×0.7 cm. The peripheral rim grew flatter and hardly projected above the surrounding tissue. On palpation the tumour could be felt to a depth of 0.5 cm. The lymphatic node on the right maintained its firm consistency but was *halved in size*, and those on the left also grew smaller, one to 0.75 cm and the other to 0.5 cm in diameter. Nodes could no longer be palpated in the mental region.

By 8 February, after 27 injections (23,900 units), the tumour had become even smaller. Examination revealed only a flattened growth covered by normal epithelium, slightly paler than the surrounding tissues.

After 42 injections (39,300 units) the area formerly occupied by the tumour consisted of a depression 0.2 cm deep and 0.8 cm in diameter, diffusely thickened and covered by normal epithelium. A fibrotic area could be felt only in the region of the biopsy scar. The lymphatic node on the right measured 1.5×0.8 cm. The nodes on the left and in the mental region were soft, mobile, and measured: submandibular 1×0.5 cm, mental 0.3 cm in diameter (Plates 42*a* and *b*).

The first course of injections of the preparation, as evidenced by the case history, led to *regression of the tumour*:

(1) tumour growth was *arrested*;

(2) there was considerable *decrease in the size* of the lesion. On the site of the former tumour, previously about 2 cm in diameter and 0.5 high, there remained only a depression measuring $0.5 \times 0.8 \times 0.2$ cm.

Before treatment the tumour could be felt to a depth of 1–1.2 cm in the thickness of the lip, but after the first course of injections the residual lesion was *superficial* in nature;

(3) the ulcerated surface became completely *covered by epithelium*;

(4) the lymphatic node on the right, showing signs of cancerous induration, was *halved in size*;

(5) there was diminution and partial or complete disappearance of the left submandibular and mental nodes.

The combination of these symptoms characterized the onset of *tumour regression*, as recorded in photographs (Plates 39–42).

At this stage the patient, because of business matters and possibly also because of the marked improvement which had occurred, started to attend irregularly for injections, and from 8 March the course was interrupted for 25 days.

SECOND SERIES OF INJECTIONS AND COURSE OF THE CONDITION

After this interval of one month there was some deterioration in the process; a thickened area of 0.5×0.8 cm, with central ulceration, appeared in the affected portion (Plate 43). In spite of this the patient still attended irregularly during the second course of injection, which was started on 1 April. For this reason he received only 45 injections in three and a half months, instead of 110.

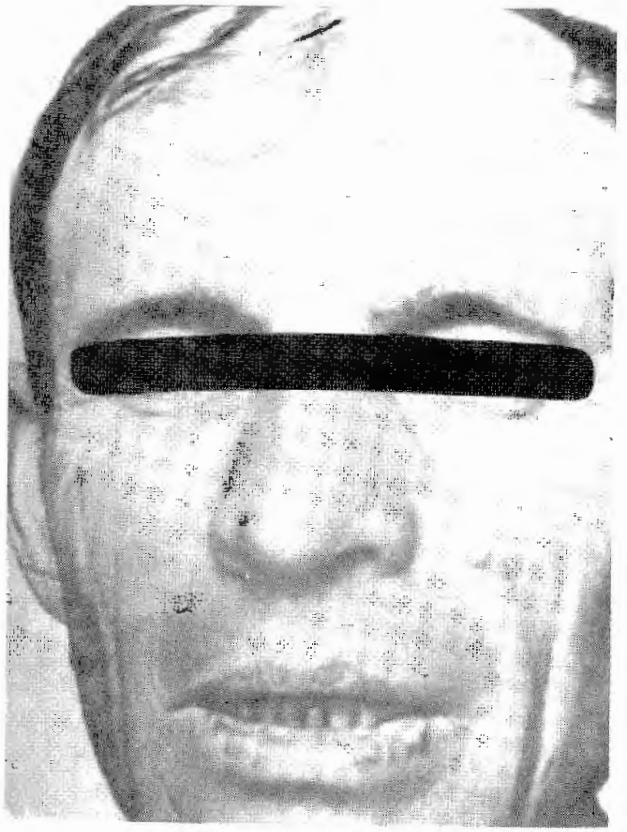


PLATE 26. Patient K., Observation No. 5. Squamous-cell carcinoma. 30 July 1947. Before the start of the first course of injections: on the left of the lower lip is a tumour measuring 1.2×0.8 cm, projecting above the level of the mucosa by 0.1–0.2 cm. The surface is covered by areas of hyperkeratosis. Histological diagnosis by three specialists: (1) chronic inflammation of the Malpighian layer; (2) epithelioma; (3) hypertrophic angioma.

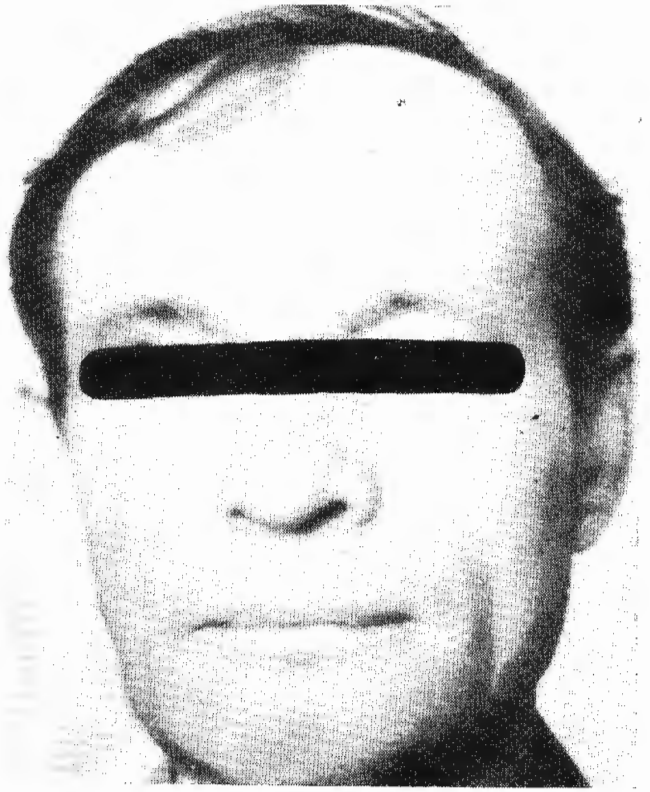


PLATE 27. Patient K., 11 September 1947. At the end of the first course, after 57 injections. The tumour has regressed. The hyperkeratosis has disappeared. The patient has gained 7 kg in weight. The course of injections was interrupted because of the divergent histological conclusions and the clinical picture of the disease.

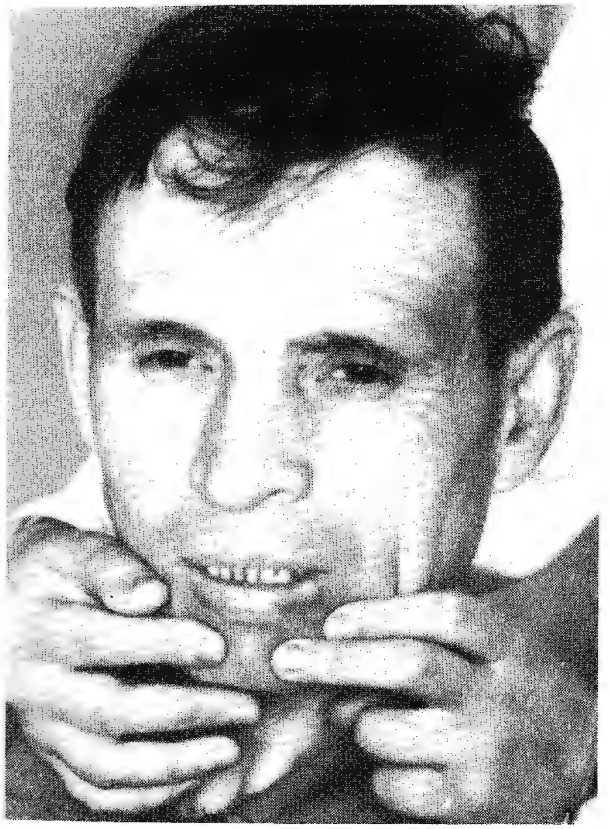


PLATE 28. 7 months after the end of the first course of injections: a recurrence. At the site of the former tumour a new tumour has appeared, superficially resembling a papilloma with hyperkeratosis and measuring 0.3×0.4 cm.

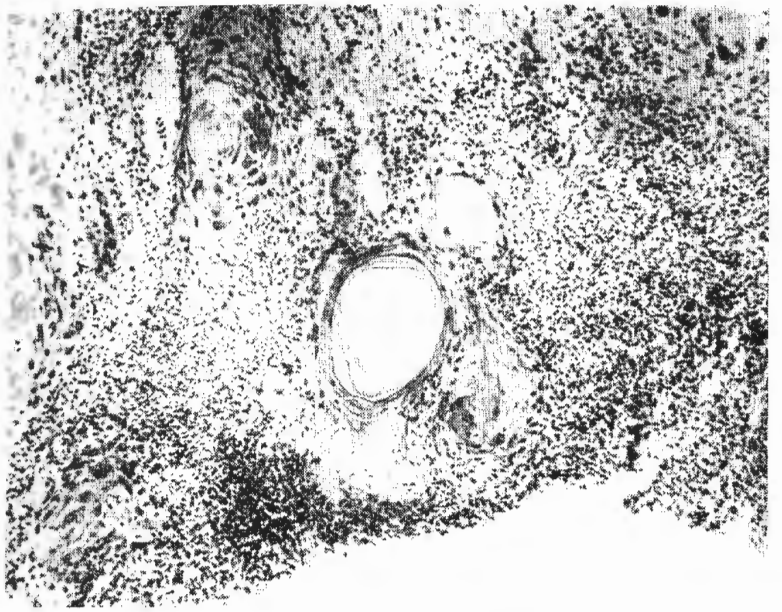


PLATE 29. Patient K., 14 May 1948. Histological structure of the recurrent tumour: squamous-cell carcinoma, a field from the depths of the preparation.

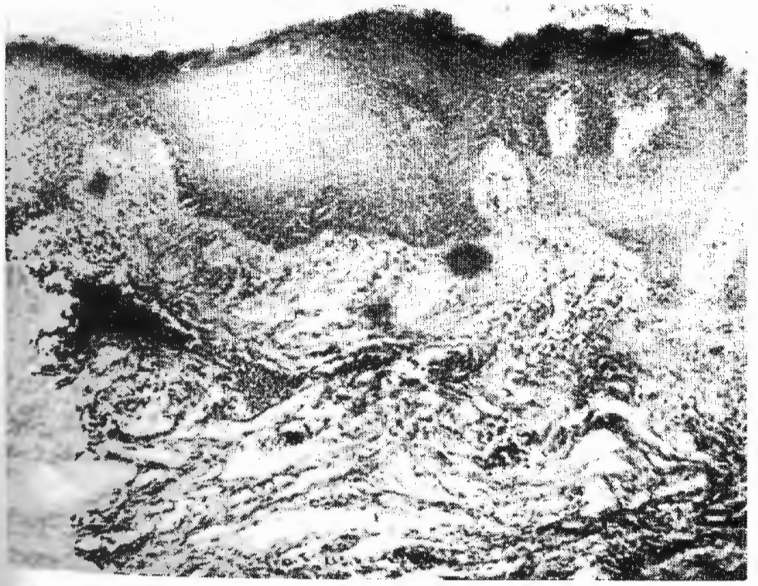


PLATE 30. Patient K., 10 August 1948. Control histological investigation carried out after 88 injections of the preparation, showing considerable thickening of the epithelial covering with hyper- and parakeratosis and extensive subepithelial infiltration. There are no signs whatever of malignant growth.

PLATE 31. Patient K., 9 October 1949. One year after second course of injections. No tumour present.

PLATE 32. Patient K., 2 April 1951. No tumour is present, and there are no signs of recurrence or metastases. Period of observation, 3 years 10 months.

PLATE 33. Patient A. Observation No. 6. Keratinizing squamous-cell carcinoma. 27 July 1947. Before the start of injections of the preparation. At the edge of the red border of the lip are ulcers, one measuring 1.7×0.4 cm, the other somewhat smaller and surrounded by a firm border. The right half of the lower lip is slightly thicker than the left.

PLATE 34. Patient A., 25 August 1947. Histological structure of the tumour at the start of treatment: keratinizing squamous-cell carcinoma.

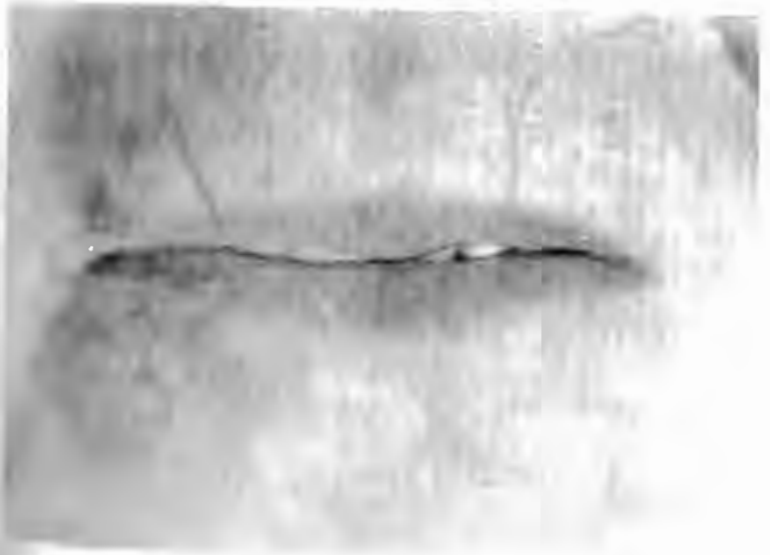


PLATE 35. Patient A., 27 December 1950. The patient has received 142 injections—28,000 units paratumourally and 181,070 units intramuscularly. There are no signs of the previous affection. The lip is soft. Period of observation, 3 years 4 months.

Here is a list of the injections and omissions during the *second course*:

in March — 5 injections
in April — 15 injections
in May — 14 injections
in 18 days in June — 11 injections

1 April	— 100 units	12 April	— missed
2 „	— missed	13 „	— 200 units
3 „	— missed	14 „	— 300 „
4 „	— 100 units	15 „	— 400 „
5 „	— missed	16 „	— missed
6 „	— 150 units	17 „	— missed
7 „	— 300 „	18 „	— 500 units
8 „	— 200 „	19 „	— missed
9 „	— missed	20 „	— missed
10 „	— missed	21 „	— 500 units
11 „	— 200 units		

In all, 16 injections were given from 8 March, and 35 days were *missed.* The tumour measured 0.6×0.8 cm, with a central fissure. Areas of cartilagenous consistency appeared. The right submandibular lymphatic node measured 1×0.5 cm, the left was 0.5 cm in diameter, and no nodes could be palpated in the mental region.

22 April	— 500 units	28 April	— missed
23 „	— missed	29 April	— 500 units
24 „	— missed	30 „	— missed
25 „	— 500 units	1 May	— missed
26 „	— missed	2 „	— missed
27 „	— 500 units	3 „	— missed

This gives a total since March of 20 injections, 43 days *missed.* The tumour had increased in size to 1×0.8 cm. The right lymphatic node was firm, 1.2×0.8 cm, the left 0.75 cm in diameter, and a node in the mental region was 0.3 cm in diameter.

4 May	— 500 unis	18 May	— 500 units
5 „	— 500 „	19 May	— 500 „
6 „	— 500 „	20 May	— 500 „
7 „	— missed	21 „	— missed
8 „	— missed	22 „	— missed
9 „	— 500 units	23 „	— 500 units
10 „	— missed	24 „	— missed
11 „	— 500 units	25 „	— 500 units
12 „	— missed	26 „	— 500 „
13 „	— missed	27 „	— 500 „
14 May	— missed	28 „	— missed
15 „	— missed	29 „	— missed
16 „	— 500 units	30 „	— 500 units
17 „	— missed		

The ulcerated area increased. In view of his frequent absences it was suggested to the patient that he should enter the clinic for further treatment, but he refused.

31 May	— missed	8 June	— 500 units
1 June	— 500 units	9 „	— 500 „
2 „	— 500 „	10 „	— 500 „
3 „	— 500 „	11 „	— missed
4 „	— missed	12 „	— missed
5 „	— missed	13 June	— missed
6 „	— 500 units	14–18 „	— 500 units each
7 „	— missed		

Between 8 March and 18 June patient received injections on 37 days and missed 67. On 18 June the patient underwent surgical removal of the affected portion of the lip. The observations on patient F. again show the fundamental relationship, noted in a number of similar cases, between injection of the preparation and tumour regression.

Systematic injections of the trypanosome preparation were accompanied by regression of the tumour. Termination of the injections at a time when tumour regression was not complete was associated with the renewal of progressive growth. Irregular administration of the substance—with intervals of one or several days between injections—*had no effect*. These facts deserve attention both from the point of view of justification of the use of biotherapy in cancer treatment and from the aspect of our understanding of the mode of action of the preparation.

The conclusion regarding the method of using the preparation with therapeutic aims is clear. For effective results, *it must be administered daily until there is complete destruction of the tumour and of tumour cells* capable of giving rise to new generations and eventually to a fresh tumour.

With regard to the mode of action of the trypanosome preparation, the observations show that there must be a degree of saturation of the body, maintained at a definite and moreover a constant level. It would be right to accept that *until a cure is achieved, interruptions are inadmissable*. This conclusion arises from the clinical observations described above, and from other similar clinical observations. It has also received concrete support in the morphological investigations described earlier. These investigations showed that administration of the trypanosome preparation brings about changes in the morphological features of malignant cells which accurately reflect profound changes in their metabolic function, which in their turn involve inhibition of their growth and multiplication. In allowing intervals between injections of the antineoplastic preparation,

we also create the opportunity for unhampered formation of new generations of malignant cells and hence progressive tumour development.

OBSERVATION NO. 9

Patient L., male, aged 45 years. Clinical diagnosis: *cancer of the lower lip, stage II*. Histological diagnosis: *basal-cell carcinoma*.

On admittance to the clinic on 21 September 1948 the patient's lower lip bore on the right a painless, ulcerated tumour measuring 1.2×1 cm, with a thickened margin, palpatable to a depth of 0.3—0.5 cm. The neoplasm was fibrous in density, its posterior half almost cartilaginous.

On both sides in the submandibular region there were firm, oval, mobile lymphatic nodes, one on the right measuring 1.5×1 cm and another 1×0.5 cm; on the left one was 0.5×1 cm and another 0.25×0.5 cm. A soft lymphatic node 0.3 cm in diameter could be palpated indistinctly in the mental region.

A *biopsy* was performed 25 September 1948, involving wedge resection of one sixth of the ulcerated area under local infiltration anaesthesia.

On the same day injections of modification VI of the preparation were commenced. The first 38 injections were paratumoural, all round the tumour, the rest intramuscular, once daily; the dose at the first injection was 300 units, the second 500, the third 1000, the fourth 2000 and the rest 3000-4000 units each.

After 61 injections the affected area was smaller and the peripheral region flatter and less firm. Half of the surface was covered by epithelium.

After 68 injections the patient interrupted the course for 49 days.

During the 49-day interval the tumour decreased slightly in size and could no longer be palpated in the depth of the lip; the peripheral rim remained in only a few places and hardly projected above the level of the mucosa; at its centre was a depression, the surface of which was covered by mucous membrane hardly distinguishable in colour from the normal mucosa surrounding the tumour. Two dry scabs measuring 0.4×0.2 cm remained on the anterior part.

From 1 February to 7 March 1949 the patient underwent a *second course* — 29 injections of 1000 units. The injections were terminated on 7 March when the patient went away.

Tumour regression continued during the second course of injections. At the time the patient was discharged, deep infiltration of the lip *could not be palpated*. A fibrous area remained along the scar left from the biopsy and there was some superficial thickening corresponding to the peripheral

rim. On the edge of the former lesion there was also a superficial scab 0.2–0.3 cm in diameter, while the central part was covered by normal mucous membrane. With these residual lesions patient L. again interrupted the course of injections, returning only four months later. During this time the tumour had grown appreciably. The patient underwent another biopsy on 28 June 1949, when a fragment of the neoplasm was excised. Histological examination revealed a picture of keratinizing squamous-cell carcinoma. The patient started a *third* course of injections.

After 30 injections only a hardly palpatable firm area remained in the region of the biopsy scar. After another month the lip bore a hard scab 0.5 cm in diameter. At this stage the patient went away. In December 1950, a year and four months later, L. wrote that he was healthy.

This observation differs from all the others described in that, as distinct from the usual train of events, the process of tumour regression which started during the first course of injections of the preparation continued also in their absence during the interruption in the treatment.

Thus, the second course of injections, carried out 49 days later, merged, as it were, with the first, and the regression process continued uninterrupted. This phenomenon is easily enough described, but to provide any rational explanation for it is not really possible. It cannot be excluded that during the first course the tumour regression was obscured by local tissue oedema caused by paratumoural injection of the preparation for 38 days. When the oedema subsided, the signs of regression were more pronounced. Another explanation, at present difficult to express, is also possible. Further observation and analysis will be necessary.

The second interruption in the course of intramuscular injections when the process was not complete was associated with progressive growth of the tumour, as seen in the other cases.

It is important to note that the *third* course of injections, carried out 4 months after the second, again caused marked regression of the tumour.

2. REGRESSION OF CANCER OF THE VOCAL CORD

OBSERVATION NO. 10

Patient K., male, aged 46 years, was admitted to Prof. Trutnev's clinic in October 1945. Examination revealed a *tumour on the vocal cord* less than 1 cm long and less than 0.5 cm across. *Squamous-cell carcinoma of the vocal cord* was diagnosed histologically.

According to the accepted clinical routine the patient should have undergone surgical treatment—excision of the vocal cord, with removal of both tumour and cord.

Because of previous observations indicating the safety of the trypanosome extract and its cancerolytic activity, it was decided to carry out pre-operatively a two-week course of injections of the preparation.

The first dose consisted of 1 ml injected subcutaneously in the arm. On subsequent days the patient received 2 ml of the preparation daily. On examination after 6 injections a slightly hyperaemic mucous membrane showing no signs of neoplasia was seen on the vocal cord at the site formerly occupied by the tumour. The injections were continued. After 2 weeks the hyperaemic mucosa had regained its normal appearance and was indistinguishable from the surrounding healthy tissues of the upper respiratory tract. The patient received a total of 20 injections. In view of the disappearance of the tumour and the normal state of the vocal cords and surrounding tissues it was decided not to operate. The patient continued to receive treatment as an out-patient. Injections of the preparation were accompanied by a rise in temperature to 38–39°C. At the site of the injection of the preparation in the right and left forearms there was an inflammatory reaction with infiltration into the surrounding subcutaneous connective tissue. These local inflammatory phenomena resolved on termination of the course of injections. No other abnormal reactions were noted.

The patient has been under observation by Prof. Trutneva for 14 months; no signs of neoplasia have been found.

The patient received no other treatment.

This completed our report on patient K. in 1946.

In December 1948 the patient underwent a detailed laryngoscopical and general clinical examination, which showed the absence of any signs of recurrence or metastases.

The progressive hoarseness previously present completely vanished during treatment and has not returned since (K. is a teacher by profession).

The patient's satisfactory clinical condition and the absence of signs of cancer for three years show that in this case injections of the preparation brought about clinical recovery from squamous-cell carcinoma of the vocal cord.

OBSERVATION NO. 11

Patient K., male, aged 49 years. Clinical diagnosis: *recurrence of cancer of the larynx.* Histologically: *squamous-cell carcinoma of the vocal cord.*

In March 1945 the patient developed increasing hoarseness, then pain on swallowing. In June of that year histological examination revealed a keratinizing squamous-cell carcinoma.

In June–July 1945 the patient received a course of X-ray therapy by Coutard's method, 31 exposures, a total of 6200 r by the continuous fractionation method.

For about a year, i.e. until July 1946, the patient felt normal. His voice was restored. Clinical examination showed the absence of a tumour.

One year after the radiation therapy, the patient again developed hoarseness, a cough and pain on swallowing. Laryngoscopy showed *recurrence of a malignant tumour of the left vocal cord at the site of the primary tumour.*

It was suggested that the patient should undergo radical operation for the removal of the larynx, as a second course of radiation therapy was impracticable, the first having been complicated by radiation epidermatitis and epithelitis. For this reason the patient did not receive further X- and radium irradiation to avoid tissue oedema, atrophy and necrosis of the skin, with the danger of acute necrosis of the skin of the lower jaw, and also in view of the marked decrease in tumour sensitivity on repeated irradiation.

The patient refused the radical operation for removal of the larynx and approached our clinic for biotherapy. Observations were made by Dr. Kuperman.

SYMPTOMS ON ADMITTANCE (15 AUGUST 1946)

The patient's voice was very hoarse. He complained of rasping in the throat, a cough and pain on swallowing. Laryngoscopy revealed a thickening of the left true vocal cord in the larynx. Its free border bore an area of proliferation, giving the cord a zig-zag shape. There was ulceration at the centre of the cord and under it on the left. Diagnosis: recurrence of laryngeal cancer.

The patient started a course of injections of the preparation on 15 August 1946.

INJECTIONS OF THE PREPARATION AND COURSE OF THE DISEASE

The injections were given daily, intramuscularly in the buttocks, and were of *modification II* of the preparation, From 15 August to 30 October 1946 the patient received a total of 65 injections, involving 14,425 units.

Laryngoscopical examinations made during the process of the injections showed the following changes: the mucous membrane assumed a pinkish colour, and the granulation along the border of the left true vocal cord gradually diminished and then disappeared. The area of proliferation could no longer be seen. The edge of the left vocal cord became freed. A delicate scar formed at the site of the previously observed ulceration in the thickness of the cord.

The larynx remained freely mobile. The voice gradually became clear. The subjective symptoms of pain on swallowing, coughing and rasping in the throat disappeared. Clinical recovery ensued.

Further observations on patient K. from October 1946 to October 1949, i.e. for three years, revealed no signs at all of recurrence of the malignant disease. His voice remained clear, and laryngoscopical examination of the vocal cords showed nothing suspicious of malignancy.

The patient's general condition remained satisfactory.

Analysis of the clinical observations on patient K. enables the following features to be noted:

(1) A squamous-cell carcinoma of the vocal cord—a recurrence following X-ray therapy in its initial stage—was *highly susceptible* to the action of the biopreparation, as shown by a positive cancerolytic effect leading to disappearance of signs of malignancy for three years, after which observations ceased.

(2) Disappearance of the malignant tumour was accompanied by tissue regeneration and the formation of a visible scar.

Hence, in both the described cases of squamous-cell carcinoma of the vocal cord clinical recovery was obtained without using the normal methods of treatment.

No biopsy was carried out in the second example. The tumour underwent gradual regression and a scar formed at its site.

The combination of these two observations enables us to assume that the trypanosome substance contains an antineoplastic factor capable of inducing a regression process in malignant tumours of the vocal cords.

3. REGRESSION OF MAMMARY CANCER

We turn now to a series of observations on patients suffering from cancer of the breast. Such observations are particularly difficult, firstly because of the poor accessibility of these tumours to visual control and secondly because of peculiarities of the course of regression. As an example, we need only mention the following anomaly, which has led to confusion even among experienced clinicians on more than one occasion: in certain

patients at one of the stages of treatment the trypanosome preparation caused enlargement of the tumour lesions, which was interpreted, naturally enough, as growth of the neoplasm and not regression. Radical operations were carried out. Histological examination showed inflammatory changes at the site of the former malignant tumour, without any signs of malignancy. The radical operation for removal of the breast was thus unnecessary. Only later was this stage of regressions studied histologically and correctly interpreted. Earlier, this was, understandably, impossible.

The following situation also made the process very difficult to understand: in some patients the trypanosome substance caused first diminution of the tumour, then a process of stabilization. Continued injections produced no changes at all. This period was sometimes prolonged—up to a year or more. Even experienced clinicians, seeing a previously rapidly-growing tumour in such a sustained state of "inhibition", assumed that this was an irreversible effect of the biotherapy and considered it adequate clinical recovery. After a time, however, the tumour—this apparently inactive focus—started to grow, the trypanosome preparation did not have its earlier inhibitory effect, and the malignant process progressed as rapidly as it had before the treatment was started. Only later, when this incomplete carcinostatic effect was recognized, did clinicians start to adopt operative removal of the tumour lesion, as limited by the extent of the visibly affected tissue, as well as the injections. When the method of using the preparation had been changed in this way, prolonged clinical recoveries were obtained in a number of patients, without radical operation or X-ray therapy. This came later, however, when some experience had been gained.

The present section deals with some of these observations.

OBSERVATION NO. 12

Patient D., aged 43 years. Clinical diagnosis: *cancer of the right breast*, stage II. Histological diagnosis: *scirrhous carcinoma*. She was admitted to the clinic on 3 March 1950. Examination showed: in the upper superficial quadrant of the right breast there was a firm area measuring 2.0×1.5 cm with a thorn-shaped projection; the thickening was fibrous in nature and the projection almost cartilaginous in consistency. A soft, flat lymphatic node measuring 1.0×0.75 cm could be palpated in the right axilla; no nodes could be felt in the left axilla nor in both supraclavicular or subclavicular regions (Plates 44*a* and *b*). Injections of the trypanosome preparation were started on 28 February 1950.

INJECTIONS OF THE PREPARATION AND COURSE OF THE DISEASE

Patient D. received a prolonged course of intramuscular injections from 28 February 1950 until 31 March 1951. In 11 months 260 injections were made, involving 83, 000 units of modification VI of the preparation—a dried product obtained from *T. cruzi* cultures grown on synthetic culture media. After 13 injections (1640 units), on 18 March 1950, a non-radical operation was carried out: a radial incision in the right breast, with removal of the tumour to the limits of the visibly affected tissue. Histo-pathological conclusion—*scirrhous carcinoma*. Injections of the preparation were given daily. Systematic examinations throughout the course of injections showed the absence of any thickened areas either in the scar or in the breast tissue. From time to time soft or somewhat firm, mobile lymphatic nodes could be palpated in the axillary region.

The patient remained in this state:

After 46 injections	— 26 April 1950
„ 107 „	— 30 June „
„ 163 „	— 13 Sept. „
„ 195 „	— 18 Oct. „
„ 241 „	— 27 Dec. „
„ 260 „	— 28 March 1951

In January 1951 the patient suffered from jaundice. The injections were suspended during the disease (February–March), and were recommenced after her recovery at the end of March. At the end of the course of injections on 16 May 1951, an examination revealed: both breasts were of the fibrous mastopathy type; the scar had no thickened areas; both axillae contained a small (0.75 cm), rather firm lymphatic node.

After 5 years (in April 1955) patient D. was in good health, the breast had been preserved and there were no signs of recurrence or metastases.

On 30 August 1955, D. was examined by a special committee from the Presidium of the Scientific Council of the Ministry of Health of the U.S.S.R. The committee stated: "The right breast is slightly smaller than the left. The border of the external quadrants bears a linear scar, soft, mobile, painless and with no firm areas. No tumour can be found in the breast.

"Conclusions: the patient is apparently in good health, there is no recurrence of the tumour and no metastases can be determined". Period of observation—5½ years (Plate 45).

OBSERVATION NO. 13

Patient G., aged 52 years. Clinical diagnosis: *cancer of the right breast*, stage I. Histological diagnosis: *scirrhous adenocarcinoma*. In October

1948 the patient discovered by chance a tumour in the right breast. At the time of admittance to our clinic on 26 November 1948 the right breast contained a diffuse firm area measuring 1.5×2 cm, at the centre of which could be palpated a nodule 0.75 cm in diameter. In the right axilla were two lymphatic nodes measuring 1×0.5 cm and 0.75×0.4 cm. Intramuscular injections of the preparation were commenced on 26 November 1948. A biopsy was performed on 3 December, involving the excision of a portion of the tumour measuring 0.8×0.4 cm (1/3 of the tumour). Histological conclusion: scirrhous adenocarcinoma: (Plates 46*a*, *b*, and *c*, 47 *a* and *b*).

INJECTIONS OF THE PREPARATION AND COURSE OF THE DISEASE

Patient G. received 3 courses of injections. The first course lasted for 4 months—from 26 November 1948 to 31 March 1949. Clinical observations made during the process of the injections showed no diminution of the tumour but, on the contrary, an increase in size: after 1 month the infiltration in the region of the biopsy scar measured 2.5×3 cm; at 1½ months, after 42 injections (92,000 units) the infiltrated area had grown to 3.5×4 cm; soft lymphatic nodes 0.5 cm in diameter could be palpated in the axillae. A second biopsy was carried out on 27 January 1949, with excision of the postoperational scar and the thickening at the site of the previous biopsy. *Histological* examination of the excised tissues revealed *no signs of malignant neoplasia*: a picture of cystic mastopathy was seen. Subsequent examinations (27 February and 31 March 1949) showed the scar to be satisfactory, without any thickening, and no lymphatic nodes could be palpated.

The first course of injections was terminated on 31 March 1949. The patient had received 283,000 units of the preparation. Nine days later the injections were recommenced, since on 4 April an area of consolidation measuring 1–1.5 cm was found in the region of the scar. This time the injections were continued until 3 August. The patient received 59,000 units. The thickening round the scar decreased and on 4 August "the scar was satisfactory". The injections were discontinued. The patient remained under observation. After 3½ months, on 18 November, the central part of the scar was found to contain a fibrous type of thickening 1 cm in diameter. A third course of injections was instituted and continued until 20 April 1950. In this third course the patient received 101,000 units of the preparation. After only a month (21 December 1949) the scar thickening had disappeared. Repeated examinations of the patient after the end of the injections (4 February 1950, 22 February 1950, 12 April 1950, 22 November 1950 and 17 January 1951) enabled the absence of any signs of recurrence or metastases to be recorded in her case-history. In March 1951, i.e. a year and three months later, a firm nodule 1.5 cm in diameter was found beneath the scar. A fourth course of injections was started and at the same time on 30 March 1951 the scar remaining from the biopsies was excised, together with the surrounding mammary tissue.

On histological analysis of the tissues *no signs of malignancy were found.* For this reason the injections were terminated. The breast was preserved (Plates 48 and 49).

On 30 August 1955 a special committee from the Presidium of the Scientific Council of the Ministry of Health of the U.S.S.R., examining the patient, reported: "the patient's general condition is satisfactory, and she has no complaints. At the border of the external quadrants of the right breast there is a linear transverse scar, 12–14 cm, extending to the chest wall. The scar is soft, mobile and painless. No tumour or lymphatic nodes are determinable. *Conclusion*: the patient is apparently in good health, there is no recurrence of the tumour and no evidence of metastases".

Period of observation—6 years 9 months.

OBSERVATION NO. 14

Patient S., aged 35 years. On admittance to the clinic on 24 February 1949 the left breast contained a mulberry-like thickening measuring 3×2.5 cm, at the centre of which was a firm projecting spike. Injections of the preparation were started on the day of admission. After 13 days a biopsy was carried out, with excision of the affected portion of the breast. Histopathological diagnosis: *cystic mastopathy with areas extremely suspicious of blastomatous* (*carcinomatous*) *transformation.*

INJECTIONS OF THE PREPARATION AND COURSE OF THE DISEASE

The patient received two long courses of intramuscular injections with an interval of 1½ months. The first course was from 24 February 1949 to 29 March 1950. During this period of 13 months she received 66,000 units of the preparation. The second course was from 18 May to 19 December 1950 and involved the injection of 15,500 units.

Examination of the patient on 12 May 1949, 2½ months after the start of the injections and 2 months after the biopsy, revealed isolated foci of mastopathy in the left breast; in the left axillary region there was a lymphatic node measuring 0.5×0.75 cm and in the left supraclavicular region there was a similar node, both nodes being of the consistency typical of a cancer

metastasis. During subsequent administration of the preparation the clinical picture changed, and examinations on 1 July 1949, 29 March 1950, 5 July 1950, 6 September 1950, 6 December 1950 and 19 December 1950 showed the scar to be in a satisfactory condition, with no thickened foci, no palpatable lymphatic nodes nor any findings indicative of recurrence or metastases. The injections were interrupted between 30 March and 17 May 1950. The course was recommenced on 18 May and on 19 December it was stopped, after 379 injections. The patient received 81,600 units of the preparation. An examination carried out 2 years 2 months after admission to the clinic (18 June 1951) showed the absence of any signs of recurrence or metastases. This position was still unchanged in April 1955. The breast was preserved. On 13 September 1955 a committee of the Presidium of the Scientific Council of the Ministry of Health of the U.S.S.R., having examined patient S., concluded: "S. is apparently in good health, there is no recurrence of the tumour and no metastases can be determined". Period of observation: 6 years 5 months (Plates 50*a* and *b*, 51).

OBSERVATION NO. 15

Patient B., aged 38 years. Clinical diagnosis: *fibroadenoma* of the left breast, suspicious of *malignant transformation*. Histological diagnosis: *adenocarcinoma*.

On admittance to the clinic on 15 August 1947 both breasts were somewhat fibromatous. In the external part of the left breast at the junction of the upper and lower quadrants could be felt a tumour of cartilagenous consistency, measuring 1.5×1 cm, oval in shape, mobile, adherent to the gland parenchyma but not to the underlying tissue.

Enlarged lymphatic nodes were palpated in the left axillary region.

A *biopsy* was carried out on 25 August 1947, with excision of half of the tumour (0.75×0.5 cm). Histological studies showed it to be an *adenocarcinoma* with slight anaplasia of the glandular tubules and a few mitoses in the epithelial cells.

The patient started a course of injections of the preparation on 20 August 1947.

INJECTIONS OF THE PREPARATION AND COURSE OF THE DISEASE

The patient received modification VI of the preparation. The injections were given intramuscularly in the buttock region once daily. The course of injections lasted from 20 August 1947 to 24 February 1948. During this period the patient received 147 injections involving 202,000 units of the preparation.

The doses given at each injection were as follows:

injections 1–39—200 units each—20 Aug.–3 Oct. 1947
injections 40–44—300–500 units each—4 Oct.–9 Oct. 1947.

After 13 injections (2,600 units) the postoperational scar had healed. There was some inflammatory infiltration of the tumour site.

After 32 injections (6,200 units) the tumour could be palpated as a firm, mobile nodule measuring 1×0.5 cm with clearly outlined contours.

After 44 injections the tumour started to be felt as a flat oval structure with a smooth surface, measuring 1.5×1 cm.

Hence in 1½ months, during which the preparation was being injected, the tumour, which had been halved in size at the biopsy, had again reached its original size of 1.5×1 cm.

Because of this the doses of the preparation were increased:

injection 45—10 Oct. 1947—800 units
„ 46–55—11–22 Oct.—1000 units each
„ 56–64—23 Oct.–3 Nov.—1600–2000 units each
„ 65–147—4 Nov.–25 Feb. 1948—2000–5000 units each

After 64 injections the tumour measurements had decreased to 1×0.8 cm. The patient gained 1.25 kg in weight.

After 76 injections the tumour size was 0.9×0.7 cm.

After 93 injections (92,800 units), on 19 December 1947 the patient underwent operative removal of the affected portion of the breast. Under local anaesthesia a linear incision was made radially over the tumour at the border of the upper and lower external quadrants of the left breast. Some of the gland parenchyma was excised along with the tumour. On section the tumour had the macroscopical appearance of a fibroadenoma.

A morphological study of the excised material was made by Prof. Rapoport, who concluded: "On histological examination, as well as a picture of fibroadenoma, at the centre of the preparation there was a nodule the size of a pin-head, consisting of markedly abnormal glandular cords and discomplex groups of epithelial cells. Diagnosis: *fibroadenoma with transformation to adenocarcinoma.*

The injections were terminated on 25 February 1948, the patient remaining under observation. Regular examinations were made until December 1950. There were no signs whatever of metastases or recurrence. Both breasts maintained their previous character of fibrous mastopathy. The scar remained soft along its whole length.

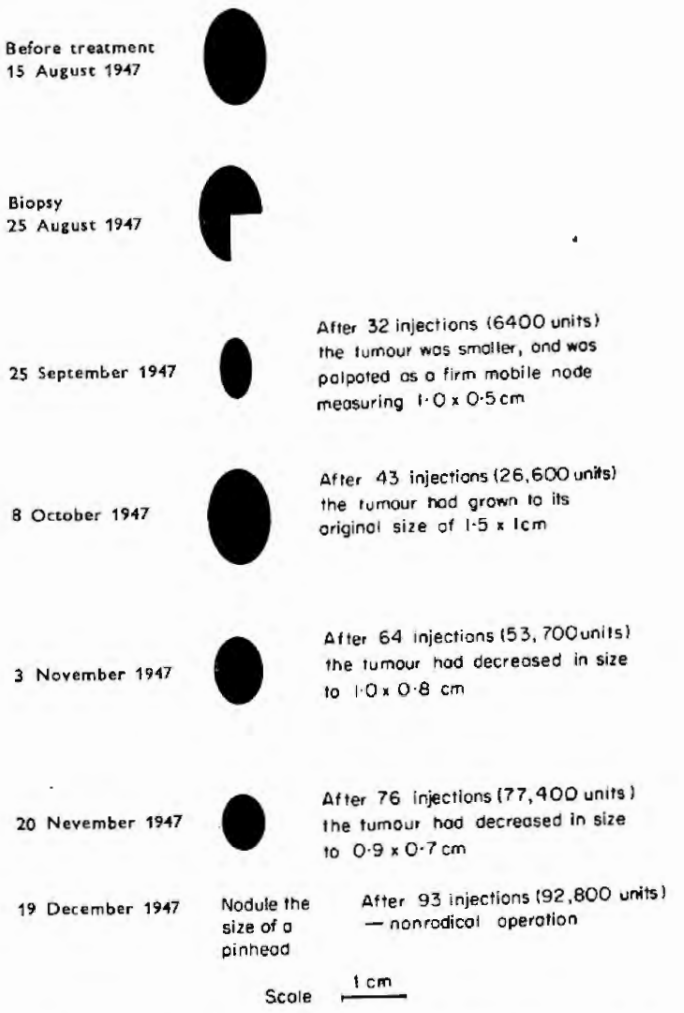


FIG. V. Diagram of tumour regression in patient B. during the process of injections of the preparation.

Thus, a process of tumour regression in patient B. became apparent after increasing the dose of the preparation to 1000–2000 units per injection: the size of the tumour decreased to that of a pin-head. This residue of the neoplasm was removed surgically on 19 December 1947.

The clinically apparent process of regression was accompanied by changes in the histological structure of the tumour. Before administration of the preparation microscopical examination revealed extensive areas of malignant tissue typical of an adenocarcinoma. After the injection of the trypanosome substance the malignant cells formed a conglomerate the size of a pin-head, surrounded and as though immured by a connective tissue capsule, as a result of which the former diagnosis of "adenocarcinoma" was changed to "fibroadenoma with transformation to adenocarcinoma". Further observations extending for three years showed no signs at all of recurrence or metastases. B. remains *clinically healthy*. The breast is preserved. Period of observation—3 years $4\frac{1}{2}$ months (Plate 52*a,b.*).

OBSERVATION NO. 16

Patient M., aged 46 years. Clinical diagnosis: *cancer of the left breast*. Histological diagnosis: *scirrhous carcinoma*.

In March 1947 the patient noticed by chance a tumour in the left breast in its external upper quadrant, with some stretching of the skin at this site. She refused the suggested operation and on 12 August 1947 approached the clinic for biotherapy.

SYMPTOMS OF THE CONDITION ON ADMITTANCE, 12 AUGUST 1947

A firm tumour measuring 2×2 cm could be palpated in the external upper quadrant of the left breast; it was painful, adherent to the gland parenchyma and at its centre adherent to the skin. In the left axilla there were three lymphatic nodes of firm consistency, mobile, painless and not adherent to the skin. One of them measured 1.5×1 cm, the two others were pea-sized (Plate 53).

On 25 August a *biopsy* was carried out, with excision of half of the left breast tumour.

Histological examination by Prof. Rapoport revealed a picture of *scirrhous carcinoma* with numerous areas of cicatricial collagenization of the stroma together with isolated cancerous nodules of *solid structure* showing signs of degeneration of the malignant cells (Plate 54, scirrhous area).

INJECTIONS OF THE PREPARATION AND COURSE OF THE DISEASE

For the first 7½ months the patient received preparation of modifications I and II, while from 16 April 1948 to the end of the observation modification VI was used—a dried preparation obtained from *T. cruzi* cultures grown on synthetic nutrient media. The injections were made intramuscularly in the buttocks, once daily, in the following doses:

1–2—25 Aug.–26 Aug. 1947	—200 units each	
3–9—27 Aug.–3 Sept. „	—300–800 units	each
10–20— 4 Sept.–16 Sept. „	—1000–2000 „	„
21–30—17 Sept.–27 Sept. „	—2000 „	„
31–51—29 Sept.–24 Oct. „	—1000–2000 „	„
52–138—27 Oct.–2 March 1948	—1500–3000 „	„
139–153— 3 March–19 March „	—1000–2000 „	„
154–177—20 March–6 April „	—1250–1800 „	„
178–385—17 April–29 December 1948	—1000–5000 „	„
386–439—30 Dec.–5 March 1949	—5000–10,000 „	„

The patient received in all 439 injections of the preparation.

After 20 injections the infiltration in the tumour region decreased. Two lymphatic nodes, rubbery in consistency, could be palpated in the left axilla.

The patient's weight was 49 kg 650 g.

After 43 injections, on 14 October 1947, the biopsy scar could be seen in the external upper quadrant of the left breast. Corresponding with the scar there was an area of fibrous thickening without distinct boundaries, and at its lower end a flattened area, very painful to the touch.

After 51 injections, on 24 October 1947, there was no apparent change from the previous position, except for the painfulness, which was now present along the whole length of the scar.

After 101 injections, on 9 January 1948, palpation of the middle portion of the postoperational scar revealed more distinctly a tumour 1.5 cm in diameter, firm, slightly painful and mobile.

After 138 injections, on 2 March 1948, no tumour could be palpated in the depth of the breast. The thickening around the scar was cicatricial in nature. Deep in the left axilla was a soft, oval lymphatic node. The supraclavicular and subclavicular spaces were free of nodes (Plate 55).

After 208 injections, on 22 May 1948, no tumour could be palpated in the region of the postoperational scar. König's symptom was negative. Palpation revealed a diffuse area, painful to the touch, firmish in consistency, poorly outlined and mobile. The left axilla contained a lymphatic

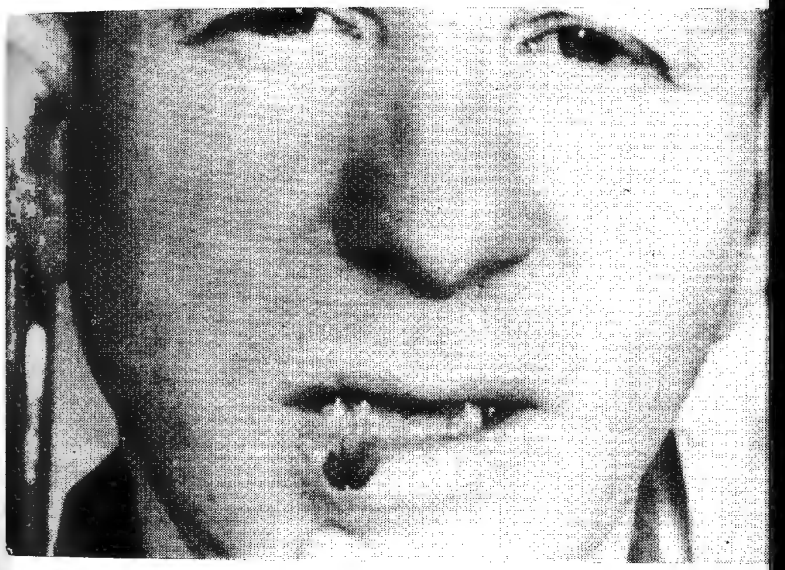
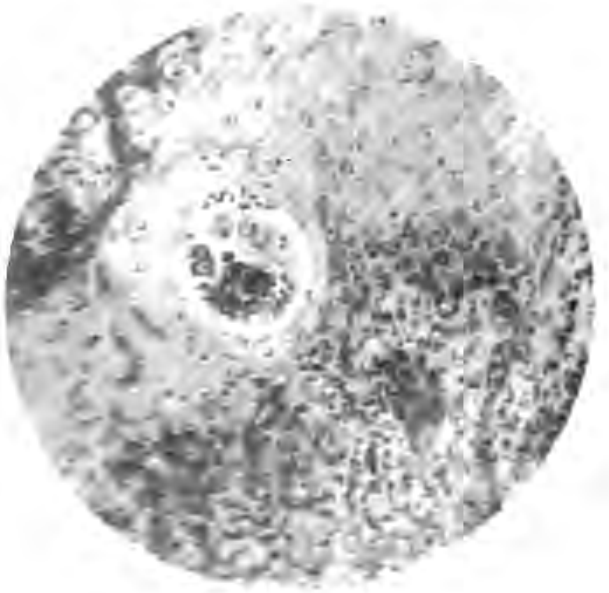


PLATE 36. Patient V. Observation No. 7. Keratinizing squamous-cell carcin
1 March 1948. The affected lip before the start of injections of the prepara
On the right of the lower lip is an ulcerated tumour measuring 1.5×1.3
At its centre is a scab. Its edge is bordered by a firm ridge. In the sub
dibular region is a lymphatic node measuring 2.0×1.5 cm.



(*a*)

(*b*)

PLATE 37, *a*, *b*. Patient V., 8 March 1948. Histological structure of the tumour before the start of injections of the preparation. The surface of the biopsied portion is partly ulcerated, and the floor of the ulcer is occupied by an abundant round-cell infiltrate containing atypical cords and groups of squamous epithelial cells with central keratinization and pearl-formation. Large multinucleated giant cells are present in the infiltrate. Diagnosis: keratinizing squamous-cell carcinoma.

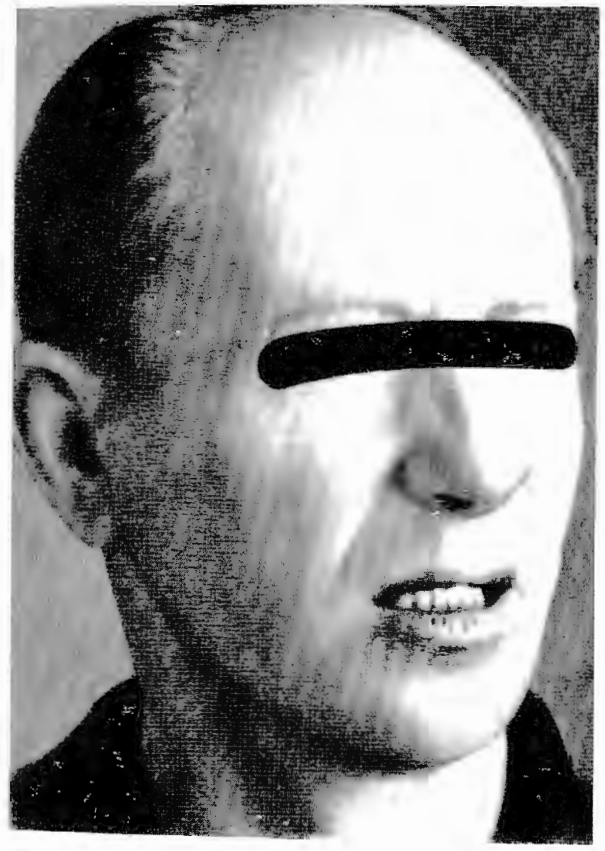
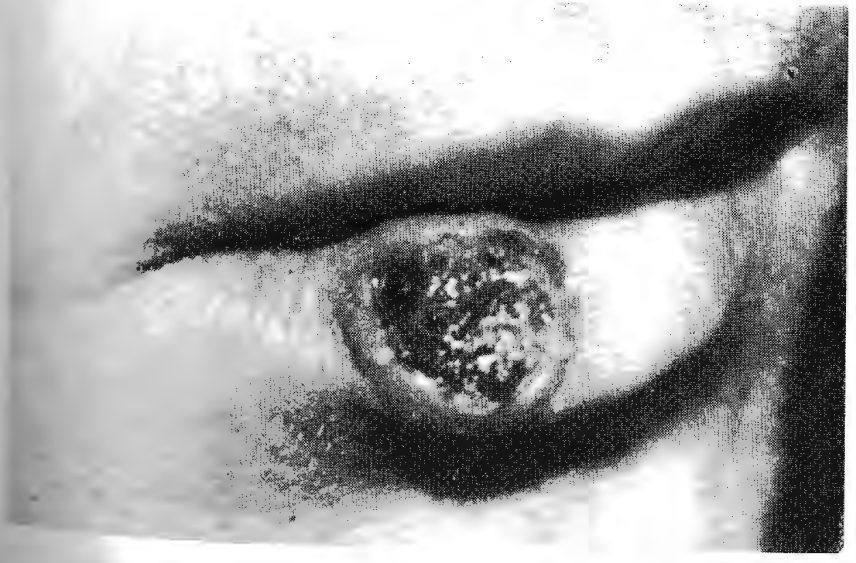


PLATE 38. Patient V., at the end of the course of injections of the preparation. The patient received 81 injections—111,000 units. The tumour has regressed, and the ulcer healed. At its external edge is a ridge-like thickening 0.2 cm in diameter.

(a)

PLATE 39, *a, b*. Patient F. Observation No. 8. Keratinizing squamous-cell carcinoma. 31 December 1948. Affected portion of the lower lip before the start of injections of the preparation. On the right of the lower lip in the region of the red border is a tumour measuring 1.7×1.7 cm with an ulcerated surface, of fibrous consistency and raised above the level of the normal mucosa by 0.5 cm. The tumour is surrounded by a firm ridge and can be palpated to a depth of 1.0–1.2 cm. Th submandibular region contains a lymphatic node, firm, cancerous and measuring 2.0×1.0 cm. On the left are two nodes, soft and measuring 1.0×0.5 cm. Also in the submandibular region is a firm lump, appearing to lie on the bone, measuring 0.5×0.3 cm.



(b)

PLATE 40. Patient F., 6 January 1949. On the operating table. Biopsy. After excision of a piece of the tumour, with the insertion of a catgut suture.

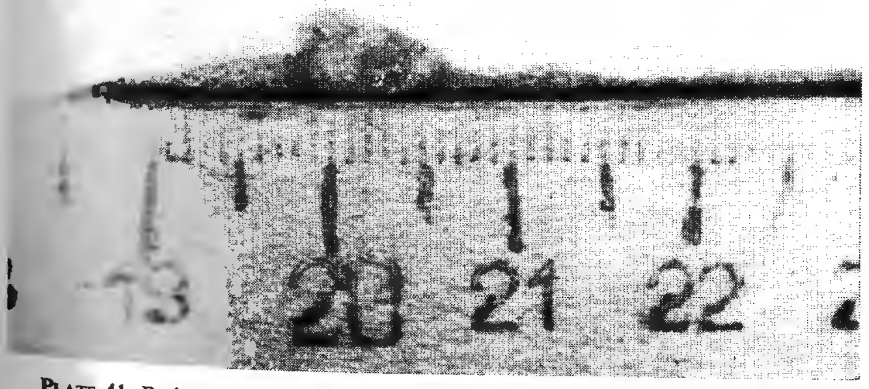


PLATE 41. Patient F., 6 January 1949. Tumour segment excised at biopsy, measuring 0.5×0.5×0.3 cm.

(*a*)

(*b*)

PLATE 42, *a*, *b*. Patient F., 7 March 1949. After 47 injections (43,300 units). At the site of the former tumour is a depression 0.8 cm across and 0.2 cm deep. The whole of this area is covered by normal epithelium. The course of injections of the preparation was interrupted.

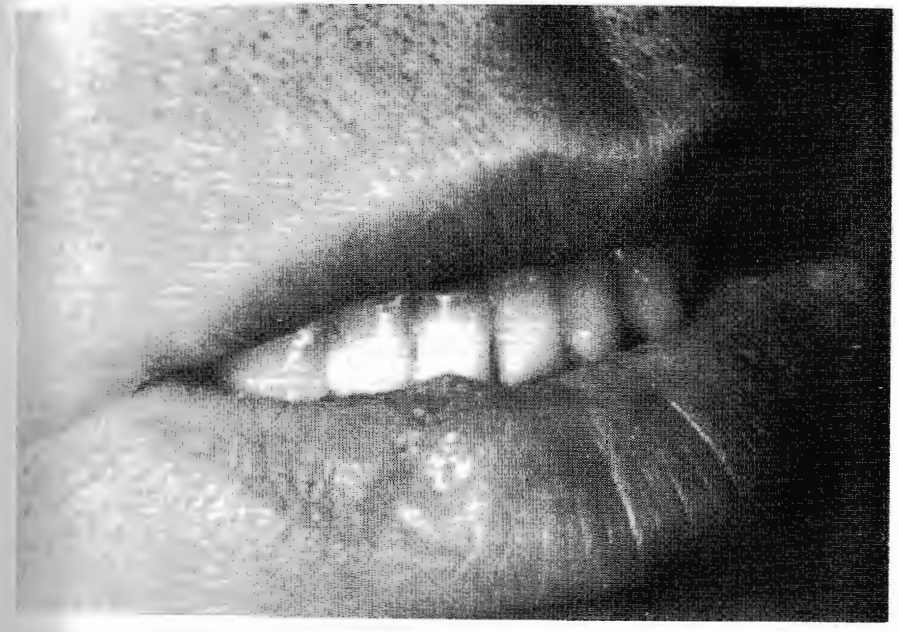
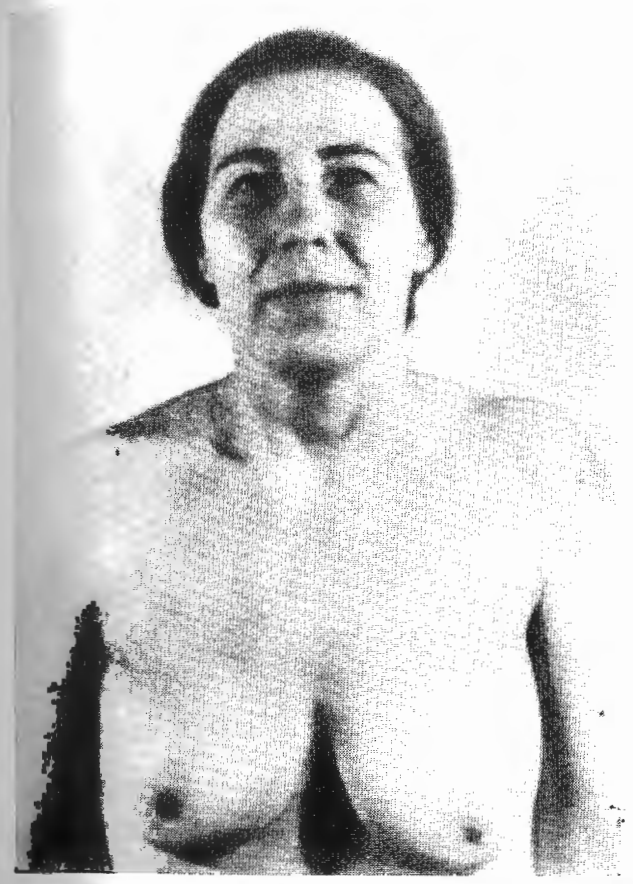


PLATE 43. Patient F., 12 April 1949. After a 25-day interval in the injections. The state of the tumour has deteriorated. At the centre of the area formerly occupied by the tumour a new thickening has appeared, measuring 0.5 × 0.8 cm, with central ulceration.



(*a*)

(*b*)

PLATE 44, *a*, *b*. Patient D., Observation No 12. Clinical diagnosis: cancer of the right breast, stage II. Histological diagnosis: scirrhous carcinoma. 28 February 1950. Before the start of injections of the preparation. In the external upper quadrant of the right breast is a tumour of fibrous consistency with a spine-shaped projection, measuring 2.0×1.5 cm; the projection is of cartilagenous consistency. In the right axillary region there is a soft, flat lymphatic node measuring 1.0×0.75 cm.

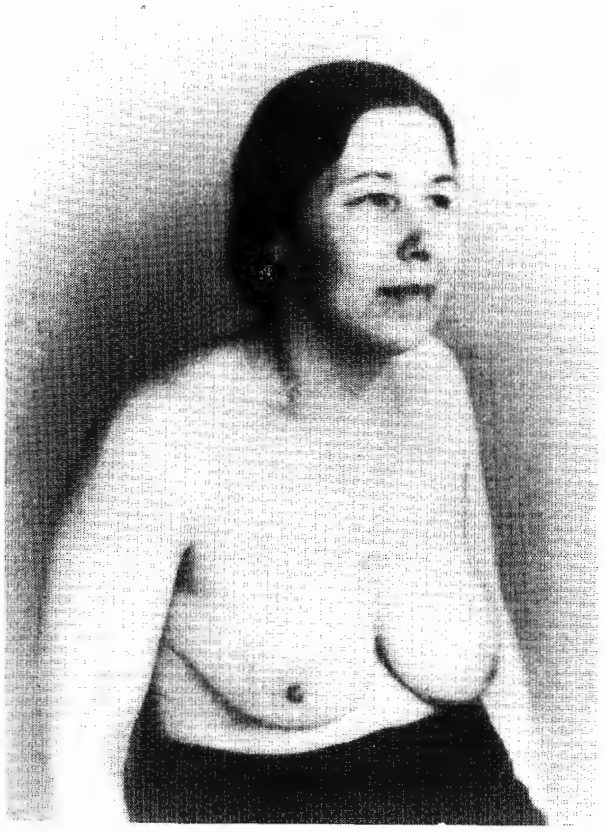


PLATE 45*a*. Patient D., 21 April 1951. After a course of injections of the preparation. The right breast shows traces of the nonradical operation carried out during the course of the injections a year previously (18 March 1950). There are no signs of recurrence or metastases. On 30 January 1955 patient D. was examined by a committee from the Soviet Ministry of Health. The committee's conclusion: "D. is apparently healthy. There is no recurrence of the tumour. No metastases can be determined." Period of observation, 5½ years.

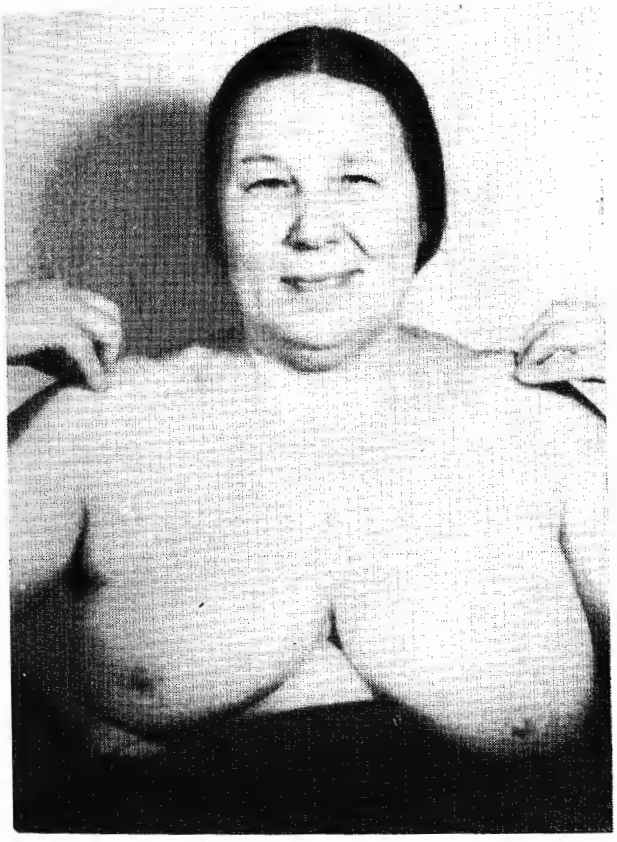


PLATE 45*b*. Patient D., 17th March 1960. Ten years after the start of the treatment and nine years after its completion. There are no signs of recurrence or metastases. The breast has been preserved.

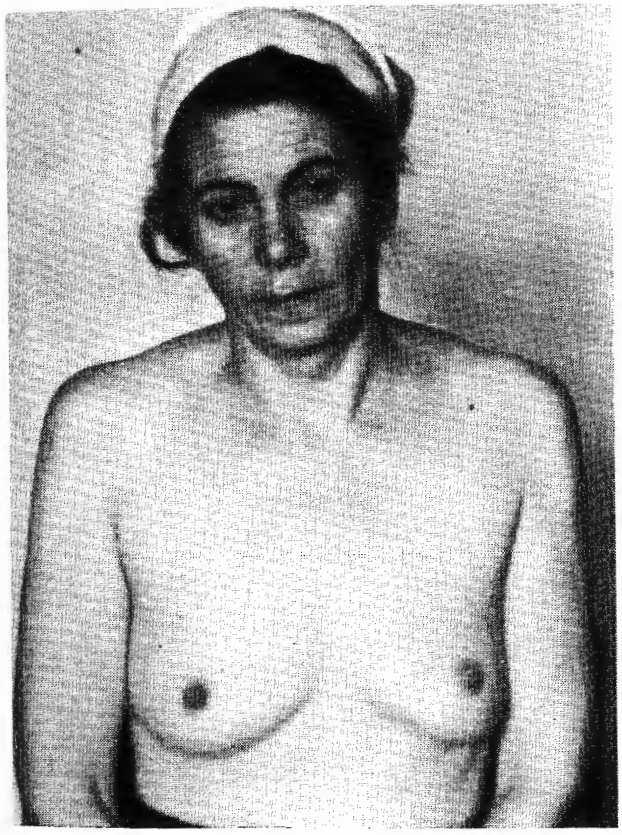
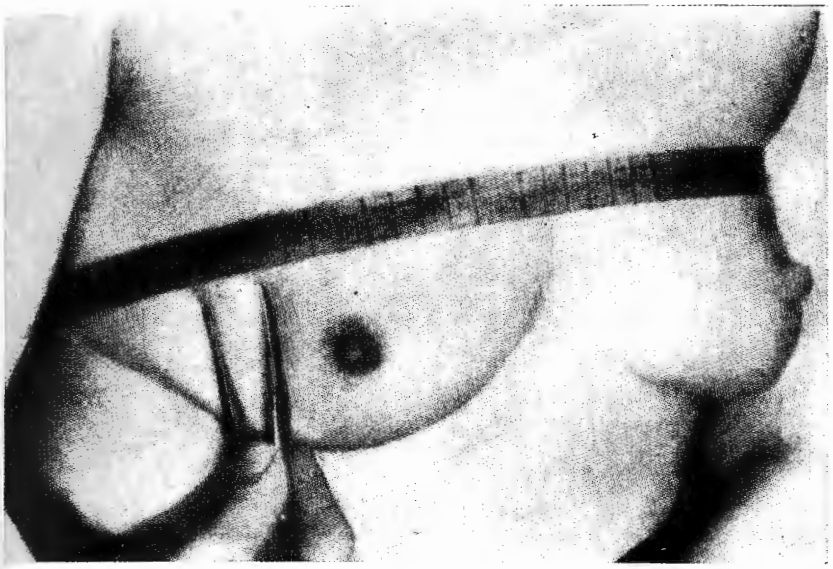
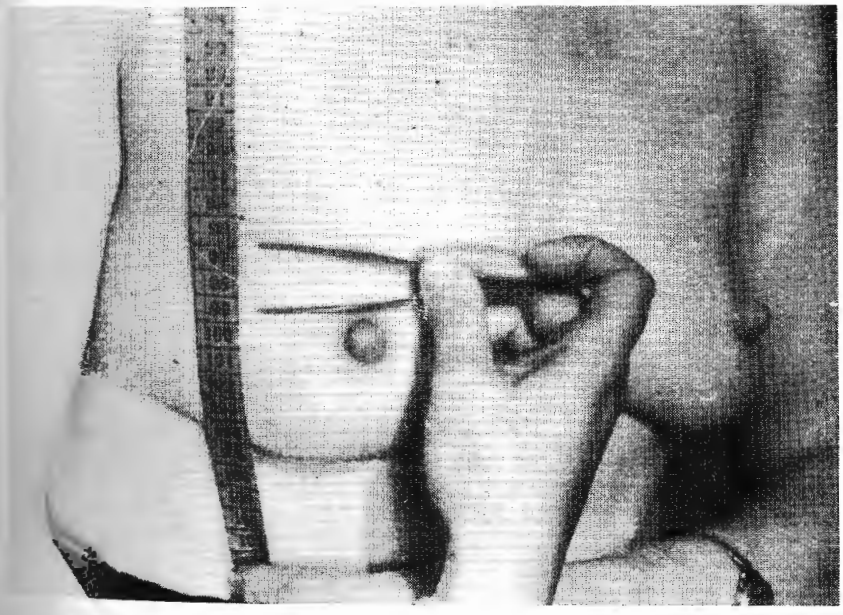


PLATE 46, *a*. Patient G., Observations No. 13. Clinical diagnosis: cancer of the right breast, stage I. Histological diagnosis: scirrhous adenocarcinoma. 27 November 1948. Before the start of injections of the preparation. The right breast contains a tumour measuring 2.0×1.5 cm, with a central nodule 0.75 cm in diameter. The right axilla contains two lymphatic nodes, measuring 1.0×0.5 cm and 0.75×0.4 cm.

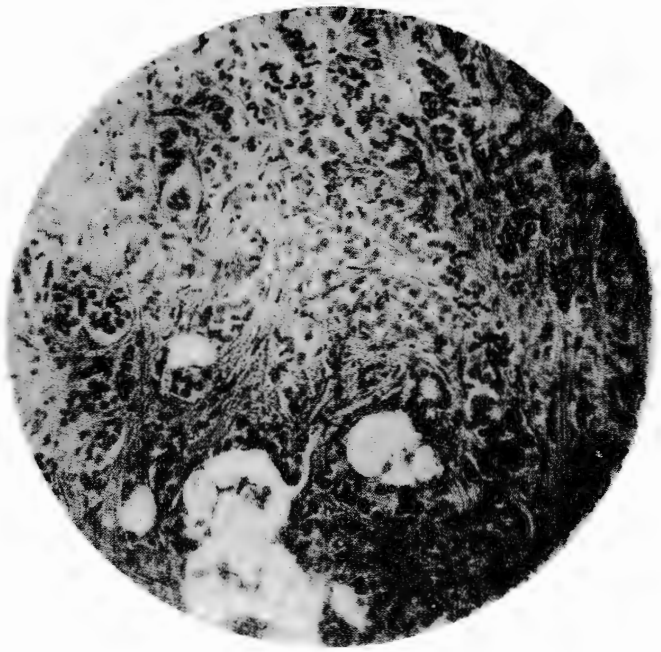


(*b*)

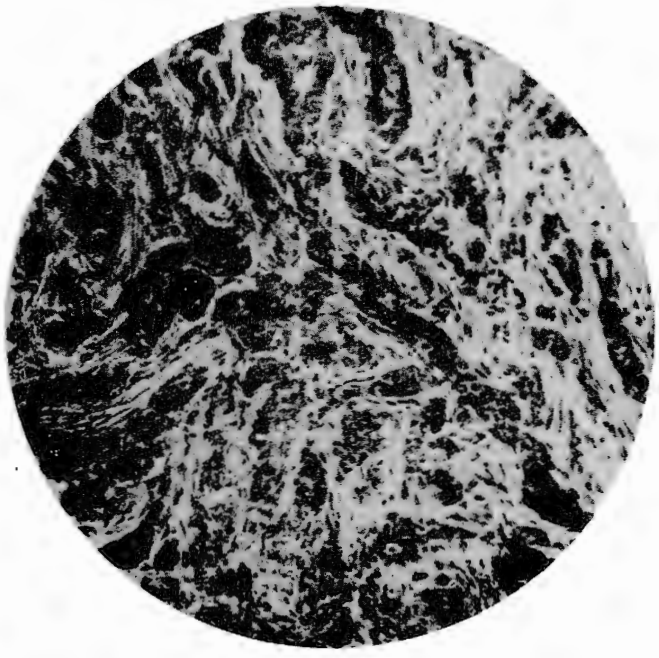


(*c*)

PLATE 46, *b*, *c*. Patient G., Observation No. 13. (For description see plate 46 *a*).



(*a*)



(*b*)

PLATE 47, *a*, *b*. Patient G., Histological structure of the tumour before the start of injections of the preparation. Scirrhous adenocarcinoma.

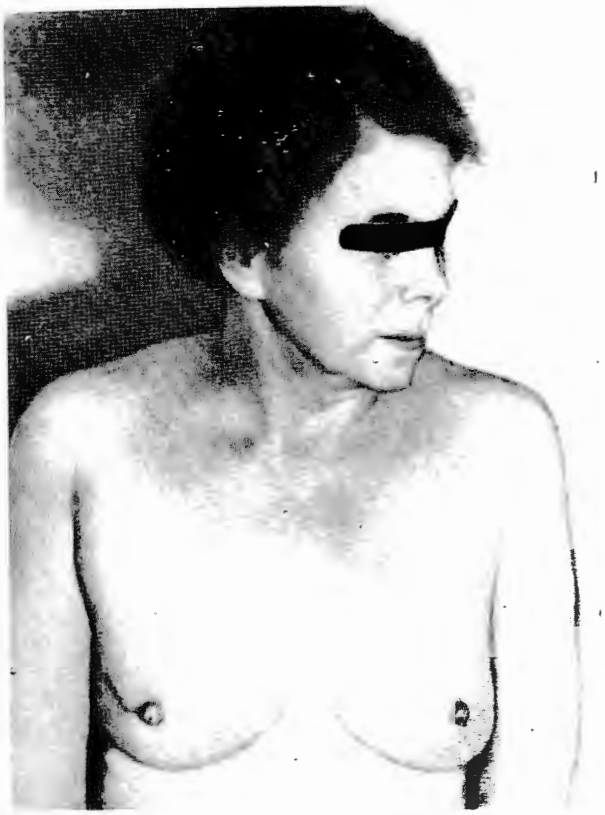


PLATE 48. Patient G., 22 February 1950. One year after a control biopsy. A histological investigation made on 27 January 1949 showed the absence of any signs of malignant neoplasia in the scar and in the glandular tissue.

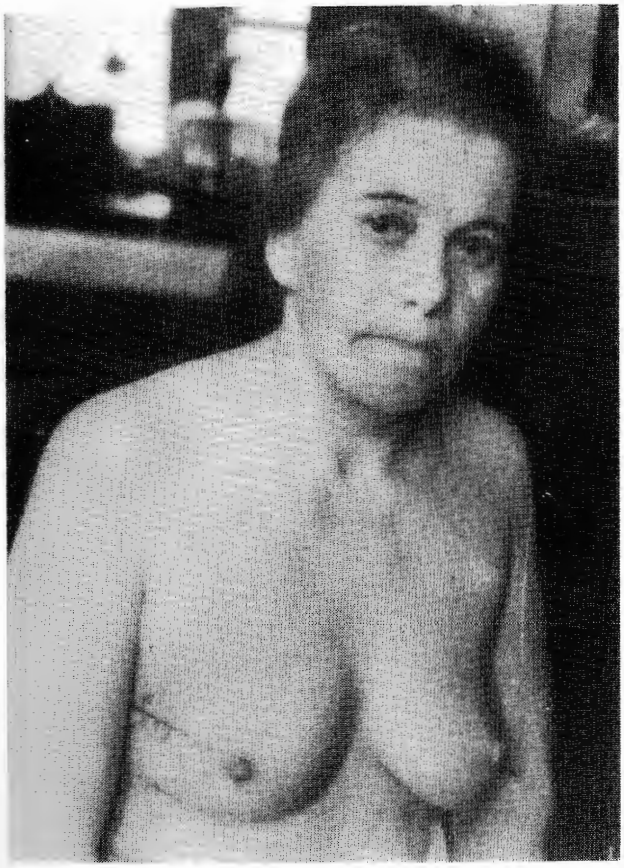


PLATE 49*a*. Patient G., 27 May 1951. 2 months after a second control biopsy. A histological analysis on 30 March 1951 revealed no signs of a malignant tumour. Patient G. received 3 courses of intramuscular injections of the preparation in connection with the appearance of thickened foci (mastopathy) in the breast. The first course was from 26 November 1948 to 31 March 1949, the second from 9 April 1949 to 3 August 1949 and the third 18 November 1949 to 20 April 1950. On 30 April 1955 the patient was examined by a committee from the Soviet Ministry of Health. Their conclusion: "G. is apparently healthy. There is no recurrence of the tumour. No metastases can be determined". Period of observation, 6 years 9 months.

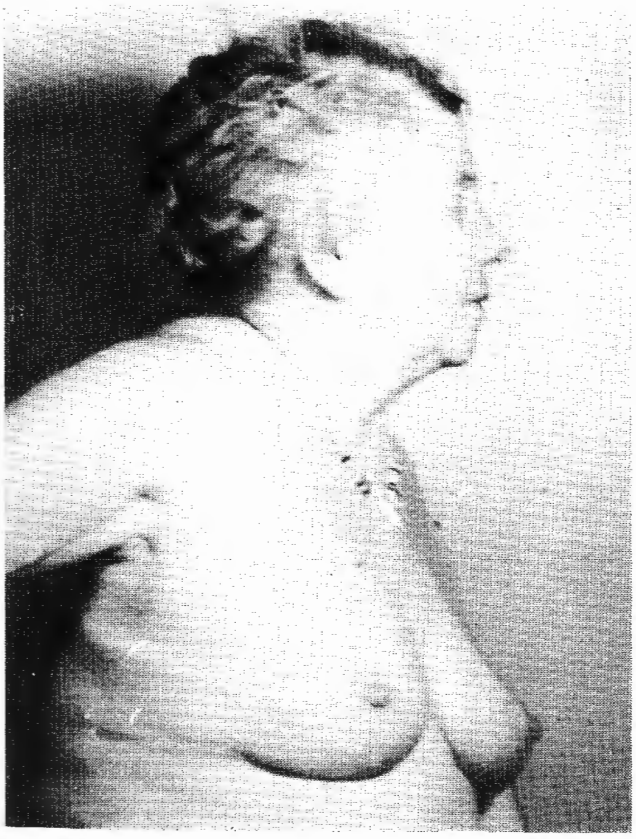


PLATE 49*b*. Patient G., 1960. After 11 years and 6 months. In good health.

node 0.5 cm in diameter, of firm rubbery consistency. The patient weighed 56.7 kg.

The thoracic organs showed no pathological changes on radioscopy.

After 248 injections, on 14 July 1948, *no tumour could be palpated in the breast.* It was decided to continue the course of injections on an out-patient basis. The patient was discharged.

After 297 injections, on 11 September 1948, there was a linear scar 6 cm long in the external upper quadrant of the left breast. In the lower part of the scar was a flattened, firm area of tissue, without distinct boundaries, measuring 2.5 × 4 cm, like an isolated portion of gland parenchyma; firmer fragments could be discerned in places in this area. The whole area showed signs of fibrous mastopathy. No lymphatic nodes were felt in the axillary or supraclavicular regions. After 354 injections, on 22 November 1948, the lower part of the biopsy scar showed some thickening. No signs of recurrence could be determined on palpation. The left axilla contained a lymphatic node the size of a lentil, mobile and painless. No nodes were palpated in the supra- and subclavicular regions. The patient weighed 55 kg.

After 370 injections, on 10 December 1948, the thickening in the lower part of the scar had not visibly changed. Somewhat medial to the upper end of the scar could be palpated a round growth with a smooth, even surface, feeling like a cyst and measuring slightly under 1 cm in diameter. No other lesions could be discovered. The patient weighed 55 kg 400 g.

After 385 injections, on 29 December 1948, the thickening in the lower part of the scar had not noticeably changed. The node in the axillary was also unchanged. A small round growth without distinct borders could be felt medial to the upper end of the scar.

After 390 injections, on 5 January 1949, the thickening in the lower part of the scar had not changed. The axillary node was also unchanged. The outline of the growth medial to the upper end of the scar could be felt indistinctly. The patient refused the suggested radical operation but agreed to the excision of the tumour and scar. This nonradical operation was carried out on 6 January 1949, with excision of the tumour and the axillary lymphatic nodes. Ether anaesthesia was employed. The old scar was outlined by an oval incision. The axillary vessels were exposed. The connective tissue of the axillary cavity, the aponeurosis of the major pectoral muscle, the connective tissue between the major and minor pectoral muscles and the aponeurosis covering the minor pectoral muscle, together with the portion of the mammary gland at the centre of which the scar and thickening were situated—were excised in one block. Skin sutures were applied.

Histological examination of the axillary lymphatic nodes revealed no signs of metastases. There was a picture of chronic hyperplasia with focal proliferation of cells of the reticulo-endothelial system. Histological examination of tissue from the thickened scar showed a picture of scirrhous carcinoma in one portion and cystic mastopathy in another (Plates 56 and 57). During the next 8 months the patient's condition revealed no signs of a neoplastic process (Plates 58*a* and *b*).

Eight months later, on 6 September 1949, an extensive control biopsy was performed, with excision of the old scar and the connective tissue of the axillary cavity. Histological examination of various areas of these tissues revealed no tumour elements.

In subsequent years of observation the patient showed *no signs of recurrence or metastases.*

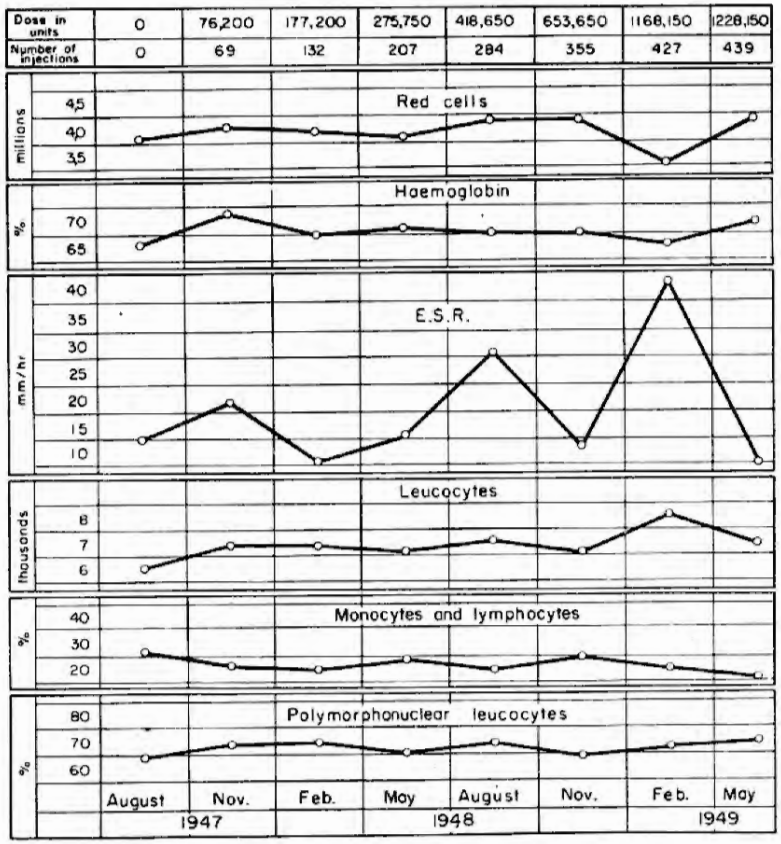


FIG. VI. Diagram of blood changes in patient M. during the process of injections of the preparation (439 injections—1,228,150 units).

On 30 August 1955 a special committee from the Presidium of the Scientific Council of the Ministry of Health of the U.S.S.R. examined patient M. and gave the following conclusions: "the patient is apparently healthy, there is no recurrence of the tumour and no metastases can be determined". Period of observation—8 years.

The above observation enables us to state that in patient M. tumour growth was halted under the influence of the trypanosome preparation. There has been a long period during which a tumour has not been clinically determinable. Localization of the carcinomatous process was accompanied by very marked improvement in the patient's general condition, she gained up to 7 kg in weight and her fitness was completely restored. Systematic haematological studies showed the absence of any pathological changes either in the blood elements or haemopoietic organs. Thus, a prolonged recovery has been achieved in patient M. by treatment with the trypanosome preparation combined with removal of the primary cancer focus, without radical surgery or X-ray therapy (Plate 59).

BLOOD ANALYSES OF PATIENT M.

Dates	20 Aug '47	23 Aug '47	30 Aug '47	2 Sept '47	9 Sept '47
Haemoglobin	68	69	70	71	71
Red cells	4,100,000	4,120,000	4,200,000	4,340,000	4,240,000
Colour index	0.8	0.8	0.8	0.8	0.8
Leucocytes	6600	8000	7100	7000	9000
E.S.R.	15	38	32	27	29
Eosinophils	2	1	3	1	3
Juvenile cells	2	6	5	4	2
Segmented cells	65	68	70	70	69
Lymphocytes	28	21	18	22	23
Monocytes	3	4	4	3	3

Dates	16 Sept '47	23 Sept '47	30 Sept '47	8 Oct '47	14 Oct '47
Haemoglobin	72	72	71	71	71
Red cells	4,320,000	4,420,000	4,260,000	4,360,000	4,220,000
Colour index	0.8	0.8	0.8	0.8	0.8
Leucocytes	6600	6000	7000	6600	7500
E.S.R.	37	30	32	27	27
Eosinophils	6	2	3	2	3
Juvenile cells	2	1	3	4	1
Segmented cells	65	66	66	69	69
Lymphocytes	22	27	23	22	23
Monocytes	5	4	5	3	4

Dates	21 Oct '47	28 Oct '47	5 Nov '47	18 Nov '47	2 Dec '47
Haemoglobin	73	73	73	74	71
Red cells	4,230,000	4,380,000	4,420,000	4,320,000	4,120,000
Colour index	0.8	0.8	0.8	0.8	0.8
Leucocytes	8800	7400	7080	7420	7000
E.S.R.	33	26	22	22	20
Eosinophils	4	1	1	3	1
Juvenile cells	1	2	2	1	2
Segmented cells	66	72	69	70	73
Lymphocytes	25	22	24	21	20
Monocytes	4	2	4	5	4

Dates	17 Dec '47	31 Dec '47	10 Jan '48	28 Jan '48	11 Feb '48
Haemoglobin	70	72	71	71	71
Red cells	4,220,000	4,420,000	4,230,000	4,240,000	4,120,000
Colour index	0.8	0.8	0.8	0.8	0.8
Leucocytes	6600	6400	7200	6800	7200
E.S.R.	26	23	28	13	6
Eosinophils	2	2	2	3	3
Juvenile cells	1	1	4	2	3
Segmented cells	73	68	68	69	62
Lymphocytes	21	25	23	22	27
Monocytes	3	4	3	4	5

Date	22 Feb '48	5 Mar '48	15 Mar '48	25 Mar '48	3 Apr '48
Haemoglobin	70	72	72	71	72
Red cells	4,180,000	4,200,000	4,340,000	4,360,000	4,260,000
Colour index	0.8	0.8	0.8	0.8	0.8
Leucocytes	7400	6400	7400	8400	7200
E.S.R.	11	14	12	18	22
Eosinophils	2	3	8	3	2
Juvenile cells	1	1	2	2	3
Segmented cells	72	68	65	68	68
Lymphocytes	21	25	21	24	26
Monocytes	4	3	4	3	3

Date	13 Apr '48	23 Apr '48	22 May '48	2 June '48	14 Jun '48
Haemoglobin	71	71	71	72	70
Red cells	4,360,000	4,120,000	4,100,000	4,400,000	4,420,000
Colour index	0.8	0.8	0.8	0.8	0.8
Leucocytes	7200	7400	7200	7400	7000
E.S.R.	24	31	16	14	21
Eosinophils	1	2	1	2	1
Juvenile cells	2	3	4	3	4
Segmented cells	71	67	66	66	68
Lymphocytes	22	25	22	24	23
Monocytes	4	3	7	5	4

Date	1 July '48	28 July'48	11 Aug '48	28 Aug '48	13 Sep '49
Haemoglobin	72	70	71	70	70
Red cells	4,300,000	4,120,000	4,180,000	4,420,000	4,200,000
Colour index	0.8	0.8	0.8	0.8	0.8
Leucocytes	8200	7400	7600	7600	7800
E.S.R.	31	22	17	31	14
Eosinophils	1	1	1	2	2
Juvenile cells	2	2	2	4	2
Segmented cells	68	72	68	69	67
Lymphocytes	26	21	25	22	25
Monocytes	3	4	4	3	4

Date	12 Nov '48	23 Nov '48	2 Dec '48	13 Dec '48	23 Dec '48
Haemoglobin	70	70	72	72	70
Red cells	4,420,000	4,360,000	4,360,000	4,340,000	4,220,000
Colour index	0.8	0.8	0.8	0.8	0.8
Leucocytes	6800	7200	7800	8000	7600
E.S.R.	16	14	31	37	28
Eosinophils	3	4	3	1	2
Juvenile cells	3	2	4	4	3
Segmented cells	67	69	68	69	70
Lymphocytes	25	27	22	21	22
Monocytes	2	3	3	4	3

Date	10 Jan '49	20 Jan '49	31 Jan '49	17 Feb '49	18 Feb '49	2 Mar '49
Haemoglobin	69	69	70	70	68	69
Red cells	4,000,000	4,200,000	4,080,000	3,900,000	3,600,000	4,080,000
Colour index	0.8	0.8	0.8	0.9	0.9	0.8
Leucocytes	7800	7400	8200	12,000	8600	8800
E.S.R.	50	50	47	54	44	36
Eosinophils	1	1	1	0	2	1
Juvenile cells	5	4	5	10	3	6
Segmented cells	69	67	69	66	69	69
Lymphocytes	22	65	19	17	21	0
Monocytes	3	2	6	7	5	4

OBSERVATION NO. 17

Patient R-e. Clinical diagnosis: *cancer of the right breast, stage II.* Histological diagnosis: *early carcinoma of the solid type against a background of marked cystic mastopathy.*

On admittance to the clinic for biotherapy on 7 May 1949 examination revealed: the internal half of the right breast contained a nodule measuring 5×4.5 cm, and in the right axillary region there was a lymphatic node 0.75 cm in diameter. A course of injections of the preparation was started on 7 May 1949 (Plates 60*a*,*b*).

INJECTIONS OF THE PREPARATION AND COURSE OF THE DISEASE

Patient R-e received a long course of intramuscular injections—from 7 May 1949 to 11 August 1950. During these 15 months 254 injections were given, involving 63,100 units, mainly of the dried, modification VI preparation obtained from *T. cruzi* cultures grown on synthetic culture media. A biopsy was performed 1 week after the start of the injections, on 14 May 1949. Histological findings: early carcinoma of the solid type against a background of marked cystic mastopathy. The course of the injections was not interrupted.

On 17 June 1949, 40 days after the start of the course, when 10,600 units had been injected, it was noted that the tumour was progressively diminishing and the nipple had become more protruberant than before. Two months later, on 17 August 1949, when 28,000 units had been injected, an examination revealed: under the scar on the right breast there was a firm tumour measuring 3×4 cm; the nipple was slightly sunken. After another 2 months, on 14 October 1949, when 37,000 units had been injected,

the tumour measurements had increased to 4×4 cm, and the lymphatic node in the right axilla measured 1×0.75 cm (Plate 61).

On 25 November 1959 the patient underwent a non-radical operation—excision of the tumour to the limits of the affected tissue. Histopathological diagnosis: solid carcinoma with background of mastopathy.

Intramuscular injections of the same modification of the preparation were given during the post-operative period and then without interruption for a further 8½ months—until 11 August 1950. Only 1 month after the operation, on 21 December 1949, when the patient had received 46,000 units since the course started and the scar was in a satisfactory condition, a thickened area 2 cm in diameter, clinically diagnosed as mastopathy, appeared external to the nipple.

About 2 months later, on 15 February 1950, when the patient had received 51,000 units of the preparation, the thickened area had disappeared. No other thickenings were noted. The scar was satisfactory.

Starting in March, a thickening 1–1.5 cm in diameter appeared in the affected breast. Its position was *inconstant:* in March (22 March) it could be felt *in the nipple region along the course of the scar,* in May (29 May) it was *separate* from the scar and in July (10 July) it was *under the scar.* These changes occurred during an uninterrupted course of injections: by 22 March the patient had received, counting from the start of the course, 53,000 units, by 29 May 54,000 units and by 10 July 60,000 units. Finally, on 9 August 1950, having been given the last, 254th, injection and having received a total of 63,100 units of the preparation, the patient terminated the treatment, still having a firm nodule 1.5 cm in diameter, not adherent to the skin, in the scar region, and in the right axillary region a firm lymphatic node (cord) measuring 0.75×1.5 cm, which could be felt and was the same size the previous February and had maintained this size, with slight variations, during the six months' course of injections.

Patient R. was examined on 21 April 1951, 7½ months after the injections were stopped. The scar was soft, without any thickened areas. Beside the scar was a nodule 1.25 cm in diameter; the lymphatic node in the axillary region had also decreased slightly in size and measured 0.75×1.25 cm. The breasts were preserved (Plate 62).

On 30 August 1955 R. was examined by a medical committee from the Presidium of the Scientific Council of the Ministry of Health of the U.S.S.R. The committee established: "the right breast is slightly smaller than the left, and its internal quadrant bears a linear scar, white, soft, mobile and painless. No tumour can be felt in the breast. Conclusion: R. is apparently healthy, with no tumour and no determinable metastases".

R. works as a conductress. During all this time, except during the period of hospital treatment (1949), she has been able to carry out her work.

Period of observation 6 years 3 months.

OBSERVATION NO. 18

Patient K-i, aged 42 years. Clinical diagnosis: *recurrent cancer of the left breast.* Histopathological diagnosis: *adenocarcinoma* (scirrhous) *with areas of solid carcinoma.*

Two years and 4 months before approaching our clinic for biotherapy, the patient had in October 1945 undergone a radical operation for cancer of the left breast, histologically determined as a "tubular scirrhous carcinoma". Eighteen months later, in 1947, a tumour appeared in the region of the postoperational scar.

At the time of admittance to the biotherapy clinic on 28 April 1948 an examination revealed: on the left breast there was an operation scar. At the level of the 4th rib on the left parasternal line there was a firm tumour 1.5 cm in diameter. At the level of the 3rd rib near the remains of the major pectoral muscle there was an area of thickening. Injections of the preparation were started on 28 April 1948.

INJECTIONS OF THE PREPARATION AND COURSE OF THE DISEASE

In 1948 and 1949 K. received a long but insufficiently regular course of injections: during the 9 months from 28 April 1948 to 25 January 1949 she was given 164 injections, and 314,000 units of the preparation, mainly of modification VI, were administered intramuscularly. During the rest of 1949 the patient received only short courses, with long intervals:

in February and March	— 11	injections
in June, July and August	— 40	,,
in September, October and November	— 18	,,

For the next 9 months the patient was not treated, receiving only 11 injections at the end of August 1950 (19 August to 1 September).

Ten days after the start of the injections, on 7 May 1948, a biopsy was carried out: excision of half of the tumour situated at the level of the 4th rib. Histopathological conclusion: *carcinoma, of the adenocarcinoma type (scirrhous) with areas of solid carcinoma.*

A month after the start of the injections, when 20,450 units had been given, the portion of the tumour remaining after biopsy had disappeared. The outlines of the second thickening at the level of the 3rd rib had become indistinct. Three months later, after 87 injections (159,900 units) it was reported on 26 September 1948: "scar in satisfactory state".

The next month, when the patient had received 207,000 units in 107 injections, a nodule measuring 0.75×0.3 cm appeared near the scar; it remained almost unchanged for the following 5 months (until February 1949). On 22 February 1949, after a month's interval in the injections, it measured 0.75×0.5 cm, and later in the period when K. was receiving no injections of the preparation this nodule took on a fibrous consistency and was regarded as "suspicious of recurrence". During the same period the thickening near the remains of the major pectoral muscle grew larger and in May 1949 had reached a size of 3×2.5 cm. The patient again started to receive injections of modification VI of the preparation, obtained from *T. cruzi* cultures grown on synthetic culture media, and from 24 May to 7 September 1949 she was given 40 intramuscular injections; 10 days later another 18 injections were given (from 18 September to 16 October). Hence 58 injections, involving 114,000 units of the preparation, were given over $4\frac{1}{2}$ months. During the course of the injections growth of the nodule and the thickened area was arrested and the thickening over the 3rd rib became soft in consistency (examination on 3 August 1949).

No change was seen in the patient's condition during the next 7 months. In October 1950 two firm nodules 1–1.5 cm in diameter were again determined. The patient had received no injections during the period commencing November 1949, except for 12 injections in August 1950, and was merely under observation until April 1955.

Thus, within eighteen months in 1948 and 1949, injections of the preparation twice brought about clinically recorded regression of the tumour in patient K. In good health and with maintained fitness, she was able to continue working until the end of the observation (April 1955) (Plates 63–65).

Period of observation—7 years.

OBSERVATION NO. 19

Patient L., aged 34 years. Clinical diagnosis: *cancer of the left breast,* stage II. Histological diagnosis: *mammary carcinoma.*

The patient was examined in Leningrad in June 1946 by Profs. N. N. Petrov and S. A. Kholdin, who diagnosed mammary cancer and suggested radical operation combined with X-ray therapy. The patient received several seances of X-ray therapy (5870 units) in the course of the preoperative preparations, but categorically refused further treatment and the

proposed operation. As a result of the deterioration in her general condition and increased growth of the tumour occurring after the passage of some time the patient was sent to our clinic on 26 August 1946 for biotherapy. At this stage the firm uneven tumour, irregularly oval in shape, had reached a size of 8.0×6.0 cm, and the axilla contained four firm lymphatic nodes (metastases). Treatment with the trypanosome preparation was commenced on 20 August 1946.

INJECTIONS OF THE PREPARATION AND COURSE OF THE DISEASE

From the start of the observation in 1946 to 1950 the patient received 4 courses of injections of the preparation, using in turn modifications I and VI.

The *first* course of injections lasted from 21 August to 10 October 1946. In these 51 days the patient received 51 injections of modification I preparation. It was injected intramuscularly in doses of from 100 to 200 units, and simultaneously intravenously (20 to 100 units) and intratumourally (10–20 units).

After 40 injections, in which 11,110 units were given intramuscularly, 1,750 intravenously and 870 intratumourally, the following changes in the state of the tumour were noted: the primary tumour lesion in the breast had decreased in size to 5×4.5 cm, as compared with the previous measurements of 8.0×6.0 cm. The consistency of the tumour remained unchanged. It was painful to the touch. One lymphatic node could be palpated in the axilla instead of four as formerly.

On 30 August the intratumoural injections were stopped. The intramuscular and intravenous injections were continued until 10 October. In all, from the start of the course to 10 October, 16,200 units were given intramuscularly, 2000 intravenously and 870 intratumourally. The tumour size had decreased from 8×6 cm to 4×3.5×3.0 cm. One lymphatic node the size of a lentil remained in the left axilla. The injections were terminated on 10 October 1946, since the patient interrupted the treatment and went home to Kiev, where she was seen by the doctors who had treated her previously. In view of the marked changes in the whole clinical picture, Prof. Yu. Yu. Karamenko considered it possible, without resorting to radical surgery, to carry out resection of the neoplasm, without removing the whole breast, or the lymphatic nodes and vessels.

A detailed histopathological analysis of the resected tumour revealed only isolated cancer cells in stages of atrophy (see Part V).

On 20 May 1947, i.e. 5 months after the operation and 6 months after the first course of injections of the preparation, the patient was again examined in our clinic.

The left breast had been decreased to one third of its size by the operation and was slightly deflected to the left. At the level of the nipple were two fresh scars, situated vertically and adherent to the tissues. The lymphatic node previously palpatable in the axilla was no longer evident.

On 21 May the patient started a *second* course of injections, using modification VI preparation, this time given intramuscularly.

The first injection was of 250 units, the second 500 and the third 800. From the fourth to the forty second injection doses of 1000–3000 units were given. In all, 96,400 units were administered in 42 injections.

Examination of the patient showed the absence of any signs of recurrence or metastases. In view of this, on 10 June the course of injections was terminated.

Eight months after the end of the second course the patient again became an in-patient at our clinic. Examination revealed the following:

The post-operational scar had grown softer during the intervening period. The left axilla contained a firm fibrous cord, like solidified adipose tissue, 2.5 cm long and 1.5 cm across. Beside it was a soft lymphatic node measuring 1.5×0.75 cm. The right axilla contained a soft lymphatic node 0.75 cm in diameter.

On 12 February 1949 the patient started a *third* course of intramuscular injections of the preparation in doses of 1000–3000 units. In all, 27 injections were given from 12 February to 15 March.

Examinations on 15 March 1949 and 4 October 1950 showed the absence of any signs of recurrence or metastases. The thickened area in the scar region remained unchanged, as did the axillary region. Further observations were made in L.'s home town, by the L'vov Oncological Officer. In February 1956 a letter was received from the patient stating that she had been found to be healthy, according to the L'vov Oncological Officer.

In analysing the observations on patient L., the following facts should be considered:

(1) The observations made during the 5 months following discovery of the tumour established that it was growing intensively, as shown by an increase in its size to that of a hen's egg. During the same period 4 firm lymphatic nodes appeared in the regional axilla. The cancerous condition in patient L. therefore belonged to that category which develops with great intensity, rapidly forming metastases in the regional lymphatic nodes.

(2) While suffering from rapidly developing, metastasizing cancer patient L. underwent a course of X-ray therapy (5870 units in all). *Radiation therapy was unsuccessful.* Tumour growth continued with an intensity

even greater than before. For this reason the patient was sent to our clinic for biotherapy.

(3) The use of injections of the trypanosome preparation was accompanied by *a gradual decrease in the tumour measurements*, which by the end of the course of injections had become less than *half* their previous value. *Three out of four* of the regional lymphatic nodes *underwent regression during the process of the injections.*

(4) Changes in the tumour associated with the regression process, expressed as a decrease in its volume, were recorded during the actual process of the injections and for a month after their termination.

(5) *Clinically* apparent tumour regression was accompanied by characteristic changes in the tumour's *structure*: degeneration of the malignant cells and the development of regenerative processes in the surrounding tissues.

(6) Finally, the *limited operative interference*, consisting of resection of the *tumour* remnants, was not accompanied in the post-operative period by recurrence of the tumour or the appearance of metastases.

The combination of all the described phenomena forces us to accept that injections of the trypanosome preparation combined with nonradical operation led to clinical recovery in patient L. for a period of 9 years 6 months—the period of observation (Plate 66).

OBSERVATION NO. 20

Patient S-s., aged 54 years. Clinical diagnosis: *cancer of the left breast*, stage I. Histopathological diagnosis: *a tuberous form of colloid carcinoma.* Examination on admittance to the clinic on 9 September 1948 showed: at the border of the upper and lower quadrants of the left breast there was a firm nodule measuring 2×2.5 cm. No lymphatic nodes could be palpated. A course of injections of the preparation was commenced on 9 September 1948.

INJECTIONS OF THE PREPARATION AND COURSE OF THE DISEASE

Patient S. received 3 courses of injections.

Course 1 lasted for 5 months—from 9 September 1948 to 15 February 1949;

Course 2—one week after the first—was from 19 February to 1 July 1949;

Course 3—$3\frac{1}{2}$ months after the second—was from 17 October 1949 to 3 April 1950.

Injections were given intramuscularly, once daily. The preparation used was mostly of modification VI—a dried product obtained from *T. cruzi* cultures grown on synthetic culture media. The three courses involved 254 injections, and 400,000 units of the preparation were administered.

Seven days after the start of the course of injections a nonradical operation was performed—excision of the tumour to the limits of the macroscopically affected tissue. Histopathological conclusion: nodular (tuberous) form of colloid carcinoma, with the formation of cavities full of mucus and the remains of cancer nodules. The postoperational wound healed after 8 days (22 September 1948). The left axilla contained a firmish lymphatic node 0.5 cm in diameter. By this time the patient had received 6200 units of the preparation. After another 2 weeks, on 5 October 1948, there was postoperational infiltration of an area 3×2 cm around the scar. The left axilla contained a lymphatic node measuring 0.75×0.5 cm. The patient had then received 40,700 units of the preparation from the start of the course of injections.

On 27 December, $3\frac{1}{2}$ months after the start of the course, when 211,000 units had been administered, there was a diffuse thickening, cicatricial in nature, along the scar. The lymphatic nodes in both axillary regions were soft and nonspecific. After another 4 months, on 5 May 1949, when the patient had received 306,000 units, it was noted that the scar was in a satisfactory state, no lymphatic nodes could be palpated and the breast was of the fibrous mastopathy type.

Further regular observations showed the absence of any signs of recurrence or metastases: on 1 July 1949, after the administration of 361,000 units; 5 October; 22 February 1950 (391,300 units); 18 March 1950 (394,000 units); 29 March 1950 and 3 April 1950.

Administration of the preparation was stopped on 3 April 1950, after 254 injections (400,000 units). Examination of the patient on 21 April 1951, 2 years and 6 months from the start of the treatment, showed the absence of any thickened areas in the scar. No lymphatic nodes were palpated.

The same findings were recorded in February 1955: no signs of recurrence or metastases.

Period of observation—6 years 9 months (Plate 67).

OBSERVATION NO. 21

Patient L.G. Clinical diagnosis: *cancer of the right breast*, stage II. Histological diagnosis: *solid carcinoma.*

Examination of the patient on her admittance to the biotherapy clinic 22 February 1949 showed: in the right breast there was a firm nodule measuring 1.5×2.5 cm. the right axilla contained a lymphatic node measuring 1.5×1 cm (Plates 68*a*, *b* and *c*).

INJECTIONS OF THE PREPARATION AND COURSE OF THE DISEASE

Patient L.G. received a long course of intramuscular injections of a dried preparation, mainly of modification VI, obtained from *T. cruzi* cultures grown on synthetic culture media. The course of injections lasted for 11 months, from 22 February 1949 to 21 January 1950. During this period the patient received 226,700 units. The injections were given once daily except on holidays.

Nine days after the start of the injections a biopsy was performed, with excision of part of the tumour within the affected area measuring 0.8×0.5 cm. Histopathological conclusion: solid carcinoma.

On 7 April, after 1½ months and when 55,000 units had been injected, there was a thickening 1.5×2 cm in the scar region. The right axilla contained a lymphatic node measuring 1.5×2 cm.

During the next two months there was no change in the clinical picture: the thickening in the scar region remained as before on palpation. The lymphatic node in the right axilla grew slightly smaller—1.5×1 cm. By this time 3½ months had passed since the start of the injections, during which L. G. had been given 104,000 units of the preparation. On 14 July 1949 a control biopsy was carried out—excision of the scar together with the thickened area and part of the gland parenchyma.

Histopathological investigation revealed *moderate fibro-cystic mastopathy*. There were no signs of a malignant tumour. The patient's breast was preserved (Plates 69*a*, *b* and *c*).

Observations during the next 6 years, until February 1955, showed that the patient's condition was permanently maintained: there were no thickened areas either in the scar or in the gland tissue.

The lymphatic node in the axilla remained present. Its consistency and measurements varied: 1½ months after the control biopsy, on 26 August 1949, when 180,000 units of the preparation had been injected, a group of lymphatic nodes 3 cm in diameter became evident in the right axillary region. After this it gradually diminished, and on 21 January 1950, after the administration of 226,700 units, it measured 1.5×1.25 cm. On this date the patient terminated the course of injections. During the next 9 months the node varied slightly in size and on palpation was sometimes softer, sometimes more firm.

An examination of the patient on 4 October 1950 showed the scar to be in a satisfactory state. There were no thickenings in either breast. The right axilla contained a firm lymphatic node measuring approximately 1.5×2.0 cm.

At the suggestion of V. I. Kazanskii, the patient underwent a course of X-ray therapy in October and November 1950, but there was no objective result: in December the right axillary region contained "a thickened area measuring 1–1.5 cm".

The last examination was made in February 1955, when there were no signs of recurrence or metastases.

Period of observation—6 years.

Thus, in patient L. G. injections of the preparation, in combination with limited resection of the primary tumour focus, led to clinical recovery for a period of 6 years.

* * *

In summing up the observations described in this chapter, the following should first be noted: it has proved to be effective in practice to use the trypanosome preparation in combination with removal of the tumour focus to the limits of the affected tissue. This has enabled clinical recovery lasting several years to be achieved in a number of patients, with preservation of the breast and without radical operation or X-ray therapy. Experience has shown that removal of the tumour focus is best carried out during the period of regression or of the cancerolytic effect caused by a course of preoperative treatment with the trypanosome preparation, without stopping the injections at the time of surgical interference or in the postoperative period.

The inhibitory effects of the trypanosome preparation were expressed as cessation of growth or retardation of the development of a formerly rapidly growing tumour in the primary focus, in regional lymphatic node metastases and also in recurrent foci, which either disappeared, or decreased in size or were stabilized in a definite condition.

In some patients the morphological picture of the tumour tissue and its stroma became changed under the influence of the trypanosome preparation (see Part V).

The process of tumour regression was accompanied by the development of temporary inflammatory foci in the breast.

Partial removal of a cancerous tumour, as in diagnostic biopsy or more extensive but *nonradical operations facilitated* the process of *regression*

produced in the malignant neoplasm by the trypanosome preparation. Observations showed that a biopsy carried out during simultaneous administration of the trypanosome preparation is converted from a growth-provoking factor to a factor *aiding the regression* of the malignant tumour (this has been confirmed experimentally).

As a whole, the clinical observations indicate that *malignant neoplasms of the breast are sensitive to the regression factor in T. cruzi.*

4. REGRESSION OF NEOPLASMS OF THE GASTRO-INTESTINAL TRACT

During the years which have elapsed since the publication of our book *The Biotherapy of Malignant Tumours* we have not devoted any special attention to malignant neoplasms of the gastro-intestinal tract. It is of interest, however, to mention some case-histories recorded by us in 1946.

OBSERVATION NO. 22 (NO. 14 IN 1946)

Patient Z., female, aged 46 years. Cancer of the oesophagus. At the end of April 1946 the patient felt pain on swallowing and obstruction of food in the *jugularis sterna* region. The pain disappeared after taking analgesics, but the patient still suffered from restricted passage and hold-up of food in that region.

On radioscopy (8 May 1946) a thick contrast mass passed slowly into the middle third of the oesophagus. On 20 May the erythrocyte sedimentation rate was 33 mm per hour. Blood was found in the gastric juice.

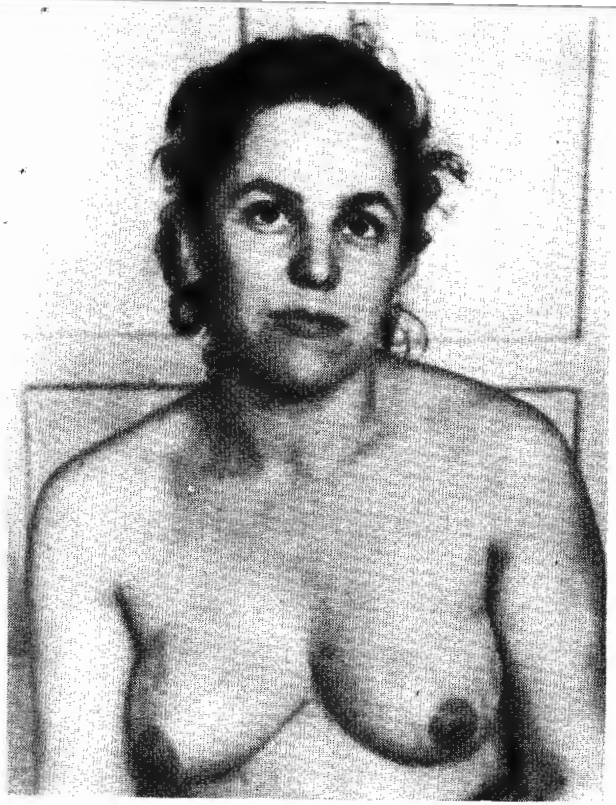
The patient was admitted to the clinic on 14 May 1946.

Injections of modification I of the preparation were started on 15 May 1946.

INJECTION OF THE PREPARATION AND CONDITION OF THE PATIENT

Date	Injections	Dose in units
15–18 May	1–4	15 each
19–25 May	5–11	20 each
26 May	12	15
27 May	13	20
28–30 May	14–16	70 each
	Total:	445 units in 16 injections.

20 May—*radioscopy*—fluid and thick contrast masses passed freely along the whole course of the oesophagus.



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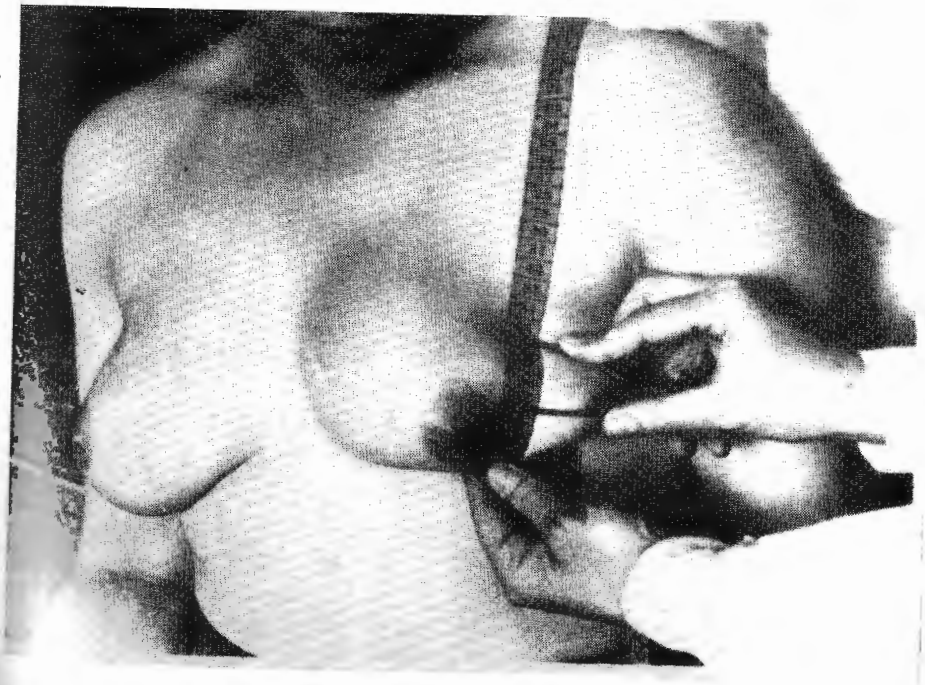


(*b*)

PLATE 50, *a*, *b*. Patient S. Observation No. 14. Clinical diagnosis: cancer of the left breast. Histological diagnosis: cystic mastopathy, with areas extremely suspicious of blastomatous (cancerous) transformation.

28 February 1949. Before the start of injections of the preparation. The left breast contains a racemose thickening measuring 3.0×2.5 cm. At its centre is a projection in the form of a firm spine.

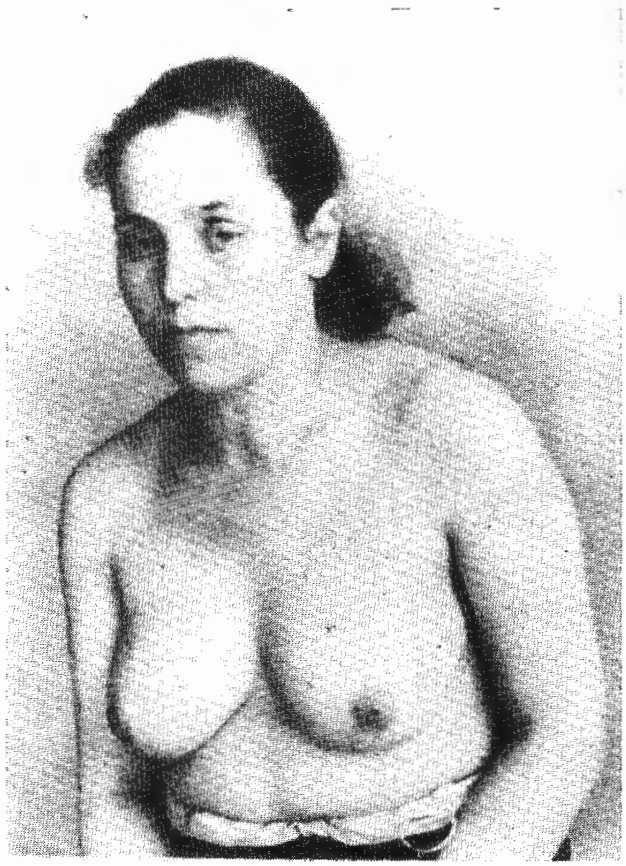


PLATE 51. Patient S., 18 April 1951. 2 years and 2 months from the start of injections of the preparation. There are no signs of the affection. The breast is preserved, and shows a slight trace of the scar from the biopsy carried out 2 years and $1\frac{1}{2}$ months previously. The patient received two courses of injections of the preparation: 1—from 24 February 1949 to 29 March 1950 (66,000 units); 2—from 18 May 1950 to 19 December 1950 (15,500 units). The last examination was on 13 September 1955, by a committee from the Soviet Ministry of Health. Their conclusion: "S. is apparently healthy. There is no recurrence of the tumour, and no metastases can be determined." Period of observation: 6 years and 5 months.

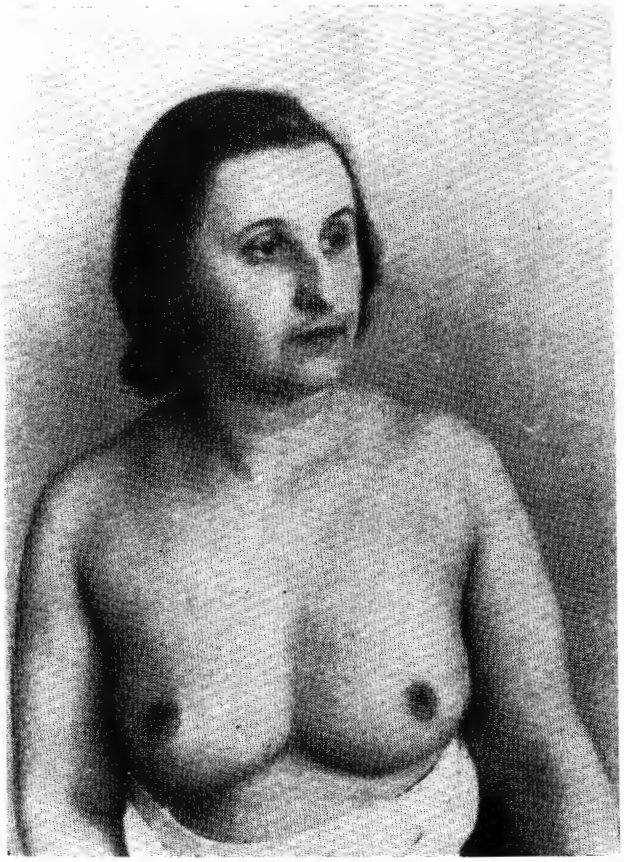


PLATE 52*a*. Patient B., Observation No. 15. Clinical diagnosis: fibroadenoma of the left breast, with suspected malignant transformation. Histological diagnosis: adenocarcinoma. 21 April 1951. 3 years 8 months from the start of the observation. The breast has been preserved. There are no signs of recurrence or metastases. The patient received 147 injections of the preparation between 20 August 1947 and 24 February 1948 (202,000 units). After 93 injections (92,800 units), on 19 December 1947 a nonradical operation was performed—removal of the tumour, which by this time had regressed to the size of a pin's head from its former size of 1.5×1.0 cm. Period of observation, 3 years 8 months.

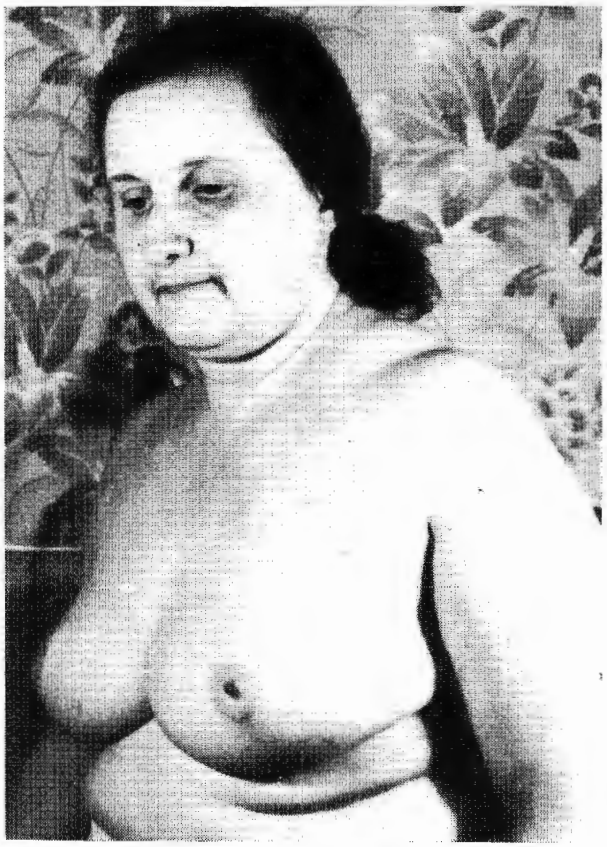


PLATE 52*b*. Patient B. Observation No. 15. 6 July 1960. Twelve years after the end of the treatment. The breast has been preserved. There are no signs of recurrence or metastases.

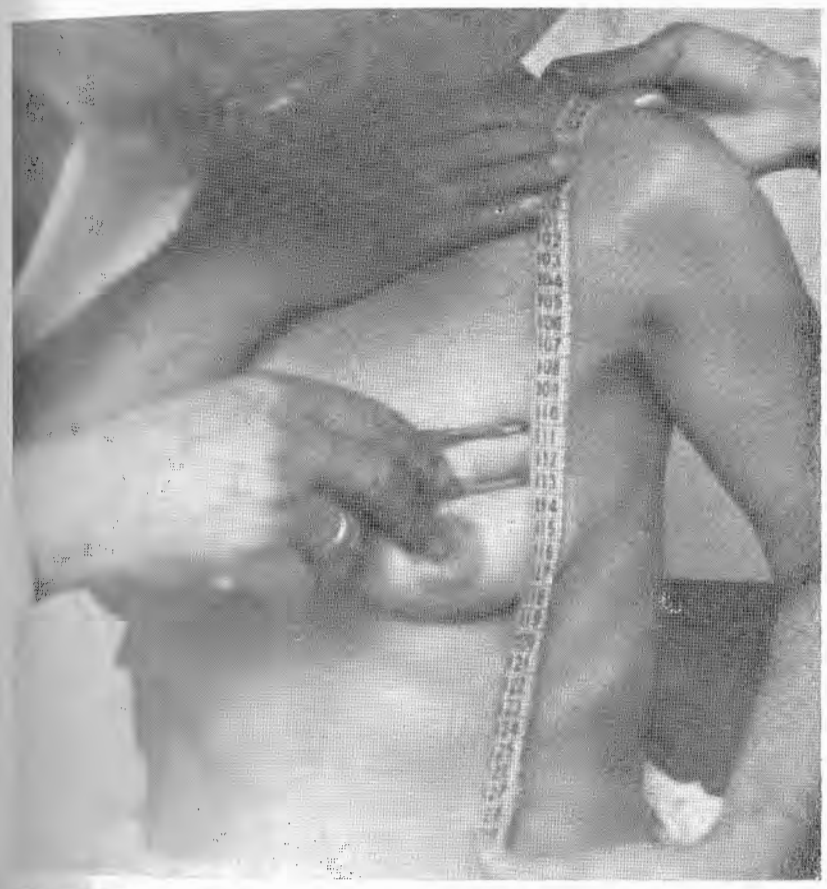


PLATE 53. Patient M., Observation No. 16. Clinical diagnosis: cancer of the left breast. Histological diagnosis: scirrhous carcinoma. 15 August 1947. Before the start of injections of the preparation. In the external upper quadrant of the left breast there is a firm tumour, measuring 2.0×2.0 cm, adherent to the gland parenchyma and at its centre adherent to the skin. The left axilla contained three lymphatic nodes, firm, mobile, one measuring 1.5×1.0 cm, the other two the size of a pea.

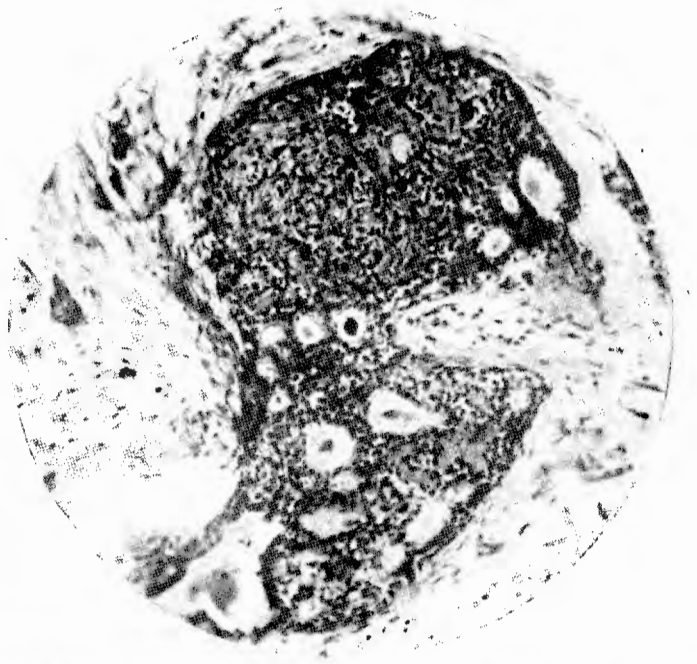


PLATE 54. Patient M., 25 August 1947. Histological structure of the tumour before the start of injections: scirrhous carcinoma with areas of cicatricial collagenization of the stroma, with isolated cancer nodules of a solid structure.

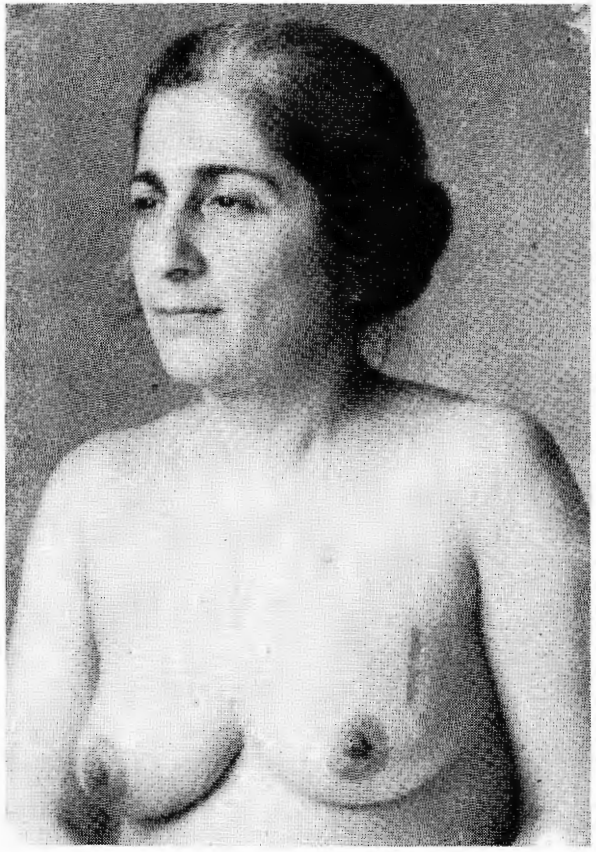
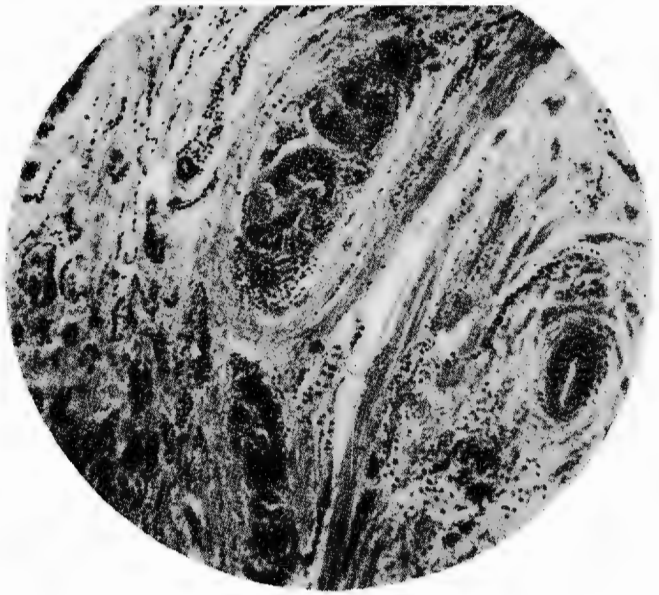


PLATE 55. Patient M., 26 February 1948 after 130 injections of the preparation. Tumour not palpatable. Lymph nodes in axilla soft.



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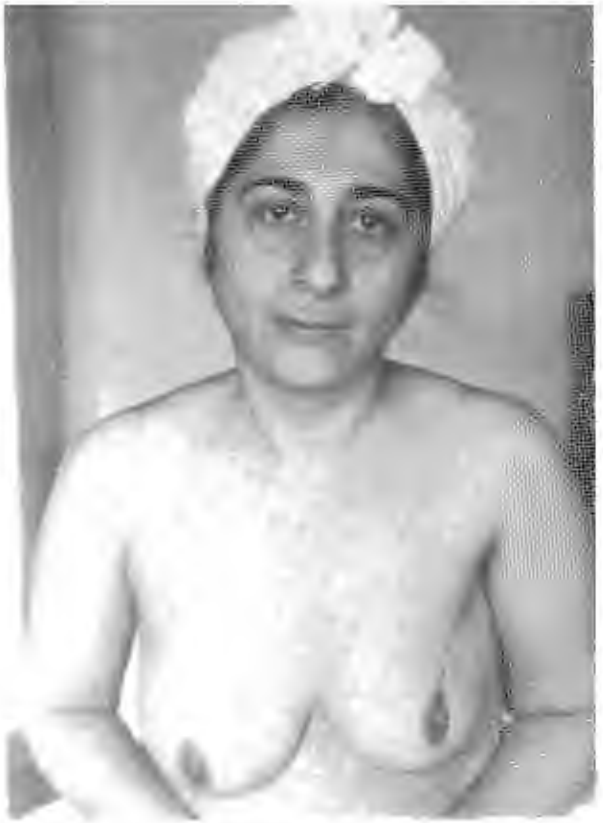
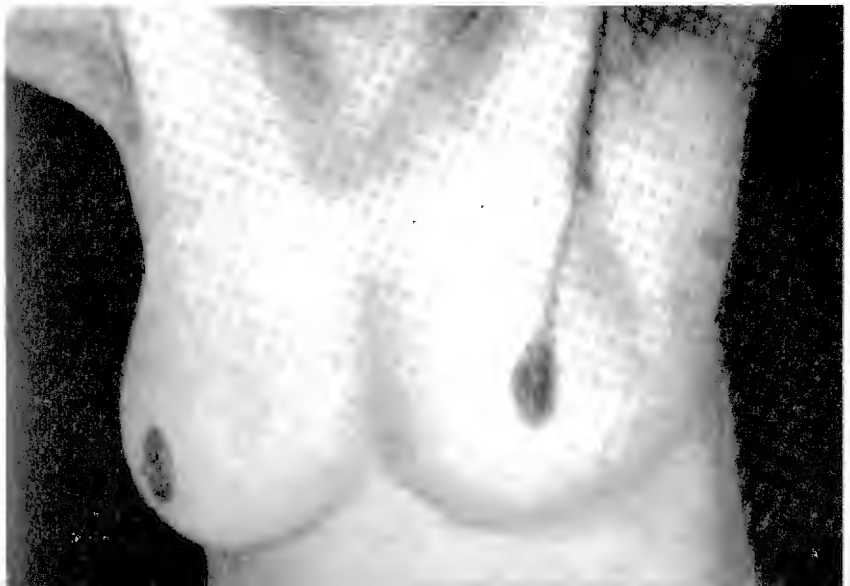
(57)

PLATES 56 and 57. Patient M., 6 January 1949. Histological structure of the tumour tissue after 390 injections. On 6 January 1949 the patient underwent a non-radical operation. No signs of metastases were found on histological examination of an axillary lymphatic node. There was a picture of hyperplasia with focal proliferation of cells of the reticulo-endothelial system. Histological examination of tissue from the scar left by the biopsy showed coıds of cancer cells among the



(*a*)

PLATE 58*a,b*. Patient M., 6 September 1949. 8 months after nonradical operation. During these 8 months clinical examinations had revealed no signs of a tumour. The photo was taken before a control biopsy involving excision of the scar and of the axillary connective tissue. No tumour elements were found on histological examination.



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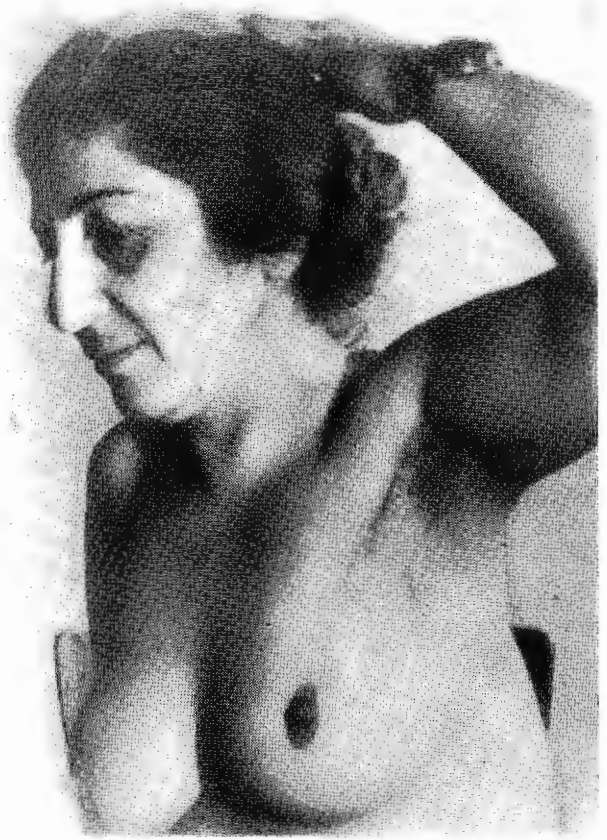
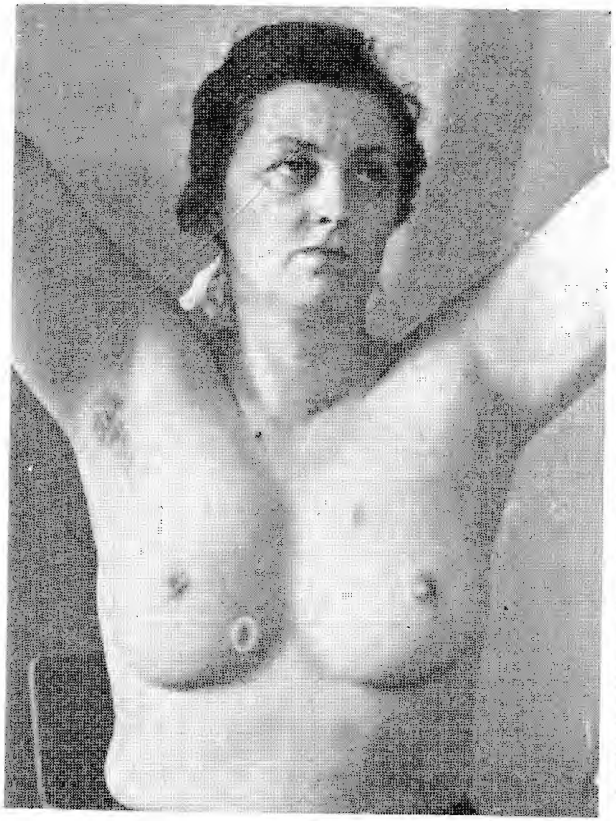


PLATE 59. Patient M., 8 April 1951. 3 years 7 months after the start of the observation. There are no signs of recurrence or metastases. The last examination was on 30 August 1955, by a committee from the Soviet Ministry of Health. Conclusion: "M. is apparently healthy. There is no recurrence of the tumour and no metastases can be determined." Period of observation, 8 years.



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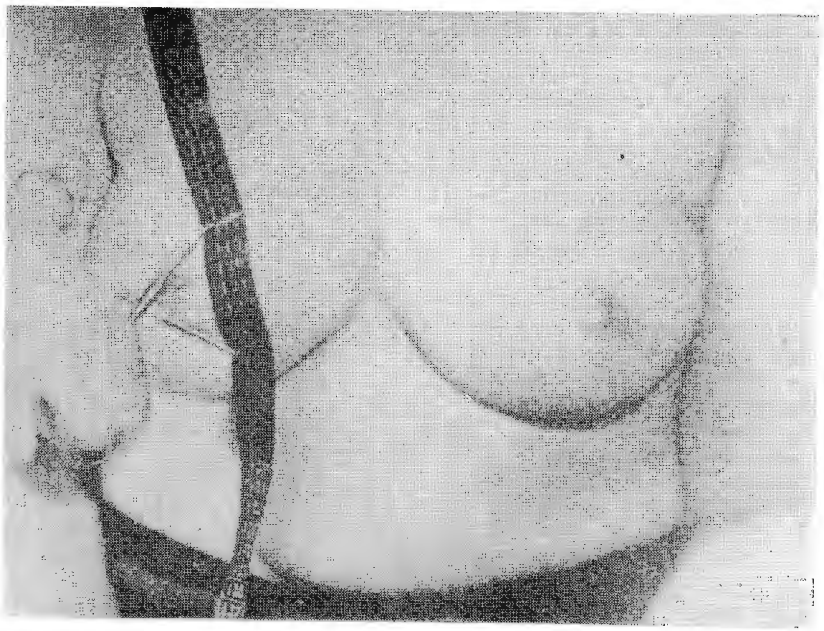


(*b*)

PLATE 60, *a*, *b*. Patient R., Observation No. 17. Clinical diagnosis: cancer of the right breast, stage II. Histological diagnosis: early solid carcinoma with a background of marked cystic mastopathy.
9 May 1949 — before the start of injections of the preparation. The internal ¦half of the right breast contains a tumour measuring 5.0×4.5 cm, and the nipple is invaginated. In the right axilla is a lymphatic node 0.75 cm in diameter.

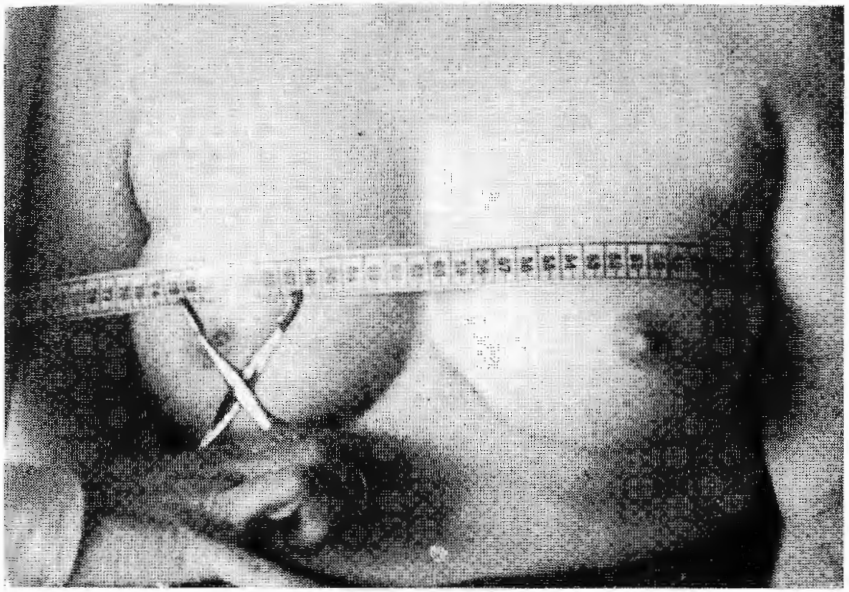


PLATE 61. Patient R., 25 November 1949. 6½ months after the start of injections of the preparation. The tumour is still large. A scar can be seen—the result of the biopsy performed 6 months previously. This photo was taken on the day that a nonradical operation was performed, involving excision of the tumour within the limits of the affected tissues. Histological examination revealed a solid carcinoma with a background of mastopathy. The course of injections was not interrupted during the postoperative period—until 11 August 1950 (8½ months).

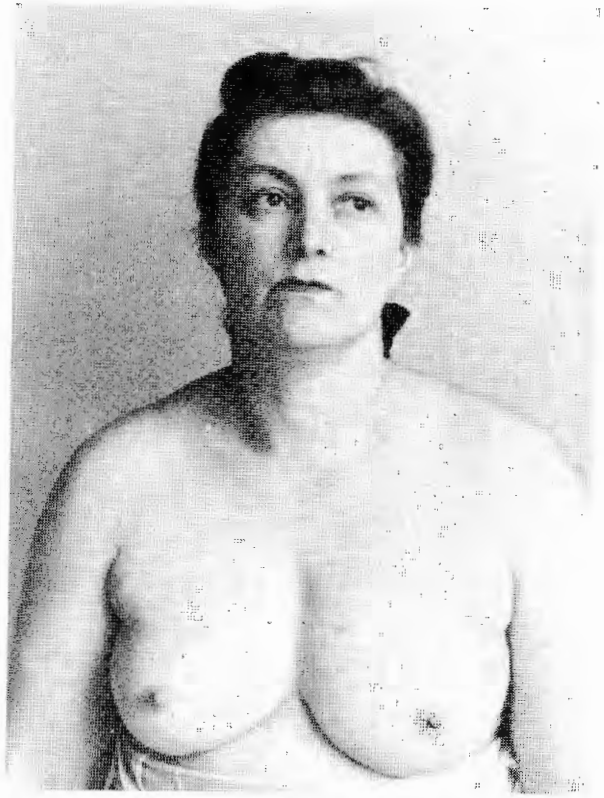


PLATE 62. Patient R., 21 April 1951. Two years after the start of observations. The patient received 254 injections of the preparation (63,000 units) in 15 months, and underwent a nonradical operation a year and 4 months previously. There are no signs of recurrence or metastases. The last examination was on 30 September 1955, by a committee from the Soviet Ministry of Health. The patient's condition was unchanged. Their conclusion: "R. is apparently healthy. There is no tumour, and no metastases can be determined". Period of observation, 6 years and 3 months.

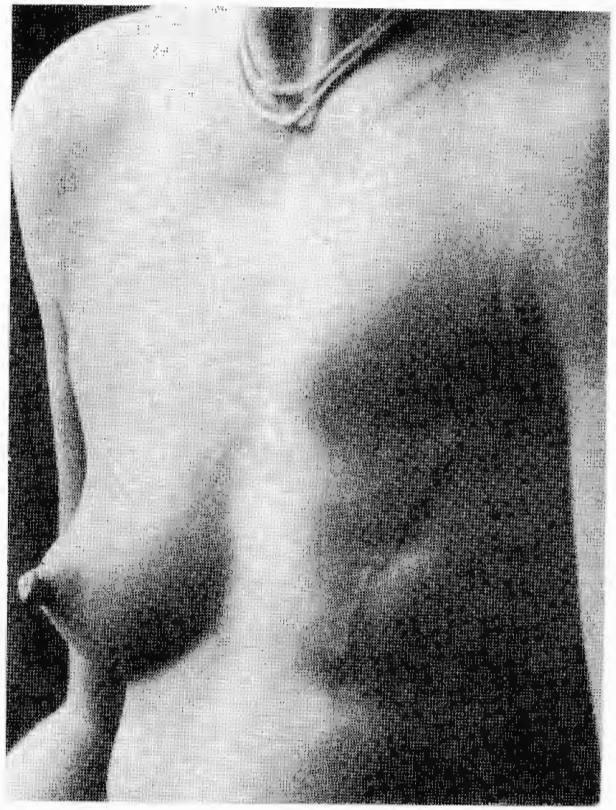


PLATE 63. Patient K. Observation No. 18. Clinical diagnosis: recurrent cancer of the left breast. Histological diagnosis: adenocarcinoma (scirrhous) with areas of solid carcinoma.

7 May 1948. At the start of a course of injections of the preparation (on the 9th day). At the site of the left breast there is an operation scar. At the level of the 4th rib, on the left parasternal line, there is a firm tumour 1.5 cm in diameter. At the level of the 3rd rib, in the region of the remains of the major pectoral muscle there is an area of thickened tissue. The photo was taken before a biopsy involving excision of the tumour at the level of the 4th rib.

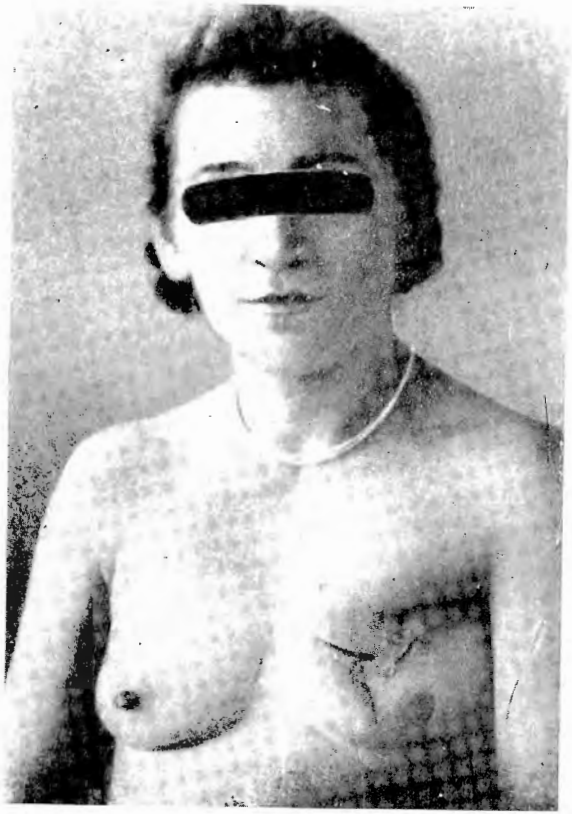


PLATE 64. Patient K., 9 July 1948. During treatment. The tumour over the 4th rib remaining after biopsy has regressed after a month of treatment (20,450 units). The outlines of the second tumour over the 3rd rib have become indistinct. The photo shows the additional scar from the biopsy carried out on 7 May 1948.

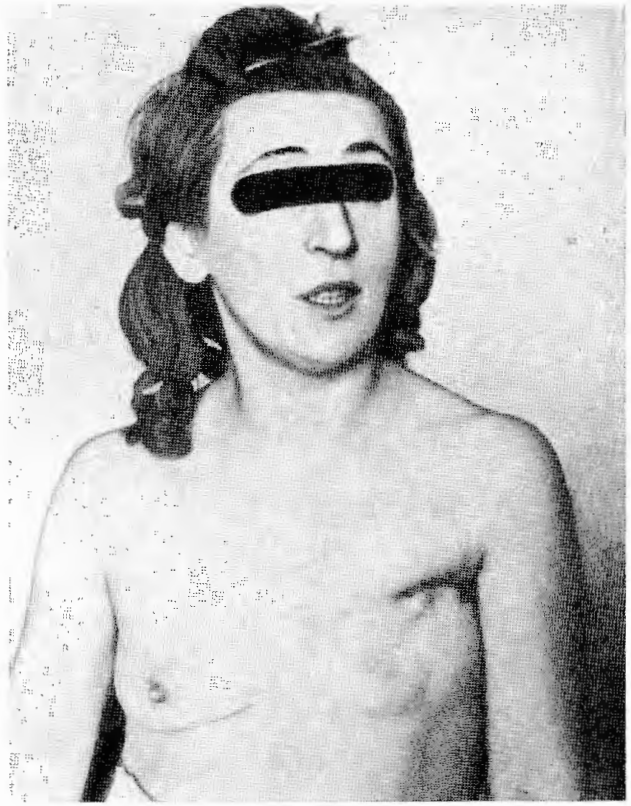


PLATE 65. Patient K., 23 May 1948. One year from the start of observations. The patient has interrupted the injections for 4 months, during which time the thickening over the 3rd rib has increased its size to 3 × 2.5 cm. The photo was taken before the start of a new course. As a result of the second course of 58 injections (114,000 units) the thickening took on a softer consistency. For the next six years the patient remained in good condition, until April 1955 when the patient left Moscow. Period of observati on,7 years.

30 May—the patient could swallow freely. She was in very satisfactory condition, and was transferred to outpatient treatment. A supplementary course was given—15 daily injections, each of 150 units of the preparation.

In August the patient began to experience subjective feelings of difficulty in swallowing, and for this reason a *second* course of intramuscular injections of the preparation was given during August and September at a dose of 500 units a day for 40 days. A radioscopical examination made at the end of this second course showed free passage of the contrast mass along the whole oesophagus, with normal outlines to the mucous membrane.

The patient remained under observation by Prof. V. M. Sviatukhin. For $2\frac{1}{2}$ months after the end of the treatment her condition was quite satisfactory. She gained 3 kg in weight. Z. returned to the teaching post which she had held before her illness.

This completed our case-history of patient Z. in 1946. After that she was under control medical observation for the whole of 1947, 1948 and 5 months of 1949. The observations established that her health remained

BLOOD ANALYSES

	29 Oct. '47	18 Nov. '47	13 Dec. '47	6 Jan. '48
Haemoglobin	67	68	69	68
Red cells	3,900,000	3,840,000	4,140,000	3,380,000
Leucocytes	7000	6600	7200	7200
Eosinophils	2	2	2	1
Juvenile cells	2	2	1	3
Segmented cells	66	67	70	68
Lymphocytes	27	26	24	23
Monocytes	4	3	3	5
E.S.R.	45	33	40	30

	3 Feb. '48	10 Feb. '48	24 Feb. '48
Haemoglobin	69	68	68
Red cells	4,170,000	3,900,000	3,880,000
Leucocytes	7700	6800	6600
Eosinophils	2	3	2
Juvenile cells	2	1	2
Segmented cells	71	69	69
Lymphocytes	23	24	23
Monocytes	2	3	4
E.S.R.	22	18	17

quite satisfactory. Regular radioscopical examinations showed free passage down the oesophagus of thick and fluid barium masses. The horizontal folds of the mucosa were well differentiated along its whole length. There was no recurrence of the feeling experienced earlier of obstruction to the passage of solid food down the oesophagus. No pathological changes were seen which could be related to Z.s previous condition.

Hence, the observations on patient Z. established the positive effect of the preparation on a condition of the oesophagus clinically interpreted as cancer and terminating in recovery. Period of observation—3 years.

OBSERVATION NO. 23

Another case-history recorded in 1946 was that of patient L., male, with a clinical diagnosis of cancer of the rectum and a histological diagnosis of an adenomatous polypus:

On 20 April 1946 blood started to appear regularly on defaecation and the patient not infrequently suffered haemorrhage without the passage of faeces. Early in May first blood, then faeces, appeared as a rule on defaecation.

Rectoscopy, carried out on four occasions—17 May, 19 May, 25 May and 28 May 1946—revealed clearly a tumour measuring 2.5×1.5 cm, with a broad base, situated 13 cm from the anus. The tumour surface was uneven, finely corrugated, with a small ulcer at its centre. Around the tumour, on the normal mucous membrane, were several small protuberances covered with normal mucosa. Conclusion of the diagnostic panel (Prof. Sviatukhin, Dr. Mironova, Prof. Mints, Dr. Baider): *cancer of the rectum.*

On 30 May 1946 injections of the "K-R" preparation were commenced.

INJECTIONS OF THE PREPARATION AND STATE OF THE TUMOUR

Date	Injection	Dose
30 May–1 June	1–3	20 units each

1 June 1946. *Rectoscopy*: findings similar to those on 28 May. Injections continued.

Date	Injection	Dose
2–3 June	4–5	30 units each
4–6 June	6–8	50 units each
7–8 June	9–10	75 units each
Total		420 units

8 June. Defaecation painless. No blood passed.

Rectoscopy. Proctoscope entered freely for 13 cm, then, having negotiated an obstruction, entered further. The ulcer at the centre of the tumour had almost healed. At its site was a bulge with radially situated scars; no attempt was made to carry out another biopsy. The tumour was damaged and slight haemorrhage occurred.

8–18 June 1946 (injections 11–19, 75 units each). A total of 1295 units had been administered.

18 June. Slight pain in the back passage on defaecation.

19–20 June 1946 (injections 20–21, 75 units each). 20 June—pain ceased.

21–25 June (injections 22–26, 75 units each). A total of 2,810 units had been administered.

25 June 1946. Rectoscopy. The proctoscope passed freely to a distance of 20–25 cm. No lesions were visible along the whole length of the mucosa. At 13–14 cm, where the tumour had previously been, there was a fresh glistening scar, covered with fresh epithelium (conclusion of diagnostic panel—Prof. Sviatukhin, Drs. Baider and Mironova).

26–29 June 1946 (injections 27–30, 75 units each). A total of 3.110 units had been injected.

29 June. *Epicrisis.* Admitted 11 May 1946 with diagnosis of haemorrhoid. Rectoscopy revealed a tumour in the rectum 13 cm from the anal opening. Histological investigation established it to be an adenomatous polypus. The patient received a course of injections of the preparation lasting a month—30 injections, 3,110 units. Repeated rectoscopy revealed a glistening scar at the site of the former tumour.

The patient's general condition remained fully satisfactory throughout the course of injections. Pain on defaecation ceased after 8 injections, as did the passage of blood and mucus. From then until the end of the course of injections the patient only once noticed slight pain on defaecation. The patient gradually gained weight during the course of biotherapy; before the injections were started his weight was 50.3 kg, after 8 injections it was 52.3 kg, after 15—53.65 kg and after 30—55.9 kg. He therefore gained 5.6 kg during the course. At the end of the injections he was discharged in good condition. As a prophylactic measure he was given 20 more injections of the preparation as an out-patient.

Further observations lasting 1 year and 3 months showed the absence of any signs of recurrence or metastases. Patient L. contracted syphilis during this period and died on 15 September 1947. On *post-mortem* examination no trace of a tumour was found in the rectum or in any other organ.

Here is the autopsy report:

Autopsy report No. 494, dated 16 September 1947. Vasilii Yegorovich L-v., aged 56 years, admitted 30 July 1947, died 15 September 1947.

Clinical diagnosis: syphilis of the brain; arteriosclerosis; cardiosclerosis; pellagroid; cancer of the rectum.

The body was of a male, undernourished but of normal physique. There were petechial haemorrhages in the skin of the left shoulder. The skin was pallid with oedema of the subcutaneous tissues of the legs and feet. The pachymeninges were tense and the sinuses contained blood clots. The brain weighed 1400 g.. The *pia mater* was thin. The vessels of the brain stem contained small atheromatous plaques. The convolutions of the brain were slightly narrow, and the brain substance was firm on palpation, with no focal lesions. The lateral and IIIrd ventricles were dilated. The ependyma of the ventricles was smooth. The lungs were spongy on palpation and grey in colour on section. The tracheal mucous membrane was pale, and the pleural cavities contained a clear fluid. The pericardial sac contained about two tablespoonsful of fluid. The heart weighed 320 g. The epicardium was covered by fat, and the myocardium was brown in colour on section. The coronary vessels showed no peculiarities. The valvular apparatus of the heart showed no lesions. The *intima* of the aorta bore atheromatous plaques. The gastric mucosa had a shagreen appearance and was markedly oedematous. *The rectal mucosa was pale, and no tumour was found.* The pancreas was thin, yellow on section and finely lobulated. The liver weighed 1050 g and was clay-coloured on section. The peritoneal cavity contained about 200 ml of a clear fluid. The kidneys weighed 120 g each. The capsule stripped freely, and the kidney surface bore cicatricial indentations. The mucosa of the bladder was normal. The spleen weighed 70 g and was rust-coloured on section. The hypophysis and thyroid gland were normal. The adrenals were thin in section and their cortices rich in lipoids.

Patho-anatomical diagnosis: emaciation; brown atrophy of the myocardium; fatty change and atrophy of the liver; hydrothorax; ascites; anasarca.

Microscopical examination of the brain: there was slight thickening of the *pia* due to fibrosis. The nerve cells were shrunken, and their cytoplasm stained deeply. Isolated nerve cells showed tigrolysis. Rarefaction of the nerve cells was seen in the cerebral cortex. There was macroglial cell proliferation in the molecular layer of the cerebral cortex. Macroglial cell proliferation was also seen subependymally, at the periphery of the *medulla oblongata,* in the region of the subcortical nuclei and the inferior olive. *Conclusion:* the histological changes in the brain were degenerative in nature; they could be associated with chronic alcoholism, starvation and avitaminosis.

5. REGRESSION OF ANGIOSARCOMA OF THE SKIN

The effects of trypanosome preparations of modifications I and II with regard to angiosarcomata have been studied by a group of workers in Kiev—Prof. Shevchenko, Prof. Shedkovaia-Rashe, Dr. Ritchenko and Dr. Kunitsa. By kind permission of the authors of these studies, we quote here a case-history describing the symptoms of the disease at the time the patient was admitted to hospital, his condition before the start of injections of the preparation, the changes taking place during the courset of injections, histological findings, and finally, the patient's condition at the end of the course of injections.

OBSERVATION NO. 24

Patient K-n., male, aged 58 years. Case-history No. 400. Admitted to the Oncology ward of Kiev Roentgeno-radio-oncological Institute on 7 June 1947. Discharged on 10 October 1947. Clinical diagnosis: *skin angiosarcoma.*

Histopathological diagnosis: *angiosarcoma* [Kaposi's disease (Xeroderma Pigmentosum)?].

Concurrent conditions: pulmonary emphysema, myocardial dystrophy, arteriosclerosis.

Biopsy—4 July and 31 July 1947.

ORIGIN AND DEVELOPMENT OF THE EXISTING PROCESS

In February 1947 a dark blood-red spot the size of a pea appeared on the skin of the middle third of the right arm. In April similar spots appeared on the right aural conchus, in May on the left aural conchus and on the skin of various parts of the body—on the legs and abdomen. The spots were painless, but sometimes gave burning and itching sensations; they did not increase in size, but became swollen, projecting above the skin surface, and new ones appeared.

The *status praesens* of the condition on 20 June 1947. On the skin of the middle third of the right arm, on its external aspect, was a spot measuring 2.5×0.8 cm; on the right aural conchus there was a spot 0.7 cm in diameter. The left cheek bore up to 14 spots measuring 0.8×0.5 cm. On the spine of the nose were 4 similar spots; the skin of both thighs bore up to 8 small spots. The *glans penis* bore 2 confluent spots measuring 2×0.7 cm. The hard palate, pharynx and gums bore several dark spots, and on the right was a swollen tumour measuring 0.4×0.7 cm. Under the right and left clavicles there were small spots measuring 0.3×0.3 cm. On the skin of the abdomen to the left of the navel was an oval spot measuring 0.8×2 cm. All the spots were a dark blood-red colour with a slate or dark brown tinge, painless, non-irritant, non-desquamating and non-exudative. They disappeared on pressure and protruded slightly above the level of the normal skin.

The patient was dwarfish in conformation and well nourished. The skin and visible mucous membranes (apart from the spots and the skin of the face) were clear and of normal colour and moistness. The regional lymphatic nodes showed no abnormalities. The musculo-skeletal system was normally and symmetrically developed. His height was 164 cm, weight 67.5 kg. Vesicular sounds with a sharp tone and marked dry rhales were heard on auscultation of the lungs. The voice was very hoarse. Heart: the sounds were sharp, with the accent of the first sound on the aorta; the left border of the heart was along the left mediastino-clavicular line. The pulse-rate was 92 beats per minute, rhythmic and soft. There was sclerosis of the peripheral vessels. The face was cyanotic and bloated. There was slight shortness of breath. The veins of both legs showed varicose dilatations.

The abdomen was rounded and somewhat protruding. The lower border of the liver could be felt on deep palpation 6 cm below the costal border, firm, smooth and painless; the liver followed the respiratory movements. Its upper border was at the level of the 5th rib. The spleen's upper border was under the cupola of the diaphragm, and the lower border could be felt 16 cm below the costal border; it was slightly painful on palpation. The various parts of the gastro-intestinal tract and the urino-genital system were without apparent pathological changes.

Treatment. On 17 June 1947 the patient started a course of the preparation given as daily intramuscular injections (into the buttocks). The dose started at 1 ml and was increased gradually by 5–10–15 ml. Sometimes for technical reasons the injections were irregular, with intervals of 2–3 days or more. The highest single dose received by the patient was 140 ml—1400 units. In all he received 67 injections—5,500 ml or 56,800 units—in 105 days.

Administration of the preparation was tolerated by the patient quite satisfactorily. Apart from a sharp pain at the injection site during administration the patient had no subjective complaints. Doses of over 50 ml were injected in two or three different parts of the body. The pulse, respiration and temperature, measured carefully during the whole observation, showed no pathological changes.

The following changes were noted in the patient's general condition: the record of his case-history from 11 July to 13 July 1947 indicates a satisfactory general condition, a decrease in the size of the liver—it protruded below the costal border by only one and a half finger-widths—and of the spleen, which extended for only 6–7 cm below the costal border. The facial swelling had disappeared and the spots on the skin of the abdomen and the spine of the nose had grown paler. The patient weighed 68 kg 400 g. By 21 July the patient had received 1500 units of the preparation.

After this the patient's condition improved, he began to look healthy, and gained weight (by the end of the treatment his weight was 70 kg 500 g). The spots gradually lost their colour and juiciness. They grew flatter, their centres becoming depressed and acquiring the appearance of normal skin. The spots which had appeared last disappeared first and most rapidly; those remaining longest were the ones round the left aural conchus, which by the end of the treatment had taken on the appearance of darkly sunburned skin.

During the treatment the patient regularly underwent urine and blood analyses, the oncotic pressure was determined (methylene blue test), and the lungs and heart were examined radiographically. There was an increase in the oncotic pressure; for example on 25 June 1947 it was 18, on 2 August it was 18, on 15 September—27 and on 15 October—24. No variations were found in the blood and urine pictures.

Date	Haemoglobin	Red cells	Leucocytes	E.S.R. mm/hr	Eosinophils
27 June	72	4,810,000	5200	4	9
15 July	74	4,830,000	5500	4	8
2 August	68	4,420,000	3800	10	4
23 August	70	4,800,000	5200	2	9
12 September	62	4,300,000	2800	5	6
27 September	66	4,500,000	6400	4	1
14 October	70	4,800,000	6200	6	4

Date	Juvenile cells	Segmented cells	Lymphocytes	Monocytes	Thrombocytes
27 June	1	72	15	3	
25 July	0	71	17	4	—
2 August	0	84	10	2	260,300
23 August	0	72	21	4	232,000
12 September	1	63	24	6	230,000
27 September	1	58	35	5	—
14 October	0	70	19	7	—

Results of histopathological examination (quoting the conclusions of the pathologist Prof. Shvedkovaia-Rashe):

(1) Skin tumour (before treatment, on 4 July 1947).

The skin tumour consisted of fantastically interwoven capillary vessels, forming nodules of various sizes, sharply demarcated from the surrounding tissues. The tumour vessels were thin-walled tubes, lined by endothelial elements outstanding in their polymorphism. As well as pale, vesiculated nuclei there were large hyperchromic nuclei with pointed ends, surrounded by acidophil cytoplasm. In some areas the angioma structure was lost, and the proliferating elements of the tumour formed bundles of closely adjacent cells with abnormal nuclei occupying the whole cell—areas of a sarcomatous nature. The tumour was situated immediately under the epithelium, and its centre was thinned down to one layer of cells. The papillary layer in this region was rich in inflammatory infiltrative elements. Granules of brown pigment lay freely in the stroma, sometimes intracellularly in the elements of the inflammatory infiltrate. Diagnosis: *angiosarcoma* [Kaposi's disease (Xeroderma Pigmentosum)?]

(2) After treatment, on 31 August 1947. The tissue separated from the epidermis by the Malpighian layer contained diffusely scattered vessels in places grouped together. Their walls were thickened, and the lumena lined by an intact endothelium. In places the vessels showed cavernous dilatations, most of them filled with debris. Accumulations of lymphoid elements and fibrous tissue proliferations rich in fibroblasts could be seen in the zone of vascular development, surrounding the separate vascular branches in the form of a cuff. Occasionally there was complete replacement of the vascular nodule by scar tissue, among which were the remains of atrophic vessels surrounded by haemosiderin granules.

Investigation of tumour foci removed after treatment revealed signs of tumour regression (of the angiomatous proliferations) and a marked mesenchymal reaction (Dr. Shvedkova).

Examination of the patient one year later on 10 October 1948. The patient was in good condition. During the past year he had not been ill or lost weight. No fresh spots had appeared and there had been no renewal of growth in the old ones. The patient was fit and able to work. The spots had completely disappeared, and could be made out with difficulty only on the left ear, where they looked like darkly sunburned skin (Dr. Kunitsa).

The patient's face before and after treatment is shown in the photographs (Plates 70, 71, 72 and 73).

The results of these examinations can thus provide answers to a number of questions:

(1) An affection of an angiosarcomatous nature underwent regression under the influence of injections of the trypanosome preparation, modifications I and II.

(2) Regression was complete, as evidenced by the absence of recurrence during further observations lasting a year, when before the injections the tumour had achieved a considerable degree of dissemination.

(3) Regression of the tumour was accompanied by changes in its histological structure.

(4) Finally, it should be stressed that the angiosarcoma on patient K. was considered a very serious condition, and that in this instance the use of the trypanosome preparation led to obvious clinical recovery.

6. REGRESSION OF PRECANCEROUS AFFECTIONS

OBSERVATION NO. 25

Patient S., aged 40 years. Clinical diagnosis: *adenofibroma* of the left breast. *Commencing cancerous process.* Histological diagnosis: *adenofibroma.*

SYMPTOMS ON ADMITTANCE TO CLINIC 24 JUNE 1947

The left breast was slightly higher than the right. On examination there was a visible swelling at its centre, over the nipple. Palpation revealed a firm tumour measuring 2.7×2.5 cm with an uneven, rough surface in the internal upper quadrant, irregularly oval in shape and painless. Its outlines were distinct except in its external upper region, where the tumour appeared to merge into the gland parenchyma. Under the nipple was a crescentic fold—the start of invagination of the nipple. König's symptom was positive. The areola was well marked above the nipple and was drawn into the fold below it. There was a milk-white secretion from the nipple (Plate 74*a* and *b*).

Two firm lymphatic nodes could be palpated in the left axilla, the first 1 cm in diameter and the second 0.5 cm.

The right breast showed no abnormalities.

On 6 August 1947 the patient underwent an exploratory *biopsy,* when half of the tumour was excised.

During the operation it was found that the tumour lying in the depth of the gland was clearly separated from the surrounding parenchyma over a large area, but in one section, in its internal upper quadrant, it was *intimately fused with the parenchyma,* as if growing into the depths of the gland.

The half of the tumour taken for histological investigation was that clearly *separated* from the surrounding parenchyma, situated on the side *opposite* to that growing into the gland parenchyma.

A histological examination carried out by Prof. V.T. Talalaev, produced the following conclusion: "adenofibroma; in some of the glandular proliferations there are signs of a precancerous character."

The same biopsy material was examined by Prof. Ya. L. Rapoport, who concluded that in patient S. only the microscopical picture of adenofibroma was shown.

The divergence between the clinical and histological diagnoses may be due to the fact that the biopsy material was taken from the least typical part of the tumour, the part *clearly* separated from the surrounding parenchyma, whereas the most typical area, as evidenced in the description of the operation given above, was on the opposite side of the tumour.

The patient started injections of modification II of the trypanosome preparation on 7 August 1947.

INJECTIONS OF THE PREPARATION AND COURSE OF THE DISEASE

The preparation was given intramuscularly in the buttock region once daily. In all, 57 injections were made, involving 96,000 units.

The doses of the various injections were as follows:

Injections	Date	Dose (units)
1	7 Aug. 1947	50
2	8 Aug. ,,	100
3	9 Aug. ,,	200
4	10 Aug. ,,	400
5	12 Aug. ,,	800
6–11	13–20 Aug. ,,	1000 each
12–20	21–30 Aug. ,,	1500–2000 each
21–57	1 Sept.–13 Oct. 1947.	2000 each

After 20 injections the infiltration round the operation scar at the biopsy site had decreased and the tumour outlines were no longer distinct.

The patient was discharged from the clinic on 30 August 1947. Injections 21–57 were given on an out-patient basis.

After 32 injections the infiltration round the operation scar had decreased further, and at the site of the former tumour there was a thickening of a consistency resembling a lobule of the gland. The left axilla contained 2 lymphatic nodes, elastic in consistency and painless.

After 44 injections the infiltration in the region of the operation scar could no longer be palpated. No tumour could be determined. The left axilla contained 2 lymphatic nodes, elastic in consistency and painless, 1 cm in diameter.

After 57 injections the tumour in the left breast could not be palpated, nor could the lymphatic nodes.

BLOOD ANALYSES

	28 July '47	2 Aug. '47	9 Aug. '47	16 Aug. '47
Haemoglobin	73	70	64	63
Red cells	4,400,000	4,200,000	3,800,000	3,700,000
Colour index	0.8	0.8	0.8	0.9
Leucocytes	7600	7000	9000	9800
E.S.R.	11	10	32	46
Eosinophils	1	1	2	1
Stabnuclear cells	3	3	5	7
Segmented cells	66	68	75	75
Lymphocytes	26	25	16	13
Monocytes	4	3	2	4

	23 Aug. '47	30 Aug. '47	6 Sept. '47	13 Sept. '47
Haemoglobin	62	64	64	67
Red cells	3,600,000	3,800,000	3,200,000	4,140,000
Colour index	0.9	0.8	0.8	0.8
Leucocytes	9600	12,000	11,000	8400
E.S.R.	51	37	39	40
Eosinophils	2	1	1	2
Stabnuclear cells	7	8	6	4
Segmented cells	69	72	75	72
Lymphocytes	18	16	15	19
Monocytes	4	3	3	3

The injections were terminated. The patient remained under observation. Clinical examinations made regularly for 1 year 7 months following the treatment, i.e. from October 1947 to August 1949 (*sic*), revealed no deviations from normal: the former tumour could not be determined and the regional lymphatic nodes were not palpatable.

	20 Sept. '47	27 Sept. '47	4 Oct. '47	2 Nov. '47	6 Dec. '47
Haemoglobin	66	67	68	69	69
Red cells	4,100,000	4,160,000	4,120,000	4,100,000	4,140,000
Colour index	0.8	0.8	0.8	0.8	0.8
Leucocytes	9000	7800	8000	9600	8600
E.S.R.	44	40	29	29	12
Eosinophils	1	2	3	1	3
Stabnuclear cells	5	3	4	5	1
Segmented cells	69	67	67	72	71
Lymphocytes	21	23	22	18	23
Monocytes	5	5	4	4	2

	19 Dec. '47	17 Jan. '48	26 Jan. '48	31 Jan. '48	12 March '48
Haemoglobin	68	69	69	69	68
Red Cells	3,880,000	3,900,000	3,800,000	3,800,000	3,680,000
Colour index	0.8	0.9	0.9	0.9	0.8
Leucocytes	7400	6800	6800	8600	8800
E.S.R.	20	12	15	8	9
Eosinophils	2	1		1	2
Stabnuclear cells	2	4	2	3	6
Segmented cells	69	69	74	71	67
Lymphocytes	24	22	21	21	22
Monocytes	3	4	3	4	3

An analysis of the observations on patient S. leads to the conclusion that *disappearance of the tumour* and similarly the *regional lymphatic nodes* took place as the result of *injections of the trypanosome preparation.*

This conclusion is based on the fact that patient S. received no other treatment apart from intramuscular injections of the preparation.

Much interest lies in the question of the nature of the tumour which underwent regression under the influence of the preparation. The histological studies force us to classify the neoplasm as benign. This is supported by its structural details as typified in the tumour fragment excised at the

biopsy. Taking it into account that only half of the tumour was taken for biopsy while the other half was left intact, we must, in accordance with the histological diagnosis, conclude that injections of the preparation led to regression of an *adenofibroma.*

This fact is in itself worthy of attention in that present-day pharmacology knows no practical means by which regression of this type of tumour can be induced. It is well known that the only *practical* way of freeing the body from an adenofibroma consists of removing it surgically.

However, having stated this we must try to analyse what sort of adenofibroma was present in patient S.

Experience has shown that 15–19 per cent of the total number of adenofibromata subsequently turn out to be malignant neoplasms. To what category did patient S.'s tumour belong—to those remaining benign indefinitely, or to the group of tumours which, having taken a benign course for a certain period, then end in malignancy?

It is, of course, impossible to give any categorical answer to this question but the clinical signs noted on examination of patient S., namely the cartilaginous consistency and roughened nature of the tumour, its uneven border, the infiltration into the gland parenchyma found on exploratory operation, the intimate adhesions between the parenchyma and one part of the tumour, and finally the presence of enlarged, firm regional lymphatic nodes—all this leads us to relate the tumour in patient S. to those which end in malignancy. The fact that histological investigation revealed a structure typical of an adenofibroma may be, as stated above, connected with the taking of a biopsy sample from the part of the tumour least suspicious in this respect.

It may be asked: what should the oncologist do, having established by clinical evidence the signs mentioned above, and having received the pathologist's report that "in some places there are signs of a precancerous character"? There can only be one answer to this question: the surgeon would be bound to undertake a radical operation for removal of the tumour-affected breast with its surrounding connective tissue and regional lymphatic nodes, especially when one considers that in patient S. they were enlarged and of firm consistency.

Without drawing any more positive conclusion, it must be stated that the use of the preparation for the treatment of a growth having the morphological structure of an adenofibroma with clinical signs of malignancy led to regression of the tumour, with simultaneous disappearance of formerly enlarged and firm regional lymphatic nodes (Plate 75).

OBSERVATION NO. 26

Patient G., aged 43 years. Clinical diagnosis: *cancer of the right breast,* stage II. Histological diagnosis: *papillary adenocarcinoma*(?)

In 1945 the patient bruised her right breast. In 1946 a clear secretion began to be discharged from the right nipple. During the same year firm nodules appeared in the breast. From June 1947 the secretion took on a blood-tinged character.

On 30 August 1947 the patient was sent to the biotherapy clinic.

SYMPTOMS OF THE CONDITION ON ADMITTANCE TO CLINIC

The right breast showed fibrous thickening. There was a blood-tinged secretion from the nipple. Palpation revealed a series of firm, round or oval nodules varying in size from 0.5 cm in diameter to 2.0×1 cm, numbering up to 24. The nodules were distributed:

(a) in the internal upper and external upper quadrants of the areola—one nodule was 1 cm in diameter, a second was 1×1.5 cm, and there were 4 nodules each 0.5 in diameter, united to form a single conglomeration, and several more, difficult to count;

(b) at the edge of the major pectoral muscle—a chain of nodules measuring 2×1 cm, 0.3×0.3 cm, 0.5×0.5 cm;

(c) along the midline from the clavicle to the nipple—a nodule measuring 1.5×1 cm.

On 15 September a *biopsy* was carried out: excision of one of the nodules, measuring 0.75×0.5 cm. Histopathological diagnosis by Prof. Rapoport: *papillary adenocarcinoma* (Plate 76).

The patient started to receive injections of the preparation on 5 September 1947.

INJECTIONS OF THE PREPARATION AND COURSE OF THE DISEASE

The injections were given intramuscularly in the buttock region once daily, using the *second* modification of the preparation. The doses were as follows:

1 — 500 units	— 5 September 1947
2–17 — 800 units each	— 6–26 Sept. 1947
18–32 — 1000–2000 units each	— 27 Sept.–14 Oct. 1947
33–155 — 1000–2500 units each	— 15 Oct. 1947–7 Feb. 1948.

After 32 injections a cyst 1 cm in diameter formed in the internal upper quadrant of the areola, and pressure on it produced a blood-tinged fluid secretion from the nipple. A diffuse area resembling fibro-cystic mastopathy, sensitive on palpation, became palpatable at the site of one of the nodules. At the border of the major pectoral muscle, also in an area where there had formerly been a clearly determinable group of firm nodules, an area of gland parenchyma formed, 2×1.5 cm in size, without distinct boundaries, diffuse and somewhat lobulated.

After 65 injections the cystic growth in the internal upper quadrant of the areola had decreased to 0.75 cm in diameter. In the region of the major pectoral muscle, as well as the cyst which had formed there earlier, there appeared a firm nodule 0.5 cm in diameter.

The lymphatic nodes measuring 1.5×1 and 0.75×0.75 cm determined earlier in the right axillary region had disappeared.

After 101 injections, on 16 January, the patient underwent an operation for *partial excision of the parenchyma of the right breast,* involving an area of 5×5 cm, together with the cystic growth.

A histological investigation by Prof. Rapoport produced the following conclusion: "the biopsy material reveals a picture of cystic mastopathy with sclerosis and slight round-cell infiltration of the connective tissue round the glandular ducts. In places the glandular lumena contain villous proliferations with a richly hyalinized stroma and marked injection of the blood vessels. This proliferation is in the nature of a papillary adenoma without obvious signs of malignancy. Diagnosis: *papillary adenoma with a background of fibrous mastopathy*" (Plate 77).

On 8 February the injections were stopped because of an intercurrent infectious disease. A total of 157,300 units had been injected since the start of the course.

A clinical examination on 22 March 1948 showed the absence of any pathological changes in the right breast. The secretion from the nipple had ceased. Three months later a number of firm, round and oval nodules measuring from 0.3 to 1.5 cm in diameter became palpatable in the internal upper quadrant of the right breast, at the edge of the major pectoral muscle.

A second course of injections of the preparation was started on 29 June. On 10 August, after 34 injections (98,500 units), the patient underwent a radical operation for removal of the right breast, together with the connective tissue of the subclavicular hollow and the axillary space, and the major and minor pectoral muscles and their aponeuroses.

Histological investigation by Prof. Rapoport revealed the following: "the material obtained by the breast operation was oval in form, measuring 16×8 cm. In the central part, near the nipple, the glandular tissue was penetrated by firm cicatricial cords in which were found areas of a honeycomb structure with colloid-like contents. On pressure a thick

mass was expressed from the markedly dilated tubules penetrating the fibrous tissue.

Microscopical examination showed a mixed picture of fibroadenoma and fibro-cystic mastopathy with massive development of hyalinized cicatricial tissue. A considerable part of the gland showed atrophy of the glandular tissue, with replacement by fresh fibrous tissue, rich in cells" (Platas 78*a* and *b*).

An examination on 20 August 1949 showed the absence of signs of recurrence. Hence, during the process of the injections there had been in patient G. a gradual disappearance of 24 nodular growths. The breast had assumed a normal character.

Three months after the first course of injections, in the interval between two courses, new nodular growths appeared, and for this reason the patient underwent a radical operation. A histological study, made, as previously, by Prof. Rapoport, this time revealed a picture of fibroadenoma and fibrous mastopathy. Prof. Rapoport then re-examined his first preparation and as a result changed his original diagnosis of "papillary adenocarcinoma" to one of "papillary adenoma".

Thus, if we consider the original diagnosis, injections of the trypanosome preparation produced in patient G. regression of a neoplasm which had been clinically and histologically diagnosed as malignant. If we accept the second diagnosis, then there was disappearance of a tumour with clinical signs of malignancy and the structure of a papillary adenoma. Even in this case the disappearance of the tumour under the influence of the biological preparation is of considerable interest, since this type of tumour could be interpreted as a precancerous state. If we refer to clinical experience, here is what we find on this subject in the works of L. M. Ratner: "...Papillomata of the breast are potentially just as malignant as those of other organs—the alimentary tract, urinary bladder, ovaries, etc." "Numerous observations confirm the possibility of transition of mammary papilloma to carcinoma. Many cases have been described where a carcinoma has developed after the persistent discharge of a secretion from the breast".

"...On histological investigation of papillomata or polycystic mastopathies excised in connection with a secretion from the nipple we frequently come across the picture now definitely recognized as the morphological expression of a precancerous state. We refer here to cellular polymorphism, unevenly staining nuclei, the appearance of abnormal forms of nuclear division, epithelial stratification, epithelial proliferations and infiltrations, abnormal villous outgrowths, and loss of the basement membrane".

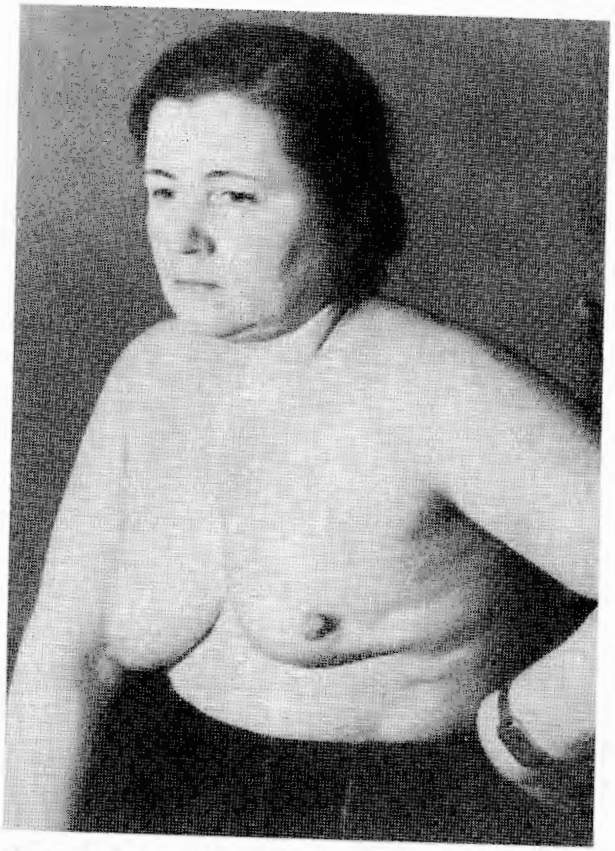


PLATE 66. Patient L., Observation No. 19. Clinical diagnosis: cancer of the left breast, stage II. Histological diagnosis: carcinoma of the breast. 23 February 1949. The photo was taken 2 years 6 months after the start of treatment. There are no signs of the malignant affection. This state was maintained for the next 7 years. Before treatment the left breast contained a pitted tumour measuring 8.0×6.0 cm, and in the axilla there were four firm lymphatic nodes—metastases. The patient underwent the following treatment:
(1) she received a course of intramuscular injections of the preparation from 21 August to 10 October 1946;
(2) after this, she underwent a nonradical operation in January 1947;
(3) she received repeated courses of injections in 1947—from 21 May, 42 injections, and in 1948—from 12 February, 27 injections; further observations from 1949 to 1956 were made at the patient's home-town, at the L'vov Oncological Dispensary, from where in February 1956 we received a note that she was in good health, with no signs of the malignant affection. Period of observations, 9 years 6 months.

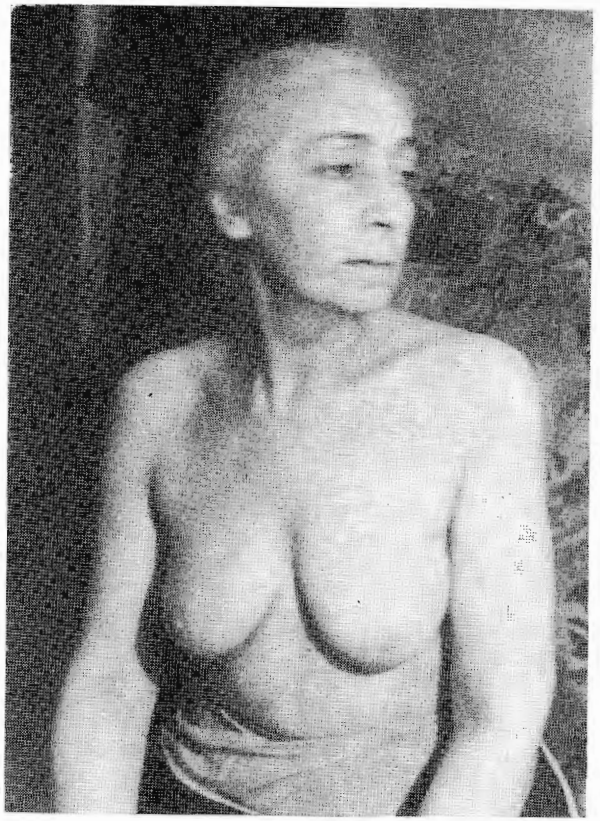


PLATE 67. Patient S., Observation No. 20. Clinical diagnosis: cancer of the left breast, stage I. Histological diagnosis: tuberous form of colloid carcinoma. 20 April 1951. 2 years 7 months from the start of treatment. There are no signs of the malignant affection. The same condition was found on examination in February 1955. Before treatment the left breast contained a pitted tumour measuring 2·5×2·0 cm. The patient received the following treatment: (1) three courses of injections of the preparation, 254 injections in all; (2) 7 days after the start of the first course of injections she underwent a nonradical operation—excision of the tumour to the limit of the visibly affected tissue. Period of observation, 6 years and 9 months.

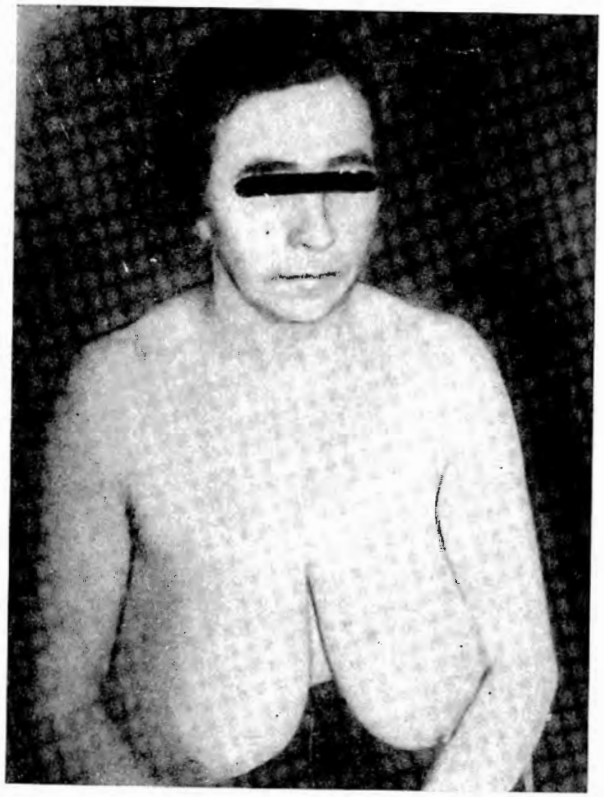


PLATE 68 *a*. Patient L., Observation No. 21. Clinical diagnosis: cancer of the breast, stage II. Histological diagnosis: solid carcinoma. Before the start of injections of the preparation. The right breast contains a tumour measuring 2·5×1·5 cm (see photos 68 *b, c)*. In the right axilla there is a lymphatic node, 1.5×1 cm (28 February 1949).

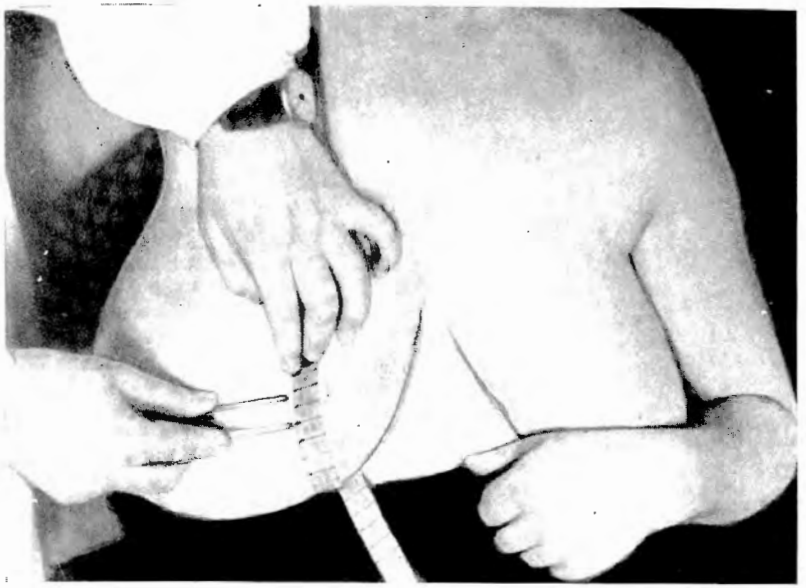


PLATE 68 *b*

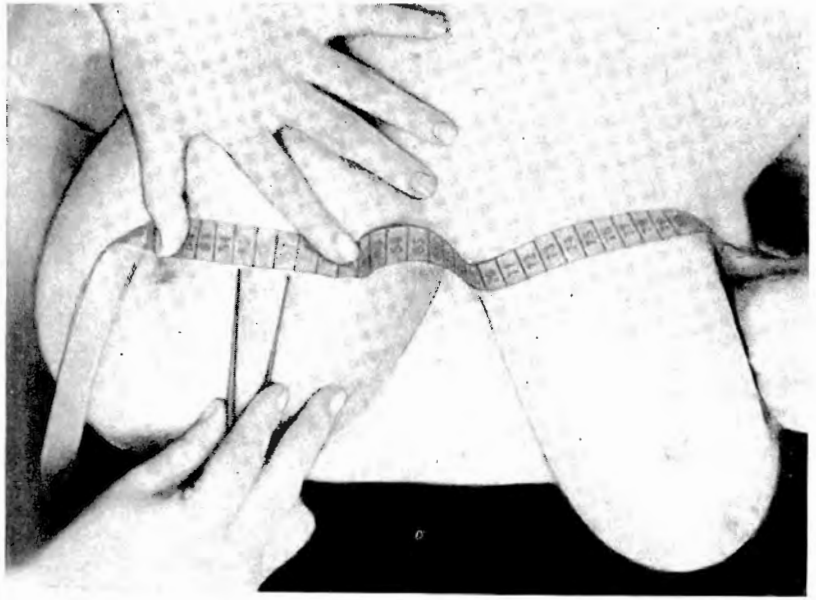
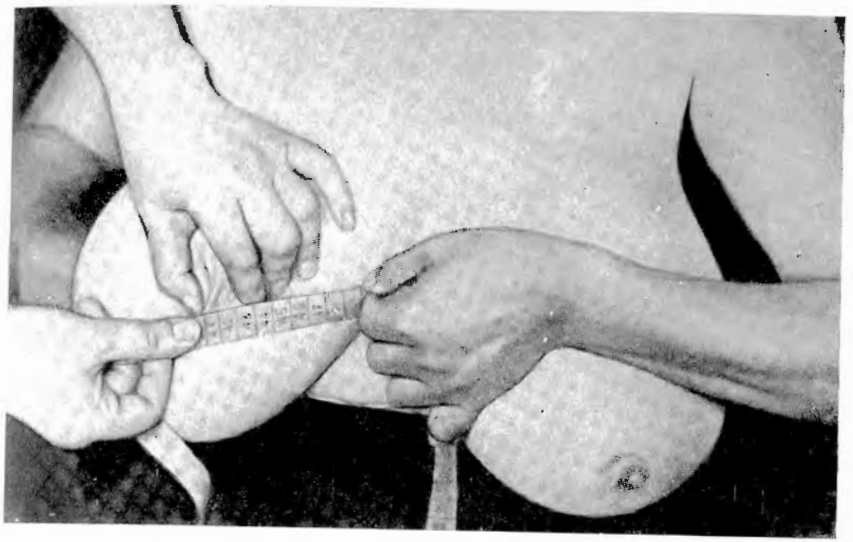


PLATE 68 *c*



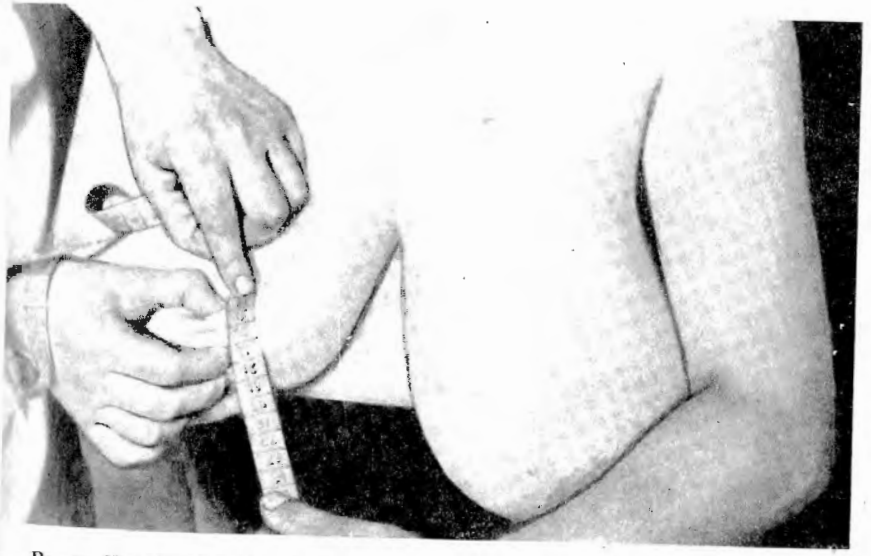


PLATE 69, *a,b*. Patient L., 8 July 1949. $4\frac{1}{2}$ months after the start of injections of the preparation. The photo shows traces of the diagnostic biopsy and also the size of the thickening in the breast. It was taken before control excision of the affected area: a histological examination on 14 July showed moderate fibrocystic mastopathy. There were no signs of a malignant tumour. The breast was preserved.

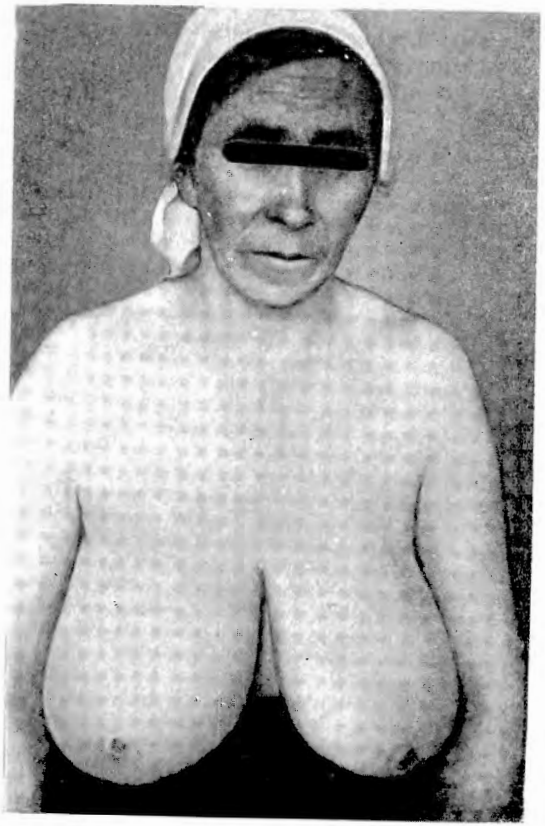


PLATE 69, *c*. Patient L., Photo taken on 8 July 1949, before control histological examination showed the absence of malignant elements. For the next 5 years and 8 months the patient's condition remained unchanged. The last examination was in February 1955: there were no signs of recurrence or metastases. Period of observation, 6 years.

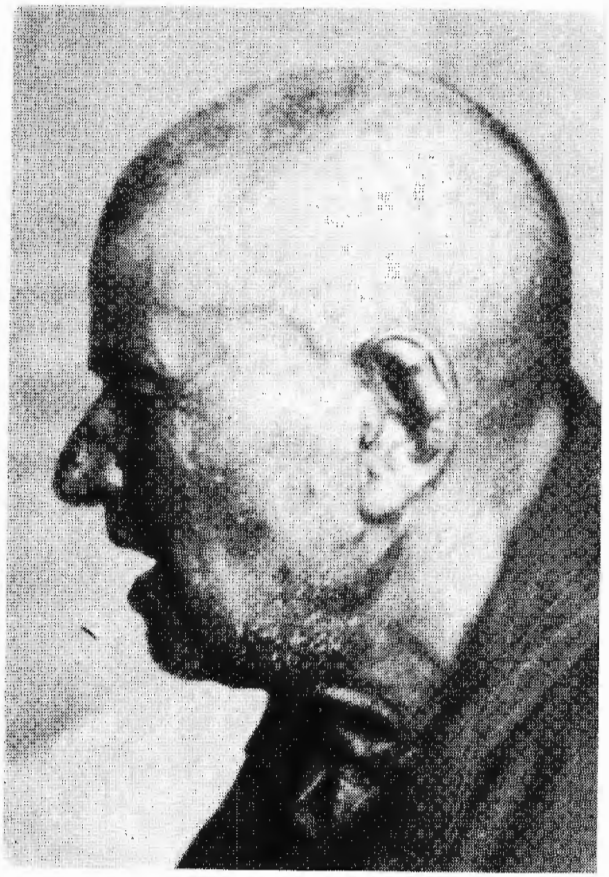


PLATE 70. Patient K., Observation No. 24. Clinical diagnosis: skin angiosarcoma. Histological diagnosis: angiosarcoma [Kaposi's disease (Xeroderma Pigmentum)?]. Left side of the face before the start of injections of the preparation. On the cheek are up to 14 spots measuring up to 0.5–0.8 cm, with 4 similar spots on the ridge of the nose.

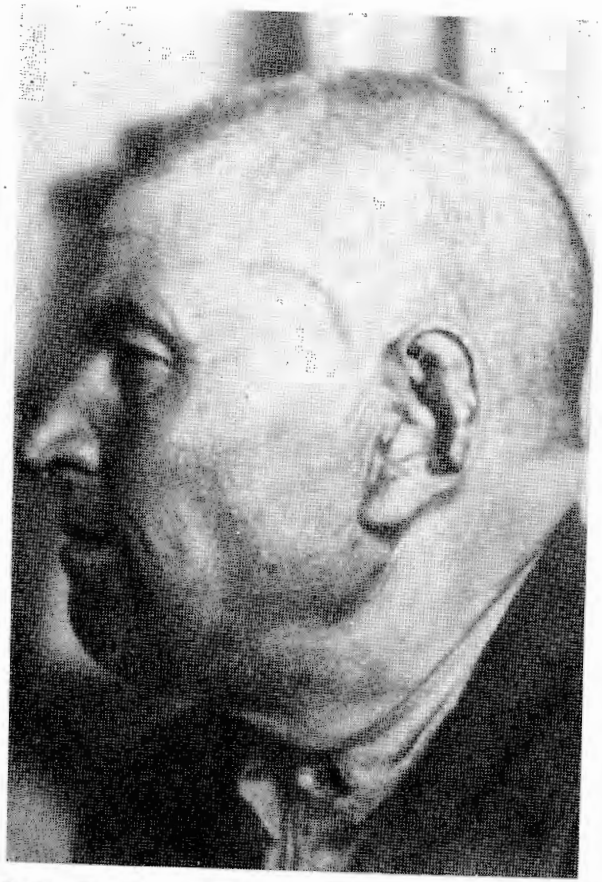


PLATE 71. Patient K., The same side of the face after injections of the preparation. The angiosarcomatous proliferations have disappeared. Around the aural conchus their traces resemble a dark sunburn. The patient received 67 injections in 105 days. One year after the end of treatment the patient wa recorded as clinically recovered.

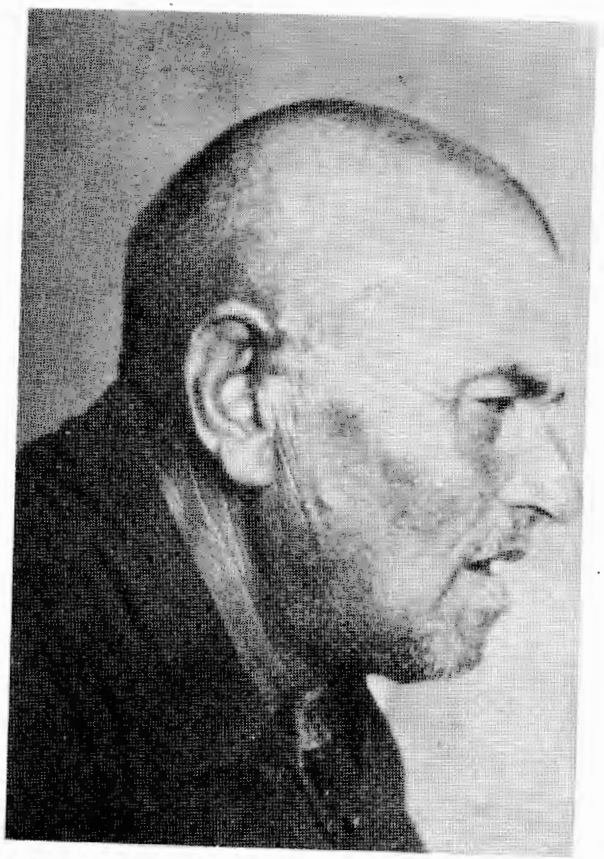
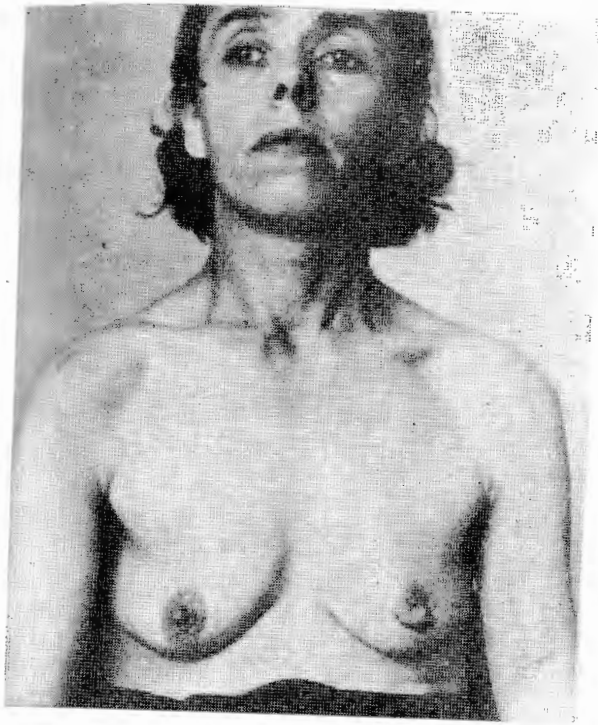


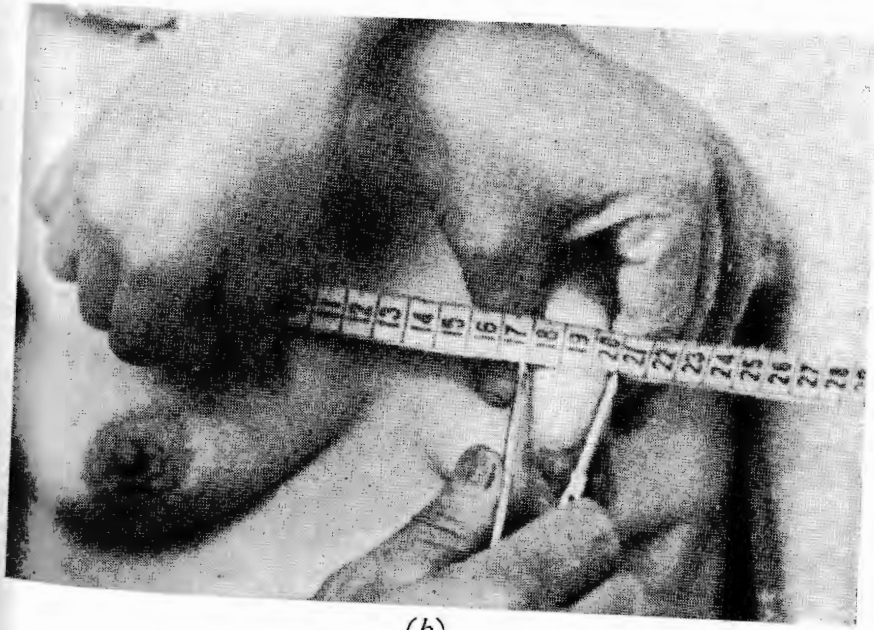
PLATE 72. Patient K., Right side of the face before the start of injections of the preparation. The aural conchus bears an angiomatou s growth 0.7 cm in diameter. The skin of the cheek and nose is also affected.

PLATE 73. Patient K., The same side of the face after injections of the preparation. The angiomatous proliferations have regressed. The patient received 67 injections of the preparation in 105 days. One year after the end of treatment the patient was recorded as clinically recovered.



(*a*)

PLATE 74, *a*, *b*. Patient S., Observation No. 25. Clinical diagnosis: adenofibroma of the left breast, the start of a cancerous process. Histological diagnosis: adenofibroma. 1 August 1947. In the internal upper quadrant of the left breast is a firm tumour measuring 2.7×2.5 cm, with a pitted surface. The external upper portion of the tumour appears to merge into the gland parenchyma. Under the nipple is a crescentic fold—the start of invagination of the nipple. The lower part of the areola is drawn into the fold. There is a milk-white secretion from the nipple. In the left axilla are two firm lymphatic nodes 1 cm and 0.5 cm in diameter.



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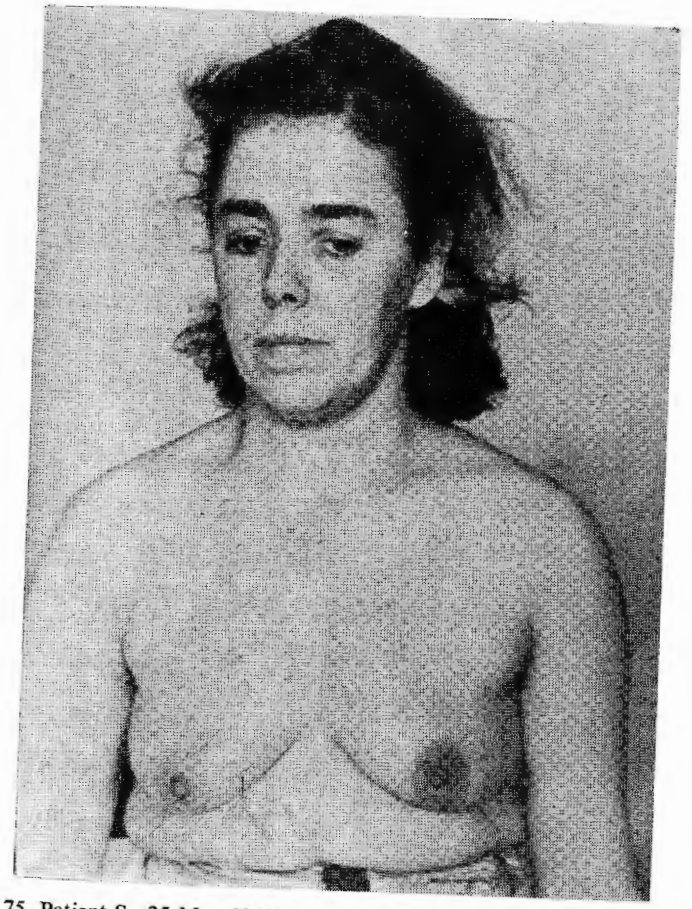


PLATE 75. Patient S., 25 May 1949. 2 years and 2 months from the start of the observation and 1 year and 7 months from the end of injections of the preparation (96,000 units). After this course the tumour could no longer be palpated, nor could the lymphatic nodes.

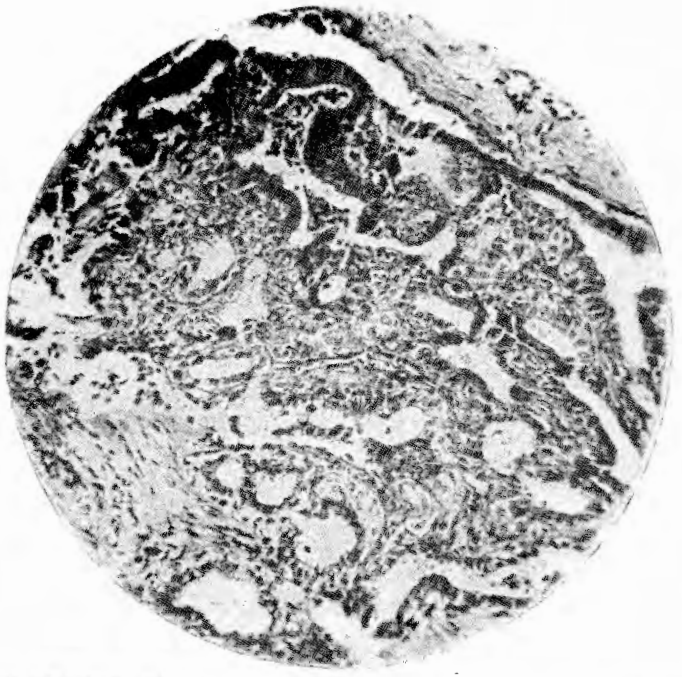


PLATE 76. Patient G., Observation No. 26. Clinical diagnosis: cancer of the right breast, stage II. Histological diagnosis: papillary adenocarcinoma, revised to papillary adenoma.

15 September 1947. Histological structure of one of the 24 nodules occupying the breast before the start of a course of injections of the preparation. In the coarse connective tissue are cavities of various sizes and shapes, occupied by extremely polymorphic papillary outgrowths covered with a cylindrical epithelium. Diagnosis: papillary adenocarcinoma, changed to papillary adenoma after a second examination of the same preparations.

The patient received 155 injections of the preparation over a period of 5 months (157,300 units). The 24 nodules which had grown in the breast during the last eighteen months underwent regression and disappeared during the course of the treatment, as did two firm lymphatic nodes in the right axilla.

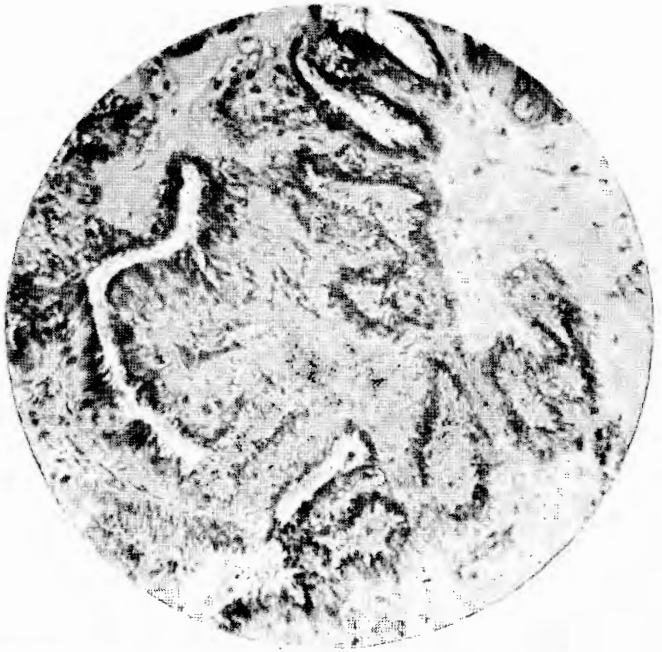
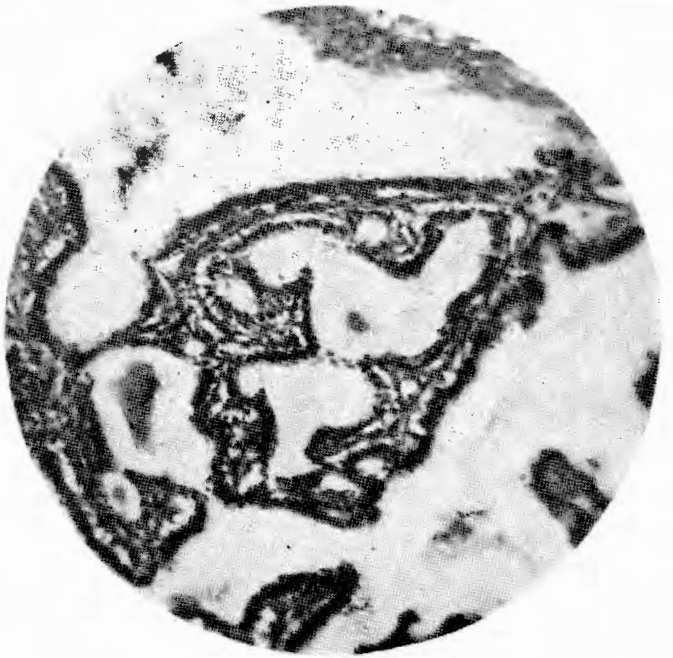
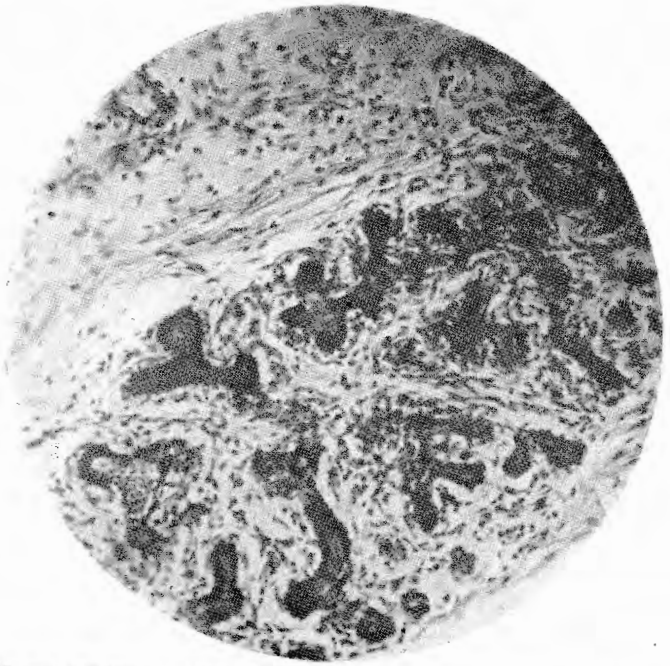


PLATE 77. Patient G., 16 January 1948.[1] Histological structure of the tumour during treatment (after 101 injections). A picture of cystic mastopathy with sclerosis and moderate round-cell infiltration of the connective tissue around the glandular ducts. In places the glandular lumena contain papillary outgrowths with a rich, hyalinized stroma and marked injection of the blood vessels: a papillary adenoma with a background of fibrous mastopathy.



(*a*)



(*b*)

PLATE 78, *a*, *b*. Patient G., Histological structure of a thickening appearing secondarily three months after the end of the first course of injections. A picture of mixed fibro-adenoma and fibrocystic mastopathy with prolific development of hyalinized cicatricial tissue. A considerable proportion of the glandular units shows atrophy of the glandular tissue, with replacement by fresh, highly cellular fibrous tissue (10 August 1948).

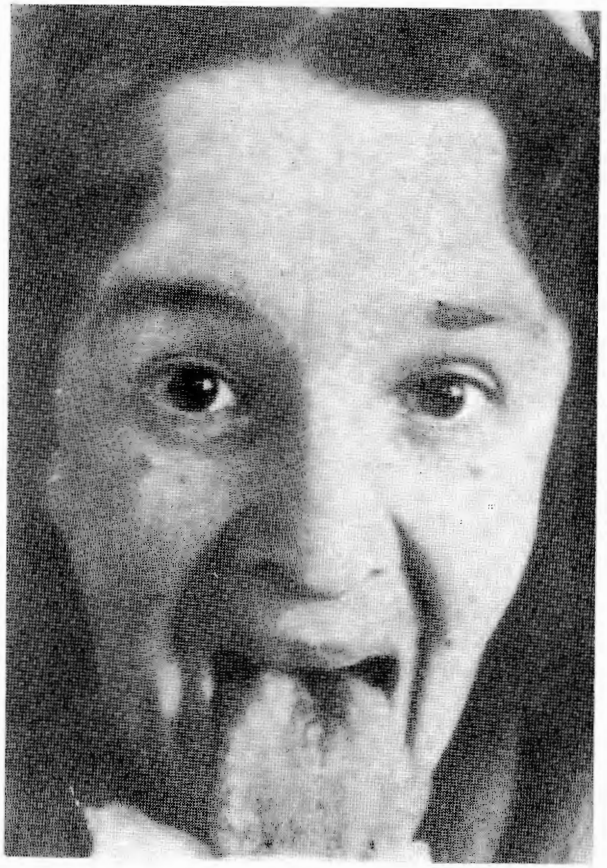


PLATE 79. Patient O., Observation No. 27. Clinical diagnosis: leucokeratosis of the tongue. Histological diagnosis: parakeratosis. Before the start of injections of the preparation. On the upper surface of the tongue along the midline is an affected area measuring 1.5 cm, irregularly oval in form, white in colour, roughened, sunken by 0.1–0.2 cm and divided from the normal mucosa by a red demarcation line 0.1 cm across.

"...While rejecting ultraradical procedures when there is haemorrhage and discharge from the breast, we also condemn the opposite, ultraconservative tactics of watching and waiting. Such tactics contradict our aims of early demonstration and rapid elimination of precarcinomatoses. In the breast, just as in the bladder or stomach, it is not always possible to decide whether one is dealing with a papilloma or in fact with a papillary carcinoma."

Our concrete analysis of patient G.'s condition does not therefore enable us to classify it, either by its clinical appearance or by its morphological signs, as a benign tumour. At best, it must be related to the precancerous, rapidly growing type of tumour.

* * *

On the subject of the regression of precancerous affections of the oral cavity, we should deal here with *leucoplakia* of the tongue. We must say that among our not very numerous observations we saw an extremely high sensitivity to the trypanosome preparation in some patients, while in others any reaction to its injection was weak or absent.

OBSERVATION NO. 27

Patient O., female, aged 44 years. Clinical diagnosis: *leucokeratosis of the tongue*. Histological diagnosis: *parakeratosis*.

In January 1947 the patient examined her throat because of a tonsillitis and found on her tongue a white area, about 1 cm in diameter. After treating it for a month with ointments the spot disappeared.

Three months later the patient again found an affected portion of similar nature in the same place. Treatment with ointments and potassium permanganate for 9 months had no effect. For this reason on 4 March the patient was sent to the biotherapy clinic.

SYMPTOMS OF THE DISEASE ON ADMITTANCE, 4 MARCH 1948

On the superior surface of the tongue in the mid-line there was an affected area 1.5 cm long, 0.5 cm wide in its upper third and 1 cm wide in its lower two thirds, irregularly oval in shape, white in colour, rough to the touch, sunken by 0.1–0.2 cm and divided from the normal mucosa by a red demarcation line 0.1 cm wide. No lymphatic nodes could be palpated (Plate 79).

On 11 March a biopsy was carried out, with excision of half of the affected area.

Histological examination revealed abnormal proliferation of the surface epithelium, with thickening and signs of parakeratosis.

On 12 March the patient started a course of injections of the preparation.

INJECTIONS OF THE PREPARATION AND COURSE OF THE DISEASE

The injections were given intramuscularly, once daily, using modification II of the preparation. The doses were as follows:

Injection	Date	Dose
1–5	12–20 March	200–600 units
6–10	22–26 March	600–1000 units
11–20	27 March–12 April	1000–2000 units

After 20 injections, involving 16,800 units of the preparation, the leucoplakic affection disappeared. An area covered by normal epithelium formed in its place.

Subsequent observation for 14 months showed the patient's condition to be satisfactory.

After 14 months an area of leucoplakia again appeared on the surface of the tongue. A second course of injections once more led to its disappearance, after which the patient was considered cured.

Thus, a leucoplakia of the tongue in patient O. which had been of a prolonged and persistent nature, not responding to the therapeutic measures normally adopted, disappeared under the influence of injections of the trypanosome preparation (Plate 80).

* * *

We have thus seen that, as with typical malignant neoplasms, the biological preparation is able to bring about a process of regression in various conditions of a nature now recognized as precancerous.

It may be that such a qualification of the pathological process will only exist until we find the true cause of cancer. The term "precancerous state" is, at the present level of our knowledge, no more than a sad prediction that such a patient may subsequently be transferred to the category of those affected by a malignant process.

Analysis of the clinical observations made during the process of biotherapy of these conditions provides a certain amount of material for an opinion regarding the question of the pathogenesis both of these conditions and of the carcinomatous process. The question arises: is not such

a state really an *early stage in a carcinomatous process*? The basis for posing such a question lies in the following facts:

(1) These affections undergo *the same process of regression* under the influence of the same microbial factors that cause regression of cancerous tumours.

(2) The mechanism of regression of a "precancerous" affection is the same as that of the destruction of a cancerous affection.

Histological investigation of a carcinoma at various stages in its regression caused by the microbial factor shows that during the regression process there may and do develop states histologically *indistinguishable* from those classified as "*precancerous*". The impression is gained that the malignant process passes in the course of its formation through a whole series of pathological states, both in its early development and during its extinction. Neither the early stages nor the stages of regression contain elements directly indicative of its malignant nature. Are these elements only found when the condition has *passed* the stage of early development and entered the next stage of morphologically apparent disease? And here is the most important question in the whole problem of the active treatment of cancer. Is not the "precancerous" condition that stage of cancer in which a therapeutic influence is most effective? *Is it in this stage that cancer is most accessible to biotherapeutic influences*?

These questions do not derive from any failure of the use of biotherapy in later stages of the cancerous process. Not at all. Active treatment, as we have seen, is also effective against developed cancerous conditions and against the far advanced process. But the question of the *timeliness* of a treatment is a rightful requirement for its effectiveness. The observations described form an objective basis to this formulation of the question, since they show that not all, but only *some* cases of adenofibroma and *some* cases of leucoplakia are sensitive to the trypanosome preparation. Consequently, the category of the so-called "precancerous" conditions is not uniform, even within a group of morphologically similar affections.

These observations also lead us to consider the pathogenesis of a malignant affection from the aspect of its connection with the "precancerous" state. This state, more than all others, indicates convincingly that the human body wages an *active battle* against the factors of malignant growth, and a cancerous condition, having lost this battle, undergoes no further development. Is this not why *only some* precancerous affections subsequently give a picture of cancer? If this position is adopted, then the effectiveness of cancer treatment is considerably increased. And this is not cancer *prophylaxis* but cancer *therapy*, and moreover *timely* therapy.

7. TEMPORARY REGRESSION AND THE PROBLEM OF REFRACTORINESS

As well as observations where the cancerolytic reaction produced by the trypanosome preparation led to persistent regression of the tumour, with a prolonged clinical effect, in a number of cases only a temporary effect was seen, after which a refractory phase ensued. Administration of the preparation no longer induced any cancerolytic reaction, the condition resumed its former course, the affection progressed and the patient passed into the category of those insusceptible to biotherapeutic treatment. In some cases, on injection of the trypanosome preparation there was no cancerolytic reaction right from the start of the course, although the affection in its histogenesis, localization and other signs appeared identical with those which reacted to the trypanosome preparation in the regular manner—by regression. However, such cases were relatively rare.

In this chapter we describe various expressions of the refractory reaction in a group of patients whose malignant affections were in the category susceptible to the trypanosome factor.

OBSERVATION NO. 28

Patient P-o., aged 52 years. Clinical diagnosis: *recurrence of carcinoma* after a radical operation on the left breast. Histological diagnosis: "*recurrence of carcinomatous condition*" (I. V. Davydovskii), "*tubular gland form of carcinoma*" (Prof. Talalaev).

In June 1945, at the Institute of Oncology, patient P. underwent amputation of the left breast because of a carcinoma. A prophylactic course of X-ray therapy had been carried out from February. In December 1945 a small intradermal nodule was noted 4 cm from the operation scar. On the advice of the doctor in charge 7 seances of X-ray therapy were given by Malet's method. After the seventh seance a large number of firm intradermal nodules appeared in the region of the operation scar and at some distance from it. The patient received another 13 seances of X-ray therapy and treatment was then stopped.

The patient was admitted to hospital on 25 June 1946 in an extremely serious condition because of cancer metastases.

Because of the character of the malignant affection, both surgical treatment and radiation therapy, having served their purpose, were discarded: a second operation was now impracticable and radiation therapy led to the formation of extensive lesions and the appearance of fresh cancer nodules. The skin of the chest at the site of the previously removed breast

and in the neighbouring areas was scattered with a large number of cancer nodules, forty seven of which could be counted and the rest could not. The affected skin was completely adherent to the underlying tissues, ulcerated in the scar region, oedematous and blue-red in colour. The firm regional lymphatic node was hardly movable. The patient was quite incapable of work, was confined to bed and was admitted to hospital in this state.

Patient P. remained under observation for $2\frac{1}{2}$ years after her admittance, during which time she was treated with the trypanosome preparation.

FIRST SERIES OF INJECTIONS AND COURSE OF THE DISEASE

The first course of injections lasted for 6 months, from 25 June to 25 December 1946. The preparation was injected intramuscularly, once daily. During the first month 12 injections were given (1800 units), and during the next 5 months 100 injections (40,400 units). Consequently, P. received the injections with only relative regularity: during this first six-month course she went untreated on 68 days.

The following signs were noted during the process of the injections: (1). Some of the firm cancer nodules disappeared, the rest decreased in size and grew flatter and softer. These signs of regression were confirmed histologically (see page 204). (2). Periodically, after the injection of certain batches of the preparation, there was a rise in temperature to 38–39–40°C, accompanied by shivering, headaches, a feeling of general malaise and marked weakness. (3). A few hours after injections an intense irritation developed in the region of the operation scar and the cancer nodules, which became swollen and flushed. (4). The patient's weight rose by $4\frac{1}{2}$ kg. Blood analysis: E.S.R.—31 mm/hr at the start of out-patient treatment, 21 mm/hr on 25 December 1946. Haemoglobin: 28 August—55 per cent, 25 December—60 per cent; red cells: 28 August—3,290,000, 25 December—4,660,000.

The patient's treatment was interrupted from 25 December 1946 to 31 January 1947. During this interval her condition deteriorated: several fresh nodules appeared, the malignant nature of which was confirmed histologically. The patient started a second course of injections of the preparation.

SECOND SERIES OF INJECTIONS AND COURSE OF THE DISEASE

The second course was started on 31 January 1947, and the doses were increased from 1000 to 3000 units. Injections were given daily except on holidays.

As before, after the injections the patient not infrequently suffered from a rise in temperature to 37.5–38–39°C, sometimes with shivering. After several injections irritation, a burning sensation and tenderness developed in the scar region.

Two months after the start of the second course some of the nodules had grown flatter, some had disappeared, some had decreased markedly in size and had grown softer. At the same time fresh nodules appeared, later disappearing and then reappearing.

From 10 April the dose was increased to 2000–4000 units per injection. Four months after the start of the second course, when the patient had received 131 injections involving 196,860 units, an examination on 4 June 1947 showed that *over the whole area lying above the level of the 4th rib the skin was soft and mobile. All the nodules previously present had disappeared completely.* A nodule situated on the anterior axillary line could now be palpated only with difficulty. Three lower nodules were *unchanged* and a fourth had grown flatter. No lymphatic nodes were palpated in the supraclavicular and axillary regions.

The patient received 3000 units daily for the next 7 days. After 8 days, on 12 June, examination revealed further flattening and diminution of the nodules.

Five months after the start of the second course, when 155 injections had been given, involving 245,800 units, an examination on 3 July showed: *no nodules could be determined over the whole area of the left half of the chest and above.* At the sites of the former nodules careful examination showed a hardly visible pigmentation. *Three lower nodules* were *without noticeable change.* No lymphatic nodes could be palpated. Radioscopy of the thorax showed the absence of any pathological elements in the lungs and heart. The patient gained 3 kg in weight.

During July and August the patient received 2000 units daily except on holidays. Her condition remained unchanged.

On 14 August 1947 it was reported: "*over the whole area above the 4th rib the skin is mobile and soft. The nodule along the axillary line is unchanged.* No lymphatic nodus can be palpated."

A month later, on 10 September, examination revealed a deterioration in the patient's condition—fresh nodules had formed along the thoracic and left axillary line. After another 20 days new groups of tumour nodules, each 0.1–0.3 cm in diameter, appeared in the scar region. By this time (29 September 1947) the patient had received 223 injections—375,600 units—since the start of the second course.

From this time onwards the disease became progressive. Fresh eruptions of nodules started to appear.

This deterioration in the disease started during the process of injections given, as before, intramuscularly, daily, with some breaks, but none more prolonged than before. Thus in August 7 days were missed, in September 6, in October 5 and in November 8. Further injections remained without result. The patient lived for another year. Her condition gradually grew worse. Lung metastases formed. P. died in December 1948, $2\frac{1}{2}$ years after treatment with the trypanosome preparation was started.

The observations on patient P. established the following:

(1) Regression of the malignant neoplasm brought about by injections of the trypanosome preparation was of a *temporary* character. Nevertheless, 43 out of 48 nodules disappeared. The postoperational scar and the tissue surrounding it, formerly firm and adherent, became soft and mobile over a considerable area. Histological study of the regressive foci confirmed the clinical picture of regression (see Part V).

The regression occurred when the patient was in a hopeless, incurable state, and was relatively prolonged. It lasted for 14 months and was so considerable that the patient could in fact return to her normal life—she was discharged from the clinic. Her fitness was fully restored. She gained 4 kg in weight, and her haemoglobin level rose to 70 per cent instead of the previous 55 per cent. Clinical and radioscopical examinations failed to reveal any affection of the internal organs.

(2) The process of regression of the cancerous tumour in patient P. was incontrovertibly caused by administration of the trypanosome preparation. This was shown by the fact that the period of the first course of injections was associated with regression, the interval in the injections with the growth of fresh cancer nodules and the period of the second course of injections with renewed regression.

The whole process of tumour regression seen in patient P. provides full justification for relating her malignant affection to the category of those susceptible to the trypanosome antineoplastic factor. And if this is so, then the cessation of regression during the course of the injections and the absence of any results from further use of the preparation force us to conclude that this state of susceptibility was replaced by a state of refractoriness. The reasons underlying such a change remain obscure. The question involves the significance of the quality of the preparation, the reaction of the tumour cells and the state of the body. Before discussing these questions we shall describe some more observations where a refractory reaction has arisen.

OBSERVATIONS NOS. 29 AND 30

Patient S., aged 51 years. Clinical diagnosis: *cancer of the right breast*, stage II. Histological diagnosis: *metastasis of an adenocarcinoma* (a lymphatic node from the axilla was taken for biopsy).

In the spring of 1946 the patient discovered by chance a thickening in the right breast the size of a lentil, mobile and painless. During the next two months the tumour grew to about twice this size. After another two months, in June 1946, the patient found a similar tumour in the right axilla. For this reason she approached the Central Institute of Oncology, where a *carcinoma* was diagnosed and an operation suggested which she refused.

On admittance to our clinic on 1 August 1946 for treatment with the trypanosome preparation, an examination revealed: on raising the right arm to the horizontal position, a deep-seated pull on the skin shown as a longitudinally situated fold was clearly visible in the region of the external lower quadrant of the right breast. Palpation in this region revealed a tumour measuring 2×2 cm, with a rough surface, firm, mobile in relation to the skin except for the area producing the tension on raising the arm.

The right axilla contained two firm lymphatic nodes. One of them was excised for histological study. Conclusion: "metastasis of an adenocarcinoma".

Injections of the preparation were given from 7 August 1946. During the first year of observation patient S. received two courses of injections with an interval of 40 days.

The first course lasted from 7 August to 20 December 1946. 121 injections were given in $4\frac{1}{2}$ months.

The preparation was injected intramuscularly, once daily, in the doses shown in the table:

Injections	Dose in units	Date
1–2	50 each	7–8 Aug. 1946
3–47	100–300 each	9 Aug.–22 Sept.
48–71	400–500 each	23 Sept.–16 Oct.
72–93	600 ,,	17 Oct.–11 Nov.
94–121	500–700 ,,	12 Nov.–20 Dec. 1946

After 47 injections the tumour surface had become less rough, measured 2×1.8 cm and was somewhat painful on palpation; after 71 injections (23,000 units) it was flatter and had decreased in size to 1.75×1.5 cm. Its surface had grown smoother.

After 93 injections (35,400 units) the tumour was elastic in consistency and flattened.

After 121 injections (50,750 units) the tumour measured 1.5×1.7 cm, had a smooth, even surface and was more mobile. The lymphatic node in the right axilla was *no longer palpatable*.

The injections were terminated on 20 December 1946.

The following changes occurred in patient S.'s condition as a result of the first course of injections:

(1) The tumour, which had previously measured 2×2 cm, decreased during the injections to 1.7×1.5 cm. Its surface grew smoother and flatter.

(2) The firm lymphatic node in the right axilla disappeared.

After 1 month and 10 days, during which time no injections were given, on 29 January 1947 a clinical examination was made. In the 40 days that the patient had been left untreated the neoplasm had acquired a firm, cartilaginous consistency, it had increased in size to 2×1.25 cm, become adherent to the skin and its surface had become roughened.

On 5 February 1947 a *second* course of injections of the preparation was started, using higher doses than previously:

Injection	Dose in units	Date
1–15	500–900 each	5 Feb.–6 March 1947
16–30	1000–3000 ,,	7 March–4 April
31–58	2000–3000 ,,	5 April–14 May
59–107	4000–5000 ,,	15 May–8 July 1947

After 30 injections (39.600 units) the tumour had decreased in size to 1.4×1.0 cm.

After 107 injections the state of the tumour remained unchanged. Its measurements, as before, were 1.4×1.0 cm, it was oval in shape and firm in consistency. It was mobile with respect to the deeper tissues, except in one area where it was, as previously, adherent to the skin. No lymphatic nodes could be palpated. The patient again refused an operation.

The *second* course of injections of the preparation was ended on 8 July.

The following changes resulted from this course:

(1) The tumour, which during the interval between the first and second courses had grown to a size of 2×1.25 cm, *diminished again* during the *second* course to a size of 1.4×1 cm.

(2) Having decreased in size, the tumour *became stabilized* and continuation of the course for another *three* months led to no further diminution.

Thus ended the first year of observation.

From this time onwards S., who lived in another town, received injections only irregularly, intervals became frequent and prolonged, the courses of injections were short, and she went for long periods without treatment,

categorically refusing any operation. Two thirds of the time in the second year of observation was occupied by intervals and only a third by injections. Here is their time-distribution and the accompanying course of the disease.

8 July–8 October 1947—an interval of 3 months. For the first 2 months the tumour maintained its former size, but then it grew to 2×1.75 cm. The remaining features were unchanged.

From 8 October to 2 December 1947—46 injections of from 1000 to 3000 injections each, a total of 81,000 units. Then there was another interval of 127 days. During the injections growth was halted. In the period following the injections the tumour also remained unchanged for more than three months. Growth then recommenced. The tumour size rose to 2×2 cm. In one of its segments there appeared a projection firmer in consistency than the remaining part of the tumour. The patient again refused an operation.

From 24 March to 24 April 1948—31 injections, 48,000 units. The affection remained without visible change. The patient was then absent for 17 days, after which time a clinical examination showed the tumour to have retained its former measurements of 2×2 cm, but changes were seen in the character of the tumour—areas appeared in the form of irregular projections of cartilaginous consistency, intimately adherent to the gland parenchyma. The patient again refused an operation.

From 11 May to 11 June 1948—26 injections, in doses of from 500 to 5000 units. There was then an interval of 18 days. From 29 June—17 injections of from 2000 to 5000 units. The tumour remained in its previous state. The patient then absented herself again, returning on 2 August. Palpation showed the tumour to have enlarged in the direction of the depths of the gland. The patient refused radical operation but consented to limited excision of the tumour. On 21 August the tumour was excised together with the surrounding adipose tissue and gland parenchyma over an area 7×5 cm. Histological investigation showed that "most of the excised portion consisted of adipose tissue, in which lay a group of confluent, irregularly outlined, small whitish nodules with an overall diameter of about 2 cm, having the structure of an adenocarcinoma, with extensive foci of solid carcinoma". The patient received injections from 2 August until February 1949, with intervals as before—in September for 10 days, in November for 15, in December and January for 45, and no further injections were given in 1950.

After operative removal of the tumour no signs of recurrence or metastases were found for a year, but then the breast tumour recurred and lung metastases appeared, from which the patient died.

The observations on patient S., and the description of the dynamics of the changes taking place in the tumour and metastases, show that during periods when the trypanosome preparation was being injected regularly into the body the tumour ceased to grow or decreased in size, whereas in periods when no injections were given the tumour, sooner or later, began to grow.

The observations also demonstrate the possibility of achieving an inhibitory influence on the growth of a cancerous tumour during both the primary and *repeated* courses of injections: in patient S. the periodically repeated inhibitory effects went on for about 3 years, as long as she was able to receive the trypanosome preparation.

From the aspect of our question regarding refractoriness, in patient S. a refractory reaction, in our opinion, was shown during the second course of injections, when the tumour, which had previously measured 2.0×1.5 cm, decreased in size to 1.4×1 cm after the first 30 injections, and then became stabilized in that state, not changing subsequently in size, consistency or mobility during the next three months of the injection course. And although later on, during the second year of treatment, there was a real deficiency in the patient's treatment consisting of frequent enforced intervals between injections, which no doubt had a highly adverse effect on the results, during the first year there were no such interruptions in either the first or second courses. During the second series of injections, two different reactions seemed to be observed in the course of the disease: the first 30 injections caused a cancerolytic reaction and were accompanied by a therapeutic effect, while the remaining 77 injections were either inactive or, more accurately, exerted a *cancerostatic* action without any subsequent cancerolytic effects, expressed as stabilization of the patient's state. In comparing this observation with that on patient P. (page 132), it should be noted that in both cases a refractory reaction arose and appeared in exchange for the former sensitivity. The real difference, however, is that in patient S. the refractory state was relative, since injections of the preparation *repeatedly halted growth* of the tumour and inhibited the overall development of the process, which advanced in the intervals between injections.

It should be remembered that before injections were started, S.'s tumour grew in 3–4 months from the size of a lentil to 2×2 cm, and the metastases which formed in the axillary region were shown histologically to be of a malignant nature. Consequently, injections of the trypanosome preparation were started when the patient was suffering from a galloping malignant process which, during the treatment and in direct association

with the inhibitory effects of the preparation, adopted an entirely different character, as evidenced by the description above.

The refractory reaction may thus occur to a varying degree and may be expressed either as a complete loss of sensitivity to the trypanosome preparation or as a partial loss, followed by the onset of only a *cancerostatic* effect without any subsequent cancerolytic reaction, as occurred in patient S.

We suggest that in analysing the problem of refractoriness particular importance lies in one of our first observations, on the course of cancer in patient E-va (observation No. 30), who approached the clinic in May 1946. Clinical examination revealed the following: in the external upper quadrant of the left breast there was a tumour of firm consistency measuring 6×7 cm. The left axilla contained several firm, enlarged lymphatic nodes. One of these was excised at a biopsy for histological investigation. Conclusion: metastasis of an adenocarcinoma. *The patient categorically refused any operation.* A course of intramuscular injections of the trypanosome preparation was started the same month.

After 80 injections (7000 units) an examination showed "at the site of the former breast tumour there is only a slight thickening" E-va was discharged from the clinic and the injections were stopped.

On 17 September 1946 the patient was re-admitted, since during the interval (about 1½ months) a tumour the size of a walnut became palpatable. Injections were recommenced.

After 62 injections (32,900 units) it was stated: *the tumour as such is not palpatable. At the site of the former tumour there is a thickening of a doughy consistency, in the thickness of which are several cyst-like spheres.* The patient stopped receiving injections for 1½ months.

During the interval the tumour grew again, and on examination on 8 January 1947 it measured 2.5 cm in diameter. Its consistency had become firm, like cartilage. The injections were renewed. However, no effects were now seen. The tumour gradually grew larger, the lymphatic nodes again became palpatable and the tumour reached the size it had been before treatment. Operative removal was without result. Metastases formed in the lungs. The patient died from generalization of the process in May 1949.

This observation is of great importance in understanding the complex process of regression of a malignant tumour under the influence of the biological preparation. On the one hand, in the first and second courses an exceptionally marked effect was achieved in this patient: an adenocarcinoma measuring 6×7 cm, with metastases, as a result of the injections could *no longer be palpated.* It cannot be denied that such a degree of

regression is extremely high. At the same time, the further course of the condition convinced us that even when the tumour had almost completely disappeared, intact malignant cells remained in the breast and in the interval between injections gave rise to a fresh tumour lesion, which was, moreover, resistant to the trypanosome factor.

Thus, in this case clinical observation provides a basis for believing that viable resistant cells may remain after contact with the trypanosome preparation, as a result of which further injections have no effect on the tumour's development.

It was this particular observation on patient E. that induced us to resort to removal of the primary focus in patients with cancer of the breast during tumour regression brought about by the trypanosome preparation. As we have seen earlier, this method has given some good results.

OBSERVATION NO. 31

(Described in 1946 as No. 11)

Patient M.-ov, aged 55 years. *Cancer of the rectum.* In May 1946 M. had difficulty in defaecation and found blood in the faeces. These symptoms increased over the next 10–15 days. Independent stools ceased early in June.

He was admitted to the clinic on 8 June 1946.

Examination by palpation. On the posterior wall of the rectum, 5 cm from the anal orifice, to the right of the midline there was a firm, irregular, necrotic tumour. The bowel wall surrounding the tumour was infiltrated. The size of the tumour could not be determined accurately by palpation, because it was impossible to reach the upper limits of the tumour with the finger. Its visible portion measured approximately 3×3 cm. A biopsy was performed on 8 June. Histological investigation established it as an adenocarcinoma of the rectum (conclusion of Prof. Talalaev).

On 14 June 1946 the patient started to receive injections of the trypanosome preparation. The injections were given daily, intramuscularly in the buttocks, thighs and lumbar region in turn.

INJECTIONS OF THE PREPARATION AND STATE OF THE TUMOUR

Date	Injection	Dose in units
14 June	1	20
15 June	2	30
16 June	3	40
17 June	4	50
18 June	5	60
19 June	6	70
	Total	270 units

State of the tumour on 19 June: on palpation the infiltration of the bowel wall had considerably decreased. The ulcer had a crater-like depression admitting the end of the finger. The visible part of the tumour had decreased in size to 1×1 cm and hung from a fold in the mucosa. Independent stools reappeared; defaecation was extremely difficult. The faeces contained shreds of mucous membrane.

Radiography. An X-ray photograph of the rectum after filling it with a contrast mass and then evacuating it showed a filling defect extending for 4 cm, situated 5 cm from the anal orifice.

CONTINUATION OF INJECTIONS

Date	Injection	Dose in units
20 June	7	80
21 June	8	90
22–27 June	9–13	100 each
28 June	14	120
29–30 June	15–16	150 each
	Total	1300 units

On 30 June defaecation was less difficult, and stools were independent on later days.

CONTINUATION OF INJECTIONS

1–5 July, injections 17–21, 150 units each, a total to date of 2110 units.

5 July. The tumour surface had become smoother (on palpation). The visible tumour measurements were still 1×1 cm.

Continuation of injections: 6–10 July, injections 22–26,150 units each, a total to date of 2860 units.

10 July. The crater-like depression was considerably diminished. The tumour had grown flatter. The infiltration of the bowel wall was markedly softer.

Continuation of injections: 17–30 July, 150 units daily.

In all, 6,010 units were given, in 47 injections.

30 July. Palpation of the bowel wall *revealed neither tumour nor infiltration.* At the site of the former tumour there was a thickening measuring less than 0.5×0.5 cm, hanging in the form of a fold. The patient's general condition was good; the stools were independent the whole time.

Haematological investigation. Four blood examinations were carried out during the course of injections of the preparation: on the day of the first injection, then after 6, 14 and 43 injections.

The blood studies revealed no deviations from the normal. The red cell count, which was low at the start of the disease, later returned to normal. The haemoglobin level stayed almost constant, with only slight variations.

The erythrocyte sedimentation rate remained at an almost constant high level.

The total leucocyte count suffered negligible changes during the whole course of injections, showing a tendency to fall towards the end of the treatment.

The lymph cytogram showed a slight lympho-monocyte reaction: after a temporary fall in lymphocytes on the sixth day of injections the number of mononuclear elements, both lymphocytes and monocytes, rose, reaching a total of 29.5 per cent instead of the earlier 25 per cent.

The polynuclear reaction remained unchanged.

GENERAL CONDITION OF THE PATIENT

The patient was admitted to the clinic when stools had been absent for 2 weeks; his overall clinical state was assessed as of one of moderate severity. For two weeks after the start of injections there were no particular changes in his condition. Independent stools occurred at times, but defaecation was painful, difficult, and was accompanied by the passage of shreds of mucous membrane, sometimes tinged with blood. The first independent stools to be more or less painless occurred after 17 injections and then became regular. From time to time the faeces contained shreds of mucous membrane and were more or less liberally tinged with blood. After a 3-weeks course of injections the clinicians noted a crisis in the patient's general condition, which was described as "quite satisfactory" and finally as "good".

His weight after 6 injections was 50.1 kg, after 21 injections 50.2 kg, and after 44 injections—52.2 kg.

The patient was treated by no other method besides the trypanosome preparation.

At this stage, for reasons beyond our control, the injections were terminated and M. went away to the Provinces without finishing the course of treatment. We were only able to renew our observations 3 months later, when on our initiative he was moved back to Moscow. Examination showed that during the 3 months the patient had been without treatment the tumour had grown again. The course of injections of the preparation was recommenced. Growth of the tumour was halted.

SECOND SERIES OF INJECTIONS AND COURSE OF THE DISEASE

In the second course the patient was given the preparation intramuscularly and intravenously. The injections were given in small doses at first, gradually increasing to 1200 units. The course lasted from 29 September 1946 to 20 January 1947.

Injection	Dose in units	Date
1	5	29 Sept. 1946 intramuscular
2	10	30 Sept. 1946 ,,

Rectoscopy. The proctoscope was admitted to a depth of 10 cm. On the anterior aspect of the rectum was a tumour measuring 5×5 cm. A piece of the tumour was removed for examination.

Injection	Dose in units	Date
3–5	24 each	1–3 Oct. 1946 intramuscular
6–8	100 each	4–6 Oct. 1946 ,,
9–12	250 each	7–10 Oct. 1946 ,,

Examination by Prof. A. N. Velikoretskii after 10 injections: examination *per rectum* revealed an oval swelling measuring 6×4 cm on the posterior wall of the rectum, with a groove down its centre.

Injection	Dose in units	Date
13–14	500 each	11 Oct. intramuscular
15–23	350 each	12–21 Oct. intramuscular

Rectoscopy after 23 injections: at a depth of 8 cm the lateral wall of the rectum bore a tumour hanging into the lower lumen. The site of the previous biopsy showed incomplete epithelialization. Digital palpation *per rectum*—the tumour was mobile, and of a firm elastic consistency.

Injection	Dose in units	Date
24–25	500 each	22–23 Oct. intramuscular
26–29	500 each	24–27 Oct. intramuscular and intravenous

Rectoscopy after 29 injections: the postero-lateral wall of the rectum at a depth of 8–9 cm bore a wide mushroom-like outgrowth on a pedicle, without signs of necrosis and at this stage bleeding slightly.

Injections 30–34, 500 units each, were given on 28–31 October, intramuscularly and intravenously.

Examination by Prof. Velikoretskii after 34 injections: on the postero-lateral wall of the rectum there was a tumour measuring 4×3 cm, less dense in consistency than previously. Its right edge was much softer and less dependent. The left edge was still firm. A crater sufficient to admit the finger tip had formed in the centre of the tumour.

Injection	Dose in units	Date
35	800	2 Nov. intramuscular
36–42	150 +100 intravenously	5–11 Nov. intramuscular
and 250 units intramuscularly every 6 hours = 4 injections per day		
43–51	1200 each	13–21 Nov. — on 4 occasions.

Examination by Prof. Velikoretskii on 22 November: the tumour measurements were the same as at the previous examination. The crater-like depression admitted one finger. The tumour was mobile.

Injection	Dose in units	Date
52	750	22 Nov.
53–54	1200 each	23–24 Nov.
55–56	1200 each	25–26 Nov., 250 units of this were given intravenously

Rectoscopy on 27 November, after 56 injections: a tumour with a rounded border was situated 8 cm from the anus. The tumour had become less papillomatous since the last examination. An area of necrosis and ulceration at the centre of the tumour formed a smooth surface, grey in colour. The edges were not covered. The tumour bled less than at the last examination.

Injections 57–59, 1000–1200 units each, were given on 27–29 November; 250 units of this was given intravenously. The faeces now contained *no blood*.

Injections 60–73—*similarly*, 30 November–13 December.

Examination by Prof. Velikoretskii after 73 injections: the size and extent of the tumour remained as previously. The edges were slightly more protruding.

Rectoscopy after 81 injections: no change. An operation was decided upon.

Injections:

82–83, 1000–1200 units, 24 and 26 Dec., 250 units of this were given intravenously
84–85, *ditto*, given on 1–2 Jan. 1947.
86–92, 500 units each, intramuscularly, 8–15 Jan. 1947.
93–95, 750 ,, ,, 16–18 Jan.
96, 250 units each, intramuscularly, 19 Jan.
97, 500 ,, ,, 20 Jan.

On 23 January 1947 an operation was carried out: amputation of the rectum with the creation of an *anus preternaturalis*. Ether anaesthesia.

* * *

The course of injections of the trypanosome preparation was terminated in the *pre-operative period.*

Further observations lasting for 2 years 9 months showed the absence of signs of recurrence or metastases.

Patient M.'s case-history shows that the second course of injections of the preparation *modified* the development of his cancerous tumour; during the process of this course the tumour: (a) decreased in size, and an area of epithelialization appeared on it; (b) some parts of the tumour became soft in consistency. However, the course of injections of modification II of the preparation in doses of from 200 to 1200 units, given intramuscularly in daily injections for 3 months, did not lead to complete destruction of patient M.'s tumour, but exerted only a cancerostatic effect, although from its histogenesis and also from the initial picture of regression this adenocarcinoma of the rectum must be included in the group of tumours sensitive to the trypanosome preparation. For this reason we were bound to record the onset during patient M.'s second course of a refractory reaction, excluding, of course, defects in the technique of the treatment.

OBSERVATION NO. 32

Patient S., male; clinical diagnosis: *cancer of the lower lip.* Histological diagnosis: *Keratinizing squamous-cell carcinoma.*

On admittance to the clinic on 21 October 1948 the patient's lower lip bore a tumour measuring 2.2×1.7 cm, with an ulcerated surface; the submandibular and mental regions contained firm lymphatic nodes.

On 30 October a biopsy was performed: one quarter of the tumour was excised. On the same day daily intramuscular injections of the trypanosome preparation were commenced, in doses of from 1000 to 3000 units at each injection. The tumour continued to grow during the process of the injections. After 17 injections (14,000 units) it measured 2.6×1.5 cm, and after 70 injections (161,000 units) 3×1.7 cm. Two and a half months after the start of the course the affected portion of the lip was excised and injections of the preparation continued in doses of 3000 units per injection.

One month later another thickening appeared in the scar region and for this reason the patient underwent a radical operation. Cytological examination of the excised tumours showed that the number of mitoses determined earlier in the biopsy sample (5 per hundred fields of vision) was unchanged. There was thus no clinical or cytological evidence that either a cancerolytic or a cancerostatic effect was produced by the trypanosome preparation in patient S. The course of the disease in this instance

was distinguished by the noteworthy peculiarity that, while affections of identical histogenesis and localization in other patients were usually sensitive to the trypanosome preparation, patient S. showed complete refractoriness, initially and subsequently.

What factors may cause a refractory reaction? Such factors may involve the preparation, the reaction of the tumour tissue and the state of the patient's body.

Refractoriness can hardly be explained in terms of loss of the antiblastic activity of the preparation. We shall later describe defects in observation in this respect (page 149). It is true that at that period, because of our inexperience, patients received preparations varying in activity. It should be remembered however that in patient P., using this unstandardized preparation, with all the defects in our therapeutic technique we obtained a cancerolytic effect twice, both to a considerable degree, and ceased to obtain it one year later, using the same technique in producing the preparation, admittedly still far from perfect but constant during the $2\frac{1}{2}$ years that patient P. received it.

In patient E. regression was so marked that a tumour formerly measuring 6×7 cm could not be palpated. After this the injections had no effect.

In patient S. there was considerable regression both during the first course and at the start of the second, and then followed a cancerostatic effect without any subsequent cancerolytic reaction. The same applies to patient M., who had an adenocarcinoma of the rectum.

We therefore have no grounds for ascribing (at least wholly) the lack of results following the use of the preparation to its quality. This circumstance forces us to conclude that in the observations described the patients showed a genuine refractory reaction.

Having adopted this viewpoint, however, we lack the facts which would enable us to discover the mechanism by which such a reaction arises—whether it is connected with resistance of the malignant cells themselves or caused by a physiological state which develops in the body in the course of the disease.

It is hard to give preference to any one of these hypotheses without special investigations. It cannot be excluded that in a mass of malignant cells there may be cells with different degrees of sensitivity to the trypanosome antiblastic factor. In such a case, during the process of the injections the sensitive cells perish and the resistant cells remain to form the basis of new, resistant generations. The refractoriness in this case is derived from a particular process of selection occurring among cells subjected to the influence of the antiblastic factor, in a manner resem-

bling that seen in microbial populations subjected to the influence of antibiotics. If this reaction were narrowly specific, the answer would be to use different antiblastic factors in turn.

Such a hypothesis is to a certain extent contradicted by the fact that repeated courses of injections of the trypanosome preparation given, with intervals, in the same patients, are able to produce a cancerolytic reaction again and again, even in cases where the previous course has been prolonged—up to several months. This has been observed in a whole series of patients. It was also seen in the patients whose cases were described in this chapter. This fact shows that the malignant cells which maintain their viability during the action of the trypanosome factor do not acquire an immunity to it and do not give rise to resistant generations.

The second hypothesis—that the cause of refractoriness lies in a particular physiological state of the body—also has some foundation. There is no doubt that each type of cancerous disease calls for a special analysis of the pathophysiological factors which leave their mark on the developmental characteristics of cancer in each case and on the different groups of malignant affections.

The best illustration of the influence of these characteristics on the development of the malignant process is provided by species resistance to heterotransplanted tumours and the possibility of inducing their growth and development in a body subjected to the effects of cortisone and irradiation. This ability to induce cancer by the destruction of one of the most stable forms of resistance—species resistance—is the best and most reliable proof of the role and significance of the body's physiological defence factors in the pathogenesis of cancer. This phenomenon of experimental oncology is in complete agreement with what has been established clinically, namely, with individual variations in the developmental features of cancerous affections of similar sites and morphology in persons of different ages and sex and accessible for the determination of other signs. Consequently, starting with species resistance and ending with the individual defences of the body, we find in every case a constant relationship between the development of cancer and the activity of physiological mechanisms.

We were able to note that the process of regression brought about by an antiblastic factor—in our observations, by the trypanosome preparation—while occurring in accordance with strictly defined principles, nevertheless showed variations of an individual and group character. We refer here, of course, to the category of affections naturally *sensitive* to the antiblastic factor. Both the progressive development of cancer and tumour regression occur under the *concrete* influence of the physiological characteristics of the body. For example, a *cancerolytic* effect may be observed at various stages of a disease, but a *cancerotherapeutic* effect is achieved the more rapidly and completely, the less intensive is the cancerous affection. This applies, at least, to regression caused by the imperfect preparation which was used in all the observations described in this book. The future will show to what extent the physiological background of the patient may be ignored when more active preparations are employed.

A question arises regarding the clinical approach to the treatment of patients who have shown some or other type of refractory reaction. As experience accumulates, of course, the biotherapeutic method will be improved. But even now our limited experience enables us, without making any definite statements, to put forward what appears to us to be some rational suggestions.

In those cases where the patient does not react to administration of the preparation by tumour regression within the first 3–4 weeks, and when none of its clinical symptoms is observed—neither a cancerolytic nor a cancerostatic effect, as seen in patient S. (observation No. 32)—injections of the preparation should be stopped, even though the patient's tumour can be related by its various signs (histogenesis and others) to a group sensitive to the cancerolytic factor. Such a patient must undergo other, more conventional methods of treatment.

If a refractory reaction arises in a patient *in place of* earlier tumour regression and is expressed as stabilization of the tumour, then experience has shown that in such cases the tumour focus should be excised to the limits of the affected tissue, and a postoperative course of injections of the preparation should be carried out. Operative removal of a cancer lesion is rationally performed at the time of a cancerolytic reaction, when regression is still evident, or in the early stages of a cancerostatic reaction, when stabilization has only just begun. The *rationale* of removal of the tumour at this time is based on cytological studies showing considerable and profound changes in the tumour. These studies are described in Part III. The grounds for limited operative interference are provided by the previously described observations in which clinical recovery was attained without radical operation or X-ray therapy.

8. DEFECTS IN OBSERVATION

The clinical observations on the regression of malignant affections reflect not only the cancerotherapeutic effects of the trypanosome factor but also the defects which are peculiar to this new direction and are as-

sociated with our lack of experience and insufficient knowledge of the principles governing the regression process in malignant tumours.

Two sources of defects may be mentioned: imperfections in the preparation and imperfections in the technique of employing it.

Three modifications of the trypanosome preparation have been used clinically. They differed in their starting material—the source of the trypanosomes from which the preparation was produced. The first and second modifications were derived from trypanosomes of animal origin, but from different species of animals. The third modification was from trypanosomes cultured on a synthetic nutrient medium.

Each of the trypanosome cultures contained an active cancerolytic agent. There are, however, no grounds for considering that trypanosomes grown under different conditions are biologically identical in this respect. On the contrary, investigations have shown that this property of *T. cruzi* is variable and depends primarily on the characteristics of a strain and its generation, on the composition of the medium, on the duration of culture and other factors. This has been established objectively in biological studies of *T. cruzi* cultures of various origin and on culturing by various methods.

The clinical observations which we have described in this book were in this sense carried out under *inconstant* conditions right from the start.

This was the first defect in observation, a defect associated with the peculiarities of the stage that work on the preparation had reached at that period. We could, in trying to standardize our experimental technique, have limited our clinical investigation to tests of the first and second modifications of the preparation, which had already justified themselves in pilot clinical observations. But such a line would be directly against the interests of practice, in that neither the first nor the second modification, while exerting an active effect, had any hope of practical application in cancer treatment, however positive were the results they had given in experimental clinical trials. Their impracticability was associated with the impossibility of obtaining in this manner an amount of the preparation sufficient even for the treatment of 3 or 4 patients. As we were aiming at developing a method of producing the preparation for the treatment of cancer patients, we were bound to attempt to study a modification having some prospects of large-scale application. The first step towards the achievement of this aim was the development of a method of obtaining sufficient and practically unlimited numbers of the trypanosomes forming the "raw material" for production of the preparation.

Therefore, having started clinical observations on the use of preparation of the first and second modifications, we were at the same time attempting to obtain trypanosome cultures, and we began testing the preparation made from them as soon as the first suitable cultures were obtained.

None of the numerous nutrient media described at that period proved suitable for the mass cultivation of cancerolytically active trypanosomes. Little's medium was best, but also needed modifications. As the result of prolonged experiments we adopted a modified fluid medium with granulated blood. The development of *T. cruzi* in this medium takes place in accordance with the principles studied fully in a number of works by A. and M. Lwoff.

Trypanosomes seeded in this medium from the blood of animals usually grow in the first generations in leishmanial or leptomonad forms. Next, in some generations sooner and in some later, crithidial forms appear which can then be cultured for long periods—up to several years.

It is extremely important to note that cultures identical in their species characteristics, grown in media of equal composition, may differ in the degree of their cancerolytic activity. Fradkina, Kats and Skorikova, in this laboratory, have investigated the cancerolytic activity of numerous *T. cruzi* cultures. Experiments were set up in the following manner. White mice were inoculated with the Crocker sarcoma. On the fourth day after this the mice were divided into equal groups (from 5 to 10 in each). One group remained as a control, and the mice in the second group were injected subcutaneously with the culture under test, usually in doses of 15 million trypanosomes in a volume of 0.5–0.6 ml. 15–17 days after implantation of the tumour and 12–15 days after injection of the culture the mice were killed and the tumours excised and weighed. The number of trypanosomes in the blood was determined on the last day of the experiment or the day before. *The ratio of the average tumour weight in the control group to the average tumour weight in the experimental group was conditionally taken as an index of the cancerolytic activity of the culture tested, or, abbreviated, "IE"* (index of effectiveness).

THE DETERMINATION OF THE CANCEROLYTIC ACTIVITY (INDEX OF EFFECTIVENESS) OF DIFFERENT STRAINS OF *T.cruzi*

(Experiments carried out 16–26 October 1946)

The weight of the tumours (Crocker sarcoma) was determined 17 days after implantation into the mice. The experimental mice were inoculated 4 days after the start of the experiment with three different strains of *T.*

cruzi (62, 64, 66). The tumour weights in the experimental mice were determined at the time of maximum trypanosome concentration in the blood.

Tumour weights in control mice in grams	Tumour weights in mice infected with various strains of *T. cruzi*, in grams		
	strain 62–13	strain 64–11	strain 66–9
2.30	0.21 — tumour soft	0.17 — structureless nodule	0 — small nodule
2.55	0.32	0.17 — tumour soft	0.17
3.47	0.55	0.15 — ,, ,,	0.57
5.40	0.60	0.60 — structureless nodule	0.40
7.40	1.30	0.19	0.30 — small nodule
8.10			0
Average weight — 4.861	0.596	0.32	0.24
Index of cancerolytic effectiveness	8.15	15.19	20.2

The tumours in the mice infected with these strains were excised on the day the mice died from trypanosomiasis, i.e. at the moment of maximum saturation of the body with *T. cruzi*. The average tumour weight in animals affected with each of the strains was much lower than the average weight in the control animals, in which the tumours had reached weights of from 2.3 g to 8.1 g in 17 days. After the same period the tumours weighed about 0.5 g or less in 14 of the 16 mice inoculated with the three *T. cruzi* strains, the tumour weighing 0·6 g in one and 1.3 g in the other. Hence all the strains were undoubtedly highly active with regard to their cancerolytic effects.

However, on calculating their index of activity, for strain 62–13 this equals 8.15, for strain 64–11 it is 15.9 and for strain 66–09 it is 20.2. It should be emphasized that these three cultures were grown on media of identical composition, were transplanted simultaneously and had identical characters; nevertheless, when tested under similar experimental conditions they were found to have differing degrees of cancerolytic activity.

This important circumstance undoubtedly had some influence on the quality of the preparation. Also, in the early period of production of the preparation we could not predict in advance the lability of the cancerolytic properties of different generations on cultivating them under uniform conditions.

Although different raw material was used the preparations for clinical trial were always produced by the same technique. The different batches,

however, did not have identical cancerolytic activities. This was the second, very real defect in our observations. It arose partly from the unstandardized raw material—the trypanosome cultures—and also from the changes which the preparation underwent during its production process, at various stages in the technological treatment of the trypanosomes.

All the clinical observations described apply to the period of our work when the technology of producing a cancerolytic trypanosome preparation had only just begun to be worked out. New, improved modifications were in the stages of thorough experimental investigation. The patients received an unrefined, unstable, unconcentrated, unstandardized preparation of inconstant activity, in the form of products consisting of the cellular contents almost in the native state, but even so the active principle was weakened during this production process. A concrete reflection of the amount of trypanosome factor preserved in the final product is provided by the results of tests of its preventative activity in experiments on white mice. These were inoculated with the Crocker sarcoma and were then injected with various doses of the preparation. One group of mice each received 1 unit daily, the second group 3 units each and the third 10 units each. Those in the fourth group were not injected and served as controls. Optimum doses had a preventative effect with regard to the sarcoma. This effect was expressed as a complete absence of tumour development, or the tumours grew more slowly than in the controls and weighed and measured less after the same period of time.

By observing tumour development in the control and experimental animals we were able to establish the *index of inhibitory activity* of the preparation, which we defined conditionally as the ratio of the average tumour weight in the control, uninjected mice to the average tumour weight in the injected animals.

In these early days it was only possible to define the dose of the preparation on the basis of the number of trypanosomes treated; the determination of the inhibitory index enables us to express the *activity* of a preparation in cancerolytic units having a *preventitive* effect on experimental sarcoma in white mice.

As an example, we include the tests on three batches of the preparation (235, 273-B, 283).

DETERMINATION OF THE INHIBITORY INDEX OF PREPARATION BATCH NO. 235

Inoculation of the sarcoma in 40 mice—24 January 1949.

Inoculated material: strain CS of 8 Jan. 1949—third passage. Fifteen-day tumour weighing 5 g.

Implantation technique: each mouse was injected subcutaneously with 0·5 ml of a 10 per cent suspension of the tumour in normal saline.

Injections of preparation batch No. 235 were started on 24 Jan. 1949.

Individual doses: 1 unit — 10 mice.
3 units — 10 mice.
10 units — 10 mice.
Control—untreated — 10 mice.

	Final result		Average tumour weight	Index of inhibitory activity
	no tumour	tumour		
Ten mice injected with 1 unit daily for 14 days	2	8	0.151	4.77
Ten mice injected with 3 units daily for 14 days	5	5	0.164	4.39
Ten mice injected with 10 units daily for 14 days	1	9	0.266	2.30
Ten control mice	0	10	0.721	—

DETERMINATION OF THE INHIBITORY INDEX OF PREPARATION BATCH NO. 283

Inoculation of the sarcoma in 40 mice—31 March 1949.

Inoculated material: strain CS of 16 March 1949, 15-day tumour.

Implantation technique: each mouse was injected subcutaneously with 0.5 ml of a 10 per cent suspension of the tumour in normal saline.

Injections of preparation batch No. 283 were started on 31 March 1949.

Individual doses: 1 unit —10 mice
3 units —10 mice
10 units —10 mice
Control — untreated —10 mice

	Final result		Average tumour weight	Index of inhibitory activity
	no tumour	tumour		
Ten mice injected with 1 unit daily for 14 days	—	10	2.282	0.840
Ten mice injected with 3 units daily for 14 days	—	10	2.377	0.807
Ten mice injected with 10 units daily for 14 days	—	10	2.189	0.877
Ten control mice, untreated . . .	—	10	1.919	—

DETERMINATION OF THE INHIBITORY INDEX OF PREPARATION BATCH NO. 273–B

Inoculation of the sarcoma in 40 mice—16 March 1949.

Inoculated material: strain CS of 28 February 1949, 15–day tumour.

Implantation technique: each mouse was injected subcutaneously with 0.5 ml of a 10 per cent suspension of the tumour in normal saline.

Injections of preparation batch No. 273-B were started on 16 March 1949.

Individual doses: 1 unit —10 mice
3 units — 9 mice
10 units —10 mice
Controls—untreated —10 mice

	Final result		Average tumour weight	Index of inhibitory activity
	no tumour	tumour		
Ten mice injected with 1 unit daily for 14 days	—	10	0.997	2.168
Ten mice injected with 3 units daily for 14 days	1	8	0.948	2.280
Ten mice injected with 10 units daily for 14 days	1	9	1.227	1.762
Ten control mice, untreated . . .	—	10	2.162	—

Remembering that the three batches, the characteristics of which are reflected in these reports, were prepared by the same technique, these three examples show clearly that the cancerolytic activity of the preparation varied in different batches. We include below the result of a check on 66 production batches (experiments involving 2640 animals). Taken as a whole they provide an objective impression of the preparation used in the clinical observations described. If a preparation showed no preventative effect in these control experiments this is shown in the "activity" table by a zero. An effect giving an inhibitory index greater than unity but less than or equal to 2, is marked "+"; greater than 2 but less than or equal to 3, "++", and greater than 3, "+++".

INHIBITORY EFFECTS OF THE PREPARATION AGAINST THE CROCKER SARCOMA

(Based on 66 production batches, in experiments on 2640 white mice)

Inhibitory effect	Activity	Number of batches	
		absolute	%
Absent	0	18	27.2
Index $>1\leqslant2$	+	26	39.3
„ $>2\leqslant3$	++	16	24.2
„ >3	+++	6	9

This investigation, carried out in our laboratory by S. S. Kasatkevich and M. N. Pavlova, shows that about one third of the batches possessed no activity. In 72·8 per cent there was some degree of inhibitory effect.

Analysis of these findings reveals a number of defects relating to the preparation used clinically in the observations described.

The main defect was the following. As, at that period, we did not know of the technique for controlling the activity developed later, we could not adopt the usual approach on issuing biological preparations, that is to *select* active batches and *reject* batches of the preparation not containing the active principle. We started to do this later on. In the clinical observations described the preparation, both because of the absence of such a technique and because of its instability, could not be controlled by laboratory experiments. Its control involved a 2-week period, and the activity of this early, fluid preparation had already decreased by the first few days after its production.

As a result of this uncontrolled administration there was, as shown on retrospective analysis, an alternation between injections of an active preparation and an inactive product. Judging from the results of the control experiments, as is quite permissible, for every 2–3 injections of an active preparation the patient received one injection of an inactive product. The clinical observations show that the preparation must be given continuously until there is complete destruction of cancer cells in the body. Interrupted or irregular administration is associated with decreased effectiveness, even when active batches of the preparation are used.

All this, however, was revealed later. During the period when the clinical observations described were carried out the derivation of these principles was still the subject of special investigations.

An analysis of the same findings by Kasatkevich and Pavlova reveals yet another serious defect associated with the dosage methods employed for the preparations used in the clinical observations.

Determination of the dosage, as we have stated, was previously based on the number of trypanosome cells treated. However, different batches, which were, in general, active following a given equal treatment of the raw material, showed their inhibitory effects *to different degrees*. This indicated that the same number of trypanosomes may give rise to greater or lesser amounts of the active principle. This was confirmed experimentally in our laboratory by E. M. Gintsburg.

In experiments 57 and 22 a dose of 5 units per injection led to practically no preventative effect, or this was very slight. Doses of 10 and 15 units produced a marked positive effect in most of the animals. In experiments

6 and 13, on the other hand, a complete absence of tumours was achieved by injections of 5 units, and in experiment 13 a dose of 5 units was just as effective as the trebled dose of 15 units.

This discovery showed the necessity for *titration* of the cancerolytic properties of each different batch, as is done with other biological products used in various other diseases.

Kasatkevich and Pavlova's studies, which did in fact involve titration of the cancerolytic activity in prevention experiments with the Crocker sarcoma, enabled the following scale of the *degree* of inhibitory activity to be given in relation to the dose injected.

THE DEGREE OF INHIBITORY EFFECT IN RELATION TO THE INJECTED DOSE OF THE PREPARATION

(48 batches, in experiments on 1920 mice)

Individual dose at each injection	Total dose in course	Number of batches having positive effect	
		absolute	%
1 unit	14	13	27
3 units	42	20	41.6
10 units	140	15	31.2

Taken as a whole, the results of all the experiments indicate that, using equal techniques, the same number of trypanosome cells may give rise to different amounts of the active principle capable of inhibiting the growth of malignant tumours. Of the production batches studied by Kasatkevich and Pavlova, in 27 per cent this amount corresponded to the number of treated trypanosomes taken as 1 unit (as we stated earlier, 1 unit represents the dose of the active principle obtained by the treatment of 1 million trypanosome cells).

In 41.6 per cent *three times* this number of trypanosomes had to be treated to obtain the same amount of the active substance. Finally, in 31 per cent it was necessary to process *ten times* as many cells.

If, on the basis of this experiment, we calculate the therapeutic dose not by the number of treated trypanosomes but by the quantity of the trypanosome preparation *able to show an inhibitory effect in preventative* experiments on the sarcoma, we find that the individual active dose for different batches varies within a ratio of 1:10.

The results given above show that the effect which was obtained in 27 per cent when 14 units were given in a course of injections was obtained in 31.2 per cent only when a dose of 140 units was given.

These variations in biological activity were not taken into account in the clinical observations described, because we lacked experience then and only found out all this later. It formed yet another defect in our observations. The case-histories show that patients were given a dose of the preparation with no allowance for its activity coefficient as determined in control experiments. Moreover, if we consider that when using a preparation containing so many impurities excessively high doses can be given just as readily as excessively low ones, it becomes obvious that for any *rational* therapy there must be some account of the units of biological activity. This method, however, only became available later.

One more defect in the clinical observations must be mentioned, and that an unavoidable one; it is associated with a period when excessively high dosages of the trypanosome preparation were used.

Having decided that the preparation was harmless in relatively low doses but still had some therapeutic effect, we naturally started to use higher dosages, firstly in an attempt to increase the effectiveness of its action and secondly to establish the relationship between the dose injected and the effectiveness of its action, the maximum tolerated dose of the preparation and the difference between the therapeutic and toxic doses. These questions were studied both experimentally and in clinical observations. Some extremely useful information was obtained by these means. In the course of the studies we could not avoid a long series of negative results, which arose from our lack of knowledge and our inability to make proper use of the biotherapeutic preparation at our disposal. These negative results did, however, have some positive significance in that because of them we found out what we should avoid and learned to some extent how to *direct* the process which we are describing here as regression of a malignant tumour.

These negative results, although unavoidable in new work, did leave their mark on the clinical observations and robbed some of them of their conviction. Now that these major faults in the clinical observations and experimental work have been partly replaced by new, positive results, based on the lessons learned from earlier mistakes, we do not regret this past work, which could not have been dispensed with because of the absence of any corresponding scientific experience.

The observations were carried out as follows: having established the safety of the preparation, we started to increase the doses gradually. From a daily dose of 100–200 units per injection we slowly progressed to 10,000 units. The preparation produced no ill effects even in such massive doses. But here we came up against a paradoxical phenomenon: when

the dose was increased above a certain limit it was not only not accompanied by any positive effect, but in a number of cases the therapeutic action ceased, and our clinic, which had previously been more like a convalescent home, became like an ordinary clinic, with the air of hopelessness that not infrequently prevails in oncological departments.

In order to analyse these phenomena, we resorted to extensive experiments. Just as in the clinical observations, we were able to confirm that increasing the dose from 1 to 5–10 and 15 units led to a rise in the effectiveness of its action. Increasing the dose to 50 units, however, caused cessation of the preventative action of the preparation in cases where parallel trials with lower doses gave a positive result. Consequently experiments on mice, as well as clinical observations, showed that the effect was produced by *optimum,* not maximum doses. This was established with regard to both mouse sarcoma and rabbit carcinoma.

In analysing the experimental and clinical findings, we came up against a very serious question: is it possible to find the optimum dosages for *different forms* of cancerous process, or would their variations when used in the human patient be of such an individual character that it would be impracticable to prescribe accurately for any one form and stage of the disease? We cannot deny that this question was very worrying. However, experience and practice has shown that it may be resolved. It has been found experimentally that the limits of the permissible variations in dosage of a preparation free of impurities are *determinable* and can be established both by experiment and during clinical treatment of patients. The observations described previously show that earlier stages of the disease call for lower doses, and the so-called "precancerous" affections may be cured in a short time by extremely low doses. The problem of establishing optimum doses is thus very real. It can be solved by clinical experience and practice combined with experimental methods.

At the same time, experiments showed the possibility of effectively increasing an already considerable dose by using a preparation *purified of ballast substances.* But all this came later. During the period to which our clinical observations relate, this was unknown, and would have not become apparent had we not carried out the clinical and experimental work described above.

There is yet another question, no less serious—that of the reasons for the ineffectiveness of massive doses. Why does over-saturation of the body with the trypanosome factor lead to the cessation of its activity against tumours? The answer to this question can only be found by work with a preparation chemically purified of ballast substances. Experiments

carried out in collaboration with E. M. Gintsburg showed that in the trypanosome cell, besides the active antiblastic fraction, there are substances not possessing this property. Injecting them into the body has no effect on the course of a malignant process: the tumour grows just as rapidly as before their injection. In the experiments, one group of sarcoma-affected mice were injected with a fraction A, purified of ballast substances, and another group with fraction B, consisting of the ballast substances in the trypanosome cell. The injection of the fraction A gave a clear inhibitory effect, whereas fraction B was inactive in this respect. It may be considered that when massive doses were used there was saturation of the body with the ballast substances which at the time had not been removed from the modifications of the preparation used. This circumstance gave rise to yet another serious defect in the clinical observations we have described. We cannot, however, exclude the possibility that there is another explanation of the irrationality of massive doses, deriving from analysis of the still undiscovered mechanism by which the trypanosome preparation acts upon malignant tissue. This field of research will be the next task for the experimenter and clinician.

One very real defect in the observations was lack of development of the *technique of administering* the preparation. It was usually injected once a day, intramuscularly in the buttock region. In a number of observations the preparation was injected paratumourally. It follows clearly from descriptions of the course of the disease that this means of administration had no advantages over the intramuscular route. On the contrary, it suffered from a number of disadvantages: (1) with this means of administration the amount of preparation was limited by the impossibility of injecting more or less significant volumes of the fluid; (2) intratumoural injection, without giving any noticeable effect, was painful and led to oedema of the tumour; (3) with more prolonged courses of injections this pain became so considerable that further intratumoural injections were impossible. In some cases the preparation was given intravenously. The number of such observations was small and for this reason we can only speak of the safety of intravenous injection—it is too early to judge whether it is more effective than the intramuscular route. Thus, we did not have enough material to *select* a technique of administering the preparation, and we can only discuss its effectiveness under conditions of intramuscular injections.

The question of the *frequency* of injections remains just as vague. The patients usually received injections once daily. The interval between injections should quite possibly be much shorter. No experiments were carried out to throw any light on this question.

We also consider it a defect that the preparation was used in the absence of any sort of additional factors which may have had some favourable effect on tumour development. The observations described show that there is an immediate need for a study involving special clinical and experimental observations under conditions where nonradical operation is employed together with the biotherapeutic method and radiation therapy. In describing the clinical observations we included sufficient facts to demonstrate that when using the biotherapeutic method nonradical operation is not only safe but useful. The general conclusion which may be drawn from the observations is that when the biotherapeutic method is used the indications for surgical interference are considerably extended. However, we did not mention observations involving questions of the relationship between radiation therapy and biotherapy.

From the descriptions of the course of the disease in patients P., L. and K. (observations Nos. 28, 19, 11) it follows that biotherapy may be of value in some cases where radiation therapy has been refused or has been ineffective, as in patient N. or patient L., or because of the risk of serious side effects, as occurred in patient K. (observation No. 11). But the relation between radiation therapy and biotherapy is not limited by this. The clinical observations show the need for a special study of the effectiveness of using the *two* methods in a definite sequence. It is appropriate to recall here two patients with cancer of the larynx, in advanced stages of the disease, who received a course of injections of the preparation. This produced positive changes in the patients, and it became possible to carry out a course of X-ray therapy. Clinical recovery was achieved as a result. One of these cases was described by us in 1946. The observation was carried out by Dr. Bongard. Here is a brief account of this case.

OBSERVATION NO. 33

Patient K., male, aged 42 years. He was admitted for observation with complaints of severe pain on swallowing. There were large groups of very firm, painful nodes on both sides of his neck. His larynx was one complete, pitted tumour, involving every part of the larynx and in places markedly haemorrhagic. Histological investigation showed it to be a squamous-cell carcinoma. The Central Institute of Oncology had declined to carry out X-ray therapy because the condition was considered incurable.

Injections of the trypanosome preparation were started. They were

given every other day, directly into the tumour tissue, using a specially designed syringe with screw-on platinum needles. The patient received 16 injections. The individual doses were of 0.5 ml, then 1 ml and the maximum of 2 ml. The injections lasted for 2 months. After 16 injections the groups of nodes had become much smaller, pain had ceased, and the tumour had shrivelled and decreased in size; the arytenoid cartilages became slightly mobile, and haemorrhage ceased. The patient gained 2 kg in weight. In view of this improvement he was sent for X-ray therapy again and was accepted for treatment.

By 25 August, after treatment by Coutard's method, there was marked improvement in the larynx. The patient maintained good condition and gained weight.

After 2½ years the patient died from another condition. No macroscopical signs of cancer were found in the larynx on autopsy.

In this case injections of the preparation had caused diminution of the tumour. This enabled X-ray therapy to be employed after this had earlier given no hope of success because of the severity of the affection.

A similar observation was made by Prof. V. K. Trutnev, also relating to cancer of the larynx.

To these cases we should add observation No. 34, concerning the course of a melanosarcoma. In patient Ya., male, aged 22 years, one year after operative removal of a melanosarcoma of the right thigh a recurrence arose in the form of multiple growths in the region of the right thigh. A course of injections of the trypanosome preparation caused regression of a number of nodular growths. The regression process then stopped. The patient received a course of X-ray therapy, using massive doses. A temporary but definite improvement was obtained as a result. We do not suggest that these observations can permit any definite assessment of the combined method and so we shall not deal with them in more detail, but we consider it necessary to refer to them because they show the need for a special study of the combined method of tumour treatment.

The idea of the possibilities of combined bio-radiotherapy, arising from a number of clinical observations, also finds support in general considerations, in that by damaging cancer cells by any one means we may thus facilitate the effects of another factor.

If we now state in clinical terms all the defects mentioned in the production and use of the trypanosome substance, we have the following:

(1) In the observations described, the patients received *unknown dosages* of the preparation. The active principle varied according to the batch of the dose in proportions of *one to ten.*

(2) In the absence of a control technique, which at that period had not yet been devised, during a course of injections batches of inactive preparation were given as well as active batches.

Retrospective analysis shows that of any three injections at least one must have been of an inactive preparation.

Clinical observations on tumour regression in patients and comparison with the inhibitory effects of the preparation in animal experiments produced the following conclusion: *to obtain a positive therapeutic effect the patient must receive daily 100–200–300 units of a trypanosome preparation having an inhibitory index not lower than three.* The index of inhibitory activity is conditionally defined as the ratio of the average tumour weight (in this case of the Crocker sarcoma) in untreated control animals to the average tumour weight in animals treated for 15 days after implantation of the tumour. The human patient must therefore be given a dose 200–300 times greater than that needed to obtain a corresponding effect in cancer-affected mice. However, experience had to be gained in order to formulate this *basic requirement* of the preparation from the clinical aspect.

(3) In a number of cases, especially in 1948 and at the beginning of 1949, patients were given *excessively high doses of a chemically unrefined preparation,* which (as shown also by experimental analysis) destroyed the positive effect obtained earlier by using the same preparation in lower doses.

(4) The patients received the preparation *without any account* being taken of the intensity of the affection—the same techniques and doses were used both in extensive malignant neoplasms and in cases of more minor affections. Experiments show that a positive effect depends on the relationship between the mass of the malignant tissue and the degree of saturation of the body with the active regression factor.

(5) The technique of using the preparation had not been specially selected and for this reason it was, quite possibly, insufficiently effective.

(6) The preparation was used without any supplementary measures which might have facilitated its effects on malignant tumours. Only later was it shown that one such favourable factor is operative reduction of the tumour mass and also, possibly, the aseptic inflammatory process caused by biopsy or nonradical removal of the tumour. Administration of the preparation in combination with radiation therapy was also not employed, although there is some foundation for this, as seen in certain observations on cancer of the breast and larynx and melanosarcoma.

We have thus named the main defects in our observations. They are, indeed, very real. It would be correct to state that the clinical observations made with the aim of revealing the cancerotherapeutic effectiveness of the trypanosome preparation *were carried out under exceptionally unfavourable conditions*. Was it right to do this? Today, on looking back, we are able to state that experience has shown the justification of this early start of clinical observations, after animal experiments *lasting several years* had enabled this, and the *safety* of the preparation had been adequately demonstrated.

We may be liable to reproach in that clinical observations were made *simultaneously* with work on the preparation, and not *after* the production laboratory was able to start issuing a standardized active preparation. We have considered this point, and have come to the firm conclusion that with regard to *cancerous* disease such a situation would be *antiscientific* and would only lead to delay in solving the problems of cancer biotherapy. The reason which induced us to collect clinical data as early as possible was that cancer, by the nature of its course, demands long periods of observation, not less than several years. It is well known that until a control period of 3–5 years has elapsed there is insufficient verification of the effectiveness of any therapeutic measure. Indeed, some forms, for example mammary cancer, may recur at even longer periods after radical operations. Moreover, it is known that the frequency of recurrence decreases considerably after a year from the operation. Consequently, the earlier could observations be started on the first group of patients under conditions of *strict clinical control,* the sooner could we expect to decide the question of the therapeutic value of the biotherapy of cancer. Experience has shown the *accuracy* of this decision: it is *only for this reason* that we are now able to carry out a critical analysis of the clinical results achieved during the early stages of the experimental and production work. Naturally, at this stage of the investigation the *number* of positive results was not great. The deciding fact is that this clinical material enables us to establish the *principles* governing the course of the malignant process, which are *different* from those usually observed. These principles can be subjected, as will be shown, to experimental analysis, and form the basis of further developments in the method of cancer biotherapy. This clinical experience could certainly not have been replaced by observations on the course of transplantable tumours of laboratory animals, or even on the treatment of spontaneous tumours with properties most closely resembling those of human cancer, because the problem of biotherapy involves primarily the question of the practical convenience of using this method in the treatment

of human cancer, with all the particular concrete manifestions seen only in man and all the characteristics peculiar to the human body.

There is no doubt that all the weak points of the preparation could and did reflect on the quality of the clinical observations. But the clinical observations also reflected all the improvements made in the preparation. We were struck more than once by the conclusion of the clinicians regarding the quality of different modifications or batches of the trypanosome preparation. It should be emphasized that only coded preparations were used in the clinic. While observing the patient's reaction to administration of the preparation, the clinicians made regular reports on the symptoms of its effects on a patient's body and on the *concrete* details attracting attention during objective recording. A review of the clinical findings led us to an analysis of the experimental-production process, an analysis which on several occasions caused surprise at the *dependence* of the clinical efiects on the technique of preparing the product.

It must also be mentioned that one of the chief difficulties in the clinical observations was that they were carried out, as already stated, *under unfavourable conditions,* and *the main danger lay in taking too little account of all the defects.* We did try to assess them, and to remove them as they became solved experimentally. This work gradually became reflected in the clinical findings. If we had started clinical observations later we would hardly have been able to avoid many ineffective results. Also, no experiment can replace the time factor—a need which only clinical observations can satisfy. Not one animal experiment could have enabled us to draw the simple and clear conclusion formulated as the result of the clinical observations: "Malignant human tumours, irrespective of their histogenesis, undergo regression under the influence of microbial factors". And, finally, not one experiment could have made it possible for us to submit to the medical profession today a series of clinical observations showing that a malignant neoplasm has in fact undergone complete regression, as a result of which clinical recovery was achieved.

9. CONCLUSIONS

(1) In human cancer, a process of regression may be induced in tumours of a malignant nature by injecting into the body a trypanosome preparation possessing antiblastic properties.

(2) Clinical observations showed regression to occur under the influence of the trypanosome preparation in the following types of malignant tumours and precancerous affections of man:

1. cancer of the upper and lower lip,

2. cancer of the vocal cords,
3. cancer of the breast,
4. a neoplasm of the rectum clinically diagnosed as cancer and histologically as an adenomatous polypus,
5. cancer of the oesophagus,
6. angiosarcoma of the skin,
7. papillary adenoma of the breast,
8. leukoplakia of the buccal mucosa,
9. rapidly growing adenofibroma with enlarged regional lymphatic nodes.

(3) Histological studies showed that regression occurred in malignant tumours and precancerous affections of different *natures, morphology and localization:*

1. squamous-cell carcinoma,
2. keratinizing squamous-cell carcinoma,
3. basal-cell carcinoma,
4. angiosarcoma,
5. adenocarcinoma,
6. scirrhous carcinoma,
7. *precancerous* growths:
   papillary adenoma,
   leukoplakia,
   parakeratoses,
   hyperkeratoses.

(4) Regression could be induced:
(a) in the primary tumour lesion,
(b) in metastases,
(c) in recurrent malignant tumours.

(5). Regression of malignant tumours and precancerous affections occurs only when the preparation is present in the body and therefore ceases when injections cease. This makes it necessary to inject the preparation continuously until there is complete disappearance of the tumour and of the individual malignant cells.

(6). After regression was complete the malignant tumours did not recur and produced no metastases.

(7). Reduction of the tumour mass facilitated the positive influence of biotherapy and enabled complete regression of a malignant tumour to be achieved more rapidly.

(8). Under conditions of regular administration of the antiblastic biopreparation operative interference could be limited to removal of the tumour within the limits of the macroscopically affected area, as shown in a number of cases of carcinoma of the breast.

(9). A study must be made of the transition of inoperable forms of cancer to operable forms, as observed in patient G. (page 126).

(10). *Clinical* signs of regression of a tumour were shown in the following:

(a) *cessation of tumour growth* for periods varying from several months to several years;

(b) *decrease in tumour size,* as recorded on measuring it;

(c) *epithelialization of an ulcerated surface,* as established by clinical examination of the affected area;

(d) diminution or disappearance of regional metastatic nodes;

(e) a clear and considerable improvement in the general condition of the patient.

(11). The clinically apparent tumour regression caused by the antiblastic biological preparation was supported by changes in the histological appearance of the affection: the structure of the tumour altered, the number of mitoses decreased, and a number of the morphological features characteristic of the malignant cell were destroyed. The next section of the book is devoted to an analysis of these changes.

(12). In some cases tumour regression was complete in nature, when the regression process ceased with the formation of a scar at the site where the tumour had formerly been present. In other cases, using the technique adopted in the observations described, only temporary regression could be induced, which then gave way to the renewal of active tumour growth. The reason for this halt in tumour regression in such cases and the onset of a refractory reaction remains obscure. In some cases an undoubted part was played by the quality of the preparation used and the imperfections of the technique of using it. There is, however, no doubt that among malignant neoplasms there exist *different degrees* of sensitivity to the trypanosome factor.

(13). A positive effect was achieved with very low doses of the antiblastic substance: it is easy to calculate that during the injections *enough* of the active substance of the trypanosome cell was given, and in a whole series of cases the malignant tumour was either reduced in size or destroyed completely, leaving a scar.

(14). Regular administration of the trypanosome preparation over a number of years, in doses of up to 5000 units per injection, had no pathological effects on the internal organs and caused no organic or functional defects in them:

(a) cancer patients, in cases where injections of the preparation had given a positive result, gained considerably in weight—from 1–2 to 10–12 kilograms—during the period of the injections;

(b) blood examinations showed complete preservation of the physiological function of the haemopoietic organs: the blood formula remained without any pathological signs; the erythrocyte sedimentation reaction, which occurred more rapidly during the effects of the preparation, the leucocytosis which increased during the injections, and also some variations in the red cell picture seen during the injections—were all restored after the end of the course, however prolonged it had been, to the state peculiar to that individual during normal physiological functioning;

(c) regular urine examinations showed that the kidneys were functioning normally during the whole period of the injections;

(d) internal organs not affected by the cancer process—the liver, kidneys, gastro-intestinal tract, musculo-skeletal apparatus, nervous system and psychic sphere remained undamaged and showed no pathological signs during prolonged and repeated courses of injections extending over several years;

(e) when cancer patients were suffering from chronic *concurrent* affections, such as myocardial dystrophy, organic affections of the heart valves, compensated and with lst degree insufficiency, chronic gastro-colitides and hepatocholecystitides, *no exacerbation was seen*, in spite of prolonged administration of the trypanosome preparation for many months and even several years;

(f) biochemical investigation of the blood revealed no signs of tissue damage associated with administration of the trypanosome preparation.

(15) Observations show that the regression of malignant tumours and of precancerous affections bore a constant and regular relationship to the degree of saturation of the body with the antineoplastic fraction from *T. cruzi*. This relationship was shown both in the fact that cessation of injections led to a halt in regression, and in the influence exerted by the preparation according to its quality and dose: *regression could only be induced by a preparation from* T. cruzi *containing the antiblastic factor with an activity index of not less than three, when each injection involved a dose 200–300 times greater than the dose causing regression of the Crocker sarcoma in white mice.*

(16) With the observation of these *fundamental* requirements, relating: (a) to the activity index of the antiblastic trypanosome preparation, (b) to the dose, (c) to the regularity and uninterruptedness of the course of injections, the cancerotherapeutic effect described in this book is *regularly reproducible.*

But before we come to the description of these experiments and observations we must deal with a number of investigations carried out by French scientists, which we learned of when this book had already been printed (Moscow, 1957). These foreign investigations answered a question which was constantly before us and which cannot be avoided by any investigator working in a new field; to what extent are clinical observations repeatable and reproducible when a trypanosome preparation is used by *other doctors in the treatment of other patients with different forms of malignant tumours.*

10. THE WORK OF FRENCH INVESTIGATORS IN THE BIOTHERAPY OF MALIGNANT TUMOURS IN MAN WITH THE AID OF AN EXTRACT FROM *SCHIZOTRYPANUM CRUZI**

In France the first work in this field was by Professor J. Coudert (1956–1961); numerous clinical observations have been carried out by Spadotto (1958), Farah (1958), Gasiglia (1960) and Maire (1960).

It should be noted that before commencing his clinical observations Coudert checked both our previously published experimental results and our first reports on the possible clinical application of the extract from *Schizotrypanum cruzi*. This demanded a number of years of purposeful study. In their latest publication Coudert and his co-workers (1960) describe a method evolved by them for determining the antitumoral activity of lyophilized extracts from *Schizotrypanum:* for this purpose they made use of the degree of inhibition of mitosis in the cells of cultured malignant human tissues (strain K. V.)

On the basis of his observations on patients of advanced years to whom neither operations nor radiotherapy had brought relief, Spadotto concludes that the most consistent therapeutic effect of the use of the trypanosome extract is a favourable effect on the pain caused by malignant tumours. This favourable effect is expressed, as a rule, after 3–7 days of treatment and does not depend on the type of tumour, and it applies not only to the primary tumour but also to metastases in the abdomen, lymphatic nodes or skin. This effect was absent if the pain was caused by secondary infection or arose from burns produced by radiation therapy.

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* This Section has been written by the authors for the English edition.

This effect on pain is shown so consistently that it enables the use of narcotic preparations to be suspended in cases where cancer patients had previously received them. Moreover, addiction has never been observed.

Together with the removal of pain there is a definite favourable effect on the general condition of the patient. In patients who had long been seriously ill and who ate with very great difficulty, appetite was renewed, the body-weight rose by several kilograms and the patients' strength was progressively restored. Apathy gave way to a feeling of well-being.

Taking into account that the preparation is quite harmless, Spadotto suggests that the trypanosome extract must occupy a definite place among the therapeutic means employed in the treatment of malignant tumours.

We include here just a few of the 100 cases described by Farah.

OBSERVATION NO. 13.

Patient S., female, aged 75 years, entered the hospital on 21 January (1958). *Icterus.* Her general condition was poor, with abdominal pain, occasional vomiting, increasing anorexia, emaciation and blood in the urine. Examination revealed a firm tumour in the epigastric region.

Blood analysis: red cells 2,300,000, leucocytes 6,900. Erythrocyte sedimentation rate: 65 (1 hour), 105 (2 hours).

Radiography: a lesion occupying one third of the lesser curvature of the stomach, with mediogastric stenosis.

Treatment: liver extract; trypanosome extract in doses of at first 2 and then 3 ampoules daily.

Within 5 days appetite was restored, the pain had completely abated and there was no sign of jaundice. There was no blood in the urine.

Blood analysis: red cells 3,300,000, leucocytes 7,200.

The patient was discharged on 29 March 1958 in splendid health. Injections of the trypanosome extract were continued at her home.

OBSERVATION NO. 23.

Patient U., female, aged 75 years, with cancer of the stomach, had lost 15 kg in weight, with aesthenia, anorexia and anaemia.

Radiography: a neoplasm of the antral region — a large tumour.

After injections of the trypanosome extract for 4 months: the pain had disappeared, appetite returned, the patient's general condition improved and she put on weight.

Radiography showed improved mobility of the stomach.

OBSERVATION NO. 28.

Patient V., male, aged 52 years, had a carcinoma of the tongue, confirmed by histopathological diagnosis.

He had been ill for 6 months, with enlargement of the submaxillary nodes. Combined treatment with radium and the trypanosome extract was carried out.

First of all the pain disappeared. Later there was cicatrization of the lesion and regression of the adenopathy, which had not been subjected to any kind of local treatment.

OBSERVATION NO. 30.

Patient Dr. S., male. Spindle-cell epithelioma of the epiglottis. Swollen lymphatic nodes in the carotid region. Painful dysphagia. The clinical diagnosis was confirmed histopathologically.

Radiotherapy gave no positive results.

Under the influence of injections of the trypanosome extract there was disappearance and cicatrization of the ulceration, disappearance of the lymphatic nodes and only slight dysphagia remained. The neuralgic pain experienced earlier was no longer observed.

OBSERVATION NO. 31.

Patient S., female. Mammary carcinoma with extensive ulceration, numerous bone metastases, especially in the femur, with severe pain. Bremer's toxoid treatment was followed by implantation of an oestrogen, without result.

Injections of the trypanosome extract were then prescribed. Under the influence of the injections the pain vanished, development of metastases ceased and by 27 December 1949 the patient's general condition had improved; the breast ulcers showed a tendency to cicatrization.

3 February 1950: the ulcers had healed, and the femur showed progressive recalcification.

28 February 1950: cicatrization of the breast.

In October 1950: examination revealed no signs of tumour development and the patient's general condition was good.

OBSERVATION NO. 37.

Patient V. Carcinoma of the *cervix uteri,* recto-vaginal fistula. Earlier, in 1952, she had received radium and X-ray therapy, In 1955 — laparotomy, with subsequent deterioration.

During 1956 and 1957 regular injections of the trypanosome extract were given.

Results: (1) marked reduction of uterine discharge; (2) narcotics were discontinued; (3) the mucous membranes, previously grey, became pink; (4) the enlarged lymphatic nodes disappeared; (5) general condition was good; (6) there was considerable gain in weight.

However, the malignant tissue was not completely cicatrized.

OBSERVATION NO. 39.

Patient V., female. Carcinoma of the left tonsil, involving the larynx, with a large lymphadenitic node. Treatment with the trypanosome extract began on 28 February 1955 and lasted for 7 months (with intervals). Results: (1) Reduction of the oedema of the wall of the buccal cavity; (2) Reduction in the thickness of the tongue; (3) Complete disappearance of the lymphadenitis; (4) the pharynx was completely cicatrized; (5) the

enlarged lymph nodes disappeared; (6) there was an improvement in the general condition of the patient and (7) her appetite was restored.

OBSERVATION NO. 47.

Patient V., aged 81 years, was operated on in September because of a carcinoma of the prostrate. In December a tumour appeared in the right testicle and enlargement of the lymphatic nodes occurred; the tumour was ulcerated, with a haemorrhagic exudate. Under the influence of injections of the trypanosome extract the wound became cleaner, the inflammation decreased, pain disappeared and the lymphatic nodes were reduced in size.

Later, the tumour grew considerably smaller. The lymphatic nodes disappeared almost completely. The right testicle is still larger than the left, however. The patient's general condition is excellent and he is no longer suffering.

OBSERVATION NO. 59.

Patient M. Inoperable carcinoma of the *cervix uteri.*

After 60 injections of the trypanosome extract there was marked diminution of the tumour and a positive effect on the haemorrhage. Distinct improvement in the general condition was seen, with restoration of sleep and appetite.

OBSERVATION NO. 82.

Patient A., male, aged 47 years, had had cancer of the liver since 1955, with severe emaciation and frequent vomiting. The patient had lost 16 kg in weight. The clinical diagnosis was confirmed histopathogically (biopsy).

After 60 injections of the trypanosome extract: (1) the vomiting ceased; (2) appetite was renewed; (3) the size of the liver was reduced; (4) the surface of the liver became smooth and not irregular, as it had been before treatment; (5) the patient gained 16 kg in weight; (6) his general condition was good.

Observations on the effects of the trypanosome extract on inoperable cancers of the stomach are described by Dr. Maire (1960).

FIRST OBSERVATION

A patient aged 68 years was admitted on 28 February 1954 because of cancer of the stomach. He was cachectic, and nearly all his food suffered vomition. The patient had already endured this for 6 months. The first radiograph showed destruction of one third of the stomach and its lumen. Clinical diagnosis was of a spongy tumour in the epigastric region, without any adenopathy.

The radiographic and clinical findings did not enable operative interference to be considered, especially as the patient categorically refused any operation. He was given a prescribed diet and directed to use daily intramuscular injections of lyophilized trypynosome extract.

After the first series of injections (March 1954) the patient was suffering less; he could eat various purées and sweet stewed fruits, and vomiting became much rarer.

After an additional two-months treatment (April and May) the patient had gained 2 kgi n weight. A second radiograph showed the presence of a large tumour embracing

the greater curvature of the stomach but leaving a definite passage through which fluid food could pass. After seven months of such treatment clinical improvement was quite definite but the patient could not be persuaded to undergo operation. He could go out for walks, and he was quite contented with his lot. He was no longer suffering, and was not troubled by vomiting as long as he did not take solid foods. In October: the patient died from influenza within 3 days.

SECOND OBSERVATION

Patient V., male, aged 47 years, sought a consultation regarding his very poor general condition and very severe epigastric pain. The patient had long suffered from dyspepsia, for 6 years. Clinical examination revealed a spongy tumour occupying the whole epigastric region. Radiography showed an enormous tumour embracing the greater curvature of the stomach and occupying its anterior wall. The surgeon could only sew up the opened lumen, since the tumour could not be extirpated, together with the affected lymphatic nodes (histopathological diagnosis — *tumeur epitheliomateuse*).

After the exploratory operation the patient received injections of lyophilized trypanosome extract. He survived for 6 months, without experiencing any pain, and taking light food; some gain in weight was observed. This patient died from cachexia, without having suffered severe pain and without resorting to morphine; he received 20 injections per month.

Dr. Maire compares his observations with two similar observations by Coudert and Farah on cancer of the stomach, and concludes that "the trypanosome extract not only relieves pain in far advanced cancer of the stomach, but its use enables the realization of a previously impossible surgical operation and, perhaps, avoids recurrences arising from microscopic metastases invisible on operation." Maire considers that this preparation "is absolutely indicated for treatment after all operations for cancer of the intestinal tract".

Gasiglia (1960) gives a detailed description of a case where an ulcerated mammary carcinoma was treated with the trypanosome extract.

14 June 1959. Patient L. M., aged 75 years, was admitted to the women's surgical ward with an enormous ulcerated carcinoma of the left breast. The tumour extended from the axillary region to the navel, occupying the anterior and lateral parts of the thoracic cage. The tumour was secreting a serous fluid, blackish in colour, with an intolerably foetid odour. The patient's skin was a straw-yellow colour, her temperature 37.9° C. She was in a subcomatose condition and delirious. Her condition was considered hopeless. The intolerably foetid wound compelled transfer of the patient from the general ward to a single room. Measures were taken to combat the patient's state of shock. Her temperature rose to 40.2°C.

Histopathological examination of a fragment of the tumour showed an infiltrating atypical epithelioma of the glandular type, consisting of fused agglomerates of polygonal cells with a clearly expressed mitotic activity. The surrounding stroma and neoplastic elements were impregnated with a substance of mucoid nature.

Blood formula: red cells 3,000,000; leucocytes 17,000; neutrophils 80 per cent, eosinophils 2 per cent, small lymphocytes 13 per cent, large lymphocytes 3 per cent and monocytes 2 per cent.

Erythrocyte sedimentation rate: 1 hour—18 mm, 2 hours—38 mm, 24 hours—180 mm.

Radiographs of the lungs and vertebral column showed no apparent metastases.

The patient was excitable, aggressive and delirious. Morphine injections quietened the patient, with some difficulty.

16 June. Treatment with intramuscular injections of the trypanosome extract commenced.

17 June. The temperature has fallen to 37°C. The patient is in a very depressed condition.

18, 19 and 20 June. The patient is dying. She does not eat or drink. A comatose condition ensues.

22 June. The patient comes out of the coma. She starts to drink. There is a change in her behaviour: the irritability and aggressiveness characteristic of the earlier days have gone.

23 June. The patient is suffering less. Slight activity is shown.

25 June. The discharge (from the tumour) has become less profuse, and the foetid odour less strong.

26 and 27 June. No injections were given.

28 June. Injections resumed. Some days later the patient starts to eat and refuses injections since the pain has disappeared. The temperature remains around 37°C. One important fact: the patient states that she has lived alone in a hut, has never been to a doctor and has never received any kind of treatment.

8 July. The patient's condition is satisfactory. Her appetite is good. She eats a normal diet. The foetid odour has almost completely disappeared. The discharge from the tumour has almost completely ceased.

18 July. Erythrocyte sedimentation rate: 1 hour — 10 mm, 2 hours — 24 mm, 3 hours — 24 mm.

The patient's condition is good and her weight has risen from 40 kg (at the time of admission to the hospital) to 43 kg. Injections of the trypanosome extract are being continued. There was a distinct and considerable reduction in the size of the tumour. The patient's general condition remains good but there is some increase in the foetid odour.

14 September. Blood formula:

red cells	— 3,820,000
leucocytes	— 10,000
neutrophils	— 73 per cent
eosinophils	— 2 per cent
small lymphocytes	— 16 per cent
large lymphocytes	— nil
monocytes	— 9 per cent.

Erythrocyte sedimentation rate: 1 hour — 23 mm, 2 hours — 50 mm, 24 hours — 81 mm.

General condition remains satisfactory. The patient's weight has reached 45.5 kg. However, the return of the foetid odour and the increased erythrocyte sedimentation rate must lead to fears of intensification of the cancerous process.

A fresh radiograph of the lungs and vertebral column again shows no apparent metastases.

Because of these findings, and taking account of the considerable reduction in the volume of the tumour, *which had now become operable*, it was decided to perform an operation. The patient had received in all 80 intramuscular injections of the extract.

The operation was carried out on 19 September, under general anaesthesia.

Operative procedure. The tumour was removed with great ease. The technical simplicity of the operation permitted the simultaneous removal of the axillary lymphatic nodes. The latter were not adherent to the axillary vein. The major pectoral muscle was preserved. Haemostasis was simple. Skin sutures were applied, with a drainage tube. The operation was simple and rapid. Blood plasma and serum with glucose was given intravenously. The patient stood up to the operation very well. The postoperative period was uncomplicated. The evening temperature was 37.8°C. Until 22 September the morning temperature was 37.5°C, the evening—38.5°C, then the temperature became normal.

23 September. Injections of the trypanosome extract resumed.

Blood formula:

red cells	— 2,900,000
leucocytes	— 14,400
neutrophils	— 92 per cent
basophils	— 1 per cent
lymphocytes	— 7 per cent
Anisocytosis, anisochromia.	

Erythrocyte sedimentation rate: 1 hour — 56 mm, 2 hours — 120 mm, 24 hours — 144 mm.

The general condition of the patient is excellent. She has a good appetite, and is happy. Nothing abnormal has been noted on subsequent days after the operation. Scar-formation is taking place normally; sutures were removed on the eighth day.

Treatment with the trypanosome extract is being continued, with regular blood analysis.

20 October. Blood formula:

red cells	— 4,620,000
leucocytes	— 8,200
neutrophils	— 58 per cent
basophils	— 2 „ „
eosinophils	— 8 „ „
small lymphocytes	— 31 „ „
monocytes	— 1 „ „
haemoglobin	— 85 „ „
colour index	— 0.9 „ „

21 October. Erythrocyte sedimentation rate: 1 hour — 12 mm, 2 hours — 32 mm, 24 hours — 92 mm.

13 November. Erythrocyte sedimentation rate: 1 hour — 10 mm, 2 hours — 26 mm, 24 hours — 84 mm.

10 December. There is a constant rise in the number of red cells up to 4,800,000 (10 December 1959); this process is taking place without the use of any measures stimulating and supplementing erythropoiesis. With regard to leucocytes, their numbers have returned to normal (at the start of treatment the count was 17,000 and on 2 July 6,000). Then there was a rise to 14,000 (23 September), after which the leucocyte count became stabilized at the normal figure.

16 January 1960. Blood formula:

red cells	— 5,000,000
leucocytes	— 10,200
neutrophils	— 65 per cent
eosinophils	— 1 „ „
basophils	— 1 „ „
small lymphocytes	— 14 „ „
large lymphocytes	— 12 „ „
monocytes	— 7 „ „

Erythrocyte sedimentation rate: 1 hour — 7 mm, 2 hours — 18 mm, 24 hours — 82 mm.

3 February 1960. General condition outstanding, weight 50 kg.

In conclusion, Dr. Gassiglia writes: A study of this observation enables a number of conclusion to be drawn. Naturally, one cannot speak of a cure and we have not had a period of observation long enough for this, but the following points may be established:

1. The patient, who had entered the hospital in a comatose, hopeless condition, reacted positively to the treatment from the very first days.

2. The pain disappeared after a few injections and it was possible to do without the morphine and dolozal which had with some difficulty relieved the patient's suffering.

3. The haemorrhagic and foetid serous discharge rapidly grew less under the influence of injections of the trypanosome extract.

4. The patient was quickly brought out of her comatose and delirious state; her physical and mental condition showed progressive improvement; appetite was restored and the patient's weight rose steadily.

5. The volume of the tumour was markedly and quite indisputably reduced.

Significantly, no signs of intolerance or intoxication were observed.

The haematological observations show a decrease in the leucocytosis and in the erythrocyte sedimentation rate. Also, the anaemia gradually improved in the process of treatment.

It is quite obvious that treatment with the trypanosome extract enabled the surgical operation to be carried out both as a result of the decrease in volume of the tumour and because of the unexpected improvement in the patient's general condition.

As a result of the treatment the tumour was clearly delineated, which facilitated its removal. In this case, as in many others, treatment converted an inoperable form into an operable one.

"This graphic observation was chosen"—writes Gasiglia—"as being exceptionally demonstrative. This single observation gives outstanding

support for the observations already published and emphasizes the fundamental effects of the trypanosome extract".

Coudert was able to observe about 200 cancer patients who had received the trypanosome extract and to analyse their case histories. One of them is given below.

Patient M. B., male, aged 54 years. Epithelioma of the laryngo-lingual furrow. The disease had commenced in February 1954 with chronic hoarseness. 16 July 1954 — biopsy. Radium therapy from 25 to 28 July, then X-ray therapy (35 sessions). X-ray dermatitis. Loss of weight by 4 kg in 2 months. From 25 October 1954 to 27 September 1955: persistent ulceration of the mucosa, pain on swallowing, disturbances in deglutition, poor general condition, progressive emaciation and loss of strength. Localised induration of the sublingual region; the mucosa was ulcerated and roughened.

Treatment with the trypanosome extract in series of 15 injections with 8-day intervals started on 27 September 1955. During this period there was improved deglutition and less pain, reduction of the oedema, cicatrization of the ulcers, which became painless, restoration of sleep and appetite, and a gain in weight.

On 20 December 1955, after 3 months of treatment: cicatrization of the affected mucosa and considerable reduction in the infiltration of the sublingual tissues. The patient had reached his normal weight; his general condition was good. Treatment was continued.

After 13 months of treatment (28 October 1956): the patient was in good condition, could drink and smoke, took exercise and had been on a journey by rail. He slept and ate normally; the lymphatic nodes were normal. There was sporadic moderate pain at the root of the tongue, which could be controlled by aspirin.

In summing up the treatment of various forms of malignant neoplasms Coudert comes to the conclusion: tumours of epithelial origin are more sensitive than sarcomata, especially the less mature ones. The first group, most sensitive to treatment with the trypanosome extract, includes carcinomata of the breast, ovary, uterus, intestinal tract (stomach, oesophagus, rectum) and also epitheliomata of the face, tongue and larynx. The Grawitz kidney tumour was also very sensitive to this method of treatment in a number of cases. Among the sarcomata, osteosarcomata are only slightly susceptible to it, though here also there is some analgesic effect and a relative decrease in the tendency of a tumour to spread. With melanosarcomata visible arrest of tumour development is sometimes achieved, but at the same time there are cases where no results at all are shown.

"An effect on the general condition of the patients was shown in almost all the cases and was expressed in many ways: gain in weight, restoration of appetite, renewed activity. This effect was in no way associated with any psychotherapeutic effect on the patient, since it was often observed in patients who did not know the nature of their illness or the purpose of the treatment given."

Coudert considers that during the course of treatment with the trypanosome extract one must not neglect supplementary methods of treatment directed against complications of the cancerous disease—against infection. In these cases also treatment with the trypanosome extract should not be interrupted. Measures directed at improving the potential defensive reaction of the body are absolutely essential, and for this reason vitamin C, the group B vitamins, magnesium salts and amino-acids should be given as supplements to the dietary regime.

Quite understandably, the first observations of the French investigators were carried out in patients with far advanced malignant affections, when all the methods of treatment normally employed had given no results. However, Coudert was able to observe the effects of the trypanosome extract on small malignant tumours that had not been subjected to any kind of previous treatment. These involved three patients with neoplasms of the tongue who had refused to undergo a disfiguring operation. In these patients, under the influence of the trypanosome extract an effect was observed which deserves *particular attention*, especially as the question of the number and size of the doses and the duration of the use of the trypanosome extract in cancer of the tongue is in the very early stages of development. Under the influence of treatment with the trypanosome extract in one patient an *apparent cure* was observed, but after 2 years there was a recurrence — then the patient underwent treatment by classical methods, with a fatal outcome. The second patient broke off the observations at a time when there was *primary cicatrization of the cancer lesion.* The third patient, writes Coudert, was cured and there have been no recurrences *in the course of the 6 years he has been under observation.* In conclusion, Coudert states that the extract from *Schizotrypanum* has the advantage that it is quite free from toxicity for normal tissues, in particular for haemopoietic tissue.

There is no need to stress that the observations of the French investigators not only confirm our observations but also supplement them, creating a wider basis for further clinical and experimental research.

The results of the clinical observations give rise to two problems:

(1) the need for *the development of methods of treating cancer* with the aid of antibiotics, since the automatic adoption into oncology of methods used in the treatment of infectious diseases by antibiotics can hardly be very fruitful;

(2) the need to employ all the achievements of microbiology, chemistry and technology used in the manufacture of antibiotics for the further improvement of the preparation from *Schizotrypanum*, since the lyophilized

extract is only the first prototype of what may be obtained from cultures of this mircobe.

We should be surprised not that one or another clinical effect was achieved in the treatment of cancer, but that this effect was obtained *on the first attempts at clinical application.*

* * *

The concepts stated here are further substantiated and developed in later sections of the book, dealing with the cytological and histological analysis of the changes occurring in malignant tumours under the influence of biotherapy, and also with experiments aimed at revealing the mode of action of the trypanosome antibiotic on cancer cells.

Part V

HISTOLOGICAL AND CYTOLOGICAL CHANGES IN MALIGNANT TUMOURS TREATED WITH THE TRYPANOSOME PREPARATION

OUR histological analysis and the conclusions arising from it are based on two main groups of observations:

(a) changes in human tumours under the influence of the trypanosome preparation;

(b) changes in tumours of experimental animals under the influence of biotherapy.

One should not overestimate the possibilities of the classical histological, i.e., strictly morphological method in assessing the changes occurring in tumours under the influence of any therapeutic agent if these changes are in the early stages. Only in cases where a powerful effect on the tumour leads to very marked general morphological changes can the histologist give *confirmation*. In all other cases the histologist must *convince*, using all the results obtained by direct observation and appropriate experiments.

This situation is explained in that:

firstly, in any tumour we may see cells in the most varied states, from actively growing and multiplying cells to cells whose life-processes have been suspended and which are in various stages of degeneration and necrosis — their numbers are infinitely variable in different malignant tumours;

secondly, the histological appearance may differ in different parts of a malignant tumour and more so at different phases of its development.

All this is well enough recognized. For this reason, the difficulties in solving the problem are many. However, a combination of the morphological method with histophysiological and microchemical methods may increase to a considerable extent the results achieved from the investigation, provided that microscopical analysis is an integral part of the experimental work and clinical experience, and is not divorced from them. We did, in fact, try to carry out our investigations within this frame.

As already stated, all the difficulties in histological and cytological analysis relate to the initial period in the process of tumour regression, when the usual staining methods are unable to reveal any changes and most histochemical reactions are not very indicative. A similar situation applies to a great extent in later phases, when the process of destruction of the cancer tissue only just prevails over the typical processes of growth and development of malignant tissue, and the tumour still consists of a mosaic of cells whose development is proceeding in different, often dianetrically opposite directions.

In order to draw all the possible conclusions from our anylasis of the material provided by clinical experimental work, we took into account, besides the general histological changes, the following factors:

(1) the reactive development of connective tissue;

(2) the macrophage reaction, and also the lymphocyte and monocyte (histiocyte) reaction;

(3) karyological changes:

(a) changes in thymonucleic acid,

(b) changes in the nucleus-nucleolus relationship

(c) changes in nuclear size,

(d) changes in the mitotic index;

(4) nucleo-cytoplasmic changes (Komuro's method): the demonstration of ageing and damaged cancer cells;

(5) changes in ribonucleic acid, the most important factor in cell growth and division;

(6) changes in the lipoid and cholesterol index of the cancer cells.

The following tests were used as auxillary histochemical tests (7) for glycogen: (8) for vitamin C; (9) for oxidases, peroxidases, oxidoreductases, succinodehydrases; (10) for arginine (histones); (11) for glutathione.

Finally, in a number of cases we carried out planimetry of growing and degenerating areas in tumours. The combination of histological, histochemical and cytological methods enabled us to consider the earliest changes in tumour tissues and also to probe to some extent into the mode of action of the trypanosome preparation on the cells of malignant neoplasms.

1. OBSERVATIONS ON CLINICAL MATERIAL

For our study of the histological and cytological changes occurring in human malignant tumours under the influence of biotherapy we were able to use material obtained from patients in various stages of the development of a cancerous process:

(a). During the period when treatment with the trypanosome preparation had no objectively recordable therapeutic effect, or when this effect was transient, as the result of which the patient underwent radical operation.

(b). During the phase of *cessation of growth*, when a tumour which had previously been showing progressive enlargement stopped its active development and appeared to enter a phase of equilibrium, with neither growth nor regression of the tumour. Clinical observations showed that such a state, once created by injections of the trypanosome preparation, may be extremely prolonged.

(c). During the phase of *regression* induced by the effects of the trypanosome preparation, at various stages in the destruction of the malignant tissue and the regeneration of normal tissue.

A. CYTOLOGICAL ANALYSIS OF TUMOURS IN PATIENTS IN WHOM A POSITIVE EFFECT OF TREATMENT WAS NOT ESTABLISHED CLINICALLY OR WAS NOT SUFFICIENTLY WELL SHOWN

We decided first of all to submit to histological analysis tumours in the fairly large group of patients where we were obliged to resort in our assessments to vague and ambiguous definitions such as "suppression of tumour growth", "temporary cessation of tumour growth", "clinical results of treatment not clear", etc. It is well known how much subjective material is used, willingly or unwillingly, by investigators in making similar assessments, how little these provide the reader with any well-founded hopes, and how many justifiable doubts there must be. For this reason we set out to find, among the many cytological methods, those which could be relatively easily performed and which would enable us to give an *objective estimate* of the effects of the trypanosome preparation on malignant cells. In evaluating the processes occurring in tumours under the influence of the trypanosome preparation, one must remember that the transformation from normal cell to malignant cell is associated with profound biological changes in a number of its properties, some of which are relatively easily demonstrable by cytological methods.

The conclusions drawn from many studies on the biology of cancer cells enable us to assume that the malignization of cells is reflected in changes in a certain complex of cytological and cyto-physiological indices: (1) in an increase in nuclear size and variability; (2) in nucleolar hypertrophy and breakdown of the nucleus-nucleolus size relationships; (3) in a marked rise in the number of mitoses. There is insufficient evidence to show that each of these signs, taken individually, is specific to malignant cells, but all these signs, considered in combination, permit the observer,

as will be shown, to follow the *initial changes* in the biological properties of cancer cells. Starting from these principles, we carried out an investigation of tumours in 19 patients who had been treated with the trypanosome preparation but who, according to the clinicians, did not show enough evidence to permit any final decision as to whether the trypanosome preparation had in these cases had any particular effect on the malignant tumour. To answer this question, the observations described below must be considered.

(1). Patient K-an. On admittance: in the external upper quadrant of the left breast there were two firm growths measuring 2.5 × 2.0 cm and 0.75 cm in diameter. The left axilla contained two firm lymphatic nodes measuring 1 × 0.75 cm and 2.5 × 1.5 cm. Clinical diagnosis: *cancer of the breast, stage II.* Biopsy; histological diagnosis: *solid carcinoma.* In this case, as in all the cases to be described, injections of the trypanosome preparation were started at the time of the biopsy. After 2½ months of treatment the tumours measured 3 × 3 cm and 1 cm in diameter. A second biopsy was carried out, but histopathological analysis revealed no noticeable changes in the tumour tissues. After another 9 months a nodule measuring 0.5 × 0.75 cm was excised, being suspected of malignancy. Histopathological findings: solid carcinoma with areas of a scirrhous nature. Hence, in this patient neither clinical observation nor histological investigation of three successive biopsy samples provided any means of answering the question: did injections of the trypanosome antibiotic have any influence on this case? However, a study of the cell nuclei showed that during the year of treatment there was a regular decrease in nuclear dimensions (Fig. VII) and simultaneously a drop in the number of dividing cancer cells (observation No. 35).

Date	Average size of nucleus (planimetric units)	No. of mitoses per 100 fields
28 March	17.9	18
1 July	16.6	12
One year after treatment started, on 22 March	12.4	In some parts of the excised tumour 5, in others 3.

These two significant indices characterizing the state of malignant cells prove that the trypanosome preparation was having an objectively measurable effect on the malignant breast cells in patient K.

(2). Patient K-ova. Examination on 7 April showed: the right breast contained a thickening 3.5 × 2.5 cm in size; at the centre of the thickening was a nodule of cartilaginous consistency measuring 1.5 × 1 cm; both axillae contained lymphatic nodes measuring 0.5 × 0.75 cm. Clinical

diagnosis: *cancer of the right breast, stage I-II.* A biopsy was performed on 19 April, involving excision of one quarter of the tumour. Histological diagnosis: *solid carcinoma with a background of fibro-cystic mastopathy.* After 2 months (13 June) examination of the patient showed: in the upper

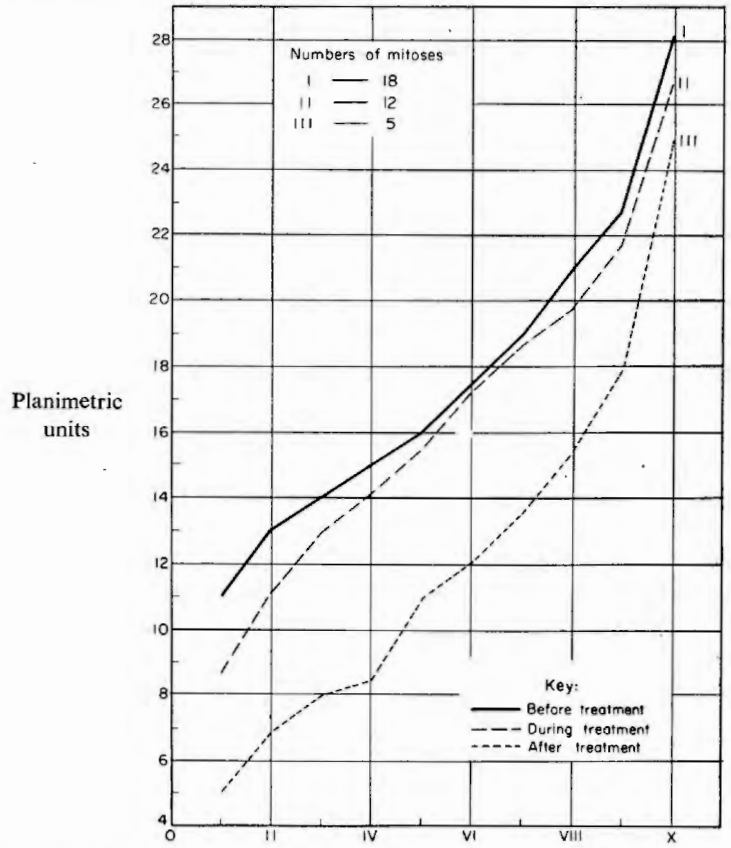


FIG. VII. Changes in nuclear size in the tumour in patient K-an during treatment with the trypanosome preparation.

half of the scar on the right breast there was a thickened area 4 × 3.2 cm. The upper portion of the thickening contained a more distinct nodule 2 cm in diameter. Both axillae contained firmish lymphatic nodes 0.75 cm in diameter. Two months later (24 August), the tumour in the right breast was somewhat smaller than at the previous examination, measuring 2.5 × 3.25 cm; the axillary lymphatic nodes were of a soft consistency. On 2 September a nonradical operation was performed—excision of the skin scar in the right breast. The thickened portion round the scar was

removed. Histological diagnosis: solid carcinoma. It may be queried whether the trypanosome antibiotic had shown any effect on the malignant cells of the solid carcinoma in this case. Cytological analysis showed that under the influence of this treatment the number of mitoses fell slightly from 17 per 100 fields of vision to 13, with a concurrent slight decrease in nuclear size from 13.6 to 12.2 planimetric units. Favourable changes in the state of the tumour were shown (Table 1) by other quantitative indices (Q%, Σ, C%, M_0); finally, there was a considerable rise in the nucleus-nucleolus ratio. This shows that certain favourable changes took place in the cells of a solid carcinoma under the influence of the preparation. The patient continued to receive injections of the preparation, and an examination after 10½ months (22 February) showed the scar to be in a satisfactory state, with no apparent areas of thickening. The lymphatic nodes in the left axilla had disappeared; the node in the right axilla was mobile and measured 0.5 × 0.75 cm (observation No. 36).

(3). Patient P-ova. Examination on admittance showed: in the right breast there was a thickened area of 4 × 3.5 cm; at the centre of this area was a thickening of fibrous consistency, 1.5 cm in diameter; the right axilla contained a lymphatic node measuring 1 × 0.5 cm, and the left axilla a node 0.5 cm in diameter. Clinical diagnosis: *cancer of the right breast, stage II* (3 February). On 10 February a biopsy was carried out, with excision of a tumour fragment measuring 0.5 × 0.5 cm. Histological diagnosis: *solid carcinoma.* As in all the other patients, treatment with the trypanosome preparation was started at the same time as the biopsy. The size of the tumour remained unchanged (4 × 3.5 cm). After 22 days from the start of the treatment partial resection of the gland parenchyma, together with the tumour, was carried out. A lymphatic node 1 cm in diameter was removed at the same time. Histopathological findings from the excised tumour—carcinoma, partly solid, partly scirrhous in type. Cytological analysis showed that under the influence of even this short period of administration of the preparation noticeable changes had occurred in the cells of the malignant tumour. The size of the nuclei had decreased markedly—the average nuclear size, at the first biopsy was 12.7 planimetric units; after using the preparation for 22 days it was 10.2 The number of mitoses had decreased from 35 to 18. Favourable changes in the state of the tumour were shown (Table 1) by other quantitative indices (Q%, M_0) a rise in the nucleus-nucleolus ratio and a shift to the left in the nuclear size variation curves in the cancer cells (Fig. VIII). Later examinations extending for 13 months revealed no signs of recurrence or metastases. Lymph nodes were not palpatable and scar-

formation was proceeding favourably. During the 13 months of treatment the patient, who had had cancer of the breast, stage II, with obvious axillary lymphatic nodes, and who had undergone a nonradical operation, was considered healthy — for the time being, of course. This situation remained unchanged at the end of 27 months (observation No. 37).

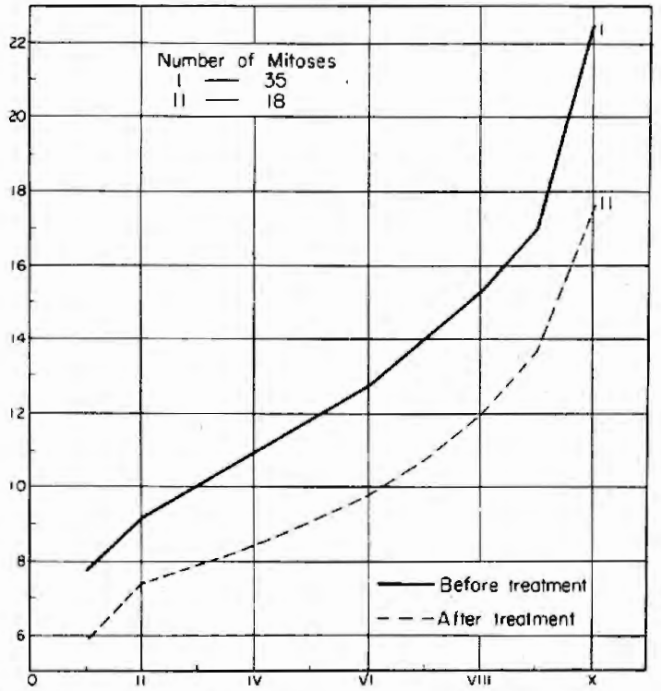


FIG. VIII. Changes in nuclear size in patient P-ova's tumour in the process of treatment with the trypanosome preparation.

(4). Patient R-ich. Clinical diagnosis: *cancer of the left breast, stage II–III.* Biopsy: excision of lymphatic nodes from the left axillary region; histological diagnosis: *solid carcinoma with areas of necrosis.* After one month of treatment, an operation was performed: removal of the tumour nodule. Histological diagnosis: multiform carcinoma with alternating areas of a medullary and scirrhous character. What changes took place in the cells of this far-advanced tumour as the result of one month's treatment with the trypanosome preparation? Cytological observations showed a decrease in nuclear dimensions from 32.9 to 27.6 units and a decrease in the nucleoli from 2.5 to 2.0 units. Simultaneously there was a rise in the nucleus-nucleolus ratio in the tumour cells (from 13.2 to 13.8). There were thus a number of cytological signs showing objectively the influence

of the trypanosome preparation on the properties of the cancer cells, resulting in definite changes in the cytology of these cells which, taken in combination, must be assessed as positive moves (observation No. 38).

(5). Patient Shch-eva. Examination revealed a firm node 2.5 × 2.5 × 3 cm with a spine-shaped projection in the left breast, against a background of fibrous mastopathy. Clinical diagnosis: *cancer of the breast, stage II.* A biopsy was performed on 21 May. Histological diagnosis: *carcinoma, in some areas medullary and some areas scirrhous in character.* Examination of the patient 3 months later showed: in the area around the scar in the left breast there was a thickening measuring 4 × 3 cm; at this time there were lymphatic nodes in each axilla, 2 on each side, measuring 0.75 × 0.5 cm. On the day following this examination the patient underwent an operation for excision of the tumour nodule as far as the macroscopically normal tissues. Histological analysis of the excised material revealed multiform carcinoma, with a preponderance of scirrhous areas. The question naturally arises: would cytological analysis enable us to establish any changes in the state of the malignant cells under the influence of biotherapeutic interference? Observations showed that the following changes occurred in this patient's tumour: there was a decrease in nuclear size from 9.9 to 7.4 units and a fall in the number of mitoses from 13 to 7. These findings show that the operation—removal of the tumour as far as the macroscopically normal tissue—was carried out at a time when cytologically favourable changes were seen in the tumour, expressed both in diminution of the nuclei and in a decreased number of mitoses. Further observations on this patient revealed no suspicious symptoms for 10 months. The post-operational scar remained in a satisfactory state, no thickened areas could be determined in the breast and the lymphatic node present before the operation was no longer palpatable. These findings permit us to assume that the trypanosome preparation had a definite effect on the course of the malignant process in patient Shch-eva.

(6). Patient O-gova. On admittance: the left breast contained a tumour measuring 3.4 × 1 cm; in the left axillary region there were several firm lymphatic nodes. Clinical diagnosis: *cancer of the left breast, stage II.* A biopsy was carried out on 8 March. Histological diagnosis: *adenocarcinoma and solid carcinoma.* After this the tumour grew slightly larger (4 × 3 cm), and on 8 June an operation was performed: excision of the tumour and surrounding connective tissue and excision of the left axillary lymphatic nodes. Histopathological diagnosis—adenocarcinoma and solid carcinoma. On 19 May, i.e. 14 months later, the left breast contained a thickening 2.5 cm in diameter. A radical operation was per-

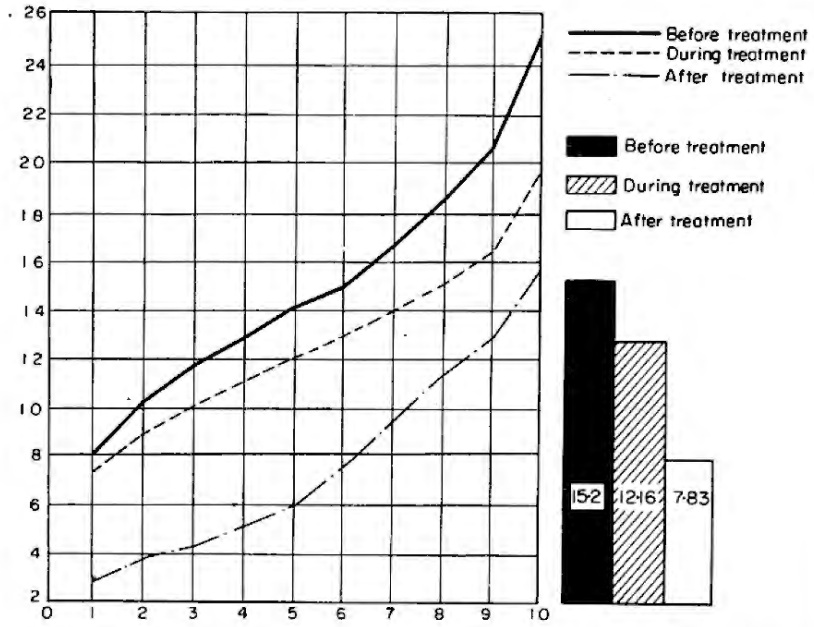


FIG. IX. Changes in nuclear size in patient O-gova's tumour during treatment with the trypanosome preparation.

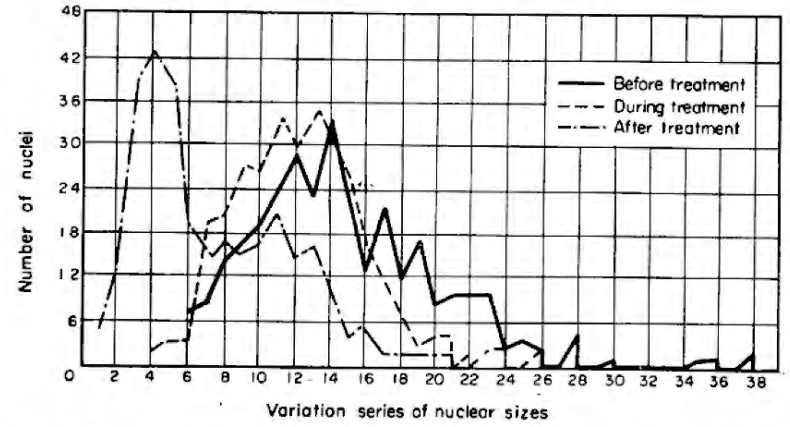


FIG. X. Nuclear size variation curves for patient O-gova's tumour before and during treatment with the trypanosome preparation.

formed. Histopathological diagnosis: solid carcinoma with areas of a scirrhous character. Cytological analysis showed that the following changes took place in the cancer cells during the course of the treatment: there was a regular decrease in the average nuclear size from 15.2 to 12.7 to 7.8 units (Fig. IX); there was a marked shift to the left in the nuclear size variation curve which was a favourable sign, but it still remained multimodal, as is characteristic for malignant tissue. The number of mitoses changed from 8 to 36 to 12, i.e. there was first a rise in the number and then a fall (Table 1, see Q%, Σ, M_0) (observation No. 40).

The changes in cellular properties seen in cancer of the breast also take place in other types of malignant tumours. This follows from an examination of the following cases.

(7). Patient E-eva. Clinical diagnosis: *cancer of the skin of the external genitalia.* A biopsy was performed. Histological diagnosis: *keratinizing squamous-cell carcinoma, with extensive round-cell infiltration of the stroma.* After one month of treatment the tumour was removed; histopathological findings: keratinizing squamous-cell carcinoma. Cytological analysis established a decrease in the average nuclear size (from 25.1 to 20.1), a decrease in the average nucleolar size (from 3.2 to 2.6), and a shift to the left in the nuclear size variation curve (observation No. 41).

(8) Patient R-band, female. Clinical diagnosis: *cancer of the rectum, stage III*; the tumour had infiltrated three quarters of the circumference of the rectum and the edge of the tumour was ulcerated. A biopsy was performed on 20 August. Histological diagnosis: *adenocarcinoma.* After $3\frac{1}{2}$ months of treatment an operation was performed: excision of the tumour of the rectum. Histopathological findings: adenocarcinoma with signs of chronic granulatory inflammation of the stroma. The patient received the preparation for a further 12 months after the operation. At the last examination, made 15 months after the operation, no thickenings could be found and the inguinal lymphatic nodes were not enlarged. We had at our disposal material obtained at two biopsies and an operation. Cytological analysis enabled us to determine the changes which had taken place in the cancer cells in the $3\frac{1}{2}$ months which passed between the biopsy and operation; the average nuclear size fell from 22.6 through 20.1 to 18.9; the average nucleolar size in the tumour cells *fell sharply* (from 2.0 to 1.8 and 0.9); there was a distinct rise in the nucleus–nucleolus ratio (from 11.3 to 11.1 then 21), which *approached the normal figure.* However, the nuclear size variation curve showed a relatively small shift to the left, and the number of mitoses, although falling considerably, still remained high (Figs. XI, XII and XIII) (observation No. 42).

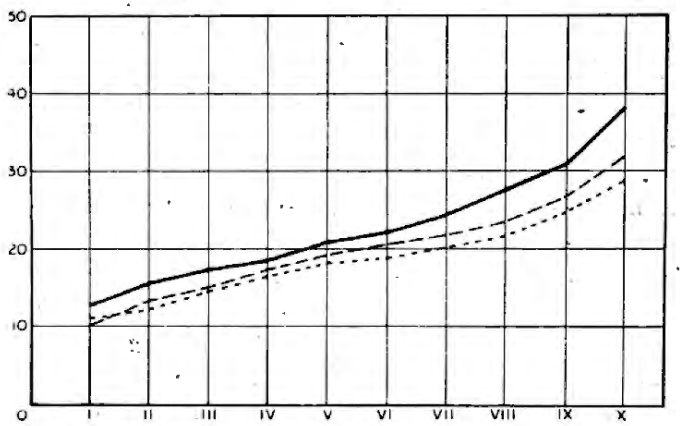


FIG. XI. Gradual decrease in nuclear size in patient R-band's tumour during treatment with the trypanosome preparation.

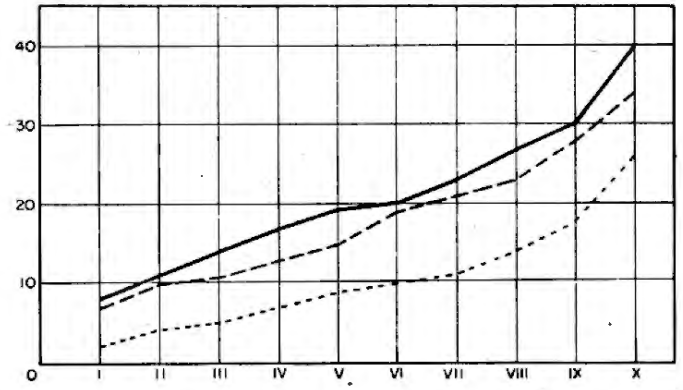


FIG. XII. Changes in nucleolar size in patient R-band's tumour during treatment with the trypanosome preparation: considerable diminution of the nucleoli.

(9) Patient V-l'eva. Admitted for treatment after panhysterectomy and removal of a tumour in the posterior Douglas's pouch; the omentum had been completely removed (20 June). Histopathological diagnosis: papillary carcinoma of the ovaries, with metastases of the same type in

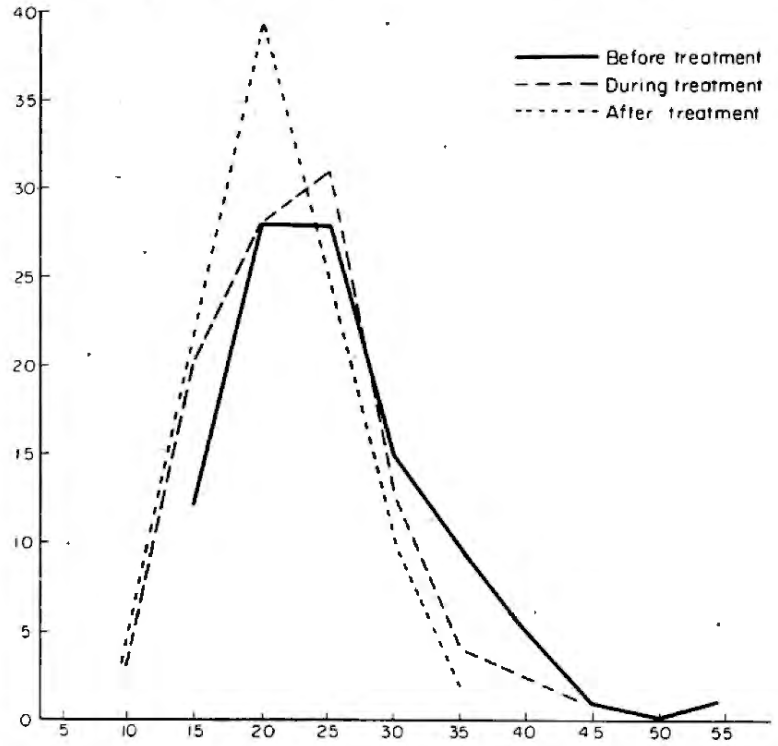


FIG. X. Nuclear size variation curve for patient R-band's tumour before and during treatment with the trypanosome preparation.

the omentum. Clinical diagnosis at the start of the treatment: *recurrence of carcinoma* (30 July). We had at our disposal material from the first operation (20 June), a biopsy (7 March) and a second operation (1 September) (removal of a tumour in the small pelvis); histopathological diagnosis: solid carcinoma. Histological examination, in this extremely seriously advanced case, supplied no information regarding the influence of the preparation on the tumour. Cytological examination, however, showed a regular decrease in the number of mitoses from 11 to 4 to 0, and a progressive decrease in the size of the cancer cell nuclei from 8.3 to 6.6 to 4.6; this is evidenced by a comparison of the nuclear size variation curves during the process of treatment (Figs. XIV and XV), though the

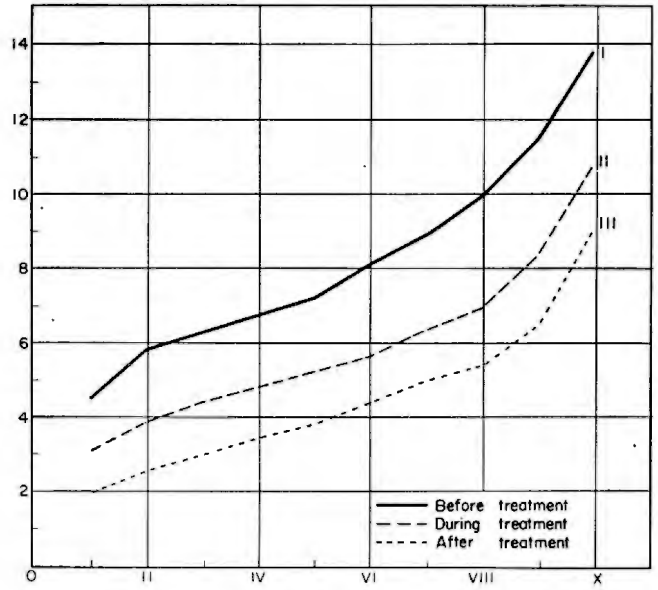


FIG. XIV. Changes in nuclear size in patient V-l'eva's tumour during treatment with the trypanosome preparation.

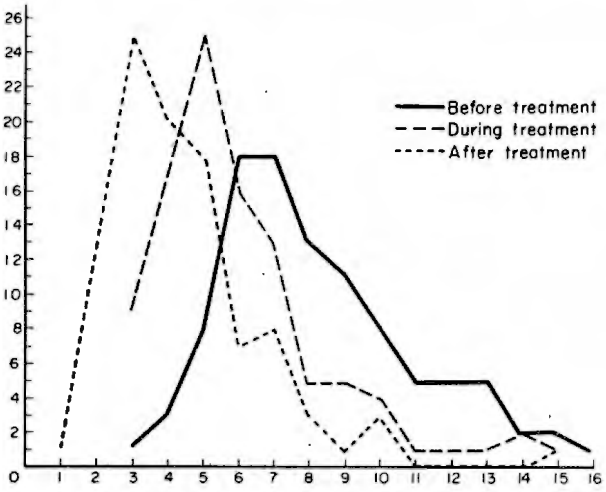


FIG. XV. Nuclear variation curves for patient V-l'eva's tumour before, during and on completion of a course of injections of the trypanosome preparation.

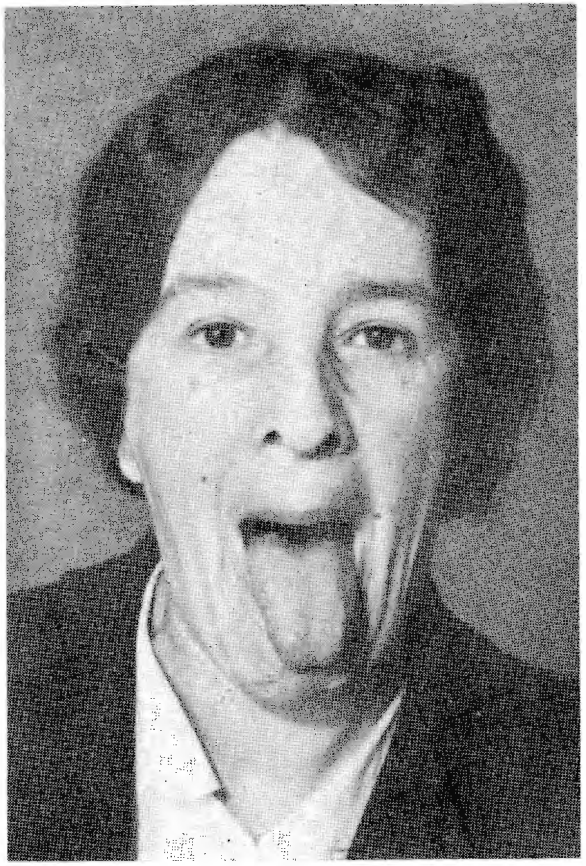


PLATE 80. Patient O., After a 20-day course of intramuscular injections of the preparations (16,800 units). The parakeratosis has disappeared. An area covered by normal epithelium has formed in its place.

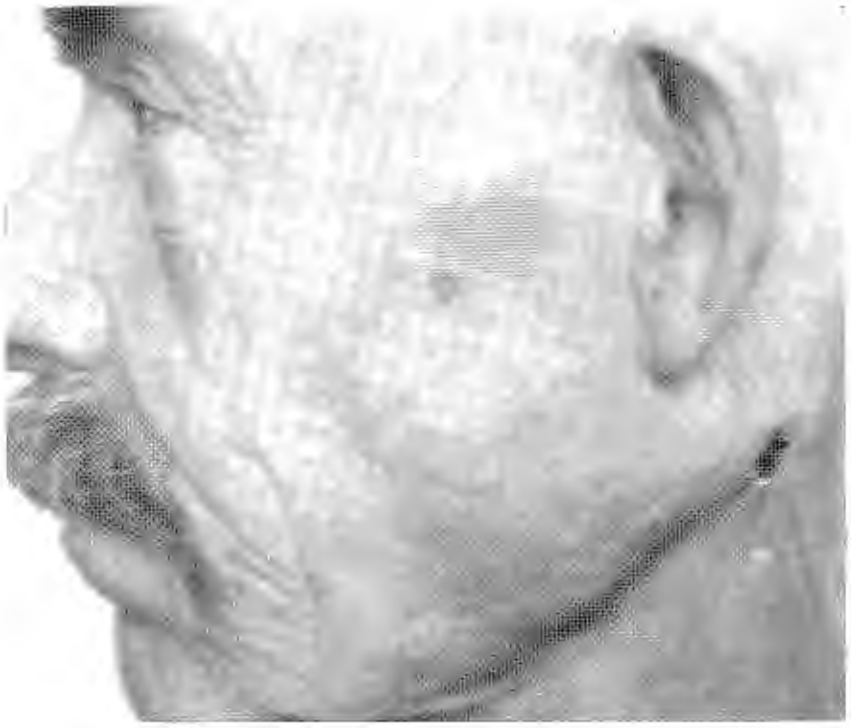


PLATE 81. Patient M., 67 years old. Basal-cell carcinoma of the skin of the neck. The tumour before treatment. The size of the tumour was 2.5×12 cm. At its centre is an ulcer, covered by a scab with an area of 1.5×0.5×0.3 cm

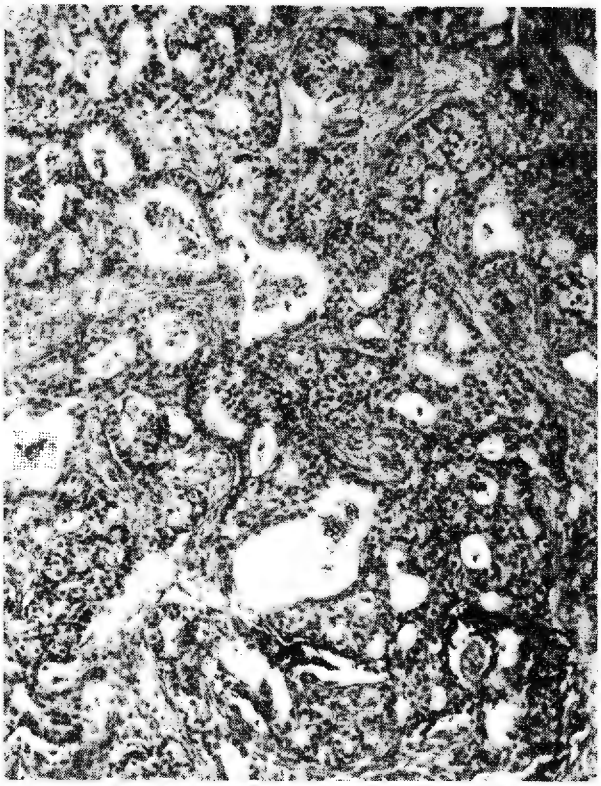


PLATE 82. Area of multiform carcinoma in patient Sh., before treatment.

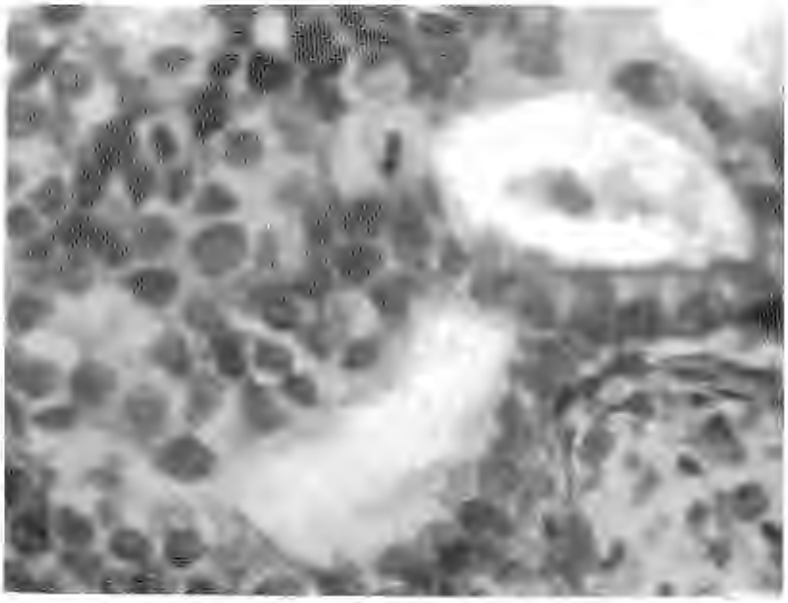


PLATE 83. Area of the same tumour under high magnification.

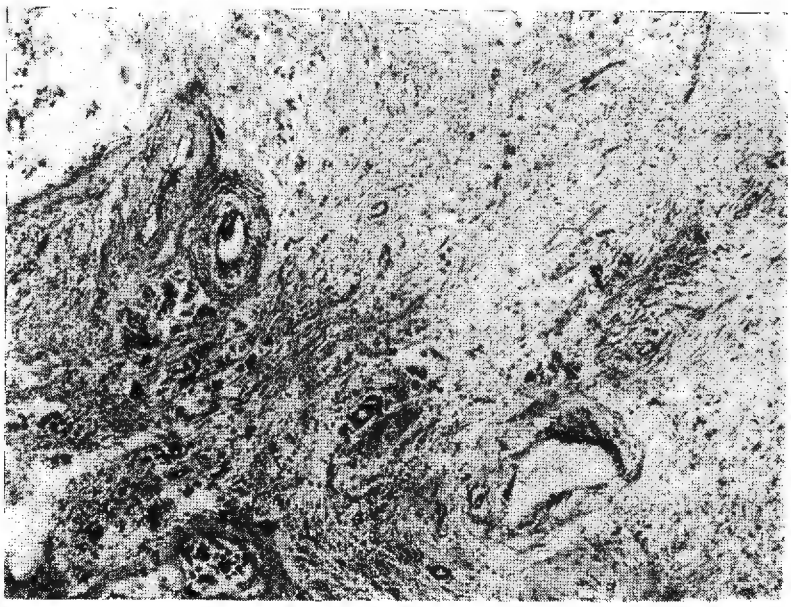


PLATE 84. The tumour in patient Sh. after treatment with the trypanosome preparation, showing extensive fields of destruction of the malignant tissue; low magnification.

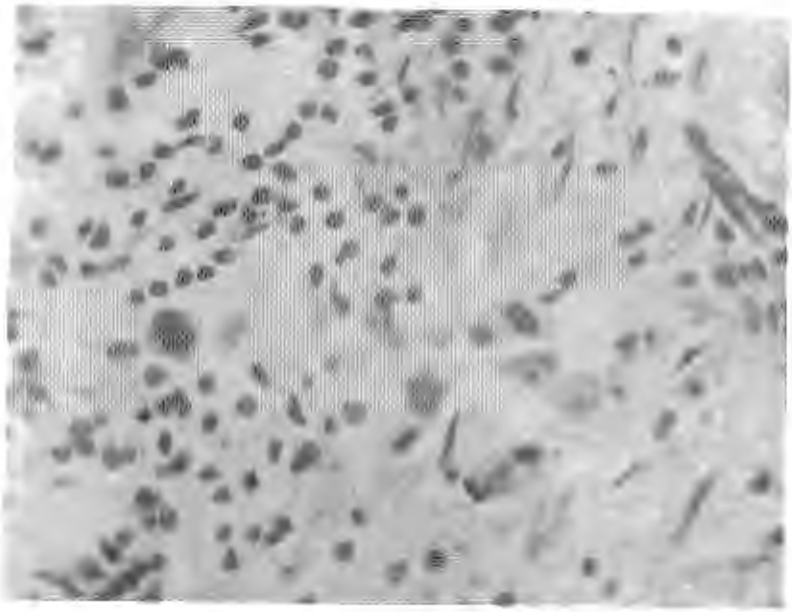
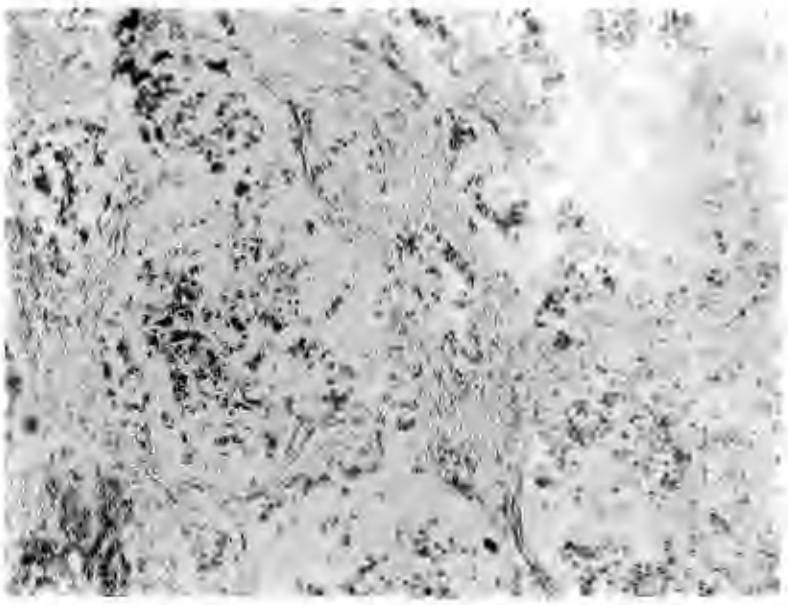
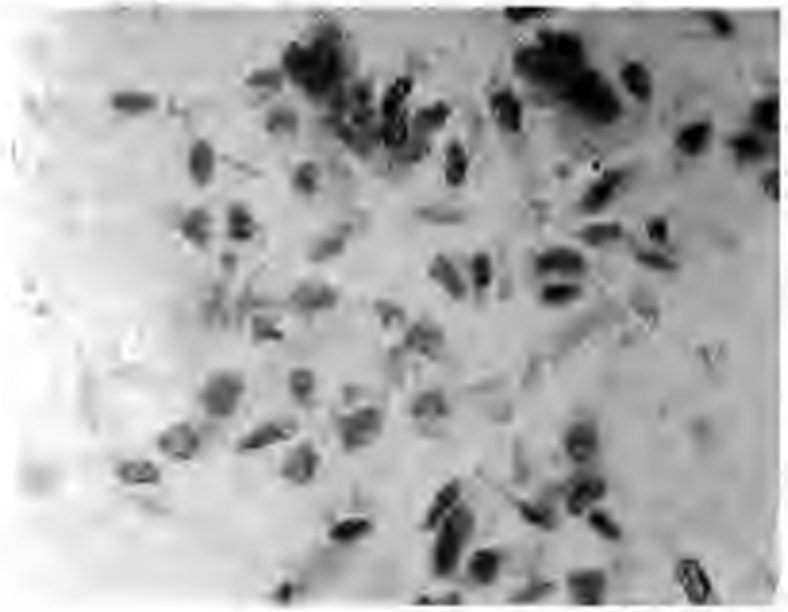


PLATE 85. The same tumour after treatment with the trypanosome preparation, showing atrophying cancer cells among a lymphocytic infiltrate; high magnification.



(*a*)



(*b*)

PLATE 86, *a*, *b*. The tumour in patient Sh. after treatment, showing nests of cancer tissue with atrophying cancer cells; low magnification.

PLATE 87. Area of the tumour in patient G., before treatment. A picture of solid carcinoma. Polymorphic nests of cancer cells situated in a poorly-developed fibrillar stroma.

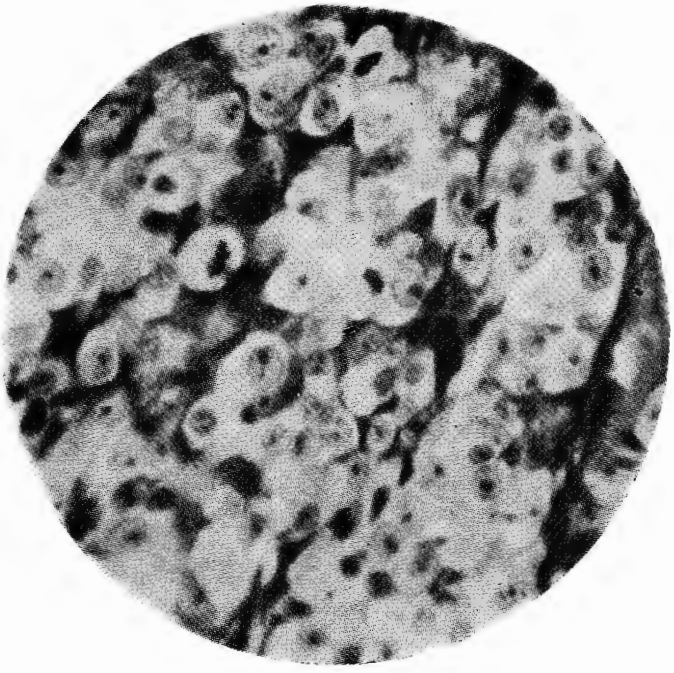


PLATE 88. Area of the tumour in patient G. before treatment. The tumour shows many mitoses. High magnification.

PLATE 89. The tumour in patient G. after treatment. Attention is drawn by the change in shape of the cancer cells, many of which are elongated—mesenchymatization of the cancer cells; ×600.

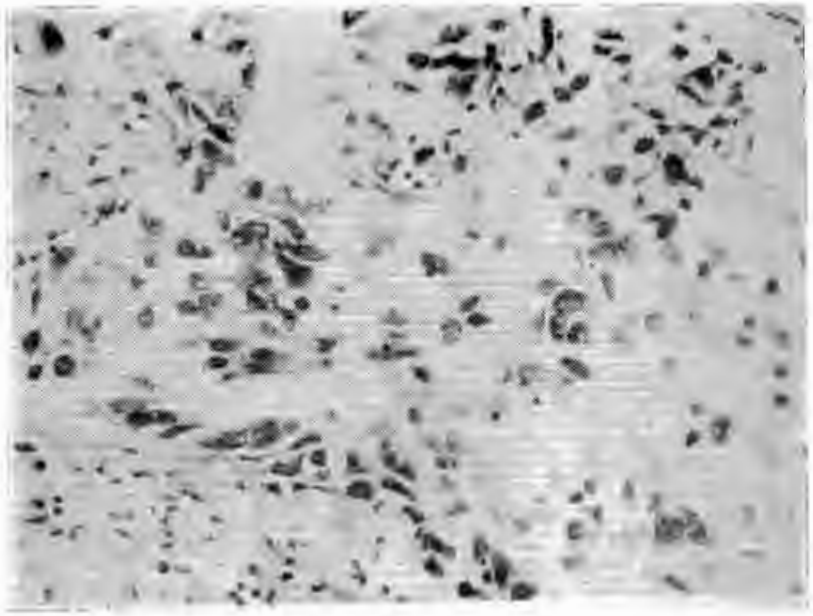


PLATE 90. The tumour in patient G. after treatment. Areas of atrophy of the cancer cells.

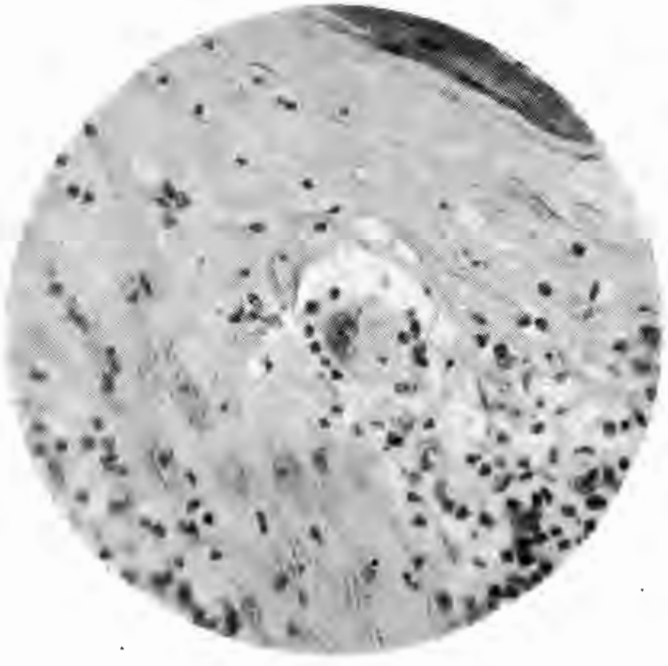


PLATE 91. Squamous-cell carcinoma of the lip in patient K. after treatment; penetration of a polyblast into the depths of the cancer tissue, with a zone of lysis around the polyblast; high magnification.

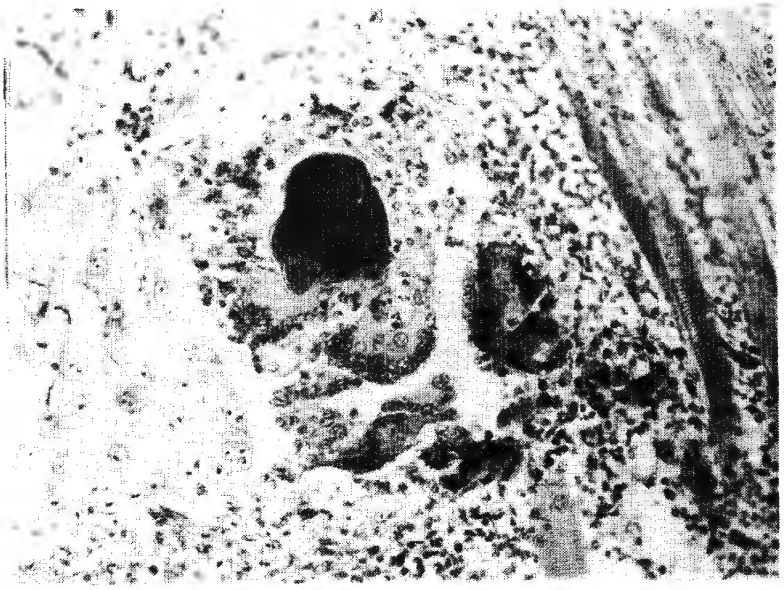


PLATE 92. Degenerating carcinoma pearls surrounded by polyblasts; patient K.'s tumour, after treatment; low magnification.

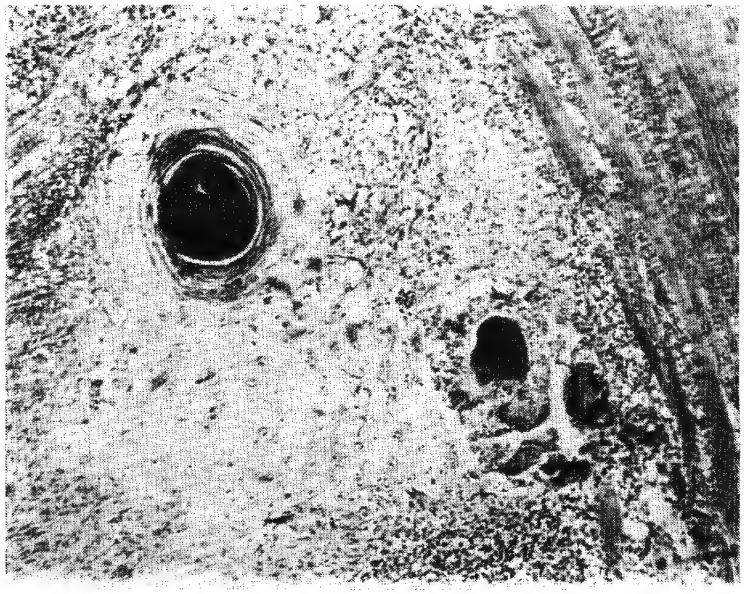


PLATE 93. Degenerating carcinoma pearls surrounded by polyblasts; patient K.'s tumour, after treatment with the trypanosome preparation; low magnification.

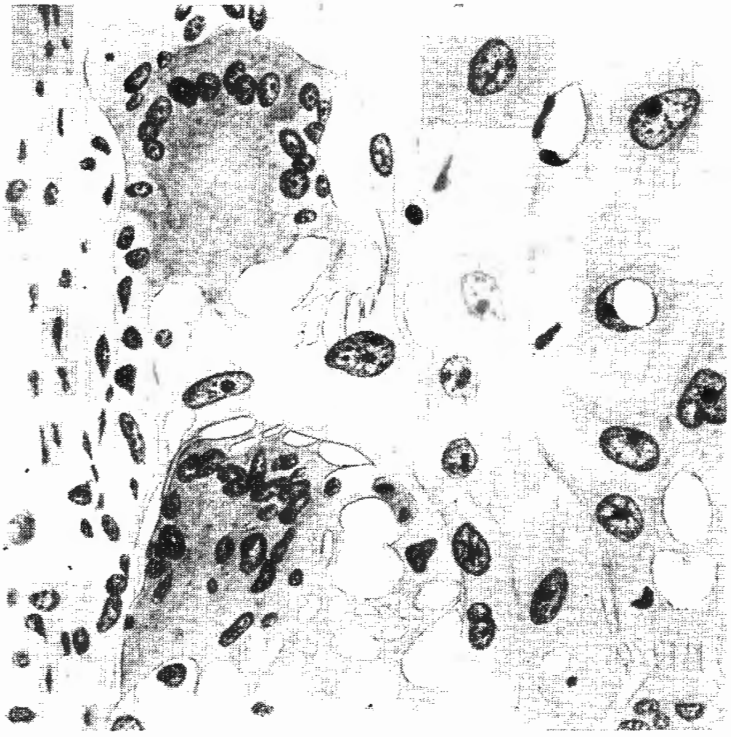
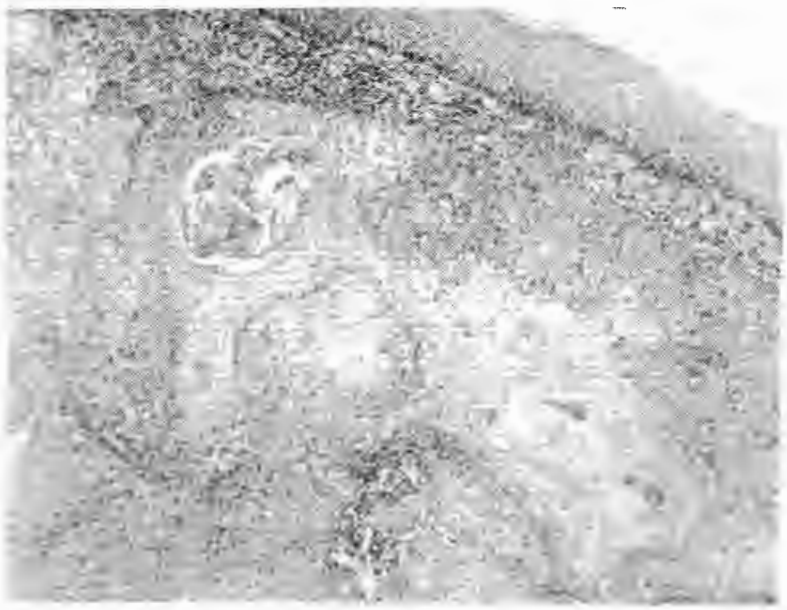


PLATE 94. Polyblasts in an area of treated squamous-cell carcinoma of the lip in patient K. (a drawing made at high magnification).

(*a*)



(*b*)

PLATE 95, *a*, *b*. Tumour of the lip in patient S. before treatment. Keratinizing squamous-cell carcinoma with prolific infiltration of the connective tissue core.

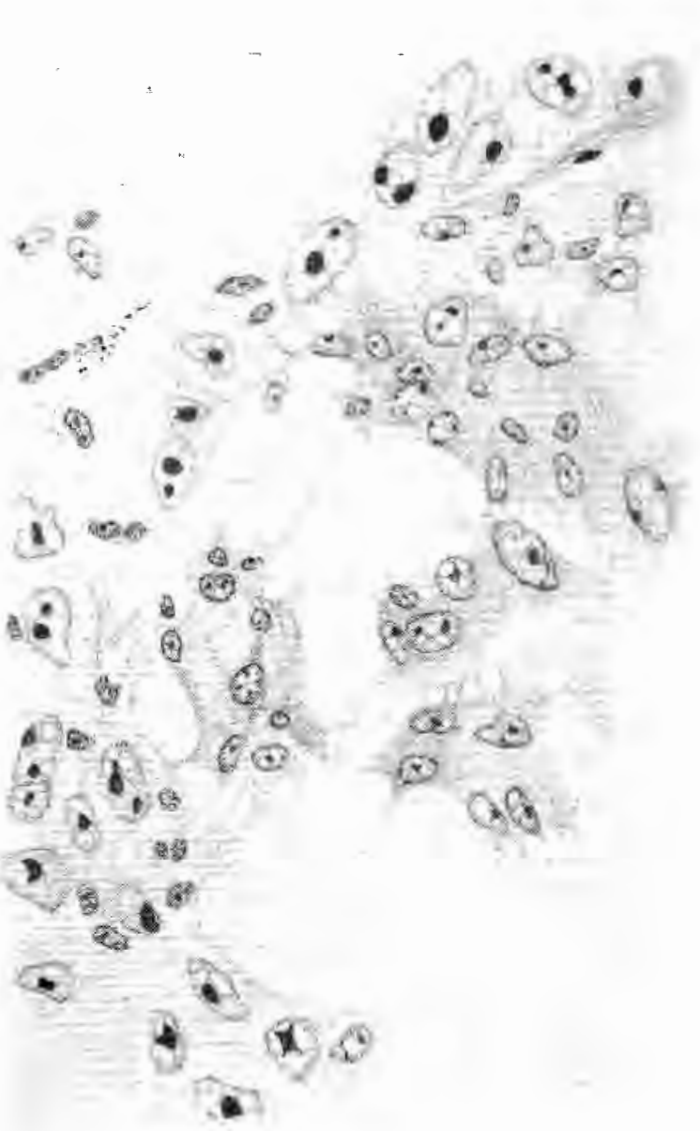


PLATE 96. Patient S.'s tumour after treatment. Polyblasts lysing the cancer tissue; a drawing made at high magniffcation.

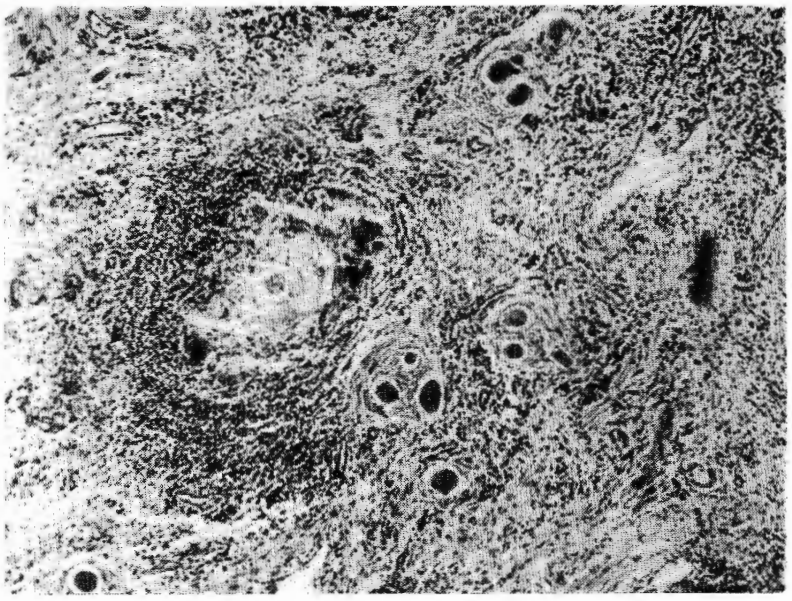


PLATE 97. Tumour in patient L. — a keratinizing squamous-cell carcinoma—after treatment. At the site of the former tumour there is cicatrization with signs of a process of diffuse lymphohistiocytic infiltration and of foreign body granuloma formation.

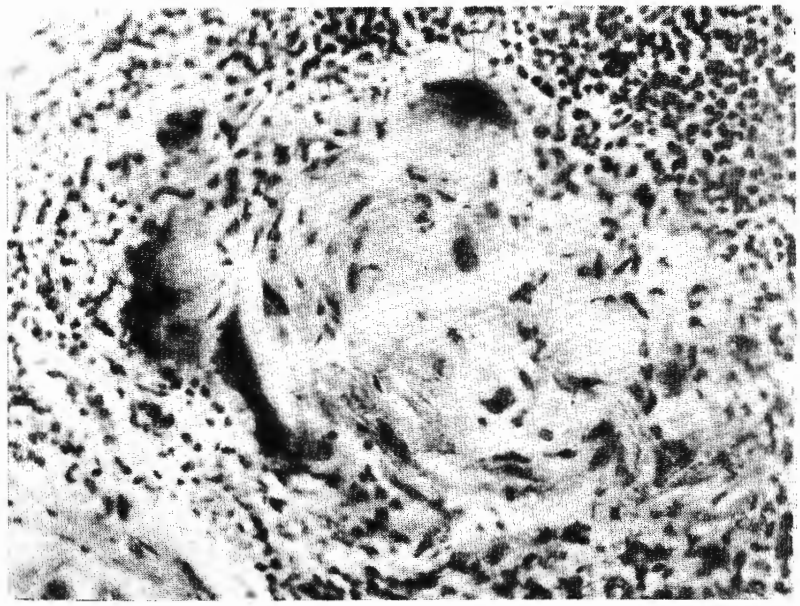


PLATE 98. Tumour in patient L. after treatment, showing a giant foreign-body granuloma; high magnification.

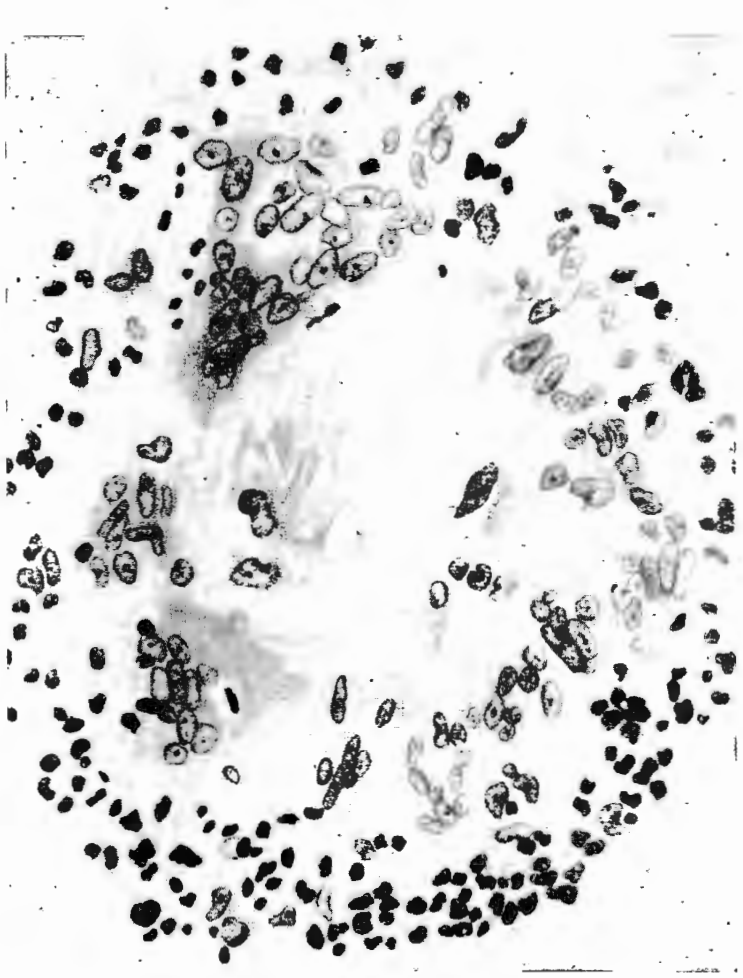


PLATE 99 *a*. Area of tissue at the site of a former lip cancer in patient L. Macrophages and lymphocytes can be seen around the formless remnant of a carcinoma pearl (drawing made at a magnification of × 600).

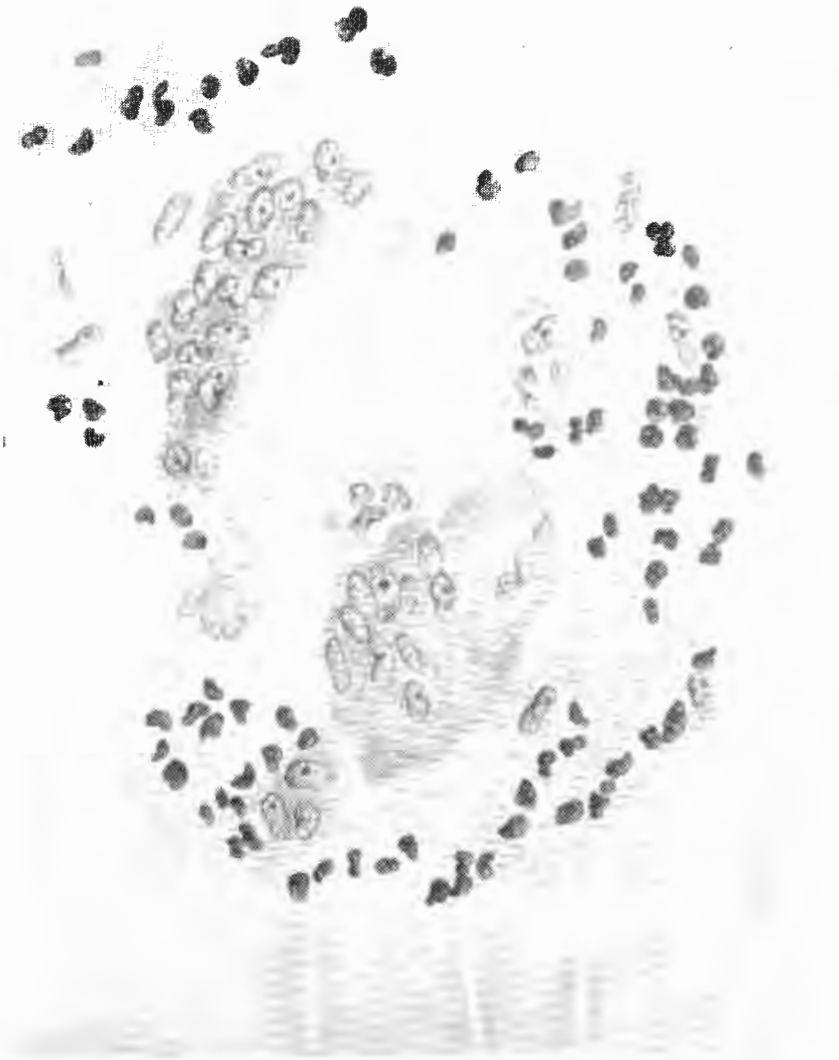


PLATE 99 *b*. Area of tissue at the site of a former lip cancer in patient L. Macrophages and lymphocytes can be seen around the formless remnant of a carcinoma pearl (drawing made at a magnification of $\times 600$).

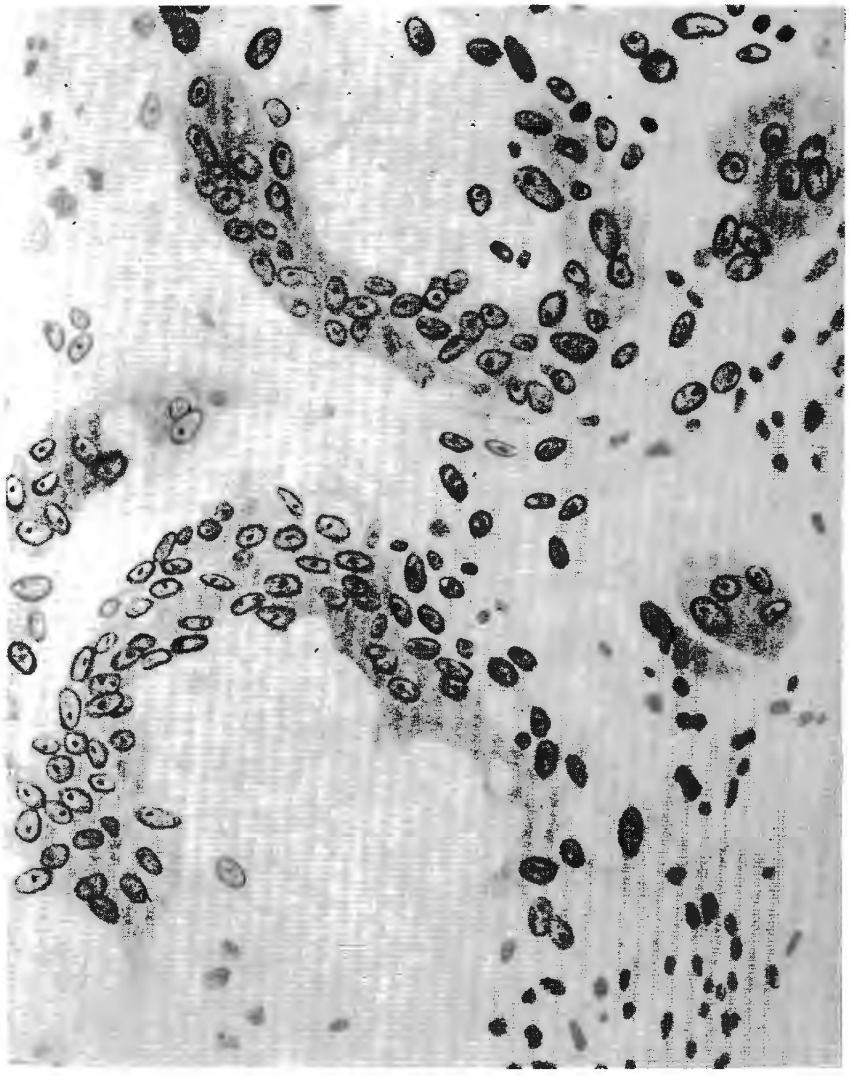


PLATE 100. At the site of a completely cured carcinoma of the lip in patient R. (observation No. 3, see pp. 62–66) there are formless remnants of former carcinoma pearls surrounded by giant macrophages; drawing made at a magnification of × 350. A similar picture to that seen in patient L. (Drawing).

multimodal nature of the curves remained unchanged, reflecting the polymorphism of nuclear size characteristic of malignant neoplasms (Observation No. 43).

Similar observations on patient T-ova are shown in Table 1.

On comparing the cytological observations on ten tumours in the patients mentioned above, treated with the trypanosome preparation, we see that in each of these tumours there were *objectively recordable* cytological changes. Taken in combination, these changes show that the processes in the cells of treated tumours appear to be *reversed* in relation to those occurring during the malignization of normal cells. This *initial stage* in tumour changes occurring under the influence of biotherapeutic interference may be called *the stage of regressive development of malignant cells*. In later analyses of histological and biochemical observations we shall attempt to rationalize this position further. When biotherapeutic action is at its most effective a second stage ensues, during which, after the changes in the cancer cells, there are changes in the tumour stroma and in the reactions of cells of the defence system—phenomena heralding a cancerolytic reaction. Lastly, it should be noted that the observations described here show that the concepts of clinical refractoriness and cytologically recordable refractoriness to biotherapeutic interference do not coincide. Cancer cells are much more susceptible to the action of the trypanosome preparation than can be judged from clinical findings alone.

Before summarizing our observations on this group of tumours, let us first deal with two questions. Have there not been analogous or similar changes in the cytological features of tumours in patients in whom the cancerous disease has been clearly progressive? Since we were unable to find the answer to this question in the literature, we had to submit to cytological investigation tumours from 15 patients in whom the use of the trypanosome antibiotic was definitely ineffective, and the cancer process, according to the clinicians, had progressed just as before the start of the treatment, and for this reason the patients had been treated by operative means. The tumours were of various types: adenocarcinoma, scirrhous carcinoma, solid carcinoma, multiform carcinoma, squamous-cell carcinoma, giant-cell sarcoma and basal-cell carcinoma. The intervals between the first and second cytological investigations varied from 20 to 90 days. The essence of these observations was: (1) nuclear dimensions were, as a rule, slightly increased; (2) the number of mitoses was unchanged or rose slightly. In those cases where we studied not only the nuclei but also the nucleoli, and the nuclear and nucleolar variation curves, it was

TABLE 1

Patient's name	Age	Histopathological diagnosis	Duration of treatment	∩	Nucleus					Nucleus-nucleolus ratio	No.of mitoses per 100 fields
					M ± m	Q%	σ ± m_σ	C% ± m_c	M_0		
T-ova	55	adenocarcinoma		300	16.17±0.352		5.75±0.217	35.5%±1.34	12.0—13.0		72
		adenocarcinoma	8 months	300	13.59±0.254	18.9%	4.4±0.166	32.3%±1.22	11.0—12.0		19
O-ova	44	adenocarcinoma and solid carcinoma		300	15.2±0.302		5.23±0.198	34.4%±1.3	13.0—14.0		8
			3 months	300	12.7±0,211	19.1%	3.66±0.138	28.6%±1.08	12.0—13.0		36
		solid carcinoma, scirrhous in places	1 month	300	7.83±0.213	61.6%	4.14±0.156	52.8%±2.0	3.0—4.0		12
P-ova	47	solid carcinoma		300	12.7±0.232		3.59±0.149	28.2%±1.07	9.0—11.0	11.07	35
		solid carcinoma, scirrhous in places	23 days	300	10.27±0.2	23.7%	3.41±0.127	33.2%±1.25	7.0—9.0	12.19	18
K-ova	50	solid carcinoma		300	13.63±0.218		3.78±0.143	27.7%±1.04	12.0—14.0	16.18	17
		scirrhous carcinoma solid in places	4 months	300	12.25±0.19	11.2%	3.31±0.125	26.9%±1.01	10.0—12.0	20.14	13

∩ — total number of variants; M — arithmetical mean; m — mean deviation, giving probable estimation of limits of deviation between different arithmetical means M, M_2, M_3 ..... of all combinations of M; O — standard deviation, giving an idea of the character and scale of variation; M_0 —mode; zone of highest incidence; Q% — percentage change from initial size; C% — variation coefficient: index of diversity or uniformity.

established that the nucleus–nucleolus ratio fell, i.e. the nucleoli increased more rapidly than the nuclei—a *sign of malignization*. At the same time, the shifts to the right of the nuclear and nucleolar size variation curves provided extra evidence of a deterioration in this picture of the cancer's progress. In comparison with these observations, the significance of the positive changes in nuclei, nucleoli, number of mitoses, etc., as seen in the first group of 10 cancer patients, is even more outstanding. The second question that the reader may legitimately raise is: how reliable, from the biometric viewpoint, are the positive cytological changes determined in the studied tumours? Because we consider this a most important question, we invited three prominent specialists in the field of biometry —P.I. Zenkevich, Dr. M.V. Ignat'ev, head of the variational statistics laboratory, and Prof. Ya.Ya. Roginskii—to express their conclusion on this material. Their conclusion was: "The number of cells present in each biopsy sample is ample for reliable statistical results. The method of treating the material raises no doubts from the variational statistics aspect. The differences between biopsy samples taken before and after treatment with the preparation may be considered statistically reliable". The facts which we determined have enabled us to draw a number of conclusions. First of all, we may note that the use of certain quantitative methods of cytological investigation makes it possible to study and assess changes which are not detectable by the usual histological and cytological techniques. These are, in fact, the primary, initial stages. They are also characterized by the fact that they do not necessarily bring about further changes in the malignant tissue, right up to a well-marked "cancerolytic reaction stage". The observations enable us to suggest that *in cases when these cellular changes do not progress to a certain level, they are reversible*. All this shows that not only normal but cancerous cells possess a plasticity, as the result of which, the cytological signs of malignization may decrease or even disappear altogether (within the scope of cytological analysis) and, on the contrary they may, following a change in conditions, reappear and increase. The first stage in the changes occurring in cancer cells under the influence of the trypanosome antibiotic may be termed the stage of "reverse development of malignant cells". Among the phenomena most typical of the process of reverse development of malignant cells under the influence of the trypanosome preparation are the following: (a) a regular decrease in nuclear size; this phenomenon undoubtedly reflects changes in the level and possibly also the character of the tumour cell metabolism (as has been shown for normal cells in a number of cytophysiological studies); (b) a regular decrease in nucleolar size and a rise in the nucleus–nucleolus

ratio, indicating the normalization of this relationship as the results of profound morpho-physiological changes in the malignant cells; (c) a progressive fall in the number of dividing cells—yet another indication of the change in the character of their metabolism, since each cell division is associated with intensive protein synthesis. This last concept has found support not only in isolated observations on tumours of treated patients but also in special experiments on the effects of the trypanosome preparation on spontaneous mouse tumours. These experiments, as will be shown, revealed that there is a considerable fall in the amount of ribonucleic acid in cancer cells under the influence of the trypanosome preparation. Since there is ample reason to suppose that there is a close link between the amount of ribonucleic acid and the level of protein synthesis within a cell, the observations described show convincingly that under the influence of the trypanosome preparation there is a breakdown in the intensive protein synthesis required for cell growth and multiplication, thus interfering with the most important factor in the rapid growth and multiplication of malignant cells. On the basis of our analysis of the tumours in this group of patients, we may form the following conclusion: *on treatment with the trypanosome preparation, objectively recordable cytological changes may occur earlier than any clinically determinable improvement.*

B. HISTOLOGICAL AND CYTOLOGICAL ANALYSIS OF TUMOURS REMOVED AT VARIOUS STAGES OF A POSITIVE BIOTHERAPEUTIC EFFECT

The study of tumours in various stages of regression was, quite understandably, much more informative. Only these observations could provide the clearest impression of the principles of the reaction of malignant cells to biotherapeutic effects. However, the accumulation of this material from clinical cases presented a certain amount of difficulty. The difficulty lay in the following. If a tumour underwent regression in the process of biotherapy we tried, naturally, to continue this process to its conclusion, since in the early period of study of the biotherapeutic method this was most important. For this reason, in successful cases of biotherapy the tumour was not, as a rule, removed operatively. The process of regression, when taken to its limits, as we shall see later, ended with the formation of scar tissue at the site of the former tumour, without any remains of the malignant affection, or in the form of mere structureless traces of its necrosis.

Histological Analysis of a Case of Basalioma

We studied a tumour in patient M-ev before and after treatment. Clinical diagnosis: *cancer of the skin of the neck;* histological diagnosis:

basal-cell sarcoma with a tendency to keratinization. The tumour measured 2.5×2 cm, with a central ulcer of 1.5×0.5×0.3 cm. From 4 September 1947, 77 injections were given. The tumour grew *slightly* smaller as a result (2×1.4 cm) and the ulcer contracted to 0.4×0.2×0.1 cm. Clinically, this case had to be interpreted as one of a weak positive effect by the trypanosome preparation (Plate 81, Fig. XVI). Observation No. 44.

The tumour was excised on 16 December 1947. Histological examination of the excised tumour: at the edge of the preparation, in one part of the depths of the connective tissue core, there were small numbers of cords of basal-type cells, infiltrating the connective tissue to a considerable depth (down to the adipose layer); in places the tumour cells were stratified concentrically upon each other and were keratinizing; in the region of the tumour cords there was proliferation of new connective tissue among

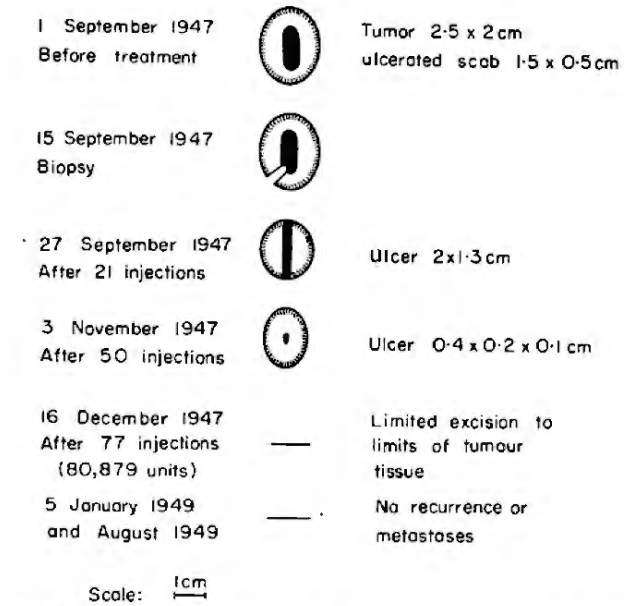


FIG. XVI. Course of injections of the trypanosome preparation and tumour measurements in patient M. (diagram).

which were the remains of atrophic cancer cords; here also were found aggregations of an inflammatory nature. Since the overall histological picture was not sufficiently indicative, we started a study of the cancer cell nuclei before and after treatment. This showed that the tumour cell nuclei had *grown smaller* after treatment. Detailed karyometric observations showed that not only had the average size decreased, but in each size

class of the nuclei there had been a shift towards a smaller size which, to a certain extent, was a sign of normalization of the cells. Here we met an extremely interesting cytophysiological phenomenon, which we subsequently met on more than one occasion in further analyses of tumours treated with the trypanosome preparation. The biological and prognostic significance of decrease in nuclear size must not be underestimated.

Histological Analysis of Five Cases of Cancer of the Breast

(1) Patient Sh-na, aged 65 years. Clinical diagnosis: *cancer of the breast, stage II.* The tumour measured 3.5×4 cm. There were two firm lymphatic nodes in the left axillary region (one 2×1.5, the other 1.5×1 cm). A biopsy of the tumour was made and treatment commenced on 23 March 1948. Histological examination of the excised fragment revealed the following: "The connective tissue stroma and adipose tissue are penetrated by compact, markedly abnormal cords, nodules and small groups of epithelial cells of a markedly anaplastic nature, with mitoses in some cells and necrosis of some of the nodules. There is round-cell infiltration of the stroma; diagnosis: *carcinoma*" (Prof. Ya. L. Rapoport). During the first few weeks after the biopsy the tumour reached a size of 4.5×3.5 cm, i.e. it became larger than before the biopsy. On the 42nd day from the start of the course of injections, however, the tumour had decreased in size to 3×3 cm (the lymphatic nodes were unchanged). The tumour remained in this state without change for the next 6 weeks. Its consistency became softer. In June, the thickened area increased to 4.5×4 cm. In connection with this, on 25 June the patient underwent segmental resection of the breast, with removal of the axillary lymphatic nodes.

Histological examination of the resected tumour by Prof. Rapoport showed: "in the white fibrous tissue are spaces of various shapes and sizes containing loose groups of cancer cells. Some fields show *atrophy of the cancer cells,* consisting of a fine fibrillar reticulum containing only *odd atrophic cancer cells and a delicate round-cell infiltration.* Considerable areas are *completely devoid of cancer cells,* consisting only of a fibrous stroma infiltrated by lymphoid elements (photomicrographs). Besides this there are isolated small foci of cancer cells in which calcareous concrements have been laid down. The lymphatic node is almost entirely infiltrated by a compact mass of cancer cells showing no signs of atrophy" (Plates 82–86); observation No. 45.

On comparing the histological pictures of the tumour before and after treatment the idea naturally arises of the way in which the tissue changes described are connected with changes in the properties of the cancer cells.

The cytologist can study these moves most easily by considering the intensity of cell division and noting the sizes of the nuclei.

We therefore made an estimate of the number of mitoses, and also took nuclear measurements, before and after treatment. Before treatment, the number of mitoses seen in one hundred fields of vision of the micro-

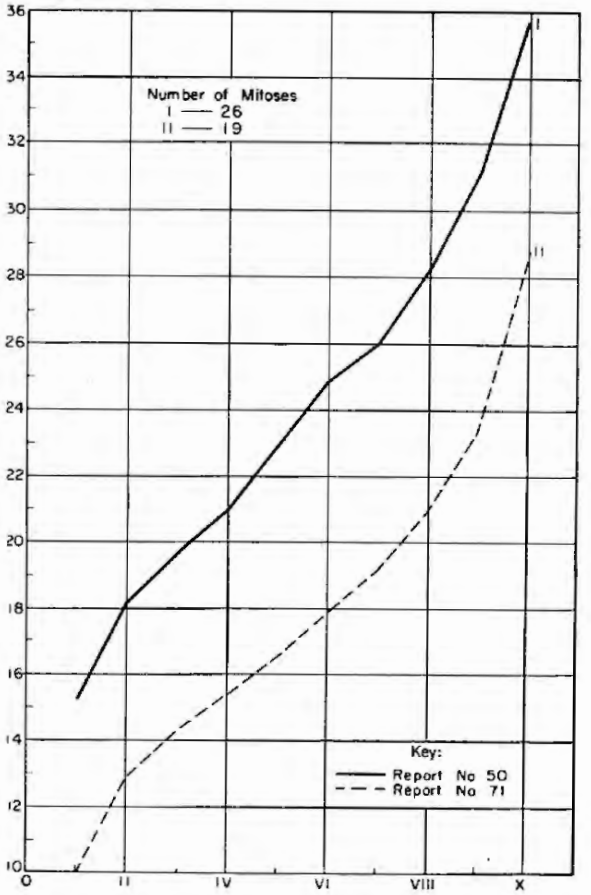


FIG. XVII. Comparison of average nuclear size in patient Sh.'s tumour before (I) and after (II) treatment. There is a considerable decrease in the size of the malignant cell nuclei under the influence of the trypanosome preparation.

scopical preparation was 74; after treatment the number of dividing cells had decreased: 49 mitoses were found in one hundred fields of vision. It may thus be taken that the rate of growth of the tumour had been slightly retarded under the influence of the trypanosome preparation. More significant changes took place in the size of the cancer cell nuclei under the in-

fluence of this treatment. Before treatment the average nuclear size of the cancer cells was 25.2 planimetric units, whereas after treatment the nuclear size had decreased noticeably—to 18 planimetric units (Fig. XVII).

Both the fall in the mitotic index and the decrease in nuclear size are interpreted by many of the authoritative histopathologists working in the field of oncology as extremely positive signs both for prognosis and for assessing the effects of therapeutic interference. It should be noted particularly that the fall in the number of mitoses and in nuclear size occurred in close association with the overall histological changes. We are thus bound to recognize the objectively recordable morphological changes established in this case in tumour tissue under the influence of biotherapy.

In comparison with two preceeding cases, patient Sh. showed more considerable and profound histological and cytological changes. However, the significance of the isolated observations described can only be assessed scientifically when considered *in association with* further histological observations carried out on clinical material and on material gathered from spontaneous and transplantable tumours of laboratory animals. For this reason we shall not rush to any conclusions but shall make a patient study of the observations collected.

(2) Patient G-na, aged 56 years. Admitted on 21 May with a diagnosis of *cancer of the left breast*, stage III. Size of the tumour before treatment: 7.0×5.5×6 cm; it was of cartilaginous consistency. There were two tumour-affected nodes in the axilla. On 25 May a biopsy was performed and treatment commenced. The tumour fragment excised at the biopsy was examined by Prof. Rapoport, who gave the following report: "Examination of the excised tumour revealed a picture of *solid carcinoma*. There were markedly polymorphic groups of cancer cells, embedded in a fibrous stroma which was generally poorly developed, giving the carcinoma a medullary appearance. The cells included many mitoses. Many extensive areas of tumour necrosis were seen". To Prof. Rapoport's description we may add the following: the borders of the cancer cells were not clearly visible. The nuclei were rounded, lightly-stained and large, *with large nucleoli*. The average nuclear size was 34.3 planimetric units (Plates 87–90, Fig. XVIII); observation No. 46.

The tumour was rapidly-growing. In 100 fields, 266 mitoses were seen, with a stage-distribution:

prophase	6
metaphase	230
anaphase	25
telophase	5

The nature of the distribution of the cells was as *completely solid, large foci*, separated from each other by fairly wide connective tissue septa. The connective tissue of the tumour contained mainly fibroblastic elements, with a few histiocytes. The surrounding connective tissue contained reaction

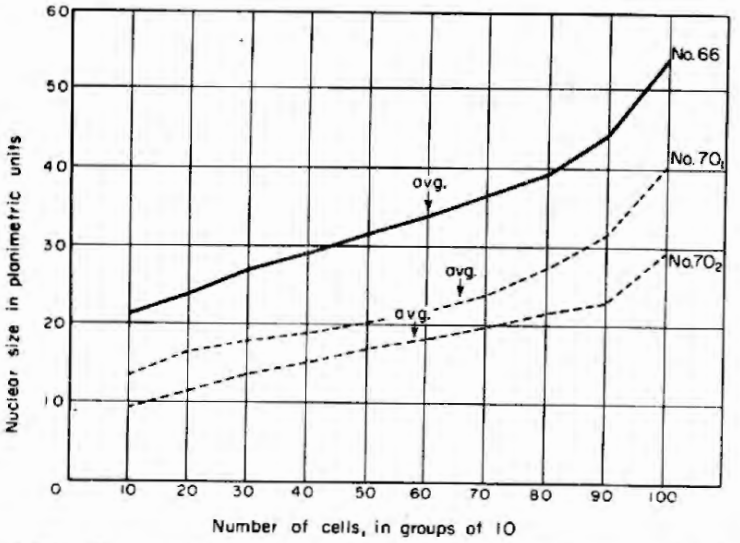


FIG. XVIII. Comparison of nuclear size in patient G's malignant tumour before (No. 66) and after treatment (Nos. 70_1 and 70_2); a considerable decrease in nuclear size took place under the influence of treatment with the trypanosome preparation.

cells, in low numbers. In places, the tumour tissue contained large necrotic areas. There were few blood vessels. On 25 June, after 25 injections of the preparation (31,330 units), a radical operation was performed—removal of the left breast and the axillary lymphatic nodes. Histological examination of the excised tumour showed an overal picture of solid carcinoma, but, *in distinction from the first biopsy, with considerable areas of atrophy of the cancer tissue, consisting of a fine, fibrous stroma, rich in elongated nuclei*, in which the atrophied cancer cells were scattered in low numbers. In the cancer foci the cell groups were more friable than in the material taken at the first biopsy. *Attention was drawn by the change in shape of the cells, which included many elongated forms, resembling a mesenchymoplastic epithelium*. Abnormal mitoses were found *in high numbers* (report by Prof. Rapoport).

To Prof. Rapoport's description it may be added that the following changes were found to have occurred in patient G.'s tumour after carrying out the course of treatment with the trypanosome preparation.

The number of mitoses in the tumour tissue had *decreased markedly*. In 100 fields, 41 mitoses were found, with a stage-distribution:

prophase	0
metaphase	34
anaphase	6
telophase	1

The number of mitoses had thus *fallen* by 6.4 times.

The size of the nuclei had changed considerably: the average nuclear size had fallen to 23.4 (in planimetric units). The ratio of nuclear size before treatment to nuclear size after treatment was 1.42—there had been a *real reduction in nuclear size*. There was a simultaneous change in nuclear structure. Before treatment, the nuclei in the preparations were lightly-staining, with *large nucleoli* (one or two nucleoli), while after treatment the nuclei stained differently with haematoxylin (more darkly) and the nucleoli in many nuclei were absent or else were very small.

The changes in the tumour were not restricted to the marked change in the number of mitoses and decrease in nuclear size and structure. *The histological structure of the tumour also changed:* in many areas the cancer cells were loosely arranged, disrupted, clearly delineated from each other, with well-defined cell borders (Plates 89,90).

There were changes in the stroma as well as in the cancer cells: the connective tissue came to include many histiocytes, lymphocytes and reaction cells. Hence, a whole series of *real and objectively recordable changes* could be shown to have taken place in patient G.'s treated tumour. A comparison of the two histological analyses, before and after treatment, shows that after treatment:

1. There appeared considerable areas of atrophy of the cancer tissue.
2. There was discomplexation of the cancer tissue — a phenomenon met frequently in treated spontaneous mammary tumours of mice.
3. There was a reduction in the size of the cancer cell nuclei, a decrease in the number and size of their nucleoli and a change in the staining properties of the nuclei.
4. The number of mitoses decreased —a factor which demands serious consideration.
5. There was a change in the cellular composition of the connective tissue stroma: a considerable rise in the number of histiocytes and the appearance of noticeable numbers of lymphocytes and reaction cells.
6. A phenomenon of mesenchymatization of the cancer tissue took place: the significance of this process—as a marked positive reaction under the influence of experimental stimuli—has recently been re-emphasized. Dobrovolskaya-Zavadskaya and Nekhorosheva (1947) state: this process of mesenchymatization "arises in purely neoplastic tissue, without apparent connection with the stroma, and leads to the more or less widespread replacement of cancer tissue with connective tissue".

At the same time, clinicians stated that as a result of the injections the patient had been brought *from an inoperable state to an operable one.*

If we compare all the *positive changes* in the tumour's histology with the clinicians' statements, the significance of the facts presented must be considered against the general conclusions of our morphological, cytological and histophysiological observations.

In concluding our description of the case of patient G., we must mention the following: the attention of the oncologist must not dwell only on the changes in this tumour which could be determined objectively, but also on the fact that the trypanosome preparation can have a definite influence on a malignant process so far advanced, so actively developing and in such a late stage.

(3) Patient T., aged 48 years. *Cancer of the right breast, stage III.* Tumour size: 15×15 cm. The tumour was adherent to the skin over a limited area (observation No. 47). A biopsy was performed on 18 May 1948, with excision of a tumour fragment measuring 1.5×1 cm. Treatment was started simultaneously. After histological examination of the biopsied fragment Prof. Rapoport wrote: "a picture of solid carcinoma was found, primarily medullary in character".

On 29 June radical removal of the breast was carried out. Examination of various parts of the tumour revealed: "In distinction from the previous biopsy, as well as normally constructed areas of solid carcinoma *there were extensive fields of atrophy and breakdown of the cancer cells*, which were infiltrated by small numbers of lymphoid cells, similarly to the earlier cases" (Prof. Rapoport's conclusion).

Even greater histological and cytological changes occurring under the influence of biotherapy were found on investigation of malignant mammary tumours in patients P-ko and L-sko.

(4). Patient P-d'ko (observation No. 28) was admitted for treatment with the trypanosome preparation while suffering from a grave recurrence after radical operation for cancer of the breast: a rapidly-increasing number of cancer nodules broke out in the region of the operation field, and by the time of admittance they numbered 48. One of these nodules was excised for histopathological examination: "a picture of *recurrence of a malignant growth* was found" (Prof. I.V. Davydovskii). Treatment with the preparation was started on 25 June 1946, and 112 injections were given by 25 De-

cember 1946. During that period *some of the firm cancer nodules disappeared and the rest grew smaller, becoming flatter and softer.* In February 1947 one of the nodules remaining after treatment was excised. The results of histological examination of this nodule were: "A sclerotic area was found in the deep layer. *No tumour elements were found*" (Prof. Talalaev, 26 February 1947). As the results of further treatment with the trypanosome preparation only 4 of the 48 nodules remained; no lymphatic nodes could be determined anywhere.

Treatment was interrupted from 25 December 1947 until 31 January 1947. At the end of this break several new nodules had appeared. One of them was excised and sent to Prof. Talalaev; his histological examination showed: "a tubular-gland form of carcinoma, growing in the fibrous foundation of the skin". A biopsy was carried out on 15 July 1947: an area of skin containing two intradermal nodules was excised. Histopathological investigation showed: "in the deep layer of the fibrous foundation of the skin of the preparation received, malignant cellular elements, *some of them showing signs of regression*, were found in the spaces between collagen fibre bundles" (Prof. Talalaev). Thus, during the period of treatment more than 40 cancer nodules disappeared. This satisfactory state of the patient continued until September 1947. On 10 and 29 September 1947 a group of tumour nodules (each 0.1–0.3 cm in diameter) was found in the region of the upper third of the scar. Further injections produced no results. Metastases formed in the lung, and the patient died in December 1948, almost $2\frac{1}{2}$ years after she started, while in a grave condition, treatment with the trypanosome preparation. Thus, in patient P. we were able to observe a *prolonged period* (1 year and 2 months) *of degeneration and disappearance of cancer cells.*

We examined Prof. Talalaev's preparations as well as material from the subsequent biopsy. In all these preparations, *taken at various stages of the action of the trypanosome substance* during a period of marked and prolonged improvement in the course of the malignant process, our attention was drawn by significant changes in the cancer cell nuclei; *the cell nuclei had minimal, hardly measurable nucleoli*—a phenomenon closely associated with changes in the whole metabolism of the malignant tissue.

(5). Patient La-skaia. Observation No. 19, 1946. It will be remembered that during the preoperative preparation the patient received several seances of X-ray therapy, but she categorically refused further treatment or the suggested operation. As a result of the gradual deterioration in her general health and increased growth of the tumour the patient was sent to the cancer biotherapy clinic. By this time the patient's tumour

was firm, uneven, irregularly oval in shape and measured 8.0×6.8 cm, while 4 firm lymphatic nodes (metastases) could be determined in the axillary region. The patient started treatment with the trypanosome preparation on 26 August 1946. After 51 injections the tumour had decreased in size to $4 \times 3.5 \times 3$ cm; only one lymphatic node the size of a lentil remained in the left axilla. On 10 October the injections were stopped, as the patient interrupted the treatment and went home to Kiev, where she attended the doctors who had treated her previously. In view of the marked changes in the whole clinical picture, Prof. Kramarenko considered it feasible, without resorting to radical surgery, to carry out resection of the neoplasm, not removing the whole breast, nor the lymphatic nodes and vessels.

A detailed analysis of the resected tumour was carried out by the patho-anatomist of Kiev Oncological Institute, Prof. Shvedkova-Rashe, who kindly passed on her findings, which we will give in their original form (the italics are ours). "The tumour is white in colour, firm in consistency, round in shape, measuring 2×3 cm, clearly delineated from the surrounding mammary tissue. In some places wide bands of fibrous tissue with few cellular elements leave the periphery of the tumour. There are areas of complete hyalinization of the tissues, with atrophic nuclei preserved in some places. Glandular ducts, cut in various planes, lie among homogeneous bundles. Their walls are thickened and consist of degenerated elastic fibres, forming a wide cuff around the lumen. The lumena of the ducts are lined by a thickened epithelium. In places they are filled with proliferations of large cells with frothy cytoplasm. Quite often the ducts are obliterated by scar tissue showing signs of calcification.

In both the central and peripheral parts of the tumour, between the fibrous tissue bundles there are a *few tumour elements*, forming cords and groups typical of the cancer *Carcinoma solidum*. The tumour elements only rarely appear more or less unchanged, the majority showing marked signs of degeneration. The cell cytoplasm is vacuolated, the nuclei either decreased in volume, pyknotic, staining diffusely with heamatoxylin, or, on the contrary, enlarged, hypochromic, acquiring the pale appearance of the so-called 'empty' nucleus, with distinct karyolysis. The border between the nucleus and the cytoplasm disappears and the cell becomes enucleated, with homogeneous or frothy cytoplasm.

In the peripheral parts of the tumour, among uniform homogeneous bundles, there are areas of areolar connective tissue, containing thin-walled vessels. Around the vessels are cellular infiltrates consisting of lymphoid and plasma elements and multinucleated leucocytes. The latter

can also be seen in the dilated lumena of the capillaries. Among the cellular infiltrates *can be found complexes of tumour elements, some of them intact, some of them showing marked changes.* Cords of proliferating epithelium which have not yet lost their structure are infiltrated by multinucleated leucocytes, situated both intra- and intercellularly, *surrounding the remains of neoplastic elements so altered that it was difficult to recognize them.*

The presence of tumour elements, partially preserved, partially necrotic, surrounded by cellular infiltrates, can also be determined in cords of fibrous tissue situated outside the tumour in the adipose tissue. The mammary tissue in these areas consists of lobules with coarse-fibred intralobular tissue indistinguishable from the interlobular connective tissue. The epithelium of the acini and milk ducts is partly atrophic, in places showing a picture of marked hyperplasia to the extent of completely filling the lumen. The internal layer of the epithelial lining consists of hypertrophic cells with markedly acidophilic cytoplasm and hyperchromic polymorphic nuclei. Infiltrates of lymphoid elements surround the lobules.

In the adipose tissue, in the areas of macroscopically apparent thickening, there are spaces surrounded by foci of cellular proliferation consisting mainly of large polygonal cells with frothy cytoplasm. The cells are in sheets and include isolated multinucleated giant cells of the foreign body cell type. These spaces are lined by cells with frothy cytoplasm. The fibrous connective tissue in the foci described takes the form of fibrous tissue with sparse, atrophic nuclei".

This concludes the histopathological report by Prof. Shvedkova-Rashe. It may be summarized thus: at the site of the malignant tumour there remained only completely *insignificant groups of cancer cells in various stages of degeneration and necrobiosis.* This histologically diagnosable picture coincides fully with the clinical observations: the tumour grew smaller, its consistency changed, and the axillary metastases disappeared. We have studied Shvedkova-Rashe's preparations and can only confirm all that is written above. At the next examination on 4 October 1950 it was stated: no signs of recurrence or metastases were found. This situation, as mentioned earlier, has not changed nine years and six months after the start of treatment with the trypanosome preparation.

Histological Analysis of Four Cases of Cancer of the Lip

Our observations on changes in malignant tumours of the lip after treatment with the trypanosome preparation may be placed in order of

the intensity of the changes in histological structure. These changes, as shown by the following cases, *parallel those seen clinically.*

1st case. Patient K., female. Histological diagnosis of tumour before treatment: "*squamous-cell carcinoma of the lower lip*". The case-history of this patient is described in our book (Klyuyeva and Roskin, 1946), but a more detailed study was made later. On commencing treatment with the preparation the tumour size was 4 × 2 cm. After 18 injections it measured 3 × 2 cm, with a soft scar on the left side of part of the former tumour. The tumour grew appreciably softer in consistency. Since the patient had to go away, the diminishing tumour was resected and sent to Prof. Talalaev for examination. His written conclusion, which he called "a preliminary *report on certain histopathological peculiarities of cancer of the lip treated with the trypanosome preparation*" is given here in its original form.

"We have examined," wrote Prof. Talalaev," a case of cancer of the lip in patient K., who had been treated with the trypanosome preparation. Biopsy material taken from this patient *before treatment showed the typical histological picture of squamous-cell carcinoma.*

In biopsy material taken after, or more accurately *during treatment* we found:

1. Groups of cancer cells deeply buried in the lip tissues as far as the transversely-striated muscles, with an intense perifocal inflammatory reaction.

2. Inflammatory infiltrates, consisting mainly of cells of the lymphoid and macrophage types.

3. Cancer cells showing signs of degeneration and necrobiosis, with complete cessation of proliferation and infiltrative growth *in places.* In such places there was an *intense macrophage reaction, with giant cells.*

4. Necrotic masses of former cancer cords, surrounded by giant cells.

5. Macrophages lying free among the giant cell infiltrates, showing that *resolution was complete there.* Hence, in this so far unique observation on cancer treated with the trypanosome preparation it may be noted that, probably *under the influence of a definite factor, degenerative and necrobiotic changes have occurred in the cancer cells, with the disappearance of signs of proliferation and subsequent resolution of the products of alterative processes in the cancer cords*".

Having studied patient K.'s tumour after Prof. Talalaev, we can only confirm the description given above (Plates 91–94. Observation No. 48. Described in 1946 as case No. 6).

Patient K.'s case was very illuminating. The following objection may, however, be raised: the signs of a macrophage reaction, so clearly visible in our preparations, do not depend on the action of the trypanosome preparation, since other similar pictures may *sometimes* be seen in malignant tumours which have not received any treatment at all. Does this objection carry any weight? No, it can be *only an apparent objection*, if considered in dissociation from the clinical observations. Here is the reason: signs of defence reactions may *sometimes* be met with in malignant tumours — because they reflect the fight which the body carries on against the cancer tissue, i.e. in cases *when this fight is unsuccessful*. In the preparations of patient K.'s tumour the same signs are seen in completely opposite circumstances, when the tumour has not only diminished by about one third, but, even more important, has been replaced by *normal scar tissue*. Thus, the macrophage reaction is seen here under conditions of a *successful fight* by the defensive cells against the malignant tissue. This in fact is the principal significance of the pictures observed and the only correct interpretation of them as given by Prof. Talalaev, basing his conclusions on a combination of the clinical findings and a morphological analysis of microscopical preparations.

It would, however, be hasty to draw any definite conclusions on the basis of the study of just one case. The question of the macrophage reaction cannot and must not be decided only from this aspect, as has already been stated. Detailed study of the preparations has shown that in this case we are concerned not only with the greater or smaller number of macrophages in the preparation but also with the fact that macrophages are present in the inflammatory infiltrates surrounding the tissues of the squamous-cell carcinoma and come into topographical contact with groups of cancer cells. The most important and significant fact is that the macrophages penetrate deeply into the basic sheets of malignant tissue. Around the penetrating macrophages are signs of heterolysis familiar to every histologist: each macrophage is surrounded by a large vacuole; moreover — and it is important to stress this — the work of the macrophages is apparent not only in areas of degenerating malignant tissue but also, *to no less extent, in zones of completely unchanged cancer cells.* Such a picture cannot be interpreted other than as a clear *expression of aggressiveness, not now by the malignant tissue but by the macrophages.*

For the morphologist, the microscopical picture seen in the tumour after treatment can only be interpreted thus: *aggressiveness by the malignant cells has given way to aggressiveness by the defence macrophage cells.*

If we wish briefly to put a name to these phenomena seen in the tu-

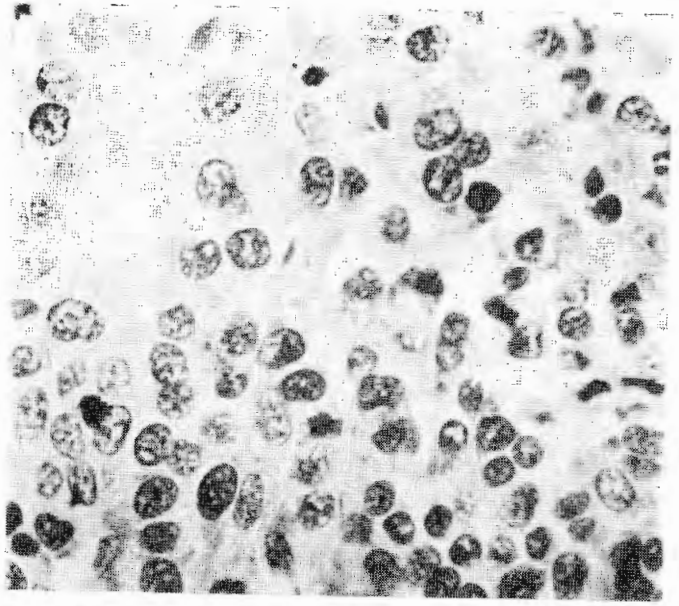


PLATE 101. Typical histological appearance of the Brown-Pearce carcinoma.

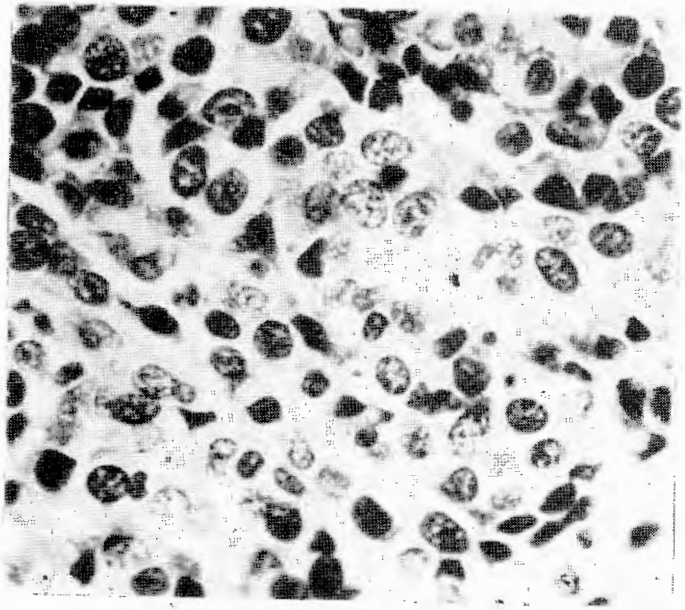


PLATE 102. Initial changes in the histological structure of the rabbit carcinoma during treatment with the trypanosome preparation; cells in various stages of degeneration may be seen.

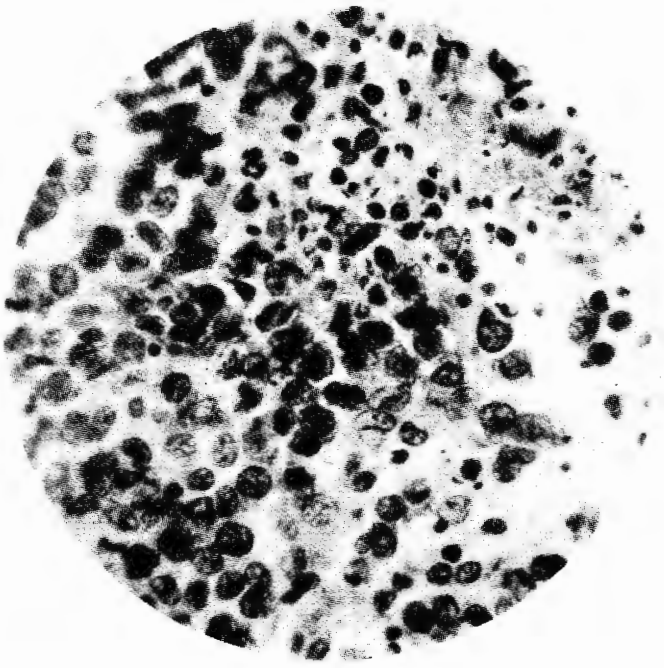
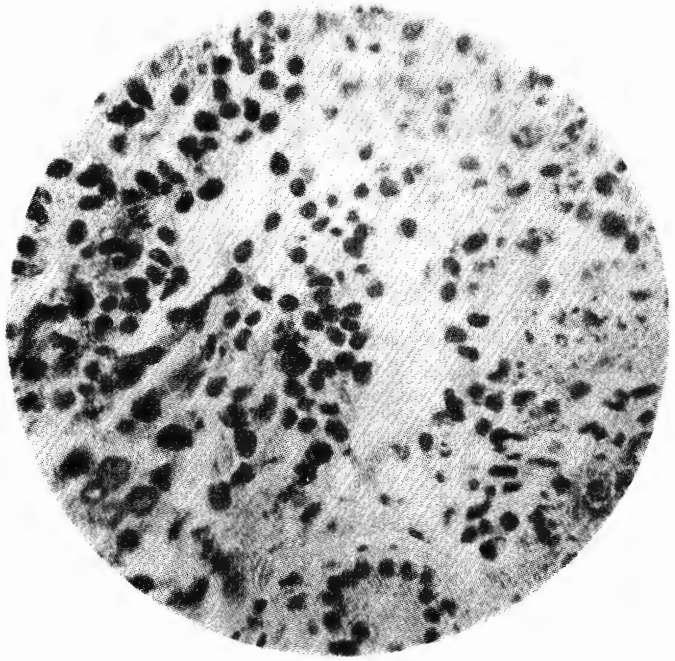
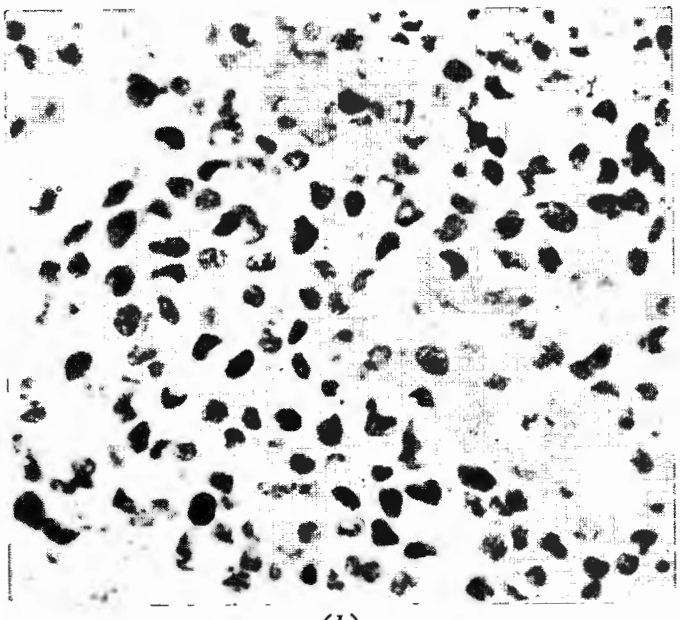


PLATE 103. Further degenerative changes in cells of the rabbit carcinoma under the influence of the trypanosome preparation.



(*a*)



(*b*)

PLATE 104, *a*, *b*. A later stage in the cellular changes in the rabbit carcinoma under the influence of the trypanosome preparation.

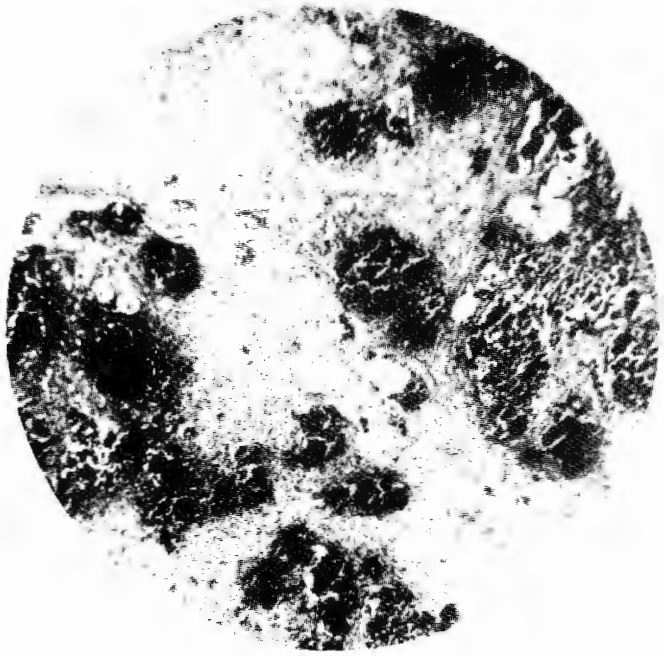


PLATE 105. Remains of the rabbit carcinoma after treatment with the trypanosome preparation, showing dark areas of completely destroyed cancer tissue; magnification × 80.

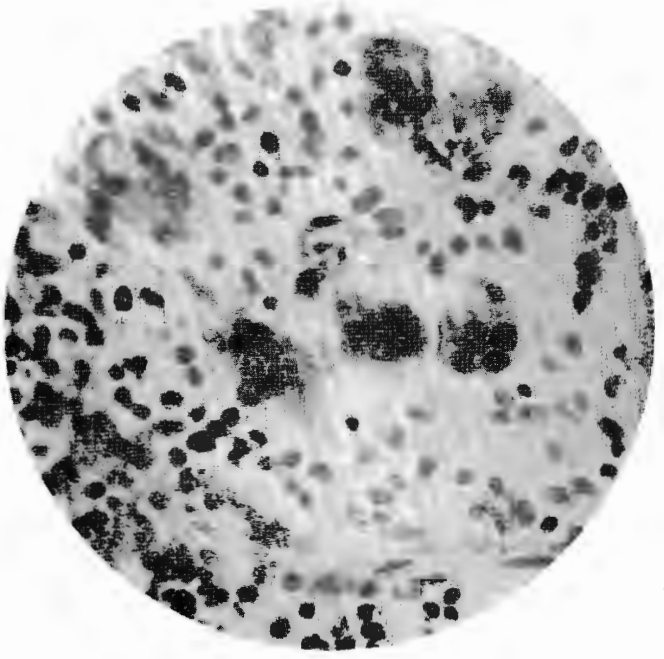
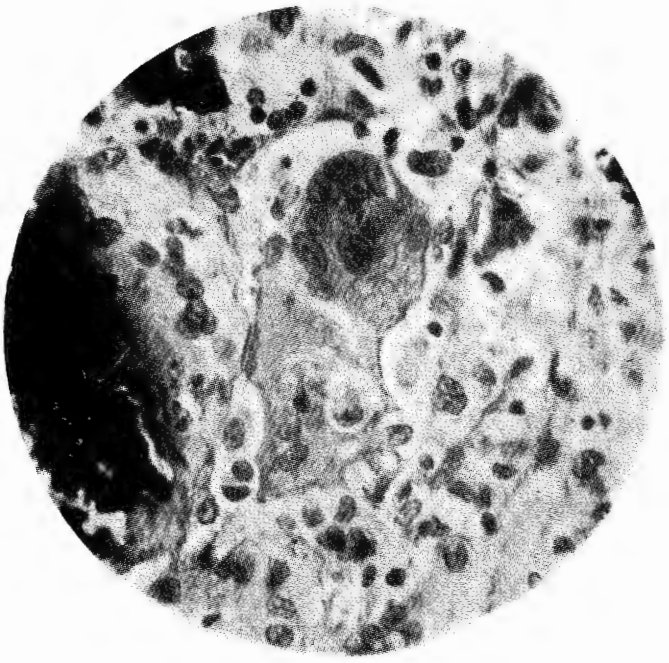


PLATE 106. The site of a former rabbit carcinoma destroyed under the influence of *T. cruzi* infection, showing polyblasts and numerous cells of the lymphocyte series.



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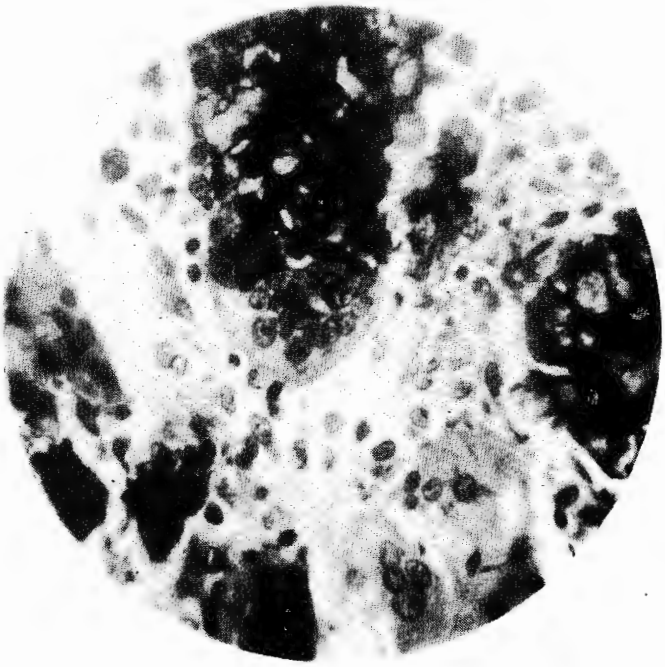


(*b*)

PLATE 107, *a*, *b*. Polyblasts among the tissue, of a destroyed rabbit carcinoma (No. 120); one of the final stages in the tumour changes occurring under the influence of the trypanosome preparation; magnification × 600.

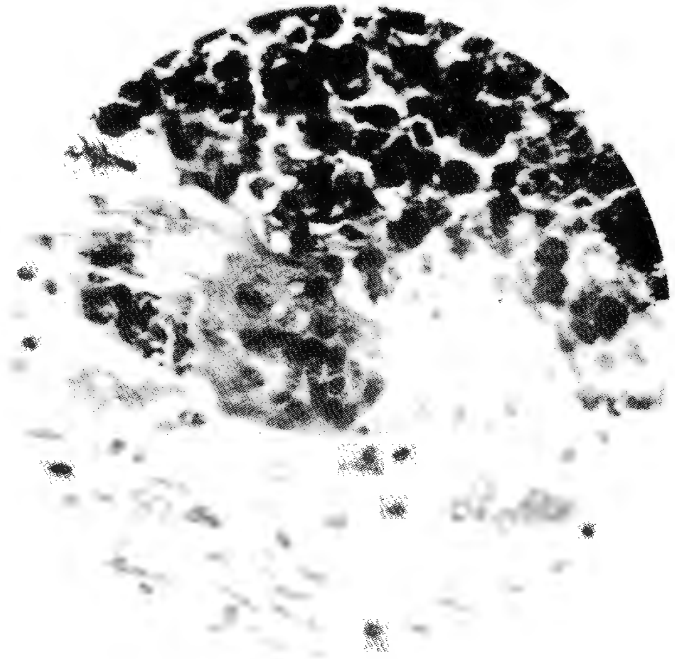


PLATE 108. Polyblasts alongside cells of a rabbit carcinoma destroyed under the influence of the trypanosome preparation; magnification × 600.

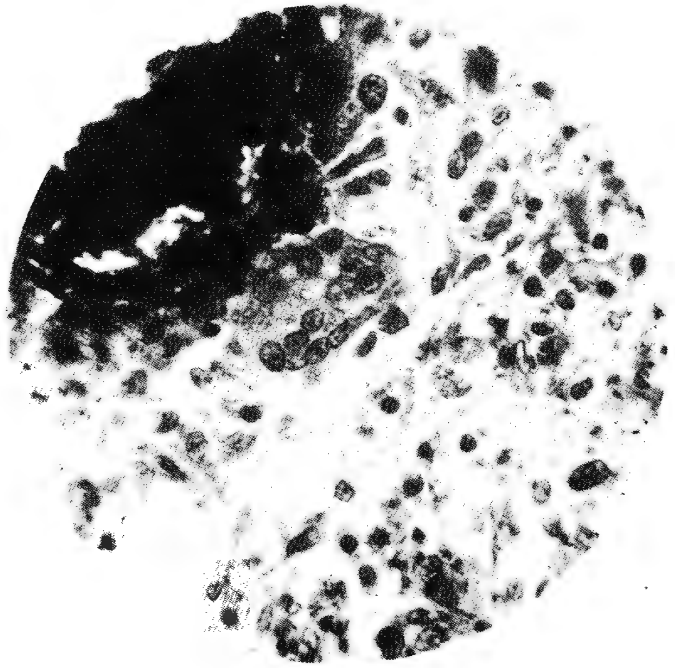


PLATE 109. A polyblast can be seen alongside the remains of a malignant rabbit tumour destroyed as a result of treatment with the trypanosome preparation; magnification × 600.

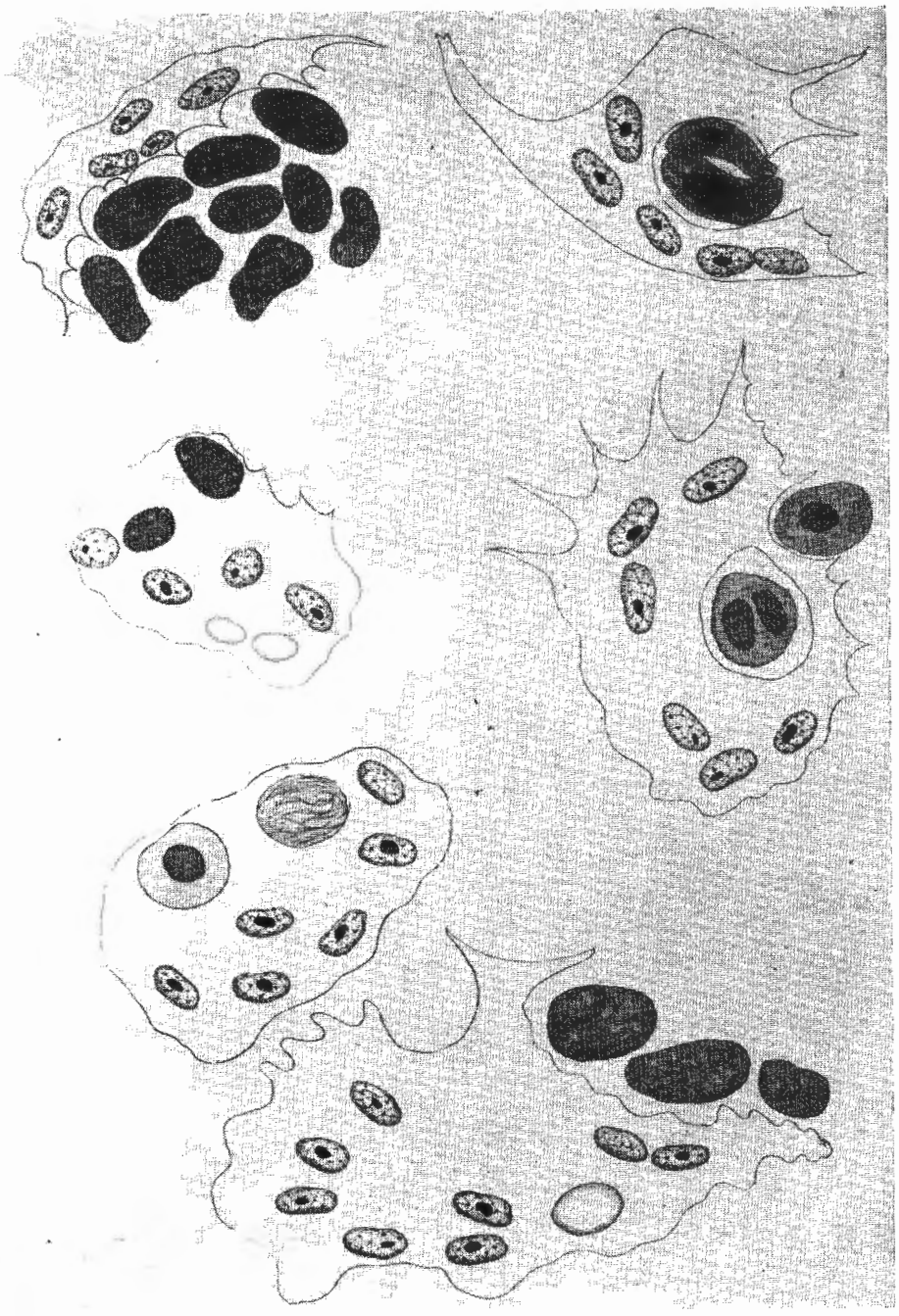


PLATE 110. A drawing of different polyblasts (macrophages) met in the terminal stages of breakdown of the rabbit carcinoma as a result of treatment with the trypanosome preparation; signs of phagocytosis of degenerating or broken-down cancer cells can be seen; (Drawing) magnification × 900.

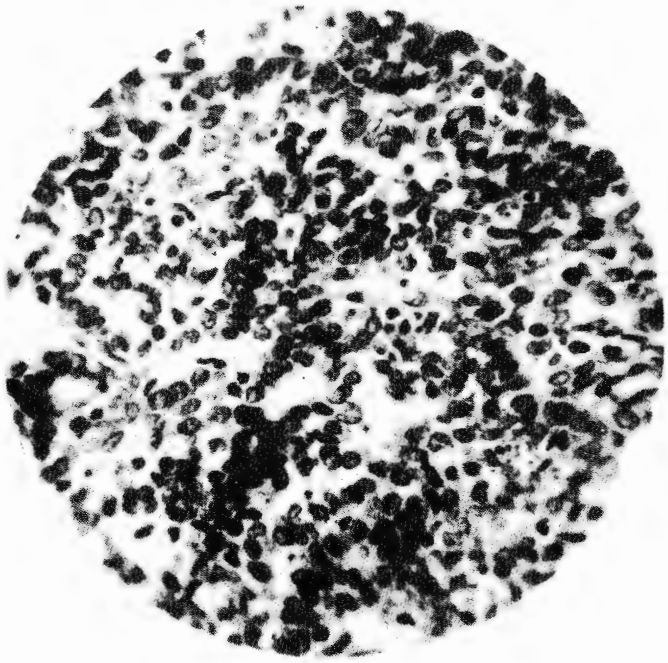


PLATE 111. Histological structure of a spontaneous adenocarcinoma in mouse 5027 before treatment; magnification × 600.

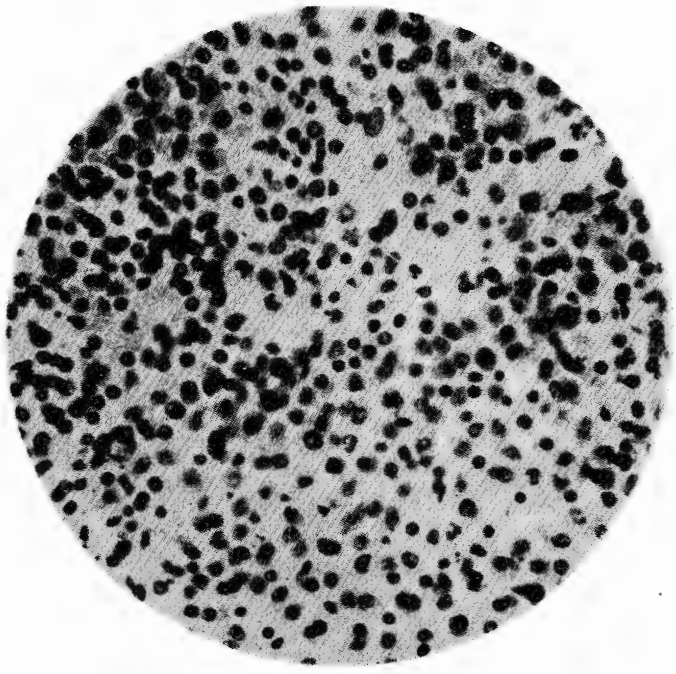


PLATE 112. Portion of the spontaneous adenocarcinoma in mouse 5027 after treatment; lympho-histiocytic cells are seen at the site of cancer tissue undergoing necrobiosis; magnification × 600.

mour after treatment, they may be classified as *canceroclastic* signs, and the macrophages, because of the particular function they perform in this case, should be called *canceroclasts*, since we are dealing here not with cells taking part in an inflammatory response but with cells carrying out a *completely new and completely specific function — the destruction of malignant cells.* We are therefore right in speaking of *canceroclasts* and *canceroclasty*. These phenomena supplement and complete the situation first put forward by the late Prof. Talalaev.

These observations on the role of macrophages and histiocytes in the process of regression of malignant tumours under the influence of the trypanosome preparation find confirmation, as we shall see later, in experiments on the treatment of the Brown–Pearce carcinoma of rabbits and transplantable and spontaneous mammary tumours of mice.

2nd case. Patient S-shkina. A tumour on her lower lip measured 3 × 1.8 cm; the submental region contained a firm lymphatic node measuring 0.5 cm in diameter. Histopathological diagnosis on a biopsied fragment taken before treatment (23 March 1948): "*Keratinizing squamous-cell carcinoma* with profuse inflammatory infiltration of the connective tissue core and extensive ulceration (Prof. Rapoport) (Plates 95 *a, b*; observation No. 49).

After 2 months of treatment (28 May 1948) another biopsy was performed. The preparations showed that significant changes had taken place in the tumour, described by Prof. Rapoport in this manner: "Histological investigation revealed that the connective tissue foundation of the skin was markedly disrupted and profusely penetrated by the cells of an inflammatory infiltrate, with a preponderance of lymphoid elements. Proliferating cords of epithelium were found to be buried deep in a tissue resembling granulation tissue. The latter had acquired a villous (papillomatous) character in places. *Findings indicating any malignant nature of the process were absent from the preparation.* Histopathological diagnosis: "*no tumour process was found. Signs of inflammation*" (Prof. Rapoport). However, there was then some deterioration in the process and after a month the whole tumour was removed. The histopathological report reads: "Resected portion of lip measuring 3.2 × 1 × 1 cm. The surface of the biopsied portion is ulcerated. The floor of the ulcer is finely granulated. The ulcer measures 2.5 × 1 cm. Histological examination of the ulcer region reveals an extremely dense inflammatory infiltration of the submucous layer, situated deep in the resected portion. The inflammatory infiltrate contains extensive foci of keratinizing and necrobiotic squamous epithelium, some of them *undergoing giant-cell resorption.*

Clearly visible in some places are *zones of reactionary inflammation around keratinizing, necrobiotic epithelial cells undergoing resorption.* There are also abnormal epithelial proliferations of a reactionary inflammatory nature, with pearl formation at the centres of the cords. *Conclusion: the findings necessary for a diagnosis of squamous-cell carcinoma are absent* from the material studied. *Attention is drawn by the picture of resorption of extensive abnormal foci of keratinizing and nonkeratinizing epithelium against the background of an intensive inflammatory process*" (Prof. Rapoport).

We have examined preparations of this tumour again and can only confirm the above description. What then was the nature of the macrophage reaction in the treated tumour in patient S.? Here we again meet the macrophage not so much as an element of the accompanying inflammatory infiltrate, not as the active cell of an inflammatory focus (this occurs independently), but as a *specialized phagocyte-carcinoclast* (Plate 96).

The histopathological observations were supplemented by special cytological studies of the cells of the first, second and third biopsies, concentrating on a comparative study of the sizes of nuclei and nucleoli and also on changes in nuclear and nucleolar sizes. In this investigation we followed the line taken earlier by MacCarty (1925–1933) and a number of other authors stating that hypertrophy of the nucleolus and especially breakdown of the ratio between nuclear and nucleolar sizes is a specific sign of malignant cells (MacCarty and Haumeder, 1934; Haam and Alexander, 1936; Fidler, 1935; Alexander and Bird, 1936).

However, the literature is not unanimous over the question of nucleolar hypertrophy as a particularly *dominating* sign of a malignant cell. The question of the extent to which this sign is a property *only* of the cells of malignant neoplasm is also not clear. To answer these questions our colleague E. Kirpichnikova carried out comparative observations on mammary cells in various states, enabling her to follow changes in the cell nucleoli in the normal state, in hypertropy of the gland during pregnancy and finally during malignant proliferation within the gland (adenocarcinoma).

Kirpichnikova's observations enabled the following conclusions to be drawn:

1. Considerable nucleolar hypertrophy is seen in malignant tumour cells;

2. Nucleolar hypertrophy is not a specific sign of cells of a malignant

tumour alone, since similar large nucleoli were found in the mammary gland of pregnant mice.

It follows from this that for practical purposes in the cytodiagnosis of malignant tumours the size of the nucleolus cannot be used in isolation from other signs of the cancer cell.

We were therefore able to state that the criterion of malignancy put forward by MacCarty and his followers is not itself of sufficient significance but may and should be used as an additional sign in combination with a general histopathological analysis.

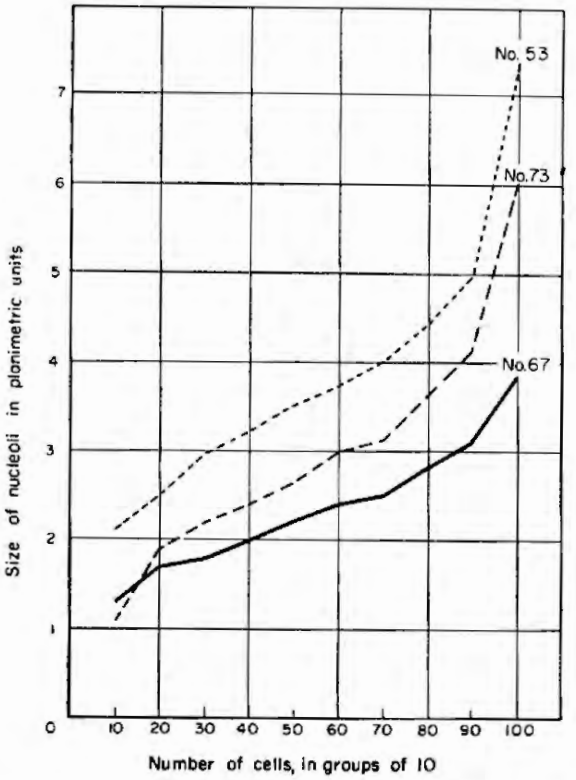


FIG. XIX. A comparison of nucleolar size in the cancer cells of patient S.'s tumour before and during treatment; analysis of three consecutive biopsies (Nos. 53, 67, 73) shows diminution of the nuclei under the influence of administration of the trypanosome preparation.

All this shows the importance of studying changes in nuclear and nucleolar size during the process of a biotherapeutic action on the tumour tissue.

Our investigations, carried out in collaboration with N.P. Dmitrieva, of three consecutive biopsies in patient S. revealed (Figs. XIX, XX) the following:

1. The average size of the nucleolus at the first biopsy was more than 1.7 times the average nucleolar size during treatment.

2. The average sizes of the cell nucleus, studied after the first and second biopsies, did not differ appreciably.

3. The nucleolus–nucleus ratio in material from the first biopsy was less than the ratio after the second biopsy, viz. 1 : 7 against 1 : 11.3.

This change indicates that after treatment with the trypanosome preparation the nucleolus–nucleus ratio moved in the direction characteristic of benign cells.

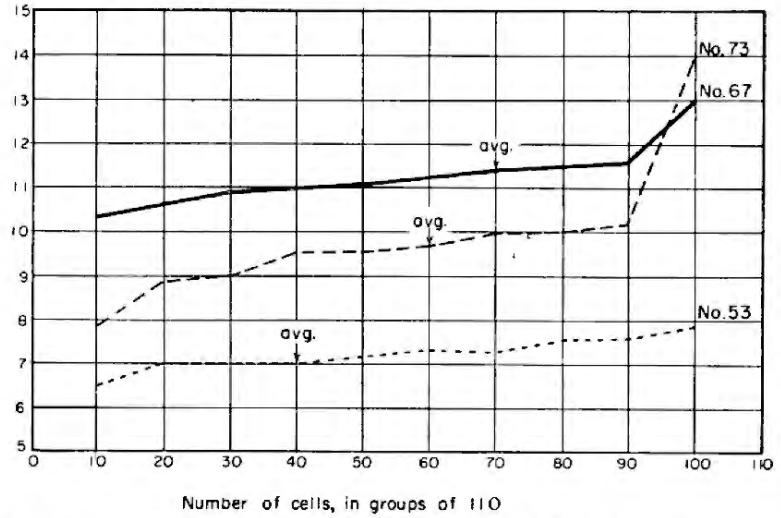


FIG. XX. Changes in the relationship of nuclear and nucleolar size in cancer cells in patient S. under the influence of the trypanosome preparation. Analysis of material from three consecutive biopsies (Nos. 53, 67, 73).

We are justified in concluding from the comparative measurements given that cells of the second biopsy showed significant changes in the nucleus and nucleolus, changes, moreover, indicating a normalization of the nucleus–nucleolus relationships.

If we now compare the findings obtained by karyometry in material from the third biopsy (when the tumour was removed surgically) with the measurements of the nuclei and nucleoli in material from the second biopsy, we must admit that in this case the nucleus and nucleolus had enlarged slightly, though the nucleolar size still did not reach its value before treatment.

Thus, the tumour cells in patient S. first showed a sharp move towards normalization of the nucleus–nucleolus relationship, followed by a move in the opposite direction, but the overall character of the changes in these relationships in the tumour cells was sufficiently obvious and characteristic.

Considering these karyometric findings in combination with the histopathological observations, we may quite justifiably state that significant histological and cytological changes took place in patient S.'s tumour.

Summing up now all the observations, histological and cytological, made on the lip cancer in patient S., we may pick out the most characteristic changes. In patient S.'s tumour, which was subjected to no therapeutic action other than that of the trypanosome preparation, we see:

1. Changes in and destruction of the cancer cells.

2. Signs of a macrophage reaction in areas where foci of keratinizing and nonkeratinizing epithelium are undergoing giant-cell resorption.

3. All these processes are taking place against the background of a clearly expressed inflammatory reaction.

It should be added that a comparison of cells from the first and second biopsies clearly indicates the change in *ribonucleic acid content characteristic of benign cells*. Finally, we see simultaneous *karyological changes* indicating normalization of the nucleus–nucleolus relationships in patient S.'s tumour.

In general, we are bound to report the presence in patient S.'s tumour of a *definite complex of cyto-histological changes*, brought about by the use of the trypanosome preparation. We must emphasize once more, however, that removal of part of the tumour was carried out at a time when the initial favourable cytological indications had deteriorated slightly.

After the operation the patient was given 61 more injections, after which she was discharged. In spite of insistent requests to continue the injections, the patient interrupted them for a long period and left town. When, 9 months later, she did return to the clinic, a recurrence of the tumour was found to be developing at the site of the operation scar.

After this a simple comparison of the two cases—the tumour in patient K. described previously and this description of the tumour in patient S.—is enough to enable us to conclude that in both cases we are dealing with what may be called successive stages of the same process: the first case was caught in an early stage and the second in one of the later stages.

The next link in our observations on the process of regression of cancer of the lip under the influence of the trypanosome preparation is provided by an observation on the changes occuring in a tumour of patient L-tsov (observation No. 4), in whom a keratinizing squamous-cell carcinoma of the

lip was completely cured solely by injections of the trypanosome preparation. Prof. L.M. Nisnevich, wishing to confirm the therapeutic result which he achieved, excised the scar which had formed at the site of the former malignant tumour. This tissue was subjected to a detailed examination by Prof. Rapoport, who, in his conclusions, having established the *complete absence* of malignant cells, found in one preparation, among the connective tissue foundation of the biopsy fragment which was infiltrated by lympho-histiocytes and plasma cells, a nodule with its centre consisting of keratinizing squames surrounded by a wall of giant cells. "This nodule—writes Prof. Rapoport—is apparently the remains of pearls which have undergone giant-cell resorption".

This remnant of the malignant tumour once present here could not fail to attract our particular attention. Photomicrographs and drawings (Plates 97, 98, 99*a,b*, 100) show that these remains of the malignant tissue are surrounded by an intensive macrophage reaction. It is quite obvious that these pictures serve to complete the previously described observations on patients K. and S., material from whom showed only the start of the process of regression of lip cancer.

4th case A study of the tumour in patient G-ov (cancer of the lower lip; observation No. 1) enabled us to determine the histological stages which must be considered the next stage in the regression of a tumour under the influence of the trypanosome preparation, following those which we were able to observe in the previously described cases of patients K., S. and L. If in patient S. the microscopical picture reflects *a penultimate stage in the process*, then material from patient G. enables us to assert that here the bistologist can observe the very final stages of a *completed process* arising under the influence of biotherapy.

A similar conclusion follows from a comparison of the histological picture described in the biopsy fragment taken before treatment with the findings of a histological analysis carried out after apparent recovery of the patient.

Let us recall briefly the state of the tumour at the start of the treatment (observation No. 1). Histological examination revealed: in the affected area there was an extremely dense, profuse infiltration of the connective tissue foundation with round cells plus a small number of polymorphonuclear leucocytes and small histiocytes. Among the infiltrate were abnormal cords and foci of squamous epithelial cells, penetrating the tissues in various directions.

The centres of the epithelial complexes showed keratinization with pearl-formation. At the periphery of the affected area there were signs of

a marked acanthosis with moderate hyper- and parakeratosis and a loose infiltration of the connective tissue core with various cells, including many plasma cells.—*Histopathological diagnosis: "early squamous-cell carcinoma"* (Prof. Rapoport).

After prolonged treatment the tumour disappeared and we were able to turn to an observation on the duration of the therapeutic effect obtained.

In this state the patient was shown to a committee of surgeons on the staff of Prof. Kuprianov, Prof. Mel'nikov and a number of other clinicians.

In spite of the findings on examination of the patient and the argumentation of Prof. Nisnevich, and also the opinions of Prof. Sviatukhin, Dr. Andreev and other clinicians, this committee did not find it possible to agree that patient G. was apparently healthy, and moreover some members of the committee, particularly Prof. Mel'nikov, specifically pointed out places where, in their opinion, there were malignant neoplasms which had not disappeared during treatment with the trypanosome preparation. In view of these circumstances, and on the insistence of Prof. Mel'nikov, the committee resolved that all areas suspicious of malignancy should be excised and submitted to histological analysis. This operation was carried out in the presence and under the direction of Prof. Mel'nikov. The operation material was fixed and sent for investigation by a special authoritative committee of Moscow and Leningrad pathologists. Here is the conclusion of this committee, in its original form (our italics).

"Histological investigation (10 December 1948) of preparations from the lower lip revealed the following:

1. One of the areas shows signs of acanthosis together with flattening of the Malpighian layer over a limited area. In this region the *development of scar tissue* can be seen in the subepithelial layers. There is profuse inflammatory infiltration of the subepithelial layers, consisting mainly of cells of the lymphoid type plus a few histiocytes and isolated plasma cells.

In the depths of the biopsied fragment there are bundles of striated muscle fibres, also penetrated by cords of scar tissue and a loose cellular infiltrate. *No signs of a tumour process can be found.*

2. The second area examined also shows acanthosis and hyperkeratosis.

Scar tissue is seen to be developing over a limited region of the connective tissue core, which also shows signs of a diffuse, chronic granulatory inflammation.

Salivary gland tissue is present in the depths of the preparation examined, with inflammatory infiltration of the stroma.

In this preparation also no signs of a tumour process can be found.

3. Examination of three lymphatic nodes reveals only a picture of hyperplasia of a *nonspecific character.*

No signs of a primary or metastatic tumour process can be seen in any of the lymphatic nodes examined.".

This conclusion was drawn by a committee of pathologists comprising: Prof. L. Shabad, Prof. A. Strukova, Prof. Grazunova and Prof. Rapoport.

It is hardly possible to add anything to their conclusion, and hardly possible to overestimate its importance. Comparison of the histopathological conclusion with patient G.'s case-history can only emphasize still further the principal significance of the established facts: in patient G. — a squamous cell carcinoma—after using only the trypanosome preparation neither clinical examination nor a microscopical histological analysis revealed cancer elements at the site of the former tumour or in the regional lymphatic nodes.

The histological analysis of patient G. 's tumour permits us to assert, on the one hand, the accuracy of our assessment and interpretation of the microscopical pictures determined in the treated tumours of patients K. and S., and on the other hand that we have quite legitimately regarded the histological changes seen in patient K., patient S. and finally in patient G. *as successive stages of a single process of destruction of malignant tissue and replacement of it by tissues of a normal process, which against the will of the experimentors was interrupted in the first two patients at various points in clinical recovery.*

Such are the findings of a histological study of a number of cases of cancer in patients subjected to treatment with the trypanosome preparation. They must be supplemented and compared with those described in Part IV of this book, where the clinical observations were presented. An analysis of all the investigations as a whole enables us to establish certain general principles of the regression of malignant tumours under the influence of the trypanosome preparation. These changes involve the behaviour of the macrophage system, characteristic changes in the connective tissue and, what is particularly significant they touch upon the basic properties of the cancer cells themselves, as reflected by changes in the mitotic index, changes in nuclear size and characteristic alterations in the nucleolus itself and in the nucleus–nucleolus relationships. For a whole series of reasons the histological analysis of clinical material is of a somewhat fragmentary nature. In parallel with these observations, however, we carried out an investigation of the histological, histochemical, and cytological changes occuring in spontaneous and transplantable

tumours of animals. The main subjects of this study were the rabbit carcinoma and spontaneous mouse tumours. We now turn to a summary of the observations made on these subjects.

2. OBSERVATIONS ON TUMOURS OF LABORATORY ANIMALS

A. THE INFLUENCE OF TRYPANOSOME INFECTION AND THE TRYPANOSOME PREPARATION ON THE BROWN-PEARCE CARCINOMA

It appeared to us that an analysis of various questions of the biotherapy of malignant tumours could only be of advantage if our experiments included, as well as spontaneous tumours of mice, a tumour so difficult to subject to experimental effects as the rabbit carcinoma. This tumour is of particular interest in deciding questions associated with the effects of the cancer antibiotic both on the process of *metastasis formation* and on *already developed metastases.* Bauer and Deckner (1935) consider the rabbit tumour particularly suitable for chemotherapeutic tests: "Anything curing this tumour has good prospects of curing other, particularly human, forms of cancer". Another author, Domagk, also points out the *exceptional resistance of the Brown–Pearce tumour to therapeutic action.*

"Of all the numerous transplantable tumours of animals obtained during the last decade, the one *closest to spontaneous human carcinoma* is the Brown–Pearce rabbit carcinoma", writes Ya. Naftol'ev (1939).

As shown by a detailed investigation carried out in our laboratory by G.S. Yumashev, *T. cruzi* infection in the rabbit takes a relatively mild course compared with that in mice, and all the rabbits recover, after which no trypanosomes can be found *in the circulating blood.* Single individuals can be found in the internal organs: lymphatic nodes, myocardium, spleen, liver, kidneys and adrenals. Nevertheless, the development of a trypanosome infection prevents metastasis formation to a great extent and causes regression of the primary tumour. The inhibitory effects seen in these experiments can only depend on factors produced by the trypanosomes situated in the various internal organs. Note that in the rabbit the trypanosome does not penetrate into the tumour tissues and is not found inside malignant cells, as we have described earlier in sarcomata and carcinomata of white mice.

On comparing Yumashev's findings on the development of the rabbit carcinoma in control and trypanosome-infected cancer-bearing rabbits, it is not difficult to establish the following important factors: (1) *T. cruzi* infection inhibits metastasis formation by the rabbit carcinoma; (2) in about

half of the experimental rabbits there was appreciable diminution of large tumours already developed in the testis; (3) in a number of cases *T. cruzi* infection also leads to the disappearance of the original tumour implanted into the testis; the original tumours disappeared in 6 of the 20 experimental rabbits; (4) trypanosome infection produced a complete cure in 30 per cent of the cancer-bearing rabbits. Subsequent observations on the effects of the trypanosome preparation showed that the principles established during the effects of trypanosome infection are repeated to a significant degree on treating the rabbit carcinoma with the trypanosome preparation. The experiments prove (see tables) that in the case of the rabbit carcinoma the trypanosome preparation *takes effect in the first instance against the process of metastasis formation.* When the preparation is used early, metastasis formation can be completely suppressed, although, as we know from the literature, in the Brown–Pearce tumour the danger of metastasis formation arises within a few hours of implantation. Analysis of reports on the treatment of cancer-bearing rabbits shows that the final effect may depend on: (1) the dosage and duration of administration of the trypanosome antibiotic; (2) the state of the body; only this can explain why different cancer-bearing rabbits react to various degrees to the injection of equal doses of the preparation. A histological investigation was made of the tumours, both those submitted to the effect of the preparation from *T. cruzi* and the controls.

B. HISTOLOGICAL AND CYTOLOGICAL CHANGES IN THE BROWN-PEARCE TUMOUR UNDER THE INFLUENCE OF THE TRYPANOSOME PREPARATION

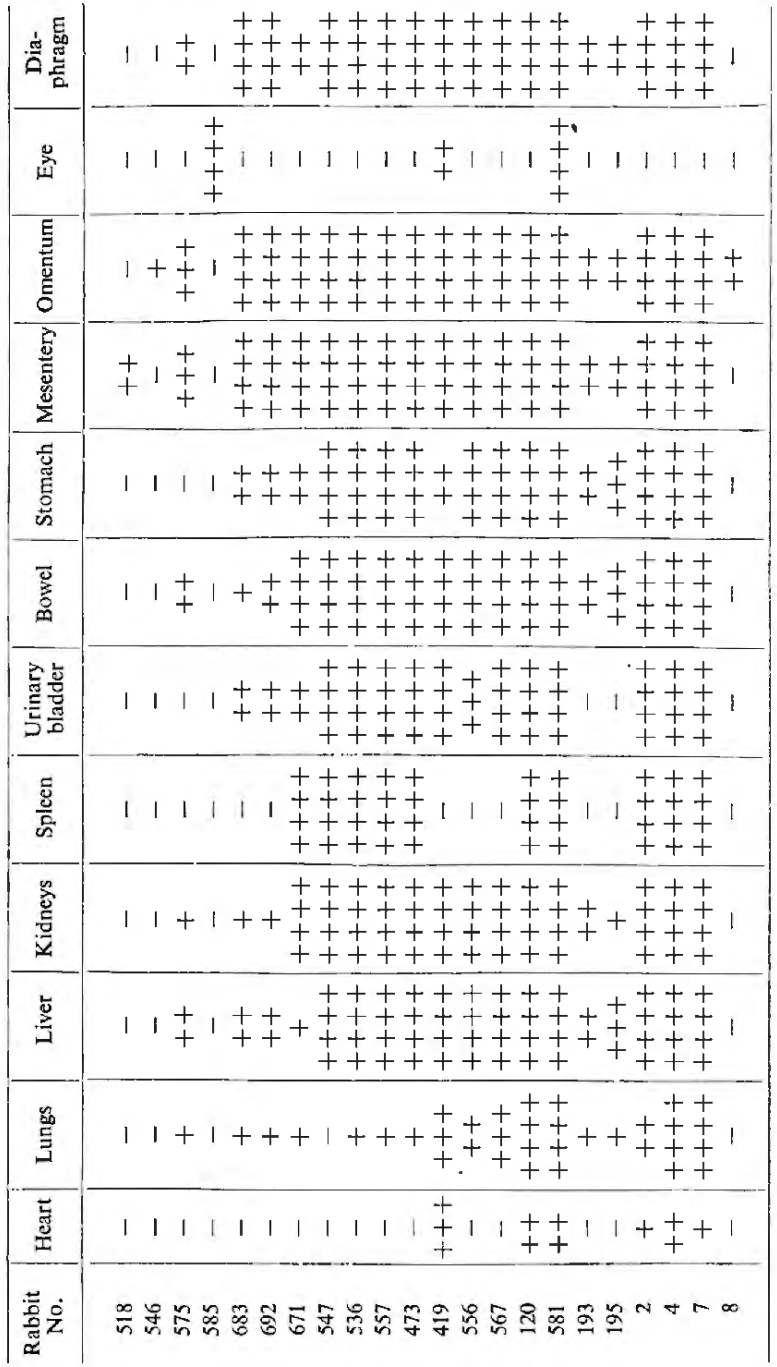
The microscopical structure of the malignant tissue of the rabbit carcinoma is not uniform in different areas and at different stages of its development. As a rule, the tumour consists of relatively large polygonal or oval cells with finely granular cytoplasm, which may include a few somewhat vacuolated areas. The nuclei are oval, often rather irregular in form, relatively rich in chromatin, with one or two nucleoli. Dividing cells may be seen throughout the tumour tissue, except in zones of necrosis, but their numbers vary with the area of the tumour and its stage of development: often 4 or 5 mitoses can be seen within a field of vision of the microscope. Sheets of typical cancer cells may alternate with zones of necrosis, the size of which varies in different tumours. Signs of necrosis may appear in the tumour very early, when it is still very small. In studying the histological, cytological and cytochemical changes occurring in the rabbit carcinoma under the influence of the trypanosome antibiotic we paid particular attention to the distribution of and changes in nucleic acids

BROWN-PEARCE CARCINOMA OF RABBITS
(Metastases in treated rabbits)

Rabbit No.	Heart	Lungs	Liver	Kidneys	Spleen	Urinary bladder	Bowel	Stomach	Mesentery	Omentum	Eye	Diaphragm
553	−	−	−	−	−	−	−	−	−	−	−	−
573	−	−	−	−	−	−	−	−	−	−	−	−
560	−	−	−	−	−	−	−	−	−	−	−	−
545	−	−	−	−	−	−	−	−	−	−	−	−
581	−	−	−	−	−	−	−	−	−	−	−	−
555	−	−	−	−	−	−	−	−	−	−	−	−
570	−	−	+	−	−	−	−	−	−	+	−	−
120	−	−	−	−	−	−	−	−	−	−	−	−
179	−	−	−	−	−	−	−	−	−	−	−	−
154	−	−	+	−	−	−	−	−	−	−	−	−
167	−	−	+	−	−	−	−	−	−	−	−	−
138	−	−	+	+	−	−	−	−	+	+	−	−
421	+	−	−	+	−	−	−	−	−	+	−	+
550	−	−	+	−	−	+	+	−	+	+	−	+
548	−	−	−	−	−	−	+	−	+	+	−	+
403	−	−	+	−	−	−	−	−	−	+	−	−
553	−	−	+	+	−	−	+	−	−	+	−	−
535	−	−	+	+	−	−	+	−	+	+	−	−
461	−	−	−	−	−	−	−	−	−	−	−	−
405	−	−	−	−	−	−	−	−	−	−	−	−
183	−	−	−	−	−	−	−	−	−	−	−	−
203	−	−	−	−	−	−	−	−	−	−	−	−
124	−	−	−	−	−	−	−	−	−	−	−	−

The influence of treatment with the trypanosome preparation on the number, size and distribution in various organs of metastases of the rabbit carcinoma; the first column of each table gives the number of the treated and control rabbits; the intensity of metastasis formation is denoted by plus signs, from one to four.

BROWN-PEARCE CARCINOMA OF RABBITS
Metastases in untreated rabbits
(Autopsy reports)



(ribonucleic and thymonucleic) in the cancer cells, considering the significance that these protoplasmic components may have in the life, development and multiplication of both normal and malignant tissues (Brachet, 1944, Caspersson and Santesson, 1942; Kedrovskii, 1942; Roskin and Kharlova, 1944; Roskin, 1946, and many others).

On the basis of these studies it must be accepted that wherever cellular activity is associated with intensive protein synthesis, there we find large amounts of ribonucleic acid. This occurs in rapidly-multiplying (for example, in Protozoa: Roskin and Gintsburg, 1944) or in regenerating cells (Roskin, 1946), or, finally, where there is intensive production of protein secretions, as takes place for example in the glandular cells of the pancreas (Brachet, 1955, 1957). By Caspersson's hypothesis, ribonucleic acid is an important link in a complex synthesis involving both cytoplasmic and nuclear proteins.

All this shows how important it is to consider the nucleic acids in the cell in all cases when it is necessary to follow the state of the cell with regard to its abilities to grow and multiply.

The cells of the Brown–Pearce tumour are rich in ribonucleic acid, which is found in the cytoplasm and in the nucleoli. The ribonucleic acid contents of different tumour cells are not equal. Frequently the main mass of a fairly well-developed tumour consists of cells containing relatively little ribonucleic acid, when the nuclei of such cells also contain relatively little thymonucleic acid. Among these cells there are sheets of cells, of various widths, or groups and even isolated cells with cytoplasm richer in ribonucleic acid; the nuclei of these cells has not such a loose structure and is, apparently, richer in thymonucleic acid. The distribution of nucleic acids changes in zones of slower growth (as judged by the number of mitoses) and, even more markedly, in areas where degenerative processes have already started. Here ribonucleic acid disappears completely from the cytoplasm or is seen in hardly detectable traces. At the same time ribonucleic acid disappears from the nucleolus, the nucleolus itself diminishes appreciably and is diffcult to make out in the preparations. In such cells considerable diminution of the nucleus occurs, sometimes by 2–3–4 times. The diminished nucleus exhibits various forms of deformation and shrinking, homogenization of nuclear structure and, finally, the onset of typical signs of karyorrhexis.

The histological appearance of tumours in rabbits infected with *T. cruzi* (investigated by G.S. Yumashev) gives varying pictures of malignant tissue undergoing destruction. Depending on the strength of the therapeutic effect, one may see in such tumours all the stages of gradual destruction

of the carcinoma, from signs hardly detectable morphologically to the formation of extensive fields of cellular detritus and, finally, enormous areas of an homogenized, structureless mass (Plates 101–103). These phenomena were also seen earlier in experiments on the effects of *T. cruzi* infection or of the trypanosome preparation on transplantable carcinomata and sarcomata of mice. It may be shown that the stages of complete destruction of the carcinomatous tissue are preceded by the disappearance of ribonucleic acid from the nucleoli, dissolution or marked diminution of the nucleoli, considerable diminution of the nuclei and of all the cancer cells as a whole, disturbances in the structure and distribution of the chromatin components of the nuclei and finally various stages of necrosis and lysis of the malignant cells.

All that has been said of the effects of *T. cruzi* infection on the histological structure of the rabbit carcinoma is to a considerable extent applicable also to observations on the effects of the trypanosome preparation. As we have already stated, the rabbit carcinoma may normally show areas of degeneration and necrosis even without treatment, but in untreated tumours these phenomena *only accompany continuous and rapid growth* of the tumour tissue, whereas *in tumours treated with the trypanosome preparation signs of regression, degeneration and necrosis predominate* and eventually lead to the complete disappearance of a considerable part of the original tumour and its metastases. These increasing signs of necrosis in treated tumours, ending in lysis of the cancer tissue, are shown in successive photomicrographs (Plates 104–105).

On studying preparations sent to us by Dr. Yumashev of a rabbit carcinoma diminishing under the influence of biotherapy, we found a considerable number of polyblasts among the necrobiotic malignant tissue (Plates 106–110). The investigator is hardly justified in ignoring the activity of polyblasts in the process of destruction of the rabbit carcinoma under the influence of the trypanosome preparation. We have already met this phenomenon in our study of histological changes in human tumours under the influence of biotherapy. The situation observed in experiments on the rabbit carcinoma not only confirms our previous observations but gives them even greater conviction, since in these cases the macrophage reaction cannot be caused by accompanying signs of a septic inflammation. We should like to emphasize once more, however, that we are not dealing with macrophage reactions in general but with the *specific activity* of multinucleated giant cells — a *canceroclastic* reaction, as a definite phase of biotherapeutic interference. Plate 110 gives an idea of the size, shape and structure of these multinucleated giant cells, the polyblasts.

They are typically amoeboid in form, often with long, clearly developed pseudopodia. Their basophil cytoplasm is markedly vacuolated in structure and is relatively rich in ribonucleic acid. The number of nuclei varies greatly, from 2 to 20 or more. The nuclei contain very large nucleoli, rich in ribonucleic acid.

The phagocytic activity of the macrophage cells is visible in a number of drawings and photomicrographs. In some areas of the preparation these giant cells surround dead cancer cells or their fragments. In other places a situation may be seen where the macrophages envelope dead cancer cells by means of their pseudopodia. The macrophages may also show large food vacuoles containing phagocytosed cancer cells in various stages of digestion.

Summing up our observations on the effects of the trypanosome preparation on the rabbit carcinoma, it may be established that in treated tumours the process of diminution and even complete disappearance of the malignant tissue takes place in stages. In the first stage there is reduction of the amount of ribonucleic acid in the cytoplasm of the cancer cells, as a result of which its basophilia is decreased. This circumstance deserves particular attention in that it reflects, according to all the signs, the initial, *fundamental* phenomena. Similarly, we may say that the reduction in ribonucleic acid excludes the most important factor in protein synthesis. The fall in nucleic acid content brings the cancer cell into an inactive state, eliminates its ability to multiply further and, by disturbing its metabolism, brings about typical degeneration and death. Almost simultaneously with the gradual disappearance of ribonucleic acid changes commence in the structure and size of the nuclei—they become smaller, and the nucleoli disappear. Next, signs of nuclear deformation appear. Extensive and ever-increasing fields of degenerating cancer cells are thus formed.

The cancerolytic effects of the trypanosome preparation are manifested by the appearance in treated tumour of "fused" cancer cells. From the moment of formation of "cell bodies" and "fused" cells multinucleated polyblasts begin to appear in the tumour, the macrophage reaction increases steadily and the phagocytosis of dying cancer cells by giant cells is seen more and more frequently. At the same time the investigator's attention is drawn by the infiltration of the necrotic zones and areas of degenerating cancer cells by fibroblastic elements, with the formation of collagen fibres and subsequent signs of cicatrization.

Since *we were unable to determine similar phenomena in any of the untreated rabbit carcinomata it is clear that they must be related to the effects of the trypanosome preparation.*

It should be noted that in some treated rabbit tumours the histological and cytological phenomena described may be expressed to a greater or lesser degree: The difference in the histological pictures may possibly be explained by the fact that the periods of treatment of the rabbits varied, as did the activity of the preparation used.

In summing up our observations on the influence of the trypanosome antibiotic on the Brown-Pearce carcinoma, two significant points should be noted: the metabolism of the cancer cells is suppressed (as judged by a fall in the level of ribonucleic acid), and there is a clearly defined stimulation of macrophage activity and processes of cicatrization.

Having reached this conclusion, we thought it necessary to compare rabbit carcinoma experiments which would parallel that part of the clinical observations where we employed a combination of biotherapy with nonradical operation.

3. THE EFFECTS OF THE TRYPANOSOME PREPARATION IN COMBINATION WITH NONRADICAL OPERATION

A consideration of the question of combining biopsy of a tumour or nonradical operation with treatment by the trypanosome preparation is of real importance for the understanding and evaluation of the clinical observations described earlier, or the results of treatment of spontaneous tumours in laboratory animals.

Some experiments devoted to this question were carried out by our colleagues Prof. Sviatukhin and Dr. Milovanova, who wished to demonstrate the influence of resection of the implanted carcinoma in the testis of a rabbit on the effects of treatment with the trypanosome preparation; is it possible, having removed the rapidly-developing main tumour, to cure the numerous metastases which, as is well known, will by that time be developing in the various internal organs of the rabbit?

In this experiment all the rabbits had the carcinoma implanted into the testicle as usual. The rabbits were then divided into two groups. In the first group of rabbits the large tumour which had developed in the testis was surgically removed, but the rabbits did not receive the trypanosome preparation. In the second group of rabbits the developed tumours were also resected (14 days after implantation of the carcinoma), after which injections of the trypanosome preparation were started (20 cancerolytic units per injection), i.e. treatment was started *two weeks after the start of the experiment.*

The results of this experiment are given in the tables.

All the rabbits operated upon and then treated gained weight, whereas rabbit No. 741, only operated upon, lost 500 g in weight.

Although this material is quantitatively not great the observations give rise to the following preliminary conclusions:

Rabbit No.	Size of resected original tumour	Duration of treatment	Duration of observation	Result of experiment
		Preparation not injected		
741	tumour 3.1×1.7 cm in left testicle	—	150 days	Recurrence in region of testicle, tumour size 2×1.5 cm
762	tumour 2.1×3.6 cm in left testicle	—	53 days	Recurrence in region of testicle, tumour size 1×1.5 cm; metastases in lungs, kidney, omentum, bowel, mesentery, spermatic cord, right testicle.
752	1.0×2.4 cm	—	30 days	Metastases in left testicle, lungs kidneys, omentum, bowel, mesentery, bladder and spermatic cord
		Trypanosome preparation injected		
765	tumour 5.7×1.5 cm in left testicle	9 days	25 days	No metastases, right testicle normal
770	tumour 5.3×2.0 cm in right testicle	13 days	34 days	No metastases, left testicle normal
760	tumour 3.7×1.8 cm in right testicle	89 days	150 days	No metastases, left testicle normal
756	tumour 4.3×2.2 cm in left testicle	87 days	150 days	No metastases, right testicle normal
728	tumour 4.5×1.2 cm in left testicle	89 days	150 days	No metastases. Spermatic cord free. Right testicle normal

CHANGES IN WEIGHT IN THREE TREATED RABBITS (728, 760, 756) AND ONE UNTREATED (741)

Rabbit No.	Initial weight, g	Final weight after 15 days, g
728	2600	3100
760	2585	2950
756	2370	2520
741	2810	2310

1. Removal of the original, sufficiently developed testicular tumour had no appreciable influence on metastasis formation, which progressed just as in the control observations, nor was there evidence that removal of the original tumour had any influence on the duration of life.

2. Removal of the original, suffciently-developed testicular tumour 14 days after implantation, when treatment with the trypanosome preparation was given after the operation, led to *complete recovery* of the experimental animals, which when applied to the Brown-Pearce tumour must be considered a very significant result, having regard to the malignant properties of this tumour.

Thus, *nonradical operation* in the case of the actively metastasizing rabbit carcinoma can give a *complete therapeutic effect* under conditions of postoperative treatment of the metastases by injections of the trypanosome preparation. Although the observations described were made on a small number of rabbits, the experiments on the treatment of spontaneous mammary tumours of mice by partial removal of the tumour combined with injections of the trypanosome preparation, as described in more detail later, confirm the twofold importance of these observations by Sviatukhin and Milovanova.

In describing these experiments, one cannot but recall the conclusions of the clinical observations and the inevitable comparisons to be drawn in this case.

This completes our account of the results of experiments on the treatment of the rabbit carcinoma, and we turn to a description of experiments on the treatment of spontaneous tumours in mice.

4. THE EFFECTS OF THE TRYPANOSOME PREPARATION ON SPONTANEOUS TUMOURS OF WHITE MICE

Most of the experiments on the treatment of spontaneous tumours—the outcome of our work in collaboration with Gintsburg and Milovanova—

were carried out *using a preliminary biopsy* in both the experimental and the control animals. This was dictated not only by the need to study the histological and cytological changes taking place in a tumour under the influence of the trypanosome preparation, but also by the desire to approximate the experiments *to the conditions of clinical treatment*, where administration of the trypanosome preparation was *always* preceeded by the biopsy essential for histopathological diagnosis.

The main questions which had to be answered by experiments on the treatment of spontaneous tumours in mice were:

(1) is there any link between the effects of treatment and the size of the tumour, i.e. what sizes of tumour can be actively affected by the trypanosome preparation?;

(2) can any regular relationship be established between the effects of treatment and the dose (daily and total) of preparation injected?;

(3) do spontaneous tumours of differing histological structures react similarly to the trypanosome preparation?;

(4) what is involved in the phenomenon of stabilization of a tumour — is there only cessation of division of the cancer cells, which can be renewed as soon as the action of the preparation is for some reason halted, or do more fundamental changes occur during stabilization, involving both the histological structure of the tumour and the cytological characteristics of the cancer cells themselves?

All the experimental tumours were in so-called *non-pure-line* mice, which were obtained from different breeding centres. Naturally, we could have carried out our experiments on the treatment of spontaneous tumours in American cancer-bearing mice from pure lines, as often demanded by Prof. Shabad in his discussions with us. However, we disagreed categorically with Prof. Shabad for the simple reason that in clinical practice there are no such "lines" of cancer patients, specially selected by genetical rules by means of prolonged crossing between siblings. Such an experimental procedure, if we followed Prof. Shabad's ideas, would not approximate the conditions of clinical observation but would undoubtedly have the opposite effect. We preferred therefore to use mice which had not been made artificially prone to cancer by genetical means. Note that for control and experiment we selected tumours without macroscopically visible necroses or ulcerations.

In our early work (1935–1946) we established that trypanosomes obtained *from the blood of animals* could, under strictly observed conditions, give rise to a lysate suppressing the growth of transplantable adenocarcinomata and sarcomata of mice, *provided that treatment was commenced*

on the day following implantation of the tumour, i.e. when experimental conditions were optimum.

A whole series of imperative considerations connected with transferring our previous methods to clinical trials forced us (a) to change from cultivation in the blood of animals to cultivation on synthetic nutrient media; (b) to change from our primitive production of the lysate from the trypanosomes, unstandardized, unstable and containing a whole complex of substances, to a more stable preparation, with a definite, if at first weak, cancerolytic activity, to a certain extent purified of its ballast components.

All this was new and therefore difficult. Under such conditions it was far easier to lose the cancerolytic trypanosome substance than to find it. There is no need to write of the number and extent of the failures which we encountered: we were concerned only with the *possibility of directing the productive capabilities of protozoan cells*—a line which was, unfortunately, somewhat new.

These failures were inevitably reflected both clinically and experimentally. However, the question of the mass cultivation of *T. cruzi* was solved. This was the first step. It was important that the trypanosomes not only lived and reproduced but also *produced* the cancerolytic substance, i.e. we were faced with the whole complex problem of the trypanosome's nutrition, respiration, stages of development and, most of all the problem of variability of the trypanosome under new and prolonged conditions of living on a synthetic medium, etc.—in short, with a multitude of questions of which so little is known in the specialist literature. We were forced to work almost in the dark, as though blindly, making use of experience in adjacent spheres of microbiology, biochemistry, protozoology and studies on antibiotics.

Only after solving certain basic problems of cultivation were we able to turn to the transition from the primitively prepared lysate to the first samples of the trypanosome preparation. This transition was associated with just such resounding failures as was the search for a method of cultivation. However, as the reader will see, we gradually became capable of obtaining a batch of a purified, *protein-free* preparation, still with a *low index of effectiveness*, about 3–4, rarely 5–6. This was the preparation used in a considerable number of our experiments on the treatment of spontaneous tumours. We had thus completely removed one possible objection — that the activity of the trypanosome preparation could be explained by a nonspecific effect of the trypanosome cell protein.

The results of some of the experiments on the treatment of spontaneous tumours are shown in the tables (Figs. XXI–XXIV).

Finally, we shall describe an experiment which is interesting in that it involved an attempt to reinforce the effects of the trypanosome pre-

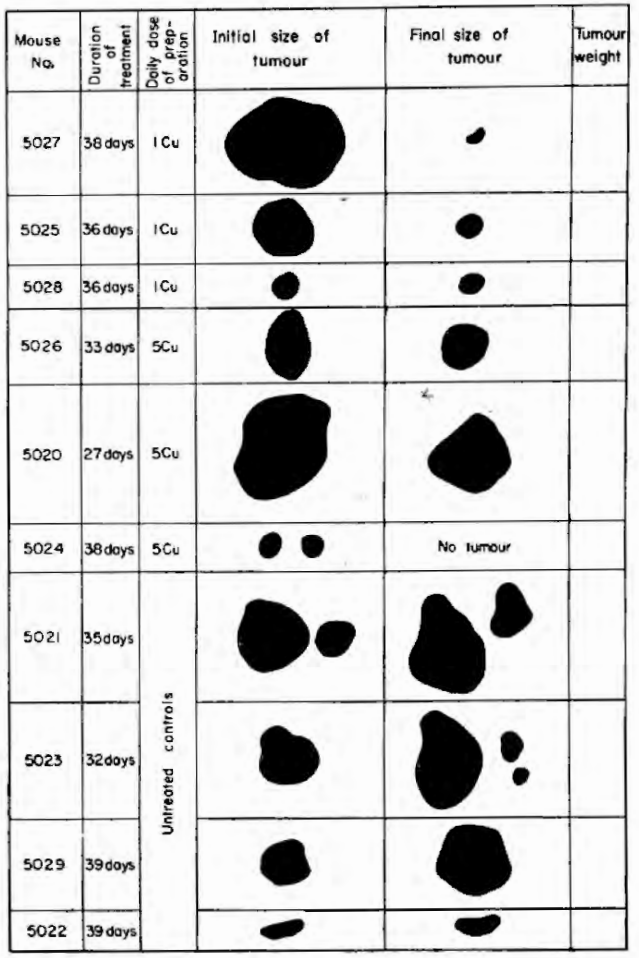


FIG. XXI. Results of treatment of spontaneous carcinomata in mice with the trypanosome preparation. CU — the unit of cancerolytic substance—that obtained from 1 million *T. cruzi* cells.

paration by the addition of preparation 221, also of microbial origin. In this case a considerable effect was achieved with a small number of injections of the trypanosome preparation. This leads in its turn to a new

and important problem: that of *combined* cancer antibiotics. The experiments on the treatment of spontaneous tumours in mice enabled the following conclusions to be drawn:

Mouse No.	Dose per injection	Number of injections	Initial size	Final size
2989	5Cu	12		No tumour
2990	"	6		
2986	"	12		
2988	"	12		No tumour
2996	10Cu	6		
2991	"	12		No tumour
2998	"	19		No tumour
2994	"	8		No tumour
2995	15Cu	9		
2999	"	12		No tumour
2997	"	6		
2992	"	19		

FIG. XXII. Results of treatment of spontaneous carcinomata in mice with the trypanosome preparation; the connection between number of injections, dose of the preparation and size of tumour.

(1). In a high proportion of cases treatment with the trypanosome preparation has a positive effect on spontaneous mammary tumours of mice.

The positive effect is expressed as stabilization of the growth of the tumour or as diminution of the tumour, right up to complete eradication of the malignant tissue. Only a relatively small number of tumours proved refractory, in a way similar to that seen during the clinical observations. According to all our results, spontaneous tumours were more sensitive to the trypanosome preparation than implanted tumours (Crocker sarcoma, Ehrlich adenocarcinoma), in spite of what may have been predicted beforehand.

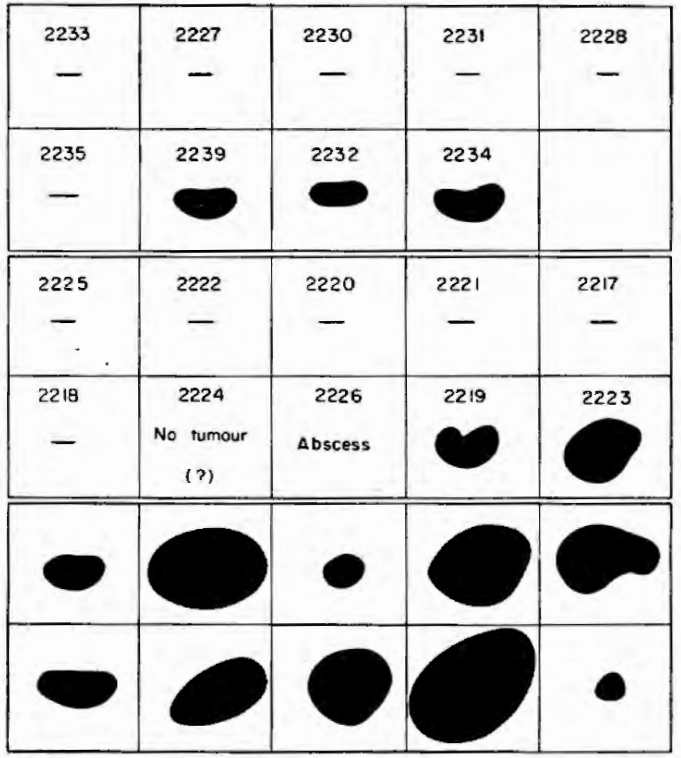


FIG. XXIII. Results of treatment of spontaneous carcinomata in mice with a highly active batch of the trypanosome preparation; in the experimental and control mice tumours of about equal size were chosen at the start of the experiment; the two lower rows represent the tumours in control mice.

(2). Removal of the protein fraction and a number of other chemical fractions does not weaken but, on the contrary, increases the cancerolytic activity of the trypanosome preparation.

(3). A preparation active with regard to spontaneous tumours may be obtained from batches of the preparation of low activity by concentration and chemical purification.

(4). The trypanosome preparation has an active effect against tumours of all sizes, including tumours up to 2.5 × 3 cm.

There may thus be considerable diminution of tumours in mice in a pre-agonal condition.

(5). There is a definite relationship between the daily dose, the total dose in a course of treatment and the size of the tumour mass acted upon.

Mouse No.	Dose per injection	Total dose	Initial size	Final size
7721	1Cu + 0·5	14Cu		No tumour
7753	"	13Cu		No tumour
7742	"	"		No tumour
7756	"	19Cu		
7746	"	18Cu		No tumour
7754	"	14Cu		

FIG. XXIV. Results of treatment of spontaneous carcinomata in mice with the trypanosome preparation in combination with another preparation of microbial origin.

(6). The many spontaneous tumours studied were varied in their histological structure, but in no instance did we note any types of mammary tumours particularly sensitive or insensitive to the trypanosome preparation. The same applies to the macroscopically visible properties of the tumours: firm or irregular, soft, haemorrhagic, with large areas of necrosis or without noticeable necroses, etc.—none showed any appreciable differences with regard to the trypanosome preparation.

(7). The use of the trypanosome preparation alters the significance of a preliminary biopsy in the final therapeutic effect. *Biopsy became a positive factor in the process of the action of the trypanosome preparation* on spontaneous tumours in mice.

The observations also enable us to answer the question of the nature of stabilization of a malignant tumour: on "stabilization" of a tumour *during the use of the trypanosome preparation* there is a certain degree of retardation of the growth of the tumour tissue, a fall in the number of

mitoses, and the establishment of an equilibrium between the processes of multiplication and death of the cancer cells in the tumour, with real and profound changes in the development of the tumour itself, in its fine structure, and in the properties of its component malignant cells. The answers to these questions are based on the findings set out in detail in the next section, where we analyse the mechanism of action of the antiblastic preparation from *T. cruzi*. There we shall consider what changes take place in the malignant tissue on diminution of the tumours. In moving on, we would note that a comparison of our clinical and experimental observations reveals clear-cut clinical-experimental parallels. Histological and cytological observations confirm this position to a great extent.

5. HISTOLOGICAL AND CYTOLOGICAL CHANGES IN SPONTANEOUS TUMOURS OF MICE UNDER THE INFLUENCE OF TREATMENT WITH THE TRYPANOSOME PREPARATION

We must first set out the main questions arising from the histological study of clinical material and of material from the treated rabbit carcinoma. These questions are: (1) is any regression seen in spontaneous tumours of mice under the influence of the trypanosome preparation? (2) what are the stages of this process and what cellular and tissue changes take place during its course?; (3) what cellular systems are stimulated or inhibited by the trypanosome preparation? (4) what principles may be established for the process of regression of malignant tumours under the influence of the trypanosome antibiotic that are common to such diverse material as that from malignant tumours of man, the rabbit and the mouse?

Material from three experiments (73, 129, 148) on the treatment of *spontaneous tumours* with various modifications of the trypanosome preparation was subjected to detailed histological and cytological analysis, with a comparison in each case between the histological and cytological structure of the tumour before and after treatment with the preparation. Before making any general conclusions, we must consider the individual cases.

Mouse 5651. The changes occuring in this tumour in the process of treatment are of undoubted interest. At the start of the experiment the tumour measured 1.3 × 1.0 cm. Before treatment a biopsy was carried out, when a portion of the tumour 0.4 cm across was removed. Treatment was started immediately afterwards. After 6 days the tumour measured

1.0 × 0.7 cm, after which it began to decrease gradually in size and on the 26th day, the last day of the experiment, it measured 0.3 × 0.3 cm. The histological and cytological analysis thus applied to a tumour which had diminished considerably under the influence of injections of the trypanosome preparation at a dose of 130 CU over 26 injections.

Histological picture of the tumour *before treatment*: mammary adenocarcinoma. *After treatment* significant histological and cytological changes had taken place in the tumour, as well as the considerable diminution. First of all we noticed a decrease in the size of the cancer cell nuclei and homogenization of their structure. The general structure of the tumour had been completely disrupted: only isolated groups of cancer cells in stages of discomplexation could be seen. Between the groups of cancer cells there were extensive spaces filled either with a homogeneous protein mass in which were individual, scattered cancer cells and lymphocytic cells, or with large haemorrhagic areas. The preparation showed extensive connective tissue proliferations. A considerable area of the preparation was occupied by large groups of lymphocytic cells, among which, although exceptionally, were individual cancer cells. Finally, there were considerable areas of the tumour where only isolated nuclei could be seen, in various stages of degeneration and necrosis.

Mouse 5642. Histological picture of the tumour *before treatment: adenocarcinoma.* The preparation included extensive fields of cancer cells separated from each other by fine connective tissue septa. The tumour contained a fair number of blood vessels with dilated lumena. Some of the vessels contained sero-sanguineous exudates. In places there were areas of necrosis and small aggregations of lymphocytes. The cancer cell nuclei were noticeably large, with hypertrophic nucleoli. The nuclei were polymorphic. The nuclear size in planimetric units varied between 4.5 and 18.3. The average nuclear size of the cancer cells (over 100 cells) was 10.4 units. The tumour was growing rapidly. In 100 fields of vision 174 mitoses were seen.

After treatment for 26 days *the tumour had decreased by more than three times:* initial size 1.8 × 1.5 cm, final size 0.5 × 0.4 cm. The histological and cytological appearance of the tumour had *changed markedly.* The preparations included extensive fields of sharply modified cancer cells, showing signs of nuclear degeneration, pyknosis and karyorrhexis. The nuclei of these modified cancer cells stained deeply and homogeneously with haematoxylin, and *no nucleoli were visible.* After treatment there was a sharp fall in nuclear size. The nuclear size in planimetric units varied between 1.9 and 5.6.

Preparations showed massive dilatations of the vascular ducts with a sero-sanguineous exudate, and also fields of necrotic tissue. Lymphocytic infiltration was encountered in places.

In sharp distinction from the tumour before treatment, after treatment its cells had ceased to divide: *there were no mitoses in preparations of the treated tumour*, whereas before treatment 170 mitoses had been seen in 100 fields of vision. We must also note the appearance of broad connective tissue septa, penetrating between the cancer cells for short distances in some places, in distinction from the poorly-developed connective tissue *stroma present before treatment.* All the details noted give an idea of the profound changes which had taken place in the tumour as a whole and its individual cells under the influence of the trypanosome preparation.

Mouse 5648. Histological picture *before treatment*: adenocarcinoma, with a relatively large number of blood vessels filled with blood. Average nuclear size: 11.2 planimetric units. Cell division was infrequent — only isolated mitoses could be seen over several fields of vision. *As a result of the use of the trypanosome preparation* the tumour diminished considerably (from 3×2 cm to 1.5×1 cm).

Microscopical appearance *after treatment*: large areas of necrosis could be seen in the cancer tissue, some of them extending to the surface of the tumour.

The cancer cell nuclei had grown noticeably smaller: the average size was 5.7 units. The surrounding connective tissue included many cells of the histiocyte type with yellow cytoplasmic inclusions. Hence, as a result of the action of the trypanosome preparation there had been not only diminution of the tumour but also a sharp decrease in the size of the cancer cell nuclei; a clearly marked histiocytic reaction appeared in the surrounding connective tissue.

Mouse 5025. Histopathological diagnosis of tumour *before treatment:* adenocarcinoma. Numerous mitoses were seen in sections; fields of cancer cells were divided by broad connective tissue septa. As a result of *treatment* for 36 days the tumour decereased in size from 1.7×1 cm to 1×1 cm. Histological preparations showed few, though rather large, areas of cancer tissue. As well as these there were extensive areas of cancer tissue showing various degrees of cytoplasmic and nuclear degeneration. Most of the nuclei stained deeply and homogeneously with haematoxylin; some nuclei had entirely lost their typical staining properties and would only stain with eosin; there was general diminution in nuclear size. Extensive haemorrhagic areas were encountered in the treated tumour. Parts of the connective tissue septa had undergone a dense round-cell infiltration.

Mouse 5029. No biopsy was performed. Histological picture of the tumour after 38 days of *treatment:* extensive fields of cicatricial connective tissue with isolated foci of cellular detritus; the connective tissue was richly infiltrated by leucocytes, lymphocytes and polyblasts, among which were isolated, very scattered small groups of suspectedly malignant cells.

Mouse 5027. Biopsy *before treatment*: the tumour had the structure of a mammary alveolar adenocarcinoma (Plate 111). As a result of 38 days of treatment of the large tumour (3×2 cm) only a very small area weighing 0.015 mg remained. The histological picture of the remaining tumour was completely different from that before treatment: sections show uniform fields consisting of cells of the lymphocytic and, mainly, monocytic and histiocytic types, infiltrated by narrow cords of newly-formed fibroblastic tissue among which were seen only occasional isolated cells suspected of malignancy; these cells were seen in very small numbers over extensive areas of the section, and were as if submerged in a mass of histiogenic elements. The whole cellular mass of the tumour, except for the fibroblastic cells, showed numerous signs of degeneration and necrobiosis. The many macrophages contained yellow granules (Plates 112, 113).

Mouse 5028. At the start of the treatment the tumour was small in comparison with the others used in the experiments (0.5×0.5 cm). After 36 days of treatment the tumour had become stabilized and had even decreased somewhat in size. Histological analysis of a small fragment of the tumour taken during a biopsy *before treatment* was started revealed the typical picture of a mammary adenocarcinoma. More or less extensive fields of necrosis were absent from the tumour; small haemorrhagic areas were encountered relatively frequently. *As a result of treatment* characteristic changes occurred in the structure and staining properties of the nuclei: many nuclei stained more deeply and homogeneously with haematoxylin. There was a simultaneous sharp fall in the mitotic index. Karyometric observations (Figs. XXV, XXVI) revealed changes in the sizes of the cancer cell nuclei — there was marked diminution of the nuclei after treatment. Although all these changes in the spontaneous tumour are relatively slight in the sense that cancer tissue was still present, they are of particular interest in that they depict that phase of the action of the trypanosome preparation where there are only degenerative changes in the cancer tissue, its destruction has not yet begun and more important, the macrophage apparatus has not come into action, with its accompanying canceroclastic and cancerolytic reactions.

Mouse 5026. Histological picture of a portion of the tumour taken at biopsy: haemorrhagic papillary adenocarcinoma. *As a result of treatment* the tumour decreased in size and its histological structure was changed. Extensive fields of cancer cells were visible, which without their former precision formed large and small conglomerates infiltrated by cells of a lymphocytic and histiocytic nature. The cancer cell masses showed degenerative changes: the nuclei stained more deeply with haematoxylin and their structure was homogeneous. Karyometric observations on the tumour nuclei before and after treatment showed very marked changes in nuclear size: after treatment the nuclei had become much smaller.

Mouse 5024. Before treatment the tumour had the structure of a haemorrhagic cyst-adenocarcinoma, measuring 0.5×0.4 cm.

As a result of treatment for 38 days an amorphous residue formed at the tumour site. Considerable histological changes had taken place in the tumour: sections showed an homogenized, structureless protein mass with areas of small nuclear fragments among which were encountered isolated fields of degenerating round-cell aggregations, and, finally, relatively very small areas of degenerating cells which were only faintly reminiscent of the cancer tissue formerly present.

Mouse 5020. Histological appearance of a portion taken at biopsy *before treatment:* mammary adenocarcinoma. *After 27 days of treatment* this huge tumour (the mouse was taken for treatment when practically in a pre-agonal state) had decreased considerably in size (from 3.2×2.2 cm to 2×1.5 cm). The following changes had taken place in its histological structure: (1) part of the tumour had undergone breakdown — microscopical sections showed extensive homogenized masses of tumour tissue lying next to no less extensive fields of typical cancer tissue; (2) the structure of other parts of the tumour tissue had changed considerably; it consisted not of sheets or ribbons of cancer cells but of masses of separate discomplex cells of the epithelioid or round-cell type with nuclei differing in their structural and staining properties from the nuclei seen previously in the cancer tissue. This peculiar conglomeration of cells, occupying extensive areas of the preparations, consisted partly of degenerating malignant cells and partly of an infiltrate of lymphocytic, monocytic and histiocytic cells in various stages of transformation and degeneration.

We turn now to a consideration of the histological analyses of the tumours in experiment No. 73. In this experiment eight of the fifteen spontaneous tumours disappeared completely, four decreased in size, one became stabilized and two tumours grew larger in spite of the treatment. In all

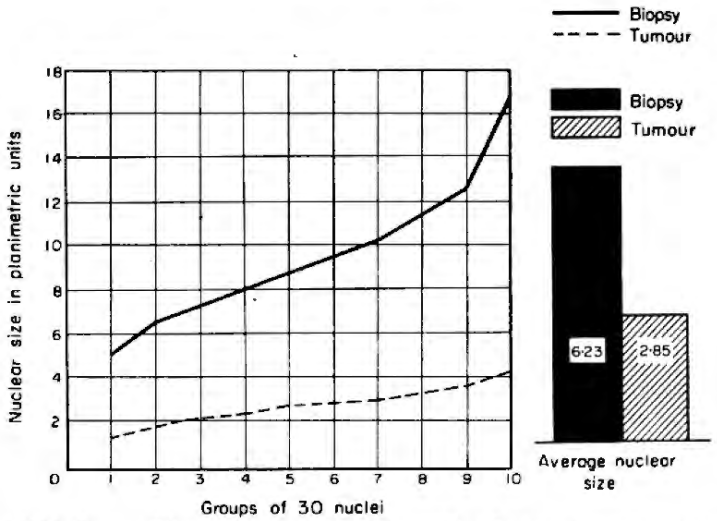


FIG. XXV. Comparision of nuclear size in the cells of a spontaneous mammary adenocarcinoma in mouse 5028 before and after treatment with the trypanosome preparation; there is a considerable decrease in nuclear size after treatment.

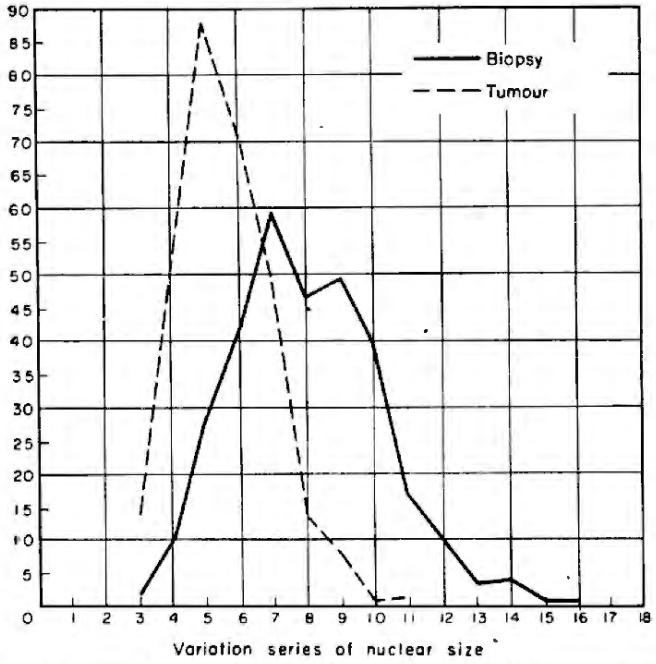


FIG. XXVI. Curves showing the variability of nuclear size in an adenocarcinoma in mouse 5028 before and after treatment with the trypanosome preparation. The curve after treatment has a single peak, as is typical for normal, but not malignant, cells.

the experimental mice a biopsy was carried out before treatment and the smallest possible portions taken for diagnostic purposes. In cases where the tumour disappeared the effects of treatment were obvious. However, in our opinion no less attention, and no more, should be given to the results obtained on microscopical analysis of the tumours growing smaller or becoming stabilized during the process of treatment. This statement is based on the following observations.

Mouse 2995. Microscopical analysis of the tumour before treatment: mammary adenocarcinoma; numerous mitoses could be seen, and our attention was drawn by the absence of any noticeable zones of necrosis; there were relatively small haemorrhagic areas (Plate 114).

As a result of treatment the tumour decreased in size from 1.7×1.5 cm to 1×0.7 cm. The changes that had occurred in *the microscopical appearance of the tumour after treatment* were particularly convincing. Serial sections from various parts of the tumour showed either huge fields (about 1 cm in diameter) consisting of a homogeneous, structureless protein mass without any recognizable cellular or nuclear remains, or similarly extensive homogeneous fields with separate areas of cellular detritus consisting of amorphous cell remnants, nuclear fragments or nuclei which had completely lost their ability to stain with haematoxylin (Plates 115, 116); finally, besides the picture of general cellular necrosis the preparations showed separate foci of cells which had preserved their features to some extent, but with nuclei in a state of pyknosis and homogenization. It would be quite fruitless to argue whether *isolated* undamaged cancer cells could be found among the studied sections of the tumour. The answer to this could hardly be provided by methods of morphological observation. It is quite obvious that under the influence of injections of the trypanosome preparation striking histological and morphological changes appeared in a spontaneous carcinomatous tumour, although macroscopically the tumour had only decreased in size.

It is well known that the cells of rapidly growing cancer tissues are rich in ribonucleic and thymonucleic acids. Using Brachet's method of demonstrating these components, we studied sections of the tumour before and after treatment. Naturally, after treatment areas of the tumour containing intact, though very modified, cancer cells were chosen for comparison.

The tumour cells taken at the biopsy were rich in ribonucleic acid, which filled the cytoplasm either diffusely, or in the form of small granules of various sizes, or as clumps lying in various parts of the cytoplasm. The nuclei of these cells were rich in chromatin granules, the nucleoli were large and contained ribonucleic acid. After treatment there was a

significant change in this situation. The vacuolated cytoplasm of the more or less intact cancer cells contained *hardly detectable traces of ribonucleic acid*. The nuclei showed hyperchromasia. The large nucleoli typical of cancer cells were absent or were seen as an exception — a particularly noteworthy situation. One very significant factor was the *disappearance of ribonucleic acid from the nucleoli*. In noting this circumstance it should be remembered that the nucleoli of all rapidly growing cells, including malignant cells, are as a rule rich in ribonucleic acid (Caspersson, 1950; Brachet, 1944; Roskin, 1945–46). Thus, the histological demonstration of ribonucleic acid in this case enabled us to note, as well as structural changes in the cells after treatment, objectively recordable *cytophysiological changes* in the nucleus and cytoplasm. The significance of these changes must not be underestimated.

Mouse 2997. Before treatment there were two tumours, 1×1 cm and 0.6×0.6 cm. The smaller tumour disappeared entirely, whereas after 6 injections (15 CU each) the size of the other was almost unchanged. *Histological picture* of the tumour *before treatment*—mammary adenocarcinoma, consisting of typically staining cells with well-developed nuclei, with very small zones of degeneration or necrosis. *After treatment* there were marked changes in the tumour's histological and cytological structure (Plates 117–120); extensive zones of degenerating cells were seen. Most of the cells showed homogenization of the nuclear structure. As a rule the nuclei stained deeply with haematoxylin. The size of the nuclei in the tumour decreased. Large foci of well-defined cellular degeneration appeared. There was a sharply increased infiltration of the malignant tissue by cells of the lymphoid and histiocyte types, which either penetrated the cancer tissue or surrounded separate small groups of modified cancer cells, where the individual malignant cells became as if lost in a mass of small-cell infiltrate (Plates 120–121).

A detailed cytological analysis of the round-cell infiltration mentioned above shows that it consists not only of cells of the lymphocyte type but may show frequent monocytes and large histiocytes with "cartwheel" nuclei and also typical macrophages. The difference between the tumour tissue before and after treatment stands out even more clearly after a comparative study of the ribonucleic acid content of the cancer cell cytoplasm. The amount of ribonucleic acid as a whole in the tumour tissue fell markedly under the influence of the trypanosome preparation. Many, if not all, of the degenerating cancer cells gave no histochemical reaction at all to the ribonucleic acid test. Along with these cells were elements of the round-cell infiltrate, rich in ribonucleic acid, which was diffusely scattered throughout

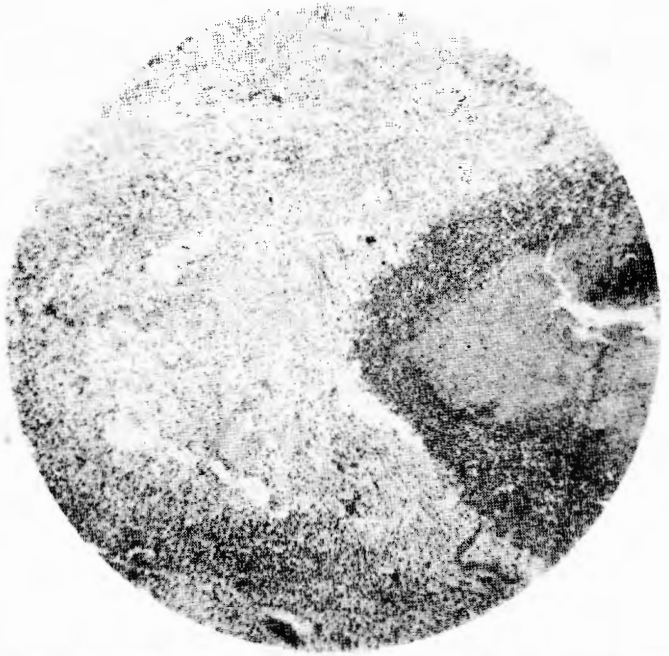


PLATE 113. Histological appearance of the spontaneous adenocarcinoma in mouse 5027 after treatment. Extensive fields of broken-down cancer tissue can be seen. Low magnification.

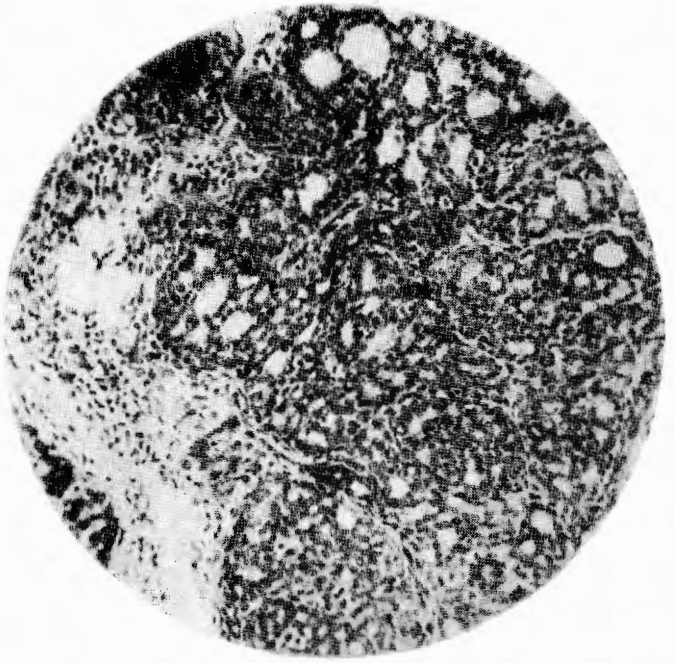


PLATE 114. Histology of a spontaneous adenocarcinoma in mouse 2995 before treatment.

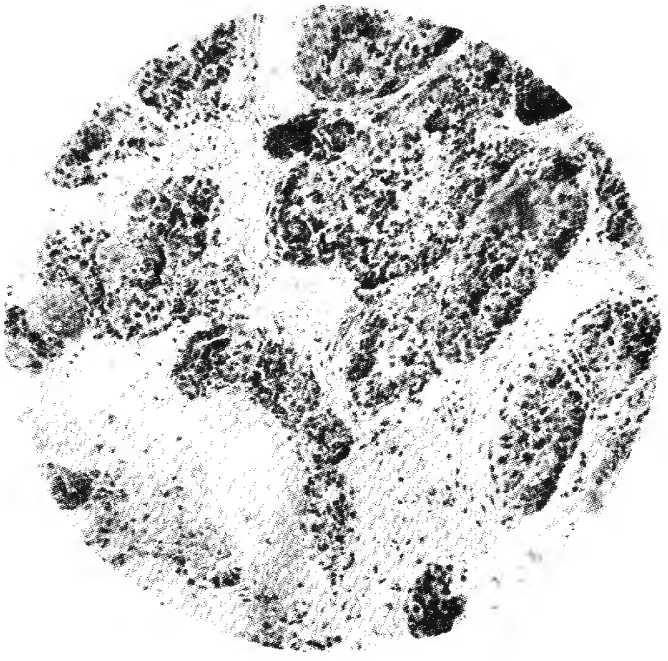


PLATE 115. Spontaneous adenocarcinoma 2995 after treatment, showing groups of degenerating cancer cells among areas of broken-down tumour; low magnification.

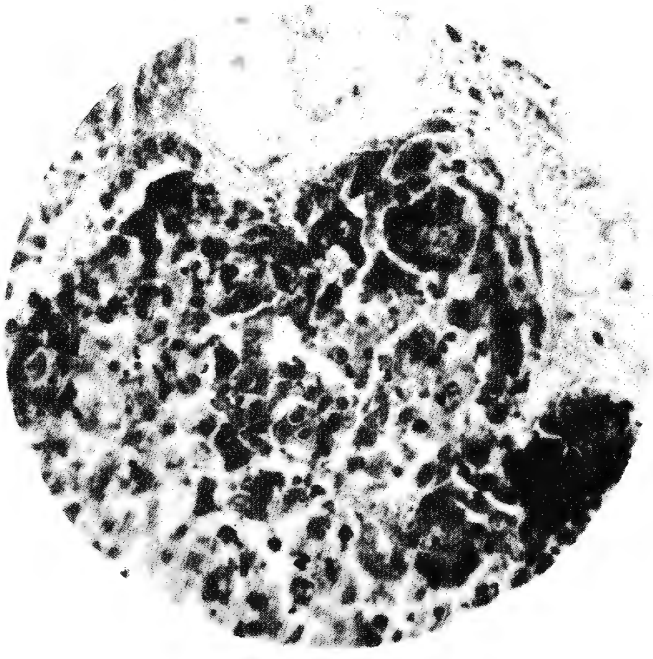


PLATE 116. Area of degenerating cancer cells in tumour 2995 after treatment; magnification × 600.

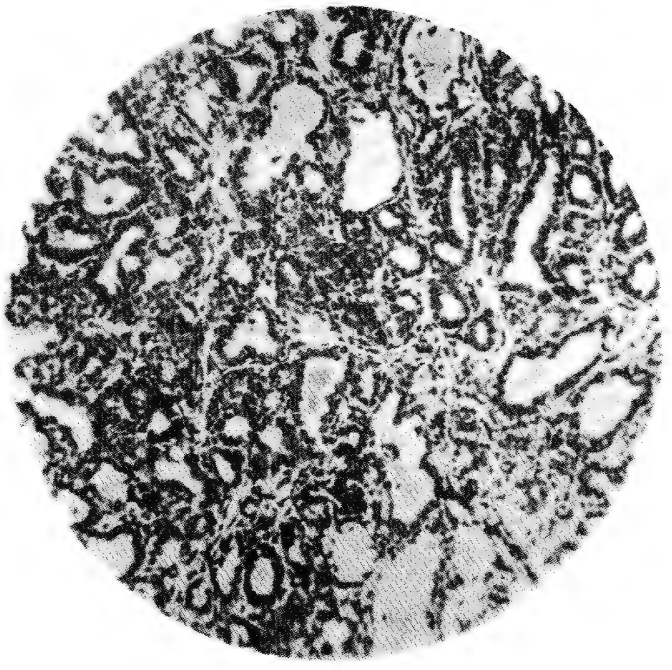


PLATE 117. Histology of a spontaneous adenocarcinoma in mouse 2997 (before treatment); low magnification.

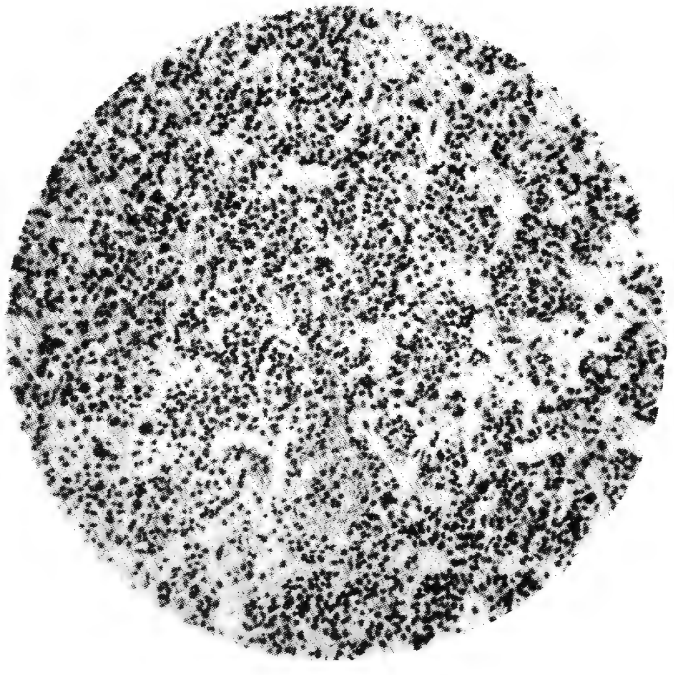


PLATE 118. Marked changes in the structure of the spontaneous adenocarcinoma in mouse 2997 occurring under the influence of treatment with the trypanosome preparation; there is degeneration of the cancer cells, diminution of their nuclei and extensive infiltration by lymphohistiocyte cells; low magnification.

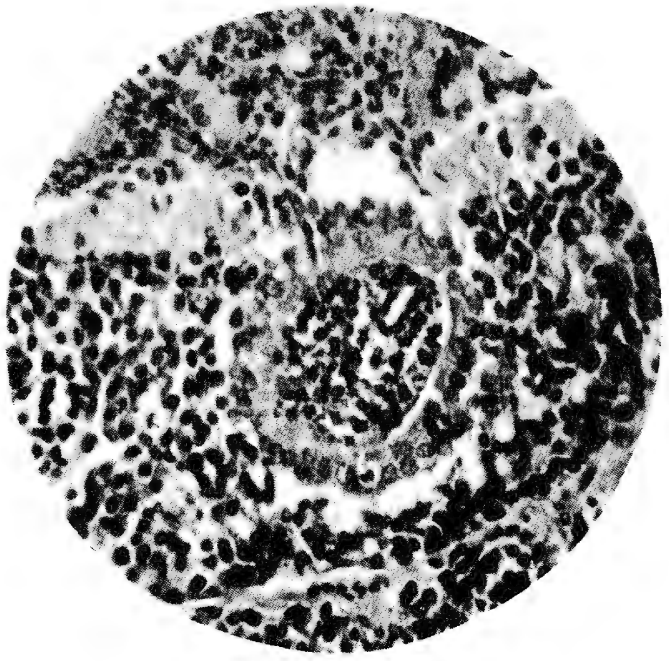


PLATE 119. Area of degenerating cancer cells in the treated tumour in mouse 2997; magnification × 600.

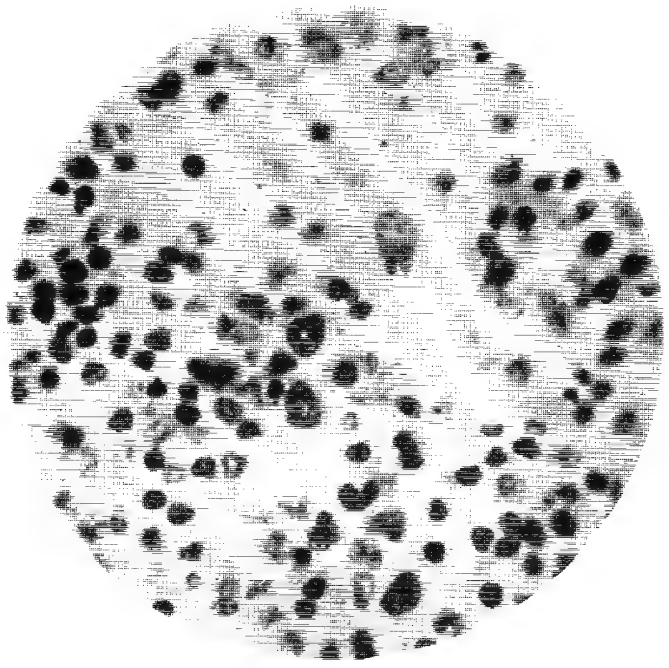


PLATE 120. Area of tumour 2997 after treatment, showing isolated cancer cells among lympho-histiocyte cells; magnification × 600.

PLATE 121. Area of tumour 2997 after treatment, showing polyblasts and lymphocytes at the site of the former cancer tissue; magnification × 900.

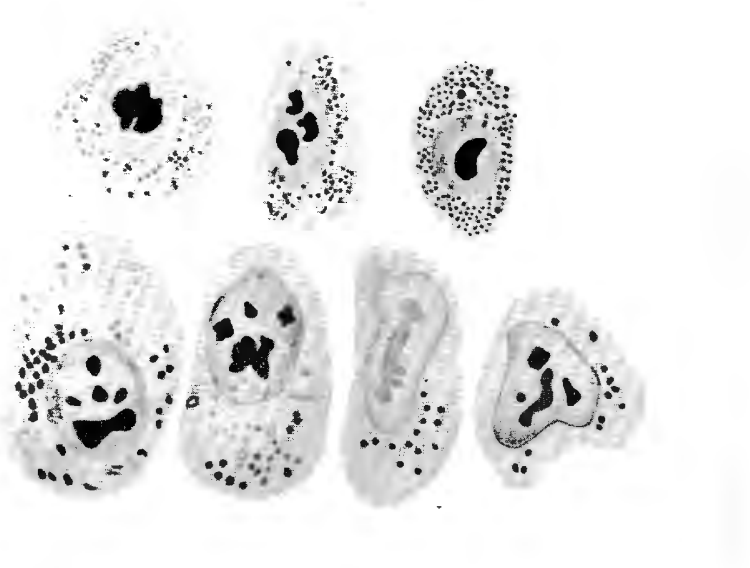
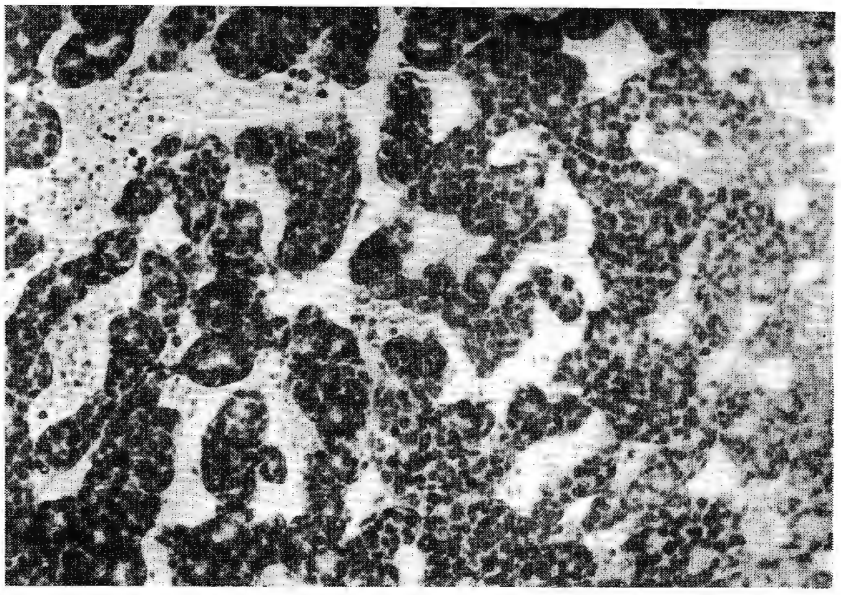
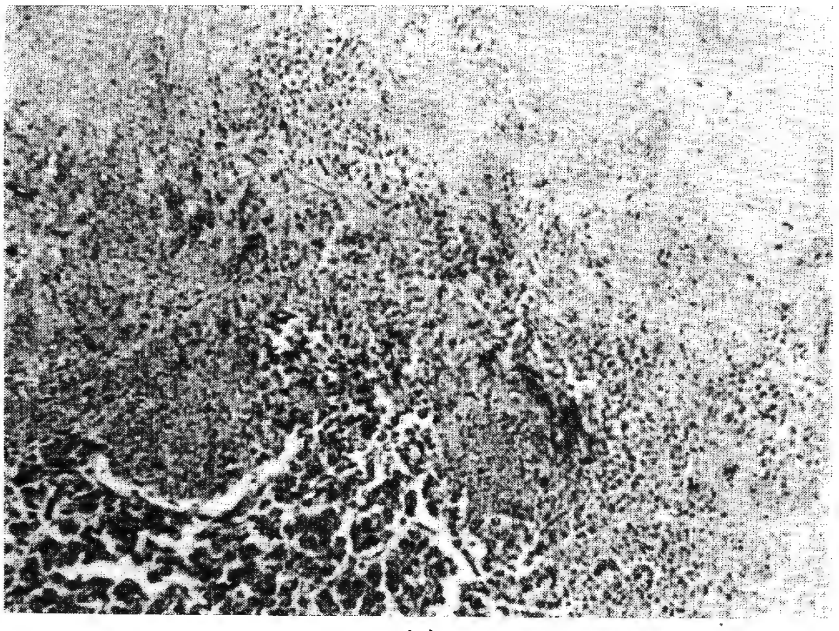


PLATE 122. Changes in the mitochondria of cells in a spontaneous mouse carcinoma under the influence of the trypanosome preparation; the upper row shows normal mitochondria, the lower row shows signs of degeneration of the mitochondrial apparatus during treatment (Drawing).

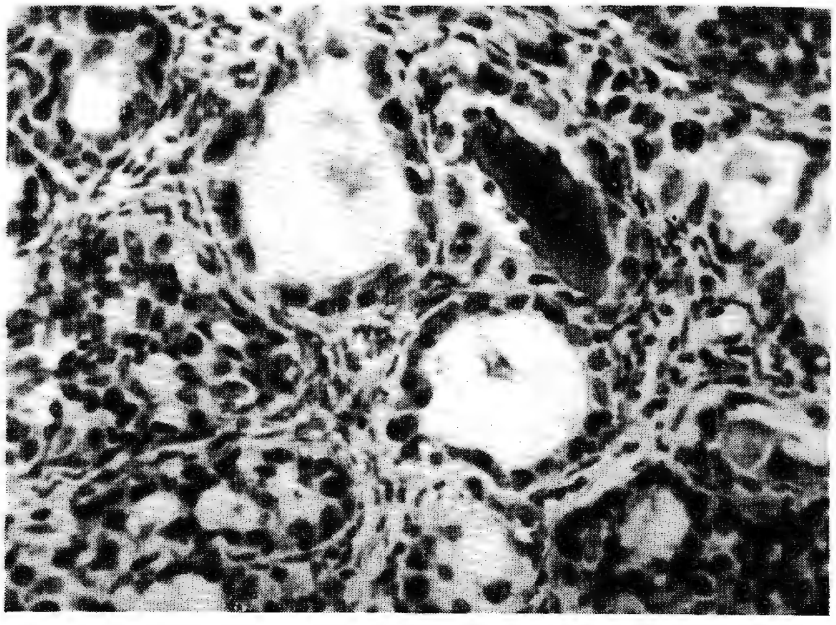


(*a*)

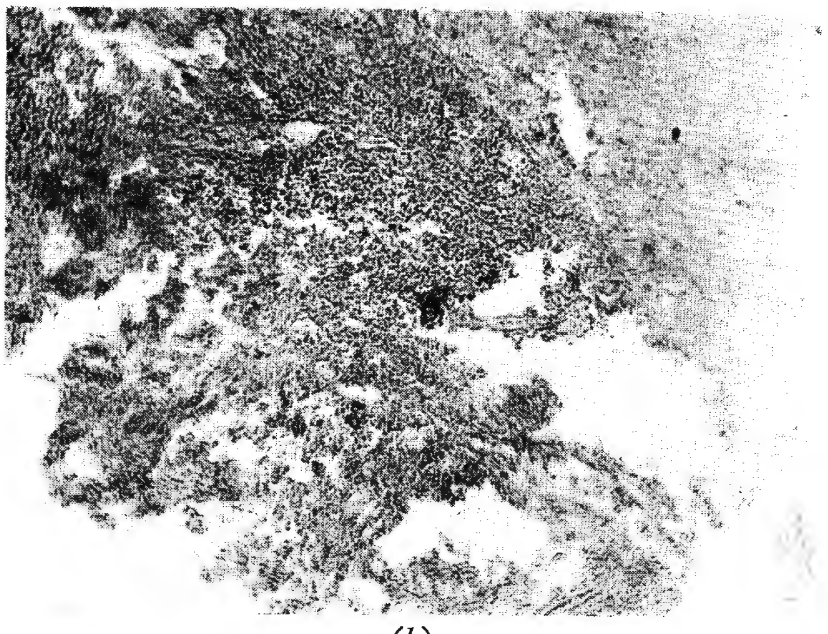


(*b*)

PLATE 123, *a, b*. Spontaneous adenocarcinoma in mouse 7718 before and after treatment with the trypanosome preparation; *a*—before treatment, *b*—after treatment—most of the tumour consists of destroyed or breaking-down cancer cells; magnification × 200.

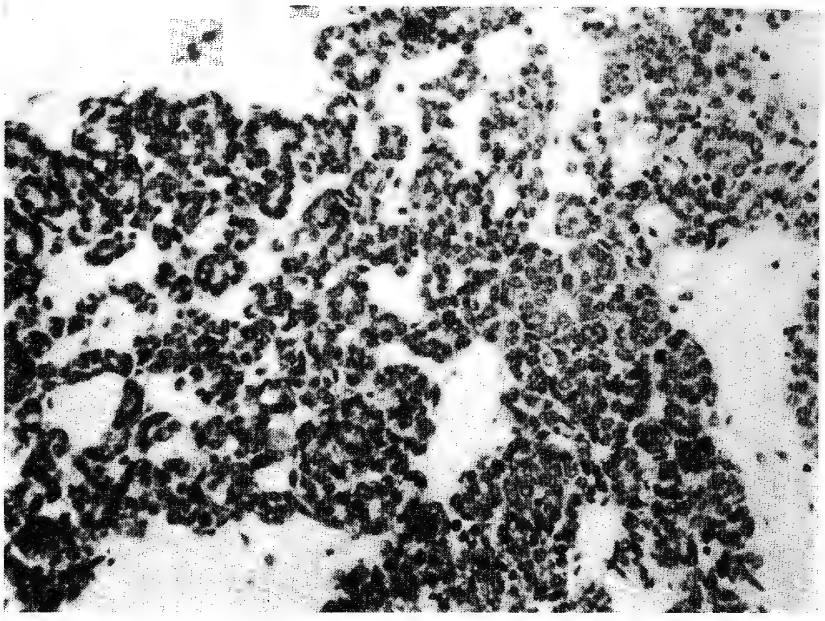


(*a*)

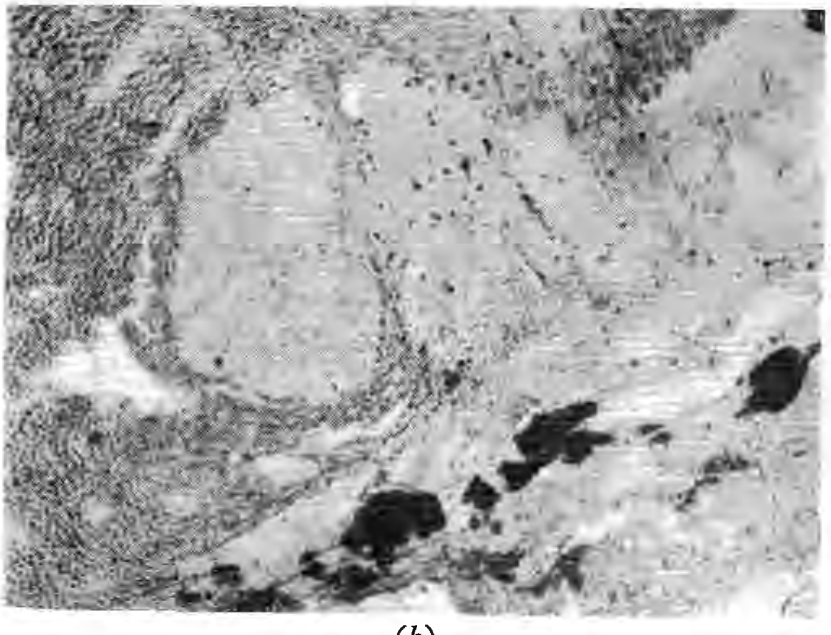


(*b*)

PLATE 124, *a*, *b*. Spontaneous adenocarcinoma in mouse 6085 before *(a)* and after (*b*) treatment with the trypanosome preparation; *a*—at a magnification of × 400, *b*—at a magnification of × 80.



(*a*)



(*b*)

PLATE 125, *a*, *b*. Spontaneous adenocarcinoma in mouse 5447 before *(a)* and after *(b)* treatment with the trypanosome preparation; *a*—at a magnification of × 200, *b*—at a magnification of × 80.

their cytoplasm and distinguished them clearly from the degenerating cancer cells.

Taken as a whole, these findings show that in mouse No. 2997, although the *macroscopically recorded effect—stabilization—could not particularly impress the observer, the internal, intimate changes in the structure of the tumour tissue, in the structure of the cancer cells and in their physiology were so significant that the interpretation of these findings must be radically changed from one of mere inhibition of the growth of the malignant tumour.*

We turn now to a consideration of the histological changes in those tumours in experiment No. 73 which decreased in size as a result of treatment.

Mouse 2986. As a result of 12 injections (5 CU each) the tumour decreased in size from 2.5×2 cm to 1.6×1.6 cm.

Histological appearance *before treatment:* typical mammary carcinoma; numerous mitoses were seen, while foci of degeneration and necrosis were almost absent and small haemorrhagic areas were seen very rarely.

After treatment there was a marked change in the appearance of this tumour. Most noticeable in the preparations were extensive homogenized, fused areas of cancer tissue. Some of these areas still contained cell and nuclear fragments, while others showed only a uniformly-stained protein mass, penetrated in places, particularly at the periphery, by a small-cell infiltrate, a considerable part of which was also in stages of degeneration and necrosis. Attempts to find groups of typical cancer cells in this tumour were in vain. Because of this, we were bound to accept that this mouse, although macroscopically still having a tumour, must in fact, within the limits of histological analysis, be considered cured. The high level of ribonucleic acid typical of cancer tissue had fallen sharply. In short, after treatment there were radical changes in the morphological and cytological characteristics of tumour tissue.

One more important factor should be noted: lysis and resorption of the remains of destroyed tumour tissue as a rule lags considerably behind the process of their degeneration and death. A particular physiological state is apparently required in order that this process may progress sufficiently rapidly. We saw earlier in transplantable tumours, and we see now in spontaneous tumours, that the processes of destruction and the processes of resorption and lysis of the destroyed tissue progress independently. In a whole series of experimental animals we were able to find these "ghost" tumours in which, in fact, the cancer cells were all, or very nearly all, destroyed. Some additional factors are apparently required for the simultaneous destruction and lysis of malignant tissue. When this

On studying the two small cancer nodules remaining from the relatively large tumour the cancer cells were seen to be lying in a disorganized mass, with only occasional duct lumena with some sort of contents. Dilated blood vessels were seen in places in these tumour nodules. *There were no dividing cells.* Among the normal cancer cell nuclei were nuclei in various stages of degeneration. The cancer cells were separated from the rest of the tumour by a thick layer of connective tissue with a lymphocytic barrier zone.

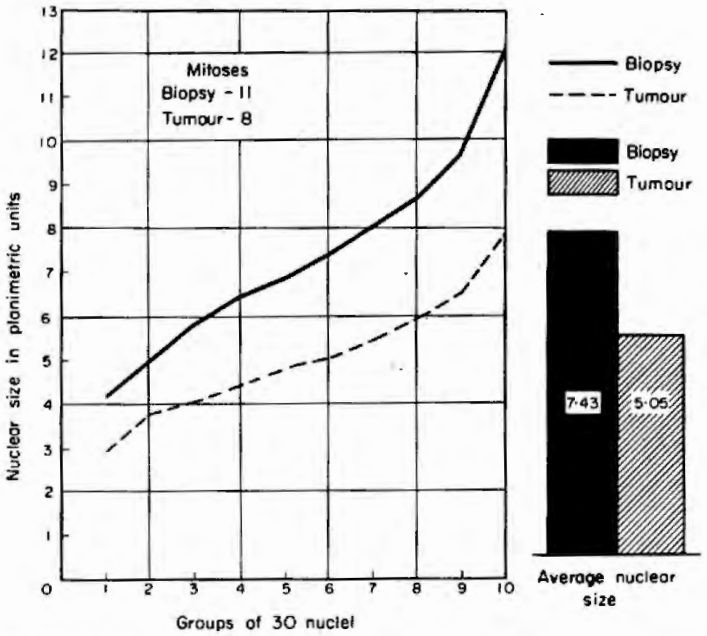


FIG. XXVII. Nuclear sizes of a spontaneous carcinoma in mouse 13 before and after treatment with the trypanosome preparation; concurrently with the diminution of the nuclei the number of mitoses fell from 64 to 1 (per 100 fields of vision).

As already stated, two thirds of the resected tumour consisted of connective and fatty tissues. The thick layers of connective tissue consisted of fibroblasts, many histiocytes and lymphocytes. Staining with van Giesen's revealed a large number of collagen fibres, in places forming wide bands. The connective tissue included many cells of the histiocyte type, with yellow cytoplasmic inclusions.

Thus we see that two small nodules remained at the site of a large, rapidly-growing tumour as a result of treatment with the trypanosome preparation while the whole remainder of the tumour was replaced by intensively developing connective tissue with massive collagenous pro-

takes place the tumour is seen to disappear rapidly, whereas in other cases, when these processes occur independently or the second process is retarded, stabilization of the tumour's size or only a relatively slight diminution is seen after treatment.

Mouse 2990. Histological analysis of a fragment taken at biopsy *before treatment* revealed the picture of a haemorrhagic cyst-carcinoma. After 6 injections (5 CU each) of the trypanosome preparation the tumour decreased in size from 0.5×0.5 to 0.2×0.2 cm.

After treatment there were substantial changes in the tumour's microscopic structure. Over the whole section there were cells with homogenized cytoplasm, fused, into large, continuous, uniformly stained masses. The cell borders were visible in only a limited number of cells. The structure of the nuclei had changed appreciably — they stained deeply with haematoxylin. The configuration of the nuclei was irregular. Many nuclei of the pyknotic type were seen, showing advanced signs of degeneration. Also visible were relatively small areas of unchanged or almost unchanged malignant tissue. Hence, in this tumour as well as macroscopically evident diminution there were considerable histological and cytological changes, but complete disappearance of the tumour had not been reached by the time the experiment on the mouse had to end.

To end this section of the book we should like to include a few more descriptions of spontaneous tumours after treatment with the trypanosome preparation.

Mouse 1–M. Appearance of tumour *before treatment:* typical adenocarcinoma; the tumour was rich in blood-vessels filled with blood, and isolated areas of haemorrhage were seen. There was a very poorly-developed connective tissue stroma between the cancer cell cords. The tumour was growing rapidly—59 mitoses were counted in 100 fields of vision. The cancer cell nuclei were extremely polymorphic and contained enlarged nucleoli.

As a result of treatment there was a considerable reduction in the size of the tumour. However, the actual decrease in the tumour tissue was much greater than that shown macroscopically, since preparations showed the excised tumour to consist mainly of connective tissue, which occupied about two thirds of the whole area of the tumour mass, while only about one sixth was occupied by two small nodules of cancer tissue. The rest of the tumour consisted of a zone of necrotic cancer cells, nuclear remnants or nuclei in a state of pyknosis or karyorrhexis, among which were isolated unchanged cancer cells.

On studying the two small cancer nodules remaining from the relatively large tumour the cancer cells were seen to be lying in a disorganized mass, with only occasional duct lumena with some sort of contents. Dilated blood vessels were seen in places in these tumour nodules. *There were no dividing cells.* Among the normal cancer cell nuclei were nuclei in various stages of degeneration. The cancer cells were separated from the rest of the tumour by a thick layer of connective tissue with a lymphocytic barrier zone.

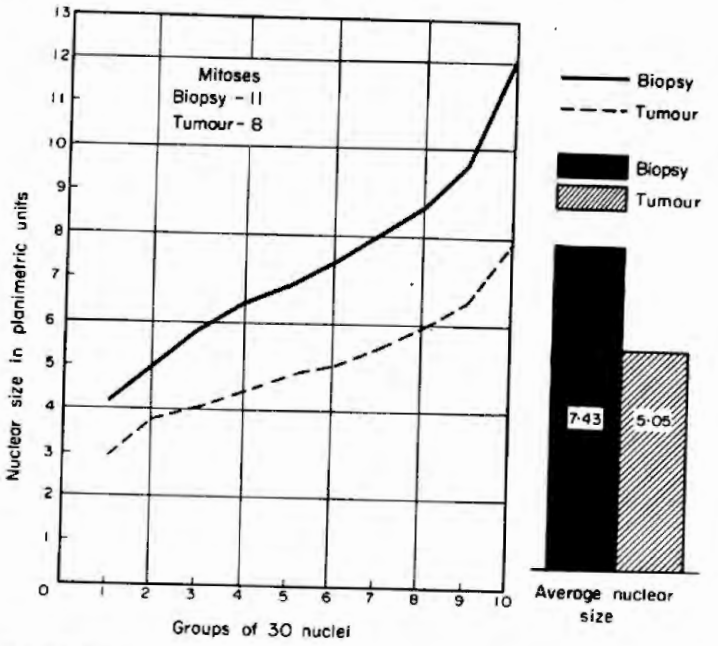


FIG. XXVII. Nuclear sizes of a spontaneous carcinoma in mouse 13 before and after treatment with the trypanosome preparation; concurrently with the diminution of the nuclei the number of mitoses fell from 64 to 1 (per 100 fields of vision).

As already stated, two thirds of the resected tumour consisted of connective and fatty tissues. The thick layers of connective tissue consisted of fibroblasts, many histiocytes and lymphocytes. Staining with van Giesen's revealed a large number of collagen fibres, in places forming wide bands. The connective tissue included many cells of the histiocyte type, with yellow cytoplasmic inclusions.

Thus we see that two small nodules remained at the site of a large, rapidly-growing tumour as a result of treatment with the trypanosome preparation while the whole remainder of the tumour was replaced by intensively developing connective tissue with massive collagenous pro-

liferations rich in histiocytes and cells of the lymphocyte type. This tumour therefore showed connective tissue activation as well as breakdown of the cancer tissue.

To conclude, we should like to devote some time to the histological pictures of certain spontaneous tumours (Nos. 52–M, 56–M, 57–M and 70-M) treated with the trypanosome preparation and removed purposely before the end of the experiment *to provide some idea of the intermediate stages in the action of the trypanosome preparation* on tumour tissue. The pictures seen in these tumours forced us to pay particular attention to the extremely powerful reaction not only by the histiocytes and lymphocytes but also by plasma cells. Histological observations showed that during these relatively early phases of treatment there is a very intensive lymphoeytic and histiocytic reaction, in which the plasma cells take an active part, many of the plasma cells being in the process of division, both mitotic and amitotic. These plasma cells penetrate between the cancer cells. As well as numerous plasma cells the preparations include large numbers of histiocytes with yellow cytoplasmic inclusions. These cells lie either in separate groups or among the cancer cells, sometimes around blood vessels, even filling their lumena. The cytoplasm of many of the histiocytes is outstanding in its sharply increased basophilia.

The areas of considerably changed cancer tissue include multinucleated giant cells, the cytoplasm of which sometimes has yellow inclusions. These giant cells give a strongly positive reaction for ribonucleic acid.

In later stages of the action of the trypanosome preparation, as seen in tumour 57, when extensive zones of fibroblastic tissue develop at the site of the former malignant tissue, the histiocytic and lymphocytic reactions are suppressed, with a simultaneous fall in the number of plasma cells.

It should be added that tumours 52, 56, 57 and 70 were the subject of a special study on the state of the mitochondrial apparatus in the cancer cells. Under the influence of the trypanosome preparation there was apparently serious disruption of the mitochondrial apparatus of the malignant cells, as reflected in its ability to stain typically by Rego's or Altman's methods.

The changes in the staining power of the mitochondria are related to changes in their physico-chemical properties. Since the mitochondria play an important part in the processes of intracellular metabolism, these changes in their properties point to changes in the metabolism of cancer cells under the influence of the trypanosome preparation. As we shall

see, this surmise is also based on experiments showing the effects of the trypanosome preparation on the respiration of malignant cells (Plate 122).

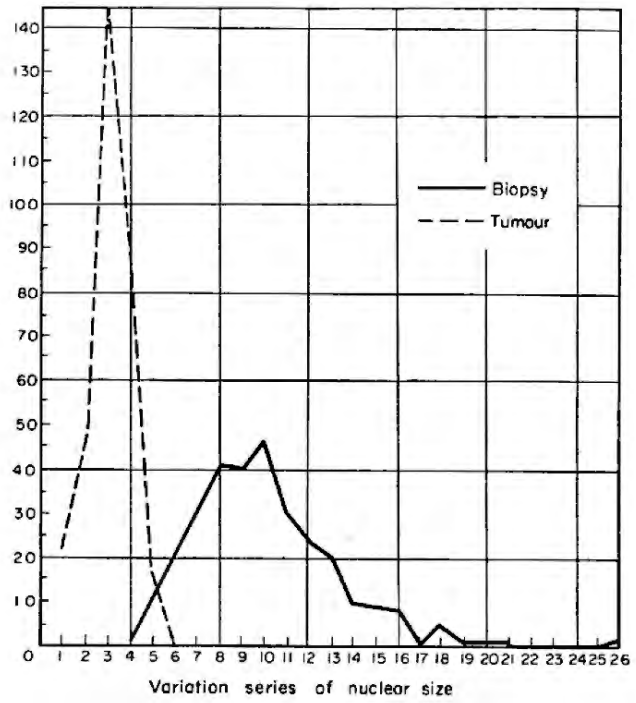


FIG. XXVIII. Curves showing the variation in nuclear size in spontaneous tumour 13 before and after treatment with the trypanosome preparation; there is a marked shift to the left and replacement of the multimodal curve by the uni-modal typical of non-malignant cells.

We could continue with descriptions of the histological and cytological observations made on the effects of the trypanosome preparation on other spontaneous tumours, e.g. 13, 7718, 6085, 5447, 5647 and 2986 (Figs. XXVII, XXVIII, Plates 123–127). We feel, however, that we have presented sufficiently clearly the definite principles which provide a conception, though only in general terms, of the processes taking place in spontaneous tumours under the influence of biotherapeutic activity. The generalization of these processes brings us to a consideration of the mode of action of this cancer antibiotic on malignant cells, which is the subject of the next section of our book.

Part VI

THE MODE OF ACTION OF THE ANTIBLASTIC PREPARATION FROM *T. CRUZI* ON CANCER

1. CYTOLOGICAL AND HISTOLOGICAL ANALYSIS OF THE MODE OF ACTION OF THE TRYPANOSOME PREPARATION

The process arising in malignant tumours under the influence of the trypanosome preparation comprises a *whole series* of moments which in combination can lead not only to stabilization or considerable diminution but also, in a number of cases, to a complete cure of spontaneous tumours far advanced in their development. The most characteristic manifestations of this process must be noted in more detail. First, we shall deal with the karyological changes.

A. CHANGES IN NUCLEAR SIZE

In our experiments, especially on analysis of the initial and middle stages of the action of the trypanosome preparation on a tumour, we have on more than one occasion resorted to karyometric investigations in order to provide objective evidence of a decrease in nuclear size. In evaluating the biological significance of these phenomena it should be remembered that the average nuclear size is a very characteristic property of the cells of any particular tissue. This property is determined by such a biologically substantial principle as the nucleus-cytoplasm ratio. It reflects the functional capabilities of the given cells, their stage of development and the nature of their metabolism. The nucleus-cytoplasm ratio is governed by the following: (1) a decrease in the nucleus-cytoplasm ratio is associated with decreased rate of growth and synthesis of living matter; conversely, the higher the nucleus-cytoplasm ratio, the more rapidly the life-processes are taking place;

(2) in the mature body the "labile", intensively multiplying cells (for example the cells of the sex glands and haemopoietic organs) have a higher nucleus-cytoplasm ratio than cells that have ceased to multiply.

Hence, the nature of the metabolism and the capability of continuous growth and multiplication are reflected in the nucleus-cytoplasm ratio and thus in the size of the nucleus. This is why the phenomenon of a decrease in nuclear size in cancer cells under the influence of the trypanosome preparation must be considered so significant—it undoubtedly indicates changes in the level and, it may be suggested, in the character of the tumour cells' metabolism.

The size of the nuclei in malignant tumours has been the subject of several special studies. As established by Epantschin (1928), Geiberg (1933, 1934) and Ehrich (1936) the nuclei of malignant tissues are usually hypertrophied. Wermel and Portugalow (1935) and Wermel and Schevschulskaya (1933) were able to confirm this, and stated that the nuclei of malignant cells are not only enlarged but show considerable polymorphism and size variability. Ehrich (1936) concluded that the observed enlargement of cancer cells (and nuclei in proportion) by 2–4 times is "*an anatomical expression of anaplasia and cataplasia in a malignant tumour.*" General biological considerations and numerous findings in modern cytology do not permit us to accept this situation without a number of reservations and limitations. Even so, change in nuclear size is a phenomenon which the cytologist must take into account, if not in the determination of the malignant nature of one or another tissue, then in any case for *an assessment of the morphophysiological changes taking place in the studied cells under the influence of certain factors, in this case the trypanosome preparation.*

B. CHANGES IN THE MITOTIC INDEX

A fall in the number of mitoses was noted repeatedly in spontaneous tumours after the action of the trypanosome preparation. This phenomenon is also fairly rapid in onset in tumours showing macroscopical "stabilization". Casey (1935, 1937) and Pearson (1936) showed the possibility of using the so-called mitotic coefficient in the determination of changes taking place in tumours. We would note that Casey and Pearson aimed at establishing not only the signs and degree of malignancy of the studied tumours but also the possibilities of clinical prognosis, based on cytological analysis of material obtained by biopsy.

In the light of present cytological knowledge Casey's basic suppositions cannot be accepted without serious discussion. It should first be noted that *it is absolutely impossible to relate the main sign of malignancy only to the frequency of division of the cells forming a tumour.* To do this would be to forget the other important properties of malignant cells: the

ability of infiltrative growth, closely related to an ability to destroy the surrounding tissues; a relative resistance to the effects of the body's defensive forces and of hormonal factors; adaptability to local conditions of respiration and nutrition. Besides these factors we should take into account the cytological findings showing that multiplication of the cells in any one tissue may not be a uniformly occurring process but may fluctuate, with its own rhythm, rising and falling according to a number of factors. The caution with which we approach any interpretation of the significance of the mitotic coefficient thus becomes understandable. For example, the mitotic activity of normal epidermis in the mouse has a characteristic diurnal rhythm.

Possible doubts in our evaluation of the biological meaning of the changes seen in mitotic activity may be partly removed by the fact that the biopsies were always carried out at about the same time of day, but the main argument lies in the work of Blumenfeld (1943), carried out on malignant tumours of mice and showing that in distinction from normal tissues, with their diurnal rhythm of division, the mitotic activity of cancer tissue *remains practically constant* during day and night. We would also mention the work of Bullough (1947) showing that cell division in normal mouse mammary tissue is a cyclic process, the highest number of mitoses occurring on the third day of the cycle and again at the end of it. Division in malignant tumours of the mammary gland proceeds in an entirely different manner; the number of mitoses is *always high, constant and cannot be changed experimentally by the administration of oestrogenic hormones.*

Considering as a whole all the changes established in the processes of division of malignant cells under the influence of biotherapy, we are bound to arrive at this conclusion: *a considerable fall in the mitotic index, right down to complete cessation of division, is a phenomenon widely observed in spontaneous tumours following the action of the trypanosome preparation and may be taken as a sign of the profound biological changes occurring in a tumour.* It should be noted particularly that in many tumours a sharp fall in the mitotic index *was seen even in the early stages* of biotherapy.

C. THE EFFECTS OF BIOPSY ON CELL MULTIPLICATION IN MALIGNANT TUMOURS

While considering the question of the mitotic index of treated spontaneous tumours of mice, we must deal with one other point. In all the mice with spontaneous tumours, both controls and those treated with the trypanosome preparation, a biopsy was carried out before the start

of the experiment. It is therefore quite natural to ask whether surgical trauma may have had an inhibiting influence on the number of mitoses. We made no special observations in this connection, since the literature contains ample evidence that the operational trauma induced by partial resection of a tumour most probably leads to increased malignancy of the process.

In oncological practice accelerated growth of a tumour is often seen after exploratory biopsy. For this reason many surgeons avoid a biopsy, or perform it immediately before a radical operation.

We know from the literature that in Nater's experiments mouse adenocarcinomata grew more rapidly in mice in which the author removed small fragments of the tumour with a sharp scalpel, then covered the operation wound with mastisol. Even by the 4th day the biopsied tumours were larger than those in control mice.

The literature also indicates that the number of metastases in tar-oil induced carcinomata can be increased by repeated biopsies.

Soboleva and Polyakov (1935), after their experiments on the influence of biopsy on the number of mitoses, arrived at the following conclusions:

1. A biopsy performed with scalpel or scissors undoubtedly influences the number of mitoses in experimental tumours; tar-oil carcinomata of mice, the Ehrlich transplantable carcinoma and the Flexner–Jobling rat carcinoma all show an *increased number of mitoses* in the great majority of cases. Surgical trauma is apparently *a direct or indirect stimulatory factor for progressive tumour growth.*

2. Nonradical operations on experimental tar-oil tumours (removal of papillomata) also apparently lead to acceleration of the transition from benign tumour to malignant. Soboleva and Polyakov finish their article: "... biopsy is a step having a powerful stimulatory effect on a neoplastic process."

We have no wish to raise here any abstract argument regarding to what degree, in what sorts of tumours and after what sorts of trauma this phenomenon occurs most or least. One thing is clear: *our experiments on the treatment of spontaneous tumours were carried out under conditions complicated by biopsies. This must be remembered when considering the general and special histological conclusions arising from the experiments we have described.*

Comparison of our clinical and experimental observations shows convincingly that *under conditions of administration of the trypanosome preparation biopsy has no negative effect on tumour regression.*

D. NUCLEOLAR CHANGES

Diminution of the nucleolus in malignant cells is a phenomenon seen widely in tumours subjected to the action of the trypanosome preparation. The prognostic and diagnostic significance of this phenomenon has already been discussed in detail. We would only add that Broders (1920, 1922), who devoted much work to the problem of specific properties of tumour tissue, also ascribes great importance to the size of the nucleolus, the ratio of nucleolar and nuclear sizes and also the variability of nuclear shape and size. Here we must emphasize once more the whole biological significance of diminution of the nucleolus. According to the hypothesis advocated by the Russian workers Bogoyavlenskii (1911) and Dogel' (1925), the nucleolus is closely associated with the process of chromatin regulation within nucleus, dealing with the transformation of chromatin substances in the nucleus. More recent observations (Caspersson, Brachet, Serra, Roskin) support this hypothesis: the nucleolus is closely related *to the metabolic processes of the nucleus and of the whole cell*, taking an active part in ribonucleic acid metabolism and possibly being the site of histone synthesis (Serra, 1944). These findings demand a careful study of the nucleoli in tumour cells before and after treatment.

As we were able to establish, under the influence of the trypanosome preparation there is *not only a sharp decrease in the size of the nucleolus but also a fall in the amount of ribonucleic acid contained in it.* Particular note should be made of these facts, with emphasis on their importance in the cytophysiological makeup of the cells of treated tumours.

E. THE LYMPHOCYTIC AND HISTIOCYTIC REACTION

On examining the histological picture of spontaneous tumours before and after treatment with the trypanosome preparation, we cannot overlook the appearance at a definite and relatively early stage of a clearly expressed *lymphocytic, monocytic and histiocytic reaction.* It must be admitted that the histological picture in different tumours varies both in the intensity of the round-cell reaction and in its cellular composition, depending on the nature of the tumour, the state of the body, the dose of the trypanosome preparation and, apparently, on certain other factors not yet discovered. Our preparations of course only show "odd frames" of the single process of a lympho-histiocytic reaction, which must be interpreted as a defensive process, since it arises regularly under the influence of an active biopreparation and only in tumours which have either ceased to grow or have started to decrease in size. It must be emphasized that in later stages of the action of the trypanosome preparation, when there

has been considerable diminution of the tumour, the processes of degeneration of the cancer cells have ended and homogenization and "fusion" of the malignant tissue have begun, there is also a gradual fading of the lympho-histiocyte reaction. Apparently most of the lymphocytes and histiocytes die along with the dying malignant tissue. Other factors, so far not definitely identified, are involved in the completion of the process—the final lysis of the destroyed cellular elements. Whatever these may be, *the stage of active participation of lymphocytic and histiocytic cells is an essential part of the mode of action of the trypanosome preparation.*

These findings may be supplemented by the relatively sparse material concerning this subject to be found in the literature. As we know, the literature contains divided opinions as to the significance of tumour infiltration by lymphocytes and histiocytes. Some investigators assert that these elements have a specific or nonspecific antiblastic effect, whereas others refute this position, although we do not understand the reasons for considering that lymphocytes, monocytes and histiocytes, the defensive significance of which is accepted in all other pathological processes, should not play a defensive role in cancer. Even if we accept conditionally that these cellular elements do not play any part in a specific antiblastic defence mechanism, how can we deny their defensive importance in the processes of neutralization of breakdown products formed on destruction and necrosis of the cancer cells? It must be stressed, however, that the presence of a round-cell infiltrate (in the broad sense of this term) is not brought about by the existence of cell necrosis in a tumour, as stated by some authors. *Lymphocytes and histiocytes also penetrate tumours in places where there are no necrotic foci of any kind*—this is not only our own opinion, but is held by a number of authoritative histopathologists specializing in oncology (for example, Hooper, 1955).

Many authors have defended the hypothesis of the antiblastic action of lymphocytes, plasma cells and histiocytes — Finogenov, MacCarty, Broders, Unna, and others. From Finogenov's early thesis (1909) on "the development of cancer in relation to the appearance of a tissue reaction in the body", the following conclusions may be drawn:

(a) the body counteracts to some or another extent the proliferation of cancer cells;

(b) the visible expression of this counteraction from the histopathological point of view is the local tissue reaction;

(c) the local tissue reaction is to some degree a granulatory, limiting and organizing chronic connective tissue inflammation;

(d) at the height of this inflammation, where all the motile mesenchymal elements are present, with leucocytic infiltration and the appearance of giant cells, a picture of destruction and organization of the cancer elements is seen which may be termed partial independent recovery;

(e) the inflammatory reaction of the connective tissue is a visible expression of the body's degree of counteraction to the development and proliferation of cancer cells and may to a certain extent serve as a criterion of it;

(f) the stroma of a malignant tumour is the product of an inflammatory reaction in the underlying tissues, and its degree of development is in relation not to the plastic properties of the tumour but to the degree of general counteractive tonus of the body;

(g) the round-cell infiltrate surrounding a tumour, although in its quantitative and qualitative make-up not inhibiting tumour growth in the clinical sense, is nevertheless a factor unfavourable to it.

A long time ago, Da-Fano (1910) showed the defensive role of lymphocytes and plasma cells in the *spontaneous resolution* of mouse tumours: these cells always surround degenerating cancer cells. We would add that Sokolov (1936) observed that after X-irradiation lymphocytic infiltration prevents the development of carcinoma implants.

Ewing (1928), and more particularly Murphy (1926) considered lymphocyte infiltration an undoubted sign of the activity of the body's defensive forces. Murphy's observations are all the more convincing in that in many rapidly growing tumours a round-cell infiltration is quite absent, and also in cases where an experimentally implanted tumour develops successfully. Murphy thought the role of the lymphocyte in anticancer immunity to be perfectly obvious.

We cannot but note, at certain stages of a cancerolytic reaction, *the active role of the plasma cells*, as seen in a number of treated tumours where plasma cells not only form dense aggregations in the stroma but also undergo intensive division.

We also cannot ignore findings on the significance of eosinophil infiltration, which holds particular interest for us since G. Khrushchov recently raised the question of whether eosinophils secrete substances inducing a macrophage reaction — its favourable prognostic significance in clinical cancer has been noted by many investigators (Rego, Rubens-Duval, Lam and many others); our material was not examined sufficiently from this point of view.

Summing up our observations once more, we are bound, irrespective of the accepted theoretical position regarding the role of the round-cell infiltrate in a malignant process, to take as experimentally proven the situation that in spontaneous tumours (which before treatment showed no significant aggregations of lymphocytes or histiocytes) *there arises a clearly expressed lymphocytic or mixed lymphocytic and histiocytic reaction which is very characteristic of the stage of tumour stabilization and the start of destructive processes in the malignant tissue.*

Support for our views on the important role of the lymphocytic reaction as a *speficic defensive* reaction is also found in recent works indicating that the lymphocytes form both the normal globulins found in blood and lymph and *antibody globulins.*

In the light of all this the lymphocytic reaction must be considered as being closely associated with *activation of the humoral factors of anticancer defence*, while the monocytes and histiocytes are concerned with the second stage of defence—*a cancероclastic function.* In short, the trypanosome preparation creates in a malignant tumour conditions under which the natural cellular factors of defence, partially or completely suppressed during normal development of a malignant tumour, *regain the ability to assert themselves.*

F. CHANGES IN RIBONUCLEIC ACID CONTENT

On summing up our histophysiological observations both on spontaneous mouse tumours and on transplantable tumours—the Brown–Pearce carcinoma and Crocker sarcoma—we are bound to accept that at certain stages of the action of the trypanosome preparation there is a decrease in the amount of ribonucleic acid in the cytoplasm and nucleoli of malignant cells, at first hardly noticeable but later progressing to its complete disappearance (within the limits of sensitivity of the Brachet test). A similar considerable reduction or even disappearance of ribonucleic acid may also be seen without any experimental interference in zones of degeneration and necrosis of malignant tumours.

Under the influence of biotherapy the size of the areas giving a weak or a negative reaction for ribonucleic acid *increases considerably*, and these areas may be found in parts of the tumour *not yet showing morphologically detectable signs of cell degeneration.* In other words, a decrease in ribonucleic acid content must be considered a *primary phenomenon* in the changes in the properties of cancer cells, *heralding changes in other protoplasmic components and organoids.*

These observations enable us to put forward the following hypothesis: the trypanosome preparation may, directly or indirectly, destroy the cellular processes regulating the accumulation of ribonucleic acid in the cytoplasm and nucleolus. In assessing the biological significance of the reduction or disappearance of ribonucleic acid it should be borne in mind that the synthesis of protoplasmic proteins is closely related to the presence of ribonucleic acid, as has been shown by numerous workers (Caspersson, Brachet, Kedrovskii, Belozerskii, Roskin, and others).

We have studied the histochemistry of ribonucleic acid in a number of tumours of various histogenesis: mammary carcinoma, cancer of the lip, cancer of the stomach, basal-cell sarcoma of the cheek, cancer of the tongue, and others. All these observations established:

(1) the cells of actively growing malignant tumours usually contain considerable ammounts of ribonucleic acid in their cytoplasm;

(2) in cancer cells which have grown appreciably in size, or in the so-called giant cells, there is relatively little ribonucleic acid;

(3) degeneration and death of cancer cells is always associated with a decrease in ribonucleic acid right up to the complete disappearance of a positive Brachet reaction.

If, in the light of these observations, we now evaluate the phenomena seen in malignant tumours under the influence of the trypanosome preparation, we are bound to accept that the *trypanosome preparation affects one of the most important components of protein synthesis in malignant cells — ribonucleic acid.* This hypothesis of the specific effect of the trypanosome preparation on the protein metabolism of the cell and in the first instance on ribonucleic acid is confirmed by observations reported to us by Prof. A. M. Kuzin: *there is a sharp fall in the amount of ribonucleic acid in living yeast cells* under the influence of the trypanosome preparation.

G. REACTIVE CHANGES IN THE CONNECTIVE TISSUE

In addition to the observations already described we feel it necessary to mention briefly the investigation by Levinson and Platonova of histological and cytological changes in the Crocker sarcoma of white mice under the influence of administration of the trypanosome preparation. This investigation, which confirms our earlier observations, provides new results essential to an understanding of the role of elements of the areolar connective tissue in the mode of action of the trypanosome preparation on a malignant tumour.

The whole process of the action of the trypanosome preparation on cells of the Crocker sarcoma, as far as can be shown by histological and cytological analysis, may be represented as follows: the preparation affects the metabolism of the sarcoma cell, as a result of which, in particular, the metabolism of nucleic acids in the cell is altered. This is expressed as a decreased basophilia of the cytoplasm, i.e. a fall in its ribonucleic acid content. Simultaneously, the mitotic index falls and the tumour's growth is retarded. The further effects of the trypanosome preparation bring about vacuolization of the cytoplasm, then nuclear pyknosis, then complete cellular breakdown. Progressive degeneration of the sarcoma cells leads to considerable enlargement of the zone of necrosis, while the growth zone becomes small relative to that in untreated tumours. The changes in the properties of the cancer cells lead to changes in the reaction of the surrounding connective tissue.

The connective tissue surrounding an untreated Crocker sarcoma differs slightly in the state of its cellular elements from connective tissue located in other parts of the body. There is an increased basophilia of its cells, admittedly very slight, and lympho-histiocytic elements are seen rather more frequently than usual. A very different picture is seen in the connective tissue surrounding a Crocker sarcoma treated with the trypanosome preparation. *The overall number of fibroblasts and lympho-histiocytic cells is considerably greater. The fibroblasts show a much greater basophilia,* i.e. they pass into a stage of activity. Between the fibroblasts lie many lymphocytes, monocytes, polyblasts and macrophages. All this shows that the connective tissue surrounding a treated tumour *is in a state of active inflammatory response.*

One of the causes of connective tissue activation may be the accumulation of products of the breakdown of tumour cells, stimulating an inflammatory reaction. When the connective tissue around the tumour has been stimulated it starts to infiltrate into the tumour. Preparations of the growth zone of the tumour clearly show a massive invasion of lymphohistiocytic cells—lymphocytes, monocytes, polyblasts and macrophages. The changes taking place in the tumour structure itself are very characteristic. In the growth zone of an untreated Crocker sarcoma the malignant cells usually lie closely adjacent to each other. The phenomenon of disintegration of the Ehrlich adenocarcinoma into isolated cells or groups of cells after treatment with the trypanosome preparation has been described elsewhere (Klyuyeva and Roskin, 1946). Similar pictures are seen consistently in the treated Crocker sarcoma; the cells of the untreated tumour, lying in a solid sheet, break apart or form small groups

(Plate 128). In this way the whole structure of the tumour tissue is altered. The breaking apart of the cells seems to start even before the connective tissue infiltration. Connective tissue then starts to proliferate rapidly in the spaces formed, and probably in its turn suppresses the development of the tumour tissue. After the polyblasts and macrophages, the tumour is invaded by fibroblasts. The increased basophilia of these cells should be given particular attention. Increased basophilia of the fibroblasts precedes the formations of collagen fibres and the participation of these cells in the formation of cicatricial tissue. The same occurs in a Crocker sarcoma treated with the trypanosome preparation: fibroblasts which have penetrated the tumour and stroma fibroblasts rich in ribonucleic acid start active production of collagen fibres. It is easily seen that an untreated Crocker sarcoma is penetrated by only a small amount of collagen fibres. A completely different picture is seen in the treated tumour. The number of collagen fibres is much greater *and the whole of the tumour tissue becomes infiltrated by collagen fibres.* It can be seen under high magnification that the separate collagen fibres lie in thick bundles, running between the cells in large numbers. Particularly thick and coarse bundles of collagen fibres form in the zone of necrosis. There is thus actual organization of normal tissue at the site of the degenerating malignant tissue, especially in the zone of necrosis. The connective tissue here fulfils a regenerative function, actively organizing and filling in the defects formed after destruction of the malignant tissue. As the result of all these processes the tumour decreases in size and cicatricial tissue forms in place of the destroyed malignant cells. These are the main factors in the action of the trypanosome preparation on the Crocker sarcoma.

* * *

As we see, the observations of Levinson and Platonova confirm and supplement the pictures of the action of the trypanosome preparation seen earlier in the Ehrlich adenocarcinoma and more recently in the rabbit carcinoma, spontaneous mouse tumours and finally in human tumours. However, the observations on the Crocker sarcoma have one peculiarity which caused us to include them here: the experiments on the treatment of this sarcoma were carried out *without preliminary biopsy of the tumours*, in distinction from the position in clinical cases and in experiments on the treatment of spontaneous mouse tumours. There are thus no longer any doubts as to whether the clearly expressed lymphohistiocytic reaction and the subsequent behaviour of elements of the areolar connective tissue — a vital stage in the mechanism of the cancerolytic reaction — is caused by operational trauma or some of its accompanying factors.

H. THE GENERAL PRINCIPLES OF THE CYTOLOGICAL AND HISTOLOGICAL CHANGES OCCURRING UNDER THE INFLUENCE OF THE TRYPANOSOME PREPARATION

In order to evaluate all the histological, cytological and histochemical changes which we have determined in tumours subjected to the action of the trypanosome preparation, we must choose for comparison the most characteristic of the properties of malignant tissue. For this, we may use the characteristics of malignant tissue put forward by Caspersson and Santesson (1942).

"*Chemical changes* characterizing malignant cells in comparison with normal cells are:

1. An increase in the amount of nucleic acids (ribonucleic and thymonucleic acids).
2. A decrease in the warm coagulation protein fraction, while the cold coagulation protein fraction remains constant or increases.
3. An increased diamino acids content.

The chemical changes are accompanied by *cytological changes:*

4. An increase in the number and change in the character of mitoses.
5. An increased variability of nuclear size, with a tendency towards larger sizes.
6. An increase in nucleolar substance — this must be the most consistent sign of malignancy.
7. At least some of the tumour cells have basophil cytoplasm."

On comparing these signs with what was seen in treated tumours, we are bound to accept that *the trypanosome preparation has a clearly determinable influence on five of the basic properties (1, 4, 5, 6 and 7) of malignant cells, out of the seven established by the work of Casperson and Santesson.*

The conclusions relating to the histological, histophysiological and cytological changes occurring in transplantable and spontaneous tumours of white mice under the influence of biotherapy apply not only to animal tumours but also to malignant tumours in the human patient. A comparison of the observations on each of these groups of tumours shows that the action of the trypanosome preparation on malignant tissue is governed by *quite definite principles*, which may, of course, be modified or complicated according to the species involved, the histogenetic characteristics of the tumour, its stage of development, the general state of the body, the time of biotherapeutic interference, etc.

Having pointed out the five basic properties of the cancer cell which change under the influence of the trypanosome preparation, we must add one circumstance, perhaps the most important — the reaction of the body. The reactions of the body comprise the lymphocytic reactions, the mixed lympho-monocytic reaction and lastly the more complex lympho-monocytic and macrophage reaction. These reactions are in many cases accompanied by mesenchymal transformation of the tumour elements themselves, or by increased development of the connective tissue stroma, or, lastly by proliferation of connective tissue from the layers surrounding the tumour.

On the basis of present scientific knowledge it may be taken that some cells, the lymphocytes, are the source of humoral defence factors; others, the histiocytes and macrophages, carry out the functions so fully described by Mechnikov that we can add nothing.

It should be emphasized that the lymphocytic reaction is, as rule, the first link in the body's general reaction, and the histiocytic reaction the second. It should by no means be assumed that the first step automatically brings about the second. This is to say in the first stage the trypanosome preparation has a *cancerostatic* effect on cancer tissue, causing corresponding changes in the tumour cells. Next, the effects of the preparation enable the lymphocytes to enter the battle, or, if that had already occurred to some extent before using the preparation, they enable the lymphocytic reaction to be reinforced. After this we see the effects of the trypanosome factor in association with the lymphocytic and histiocytic reactions. Their combined activities apparently bring about a *cancerolytic reaction* — the formation of extensive fields of broken down tumour tissue, which grow larger and larger as administration of the trypanosome preparation is continued.

The plasma cells are also of undoubted significance in the complex humoro-cellular processes bringing about the final cancerolytic effect, but it is still difficult to give a precise definition of their function.

It can easily be believed that *the trypanosome preparation plays a part in each of the stages mentioned.* It needs only experimental interruption of administration of the preparation for the malignant process to adopt its normal course, and after a time the observer finds only mere traces of all the former cytological changes in the cancer cells and the remains of the lymphocytic and histiocytic reaction, i.e. there occurs what we see in those tumours where a marked acceleration of malignant growth follows temporary regression or stabilization.

Experimentally and clinically we often saw the phenomenon of so-called *tumour stabilization.* According to all our observations, the cessation of the development of a malignant tumour under the influence of the trypanosome preparation must not in any circumstances be considered as a temporary interruption of the growth of the malignant tissue. In such a case *cessation of tumour growth is a result of the profound morpho-physiological changes which have taken place in the cancer cells.* This is reflected by the changes in nuclear size, diminution or disappearance of the nucleoli, breakdown in the structure and staining properties of the nucleus, a fall in the ribonucleic acid content of the cytoplasm and nucleolus and finally by degenerative changes in the mitochondria. This situation finds support in the observations by Prof. A. M. Kuzin on the effects of the trypanosome preparation on the respiration of sarcomatous tissue (see below). Taken as a whole, the changes established in treated tumours give us some idea of the cytological and histological moves *characterizing the regression of malignant tumours under the influence of the trypanosome preparation.*

Summing up all the cytological and histological observations, we may define the action of the trypanosome preparation thus: *the trypanosome preparation disturbs the metabolism of cancer cells to an extent sufficient to cause reduction or disappearance of the aggressiveness of malignant tissue and thus clears the way for the whole humoro-cellular sytem of the body's defensive powers.*

The trypanosome preparation induces diverse but completely regular changes in tumour tissue. The question naturally arises as to whether all these histological and histo-physiological changes caused by the cancer antibiotic correspond with our aims in the modern, rational therapy of malignant tumours. The answer to this question would appear to have been given by F.I. Pozhariskii as long ago as 1940, when he wrote: "We now have a search for a *rational cancer therapy*, progressing basically in two directions: a search for a substance which, on the one hand, will damage cancer cells and lower their resistance, and which on the other hand will stimulate the activity of elements of the reticulo-endothelial system along the lines of increased phagocytic powers of the mesenchymal elements, their proliferation and the acceleration of enzyme processes."

* * *

Cytological and histological analysis can only reveal part of the mode of action of the cancer antibiotic on the malignant tumour. Considerable help in the solution of this problem has been provided by the work of Prof. Kuzin and his colleagues on the influence of the trypanosome preparation

on the respiration of cancer tissue, and also by the experiments of Fradkina and Katz on the selective absorption of the trypanosome preparation by malignant tissue, observations on the effects of the trypanosome preparation on cancer cells under conditions of tissue culture, experiments on the specificity of the antiblastic properties of *T. cruzi*, and finally a comparison of the mode of action of the trypanosome preparation with certain other anticancer chemo- and biotherapeutic preparations.

2. THE INFLUENCE OF THE TRYPANOSOME PREPARATION ON THE RESPIRATION OF CANCER TISSUE

(PROFESSOR A.M. KUZIN'S FINDINGS)

As shown by O. Warburg, one of the characteristic features of cancer tissue is the decreased activity of its oxidative enzyme systems. Decreased respiration, with intensive anaerobic glycolysis, has been noted in cancer tissue by many investigators, particularly in advanced cases.

Findings regarding the normalizing influence of *T. cruzi* lysates on cancer tissue, with cessation of its growth and fall in the number of mitoses, with subsequent necrosis and replacement by normal tissue have lent probability to the theory that the active substance in *T. cruzi* lysates may influence the degree of respiration of cancer tissue. To check this theory a study was made of the influence of *T. cruzi* preparations of various modifications on the respiration of cancer tissue.

The investigation related mainly to the rapidly growing Crocker sarcoma. A few experiments were carried out on spontaneous mouse tumours and the Brown-Pearce tumour. Tumour respiration was studied using Warburg's apparatus and method.

Sections 0·5 mm thick were made with a razor from fresh material obtained by operation, free from necrosis and kept in sterile Ringer's solution. Each section was divided accurately in half, one half being used for the experiment and one serving as a control.

Because of the difficulty of preparing several sections from one tumour, several tumours were taken for each series of experiments. The absolute amounts of oxygen utilized for respiration by different tumours and even by different sections of the same tumour varied appreciably. We can therefore only compare the respiratory values obtained for two halves of the same section. Special observations showed that such halves always gave similar values, varying only by 5–10 per cent.

The prepared sections were placed in Warburg flasks containing 2 ml of fluid. In the control containers these 2 ml consisted of 1 ml Ringer's solution and 1 ml of a 1/15 M phosphate buffer with a pH of 7·0. In the experimental flasks were 1 ml of the same phosphate buffer, 0·5 ml Ringer's solution and 0·5 ml of a solution of various concentrations of the studied preparation, with a pH of 7·0. All the flasks contained alkali to absorb the CO_2 produced on respiration. Estimations were started after the air in the flasks had been displaced by an oxygen-enriched gas mixture and the flask had been kept in a bath or thermostat at 37°C for 20 minutes. Observations were made every 15 minutes for 2 hours. After this period the sections were removed, put into distilled water and dried to constant weight. After correcting for temperature variations as shown by a thermobarometer (not exceeding hundredths of a degree) the volume of oxygen utilized by 1 mg of the studied tissue was calculated by Warburg's formula. Comparison of this volume in the experimental flasks with the volume of oxygen utilized by the half-section in the control flask gave a measure of the inhibitory or activating influence of the preparations studied.

A series of experiments was set up for trypanosome preparations of various modifications showing a significant activity in tests on the Crocker sarcoma in white mice.

The results appended show that there is *distinct activation of respiration* under the influence of the trypanosome preparation.

In all the experiments, using various concentrations, there was a clearly expressed optimum of activation, this optimum lying among the high concentrations in the case of preparations with a low activity and changing to very low concentrations for extremely active preparations (for example Table A, batch 255). The existence of this optimum enables a preparation to be characterized quantitatively as well as qualitatively.

The results obtained are given in Tables A, B, C and D.

To confirm this possibility we used two preparations of coded activity. The results of testing them are shown in Table B.

The marked differences in the behaviour of these preparations enabled us to conclude that the first was inactive and the second had a considerable activity. A biological test for activity showed the first to be completely inactive, while the second had an index of effectiveness of 2·68.

After definite results had been obtained on activation of the respiration of cancer tissue by *T. cruzi* preparations, the following question was raised: is the substance which activates respiration also the substance responsible for the inhibitory and cancerolytic action of the preparation, or is this an expression of different agents existing in one lysate? It is quite clear

TABLE A

Expt. No.	Tumour	Preparation Batch No.	Index of effectiveness with regard to Crocker sarcoma	Concentration of preparation in 0.5 ml (in CU)	Oxygen uptake in mm³ per 1 mg tissue: with preparation	Oxygen uptake in mm³ per 1 mg tissue: control	Effect of preparation as % of control
1	Crocker sarcoma	2	3.7	100	157	388	40
				50	202	292	70
				25	333	337	100
				10	289	212	139
2	Spontaneous mouse tumour	2	3.7	100	379	424	89
				10	808	519	155
				5	813	615	132
					1 hour		
3	Crocker sarcoma	255	5.0	50	214	215	99
				10	603	263	229
				5	635	199	319
					2 hours		
				50	357	430	80
				10	512	293	174
				5	684	365	187
4	Crocker sarcoma	317	2	100	219	271	80
				50	755	467	161
				10	747	406	183
5	Crocker sarcoma	360	2.2	10	1113	879	126
				5	982	772	127
6	Brown-Pearce carcinoma	251	—	200	224	237	94
				100	430	225	169
				50	450	263	171

TABLE B

Preparation studied	Tumour	Concentration of preparation in 0.5 ml (in CU)	Oxygen uptake in mm³ per 1 mg: with preparation	Oxygen uptake in mm³ per 1 mg: control	Effect of preparation as % of control
Preparation X	Crocker sarcoma	200	448	346	123
		100	468	609	109
		50	339	275	123
		10	523	482	108
Preparation 2X	Crocker sarcoma	200	202	250	80
		100	280	285	99
		50	543	207	262
		10	969	234	414

that elucidation of this question is essential if observations on the effect on respiration are to be used for characterizing the cancerolytic activity of a preparation. The question is answered to some extent by a comparison of the figures in the above tables.

After this we studied the respiratory effects of preparations which had been subjected to various treatments.

As is known, active *T. cruzi* lysates in liquid form quickly lose their cancerolytic activity. Keeping a lysate at 37°C in an incubator inactivates it within 24 hours. We were interested to see how the effects of a preparation on the respiration of cancer tissue would be affected by such conditions. Active preparations were taken, and after investigation of their influence on respiration the (sterile) preparations were kept at 0°C and 37°C. The tests were repeated after 24 hours. The results are given in Table C.

TABLE C

Preparation	Conditions of storage	Concentration of preparation in 0.5 ml (CU)	Oxygen intake in mm³ per mg: with preparation	Oxygen intake in mm³ per mg: in control half	Effects of preparation as % of control
Batch 555	Freshly prepared	10	603	263	229
	Stored in solution at 0°C for 5 days	10	274	312	89
Batch 350	Freshly prepared	100	670	720	96
		50	504	763	66
		10	1203	572	210
	The same lysate after keeping at 37°C	100	658	747	88
		50	936	841	111
		10	762	659	115

These figures show that both storage on ice for 5 days and storage of the original lysate at 37°C for 1 day remove its active effects on respiration, indicating an instability of the active principle resembling that of the cancerolytic principle.

Earlier work by Kuzin, Poliakova, Gintsburg and Rodionov, and also by Kuzin, Kuzina and Gintsburg, has shown that the substance possessing cancerolytic properties does not pass through a semipermeable membrane and is not a free protein or lipoid.

We were interested in testing active preparations after dialysis, and also deproteinized preparations.

Experiments showed that dialysis of a preparation, i.e. the removal of all substances of low molecular weight, does not result in disappearance of its activating influence on the respiration of malignant tissue. Purified fractions of a preparation freed of protein by precipitation with trichloracetic acid and of low-molecular-weight substances by dialysis retained completely their initial effect: to retard respiration in high concentrations and to stimulate it markedly at optimum concentrations.

The experimental results show that at the optimum concentration respiration is increased by 50–100 per cent, while with high doses it is retarded to 40–60 per cent of the normal level for the studied malignant tissue. We cannot, therefore, fail to notice a considerable similarity between the effects of the trypanosome preparation on a malignant tumour and its effects *in vitro* on the respiration of malignant tissue.

We are as yet unable to give any definite opinion on the identical nature of the substances inhibiting tumour growth and those influencing respiration in the preparations studied, but the similarities noted above *make this position extremely likely*, though large-scale testing of the preparation's influence on the growth and respiration of malignant tissues is essential before a final decision is made.

The last question arising naturally from our results relates to the effect of active preparations on the respiration of *normal, non-malignant tissue.* We have made a few investigations, the results of which are shown in Table D.

Taken as a whole, these results enable us to conclude that preparations from *T. cruzi* having an inhibitory action on the Crocker sarcoma are able, in high concentrations, to suppress, and at optimum concentration to stimulate the respiration of malignant tumour tissue.

After various treatments of the active preparations, leading to their inactivation or to refination and the isolation of the active principle, similarities were seen to exist between their cancerolytic activity and their ability to activate the repiration of malignant tumours.

The figures given above show that when the preparation acted on normal tissues we were unable to observe the principles shown so distinctly in experiments with malignant tissues.

Preparations activating respiration in malignant tumours had no regular influence on the respiration of muscle tissue from healthy animals.

* * *

TABLE D

Preparation tested	Tissue studied	Concentration of preparation in CU	Oxygen uptake in mm^3 per mg		Effects of preparation as % of control
			with preparation	in control section	
Batch 261	Muscle from normal mouse	200	252	249	101
		100	343	327	104
		50	328	303	108
Batch 252	Diaphragm from normal mouse	200	1661	1253	133
		100	1854	2177	84
		50	1689	1765	95
Batch 317	Mouse liver	50	1299	1097	118
		10	1187	1106	107
,,	Muscle from normal mouse	50	1187	1200	96
		10	1133	1391	81

This concludes Prof. Kuzin's report. We can only add the words of Warburg, taken from his paper to the Berlin Academy of Sciences (1947): "Since there is no malignant tumour not possessing the powers of anaerobic glycolysis, it is very probable that tumour growth may be inhibited by inhibiting anaerobic glycolysis. Obviously, a cancer cell deprived of its power of anaerobic glycolysis would cease to be a malignant cell."

3. THE DISTRIBUTION OF THE TRYPANOSOME PREPARATION IN THE NORMAL AND IN THE CANCER-AFFECTED BODY

To shed some light on the complex mode of action of the trypanosome preparation on the cells of malignant tumours, R. V. Fradkina and E. Katz undertook a detailed investigation of the fate of the preparation after its injection into the body and of its distribution both in the blood and in various organs and tissues. A highly sensitive method was used in carrying out this task—the complement fixation test in the cold. This method enables very small amounts of an antigenic substance to be discovered. In each experiment, mice were divided into two groups. The mice in one group were inoculated with the Crocker sarcoma, while the uninoculated

mice of the second group served as controls. When the tumour had reached a considerable size (after 10–12 days) the mice in both groups were injected intravenously with an accurately measured dose of the trypanosome preparation. Mice were then killed after 1, 3, 6, 9, 12, 18 and 24 hours and the blood, organs and tumours taken for analysis. The test was interpreted by the accepted methods. The experiments on the distribution of the trypanosome preparation in the bodies of normal animals (white mice) showed that 1–3–6 hours after intravenous administration

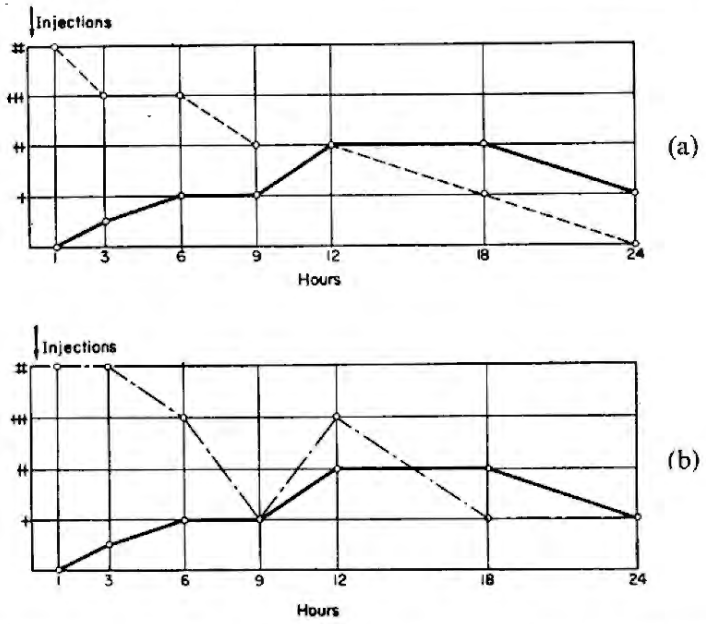


FIG. XXIX. Above: distribution of the trypanosome preparation in the tumour (dotted line) and blood (unbroken line) of a cancer-affected mouse; below: distribution of the preparation in the blood of a normal mouse (dotted line) and in the blood of a cancer-affected mouse (unbroken line).

of the preparation the complement fixation test gave a marked positive result; after 9–12 hours the intensity of the reaction decreased and after 24 hours no antigen could be demonstrated in the serum. A different situation was seen on investigation of extracts from the organs (liver, spleen) of these healthy mice: as the intensity of the blood serum reaction fell, the amount of the antigen in the liver and spleen rose. A completely different picture was seen on studying cancer-affected mice. The most important circumstance in this case was that 1–3–6 hours after the injection of the trypanosome preparation most of the antigen was found *not in the serum but in the tumour tissue, in marked distinction from what was found in normal mice*. The amount of antigen in the tumour fell sharply after 9–24 hours, while the reaction gradually grew stronger in the serum, liver and, primarily, the spleen (Fig. XXIX, *a*, *b*).

These findings enable us to assert that *the trypanosome preparation entering the body of a cancer-affected animal accumulates rapidly and intensively in the tissues of the malignant tumour*. Fradkina and Katz' results are certainly of no small importance in an analysis of the mode of action of the trypanosome preparation on malignant tumours. Comparison of these serological observations with the results of histological analysis of the tumour tissue and spleen and liver tissues shows that in the malignant tissue the trypanosome preparation causes marked changes, whereas in the normal tissues no morphologically determinable changes are present. This circumstance agrees closely with the results of earlier biochemical experiments by Kuzin and his co-workers on the selective effects of the trypanosome preparation on the respiratory metabolism of cancer cells.

* * *

As is known, experiments with cultures of malignant cells outside the body may be a real help in the analysis of antiblastic substances. With this aim the experiments described below were carried out.

4. THE EFFECTS OF *T. CRUZI* AND THE TRYPANOSOME PREPARATION ON MALIGNANT TISSUE UNDER THE CONDITIONS OF TISSUE CULTURE

A. THE EFFECTS OF *T. Cruzi* ON CULTURES OF MALIGNANT TISSUE

Our experiments (carried out in collaboration with Z. Kanarskaya) on the cultivation of *T. cruzi* in cultures of malignant tissue (Crocker sarcoma) outside the body were designed to answer four important questions:

(1) what type of cells—sarcomatous or stroma cells—are most affected by *T. cruzi;*

(2) whether the phenomenon of *tumourotropism* of *T. cruzi* could be shown experimentally in cultures of malignant tissue;

(3) the intensity with which this property is expressed by the sarcoma cells;

(4) *what changes are undergone by malignant cells under the influence of T. cruzi.*

The last question is closely related to the problem of the mode of action of *T. cruzi* on malignant tissues.

The experiments were arranged in the following manner. Mice were inoculated with the Crocker sarcoma, and on the following day they were infected with *T. cruzi*. When the number of trypanosomes in the peripheral blood had reached 15–20 per field of vision the tumour was explanted and used to set up cultures. The culture medium was rabbit plasma. The cultures were usually observed for 3–4 days, after which they were fixed and stained in the normal manner. The culture investigations concerned mainly the cells of the growth zone. The observations gathered from six series of experiments enabled the following conclusions to be made:

(1). The tumourtropism of *T. cruzi* may be seen quite convincingly in cultures of sarcoma tissue.

(2). The cells affected are almost exclusively the large, round sarcoma cells, and not the spindle-shaped stroma cells.

(3). In different cultures the number of sarcoma cells affected by *T. cruzi* varies from a few per preparation to a considerable number—5–7 per cent of all the cells in the growth zone.

(4). The number of trypanosomes in one sarcoma cell varies from 1 to 15 individuals (Plate 129).

These observations refute Hauschka's (1947) too categorical assertion that *T. cruzi* shows no tumourtropism.

(5). As a result of the penetration of the trypanosomes, the sarcoma cells show signs of degeneration. *This degeneration is expressed primarily as cytoplasmic changes: vacuolization, changes in the microstructure, signs of plasmolysis* (Plate 130).

(6). Following the cytoplasmic changes, there are *degenerative changes in the nuclei of affected cells: the normal staining properties of the nuclei are modified, with nuclear deformation and homogenization.*

The situations observed in cultures of malignant cells coincide to a considerable extent with those seen in microscopic preparations from tumours of mice infected with *T. cruzi*.

One highly important question remains unanswered: how does the presence of *T. cruzi* influence the general growth of cultures of sarcoma tissue — is it retarded or are the trypanosome's effects limited to destruction only of the cells actually infected? The solution of this problem was complicated by the technical difficulty of setting up suitable experiments with appropriate controls.

It should also be mentioned that *T. cruzi* is able to penetrate not only the cancer cells in a tissue culture. As shown by Moshkovskii, Kofoid (1935), Zud and McNeil, and then by Meyer and de Oliveira (1948), *T. cruzi* is easily cultivated in chick embryo cells (although chickens cannot be infected with *T. cruzi*).

The observations described are of definite importance in understanding the changes brought about in cancer cells by the trypanosome preparation. We have seen, however, that the effects of *T. cruzi* are much wider and are by no means limited to the cells they parasitize. Moreover, in some tumours, for example the rabbit carcinoma, as has been shown by Yumashev, actual trypanosomes are rarely seen during *T. cruzi* infection but the effects on the main tumour and on its metastases are very great. It follows from this that in the action of *T. cruzi* on a tumour the primary role is played by a humoral factor formed by the trypanosome or forming on its breakdown. For this reason, experiments on the effects of the trypanosome preparation on malignant cells under conditions of tissue culture are of particular importance for an understanding of the preparation's mode of action on malignant tissue. We turn now to the results of these observations.

B. THE EFFECTS OF THE TRYPANOSOME PREPARATION ON THE CROCKER SARCOMA *in vitro*

The effects of the trypanosome preparation ware studied by adding it directly to the culture medium (in equal quantities with rabbit serum); a parallel study was made of the influence of preliminary treatment of the preparation on the tumour tissue (storage in a refrigerator for 24 or 40 hours) and on its subsequent growth in tissue culture. Controls were set up in a similar manner, but with normal saline in place of the trypanosome preparation. In each case we studied the effects of different concentrations (5 CU, 20 CU, 50 CU, 100 CU) of the same batch of the preparation under equal experimental conditions. Cultivation was by the hanging drop method. The effects of the preparation were noted after 2, 3 and 4 days of culture growth by comparing the area of the growth zones in the control and experimental Crocker sarcoma tissue cultures

The investigation established:

(1) all the batches of preparation studied (Nos. 252, 255, 318, 322) retarded growth of the sarcoma by several times in comparison with the controls;

(2) the retardation of growth may be slight (1.5–2 times) or considerable (6–7–13–14 times) depending on the quality of the batch tested, its concentration, period of action etc.

(3) in some cases a clear effect of retardation of growth of the sarcoma cell culture changed after 3–5 days to intensive growth of the culture. This circumstance leads us to believe that no single, but only the repeated action of the trypanosome preparation may lead to complete cessation of the growth of malignant tissue and to its death.

Particularly clear-cut results regarding the influence of the trypanosome preparation on sarcoma cultures were obtained with batch No. 252. This preparation was one of those well studied experimentally in the treatment of mice with the Crocker sarcoma, mice with spontaneous tumours and finally the rabbit carcinoma.

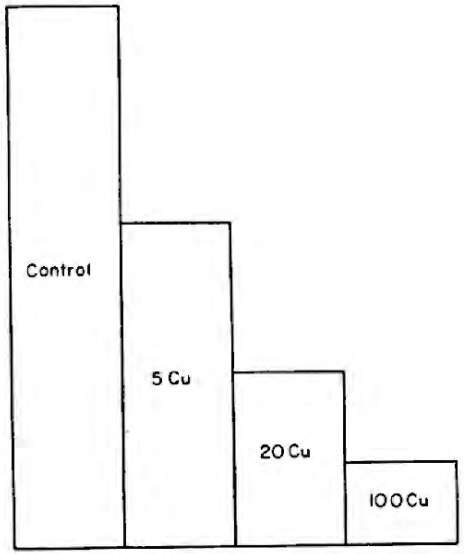


FIG. XXX. Size of the growth zone of the Crocker sarcoma (under tissue culture conditions) after previous treatment of pieces of the tumour with the trypanosome preparation in various concentrations for 24 hours; with higher concentrations of the preparation the growth zone of the sarcoma decreased by more than 6 times in comparison with the control tissue cultures.

Figure XXX gives an idea of the relation between the effects and the concentration of trypanosome preparation batch No. 252. It follows from these experiments that the trypanosome preparation, when acting on sarcoma tissue cultures outside the body, or after preliminary treatment of the piece of tumour intended for cultivation, *produces a clearly expressed retardation of the growth of the malignant tissue*. However, in 24–40 hours the trypanosome preparation does not kill all or even a considerable proportion of the cells involved in the experiment. The experiments described show that a *cancerostatic effect* is the main factor in the action of the trypanosome preparation on cancer tissue.

5. THE SPECIFICITY OF THE ANTIBLASTIC PROPERTIES OF *T. CRUZI*

The logical development of our experiments brings us to the question: what is the specificity of the inhibitory effect of *T. cruzi* infection or of the components of the trypanosome's protoplasm on the cells of malignant tumours—is this phenomenon an exception or are *other members of the Protozoa or certain parts of their cytoplasm* able to show an inhibitory influence on the growth of malignant tumours?

The theoretical, and not only the practical significance of the question of the position occupied by *T. cruzi* as a source of antiblastic substance is self-evident.

This question cannot be answered speculatively one way or the other. On the one hand, it was well known that certain species and even races of microbes may possess quite exclusive biochemical components, and therefore although such a "biological exception" as *T. cruzi* was rather surprising to the investigator, the existence of such exceptional properties is not an insurmountable contradiction to our general knowledge of the microbial cell, its components and its biochemical properties. On the other hand, it was no less well known that certain properties of microbial cells, associated with definite protoplasmic components, may be common to more or less extensive groups, i.e. it may be a species property, or it may apply to a genus or even to larger systematic groups. This is why we must return to the investigation of a whole series of protozoans within our range of interest.

In the early stages of our investigations (1932–1939) we tried to approach the solution of this task, and to this end we arranged a number of experiments which may be recalled here: (1) a series of experiments on the influence of certain protozoan infections on experimental cancer; (2) a series of experiments on the influence of preparations obtained from certain protozoans on experimental cancer.

All these experiments in turn *gave a negative result*, doubly emphasizing the exceptional position of *T. cruzi* in possessing its peculiar inhibitory effect on tumours on infecting animals with experimental cancer and the clearly expressed antiblastic effect in cases where a lysate had been prepared from the trypanosome cells.

Now, to provide a solution to this problem we have carried out a new series of studies on representatives of different groups of the extensive phylum of the Protozoa: (a) Amoebida—*Entamoeba moschkowskii;* (b) Infusoria—*Paramaecium caudatum;* (c) Haemosporidia—*Plasmodium gallinaceum;* (d) Trypanosomidae—*Trypanosoma equiperdum, T. gambiense T. kohl-jakimov, T. lewisi;* (e) *Leishmaniidae—Leishmania tropica,* 2 strains; (f) *T. cruzi*—two new Chilean strains.

The description of these experiments starts with those on amoebae.

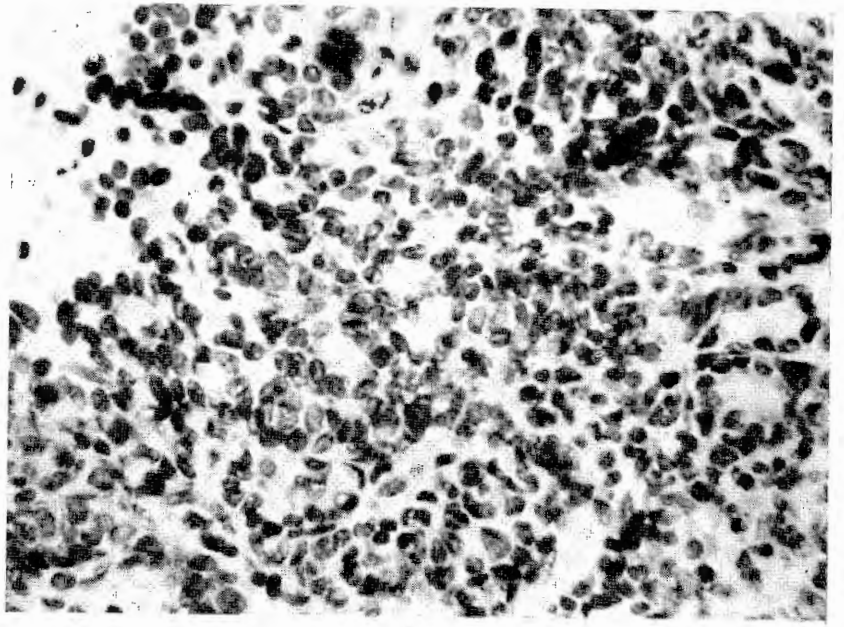
EXPERIMENTS WITH THE AMOEBA *Entamoeba moschkowskii*

Levinson, in our laboratory, set up experiments on the treatment of the Crocker sarcoma with lysates from cultures of the amoeba. To elucidate the question of whether components of the protoplasm of *Entamoeba moschkowskii* have any influence on the development of the Crocker sarcoma, fluid was taken with a Pasteur pipette from the bottom of an active culture (75–125 amoebae per field of vision) and heated in a water bath to 55°C for 20 minutes, after which 1 drop of 1 : 1000 rivanol was added to each millilitre of the fluid.

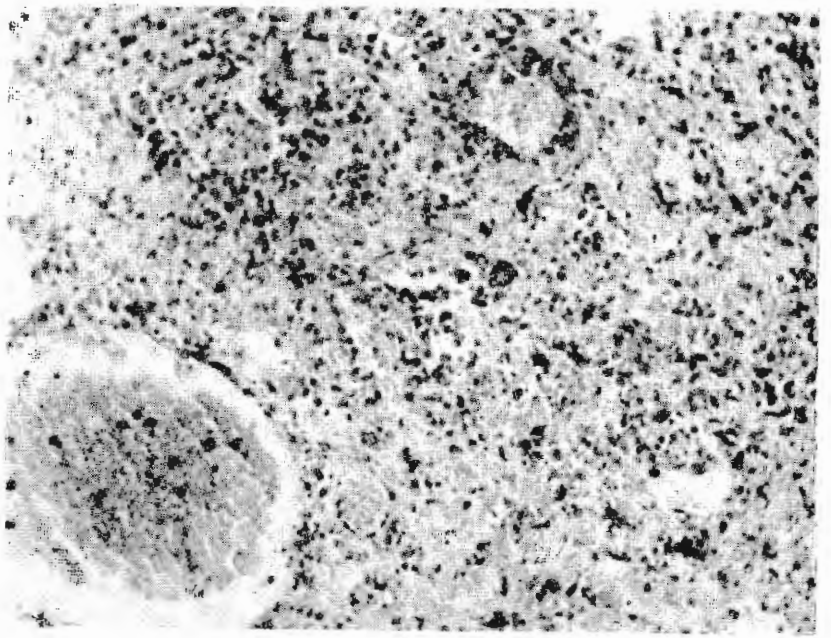
To decide whether there was any effect on the Crocker sarcoma by the metabolic products of the amoebae excreted into the cultural fluid, experiments were also set up with fluid from four-month cultures, only not containing amoebae. In these experiments thirty mice were inoculated with the Crocker sarcoma under the skin of the back; ten of them were given an amoeba lysate subcutaneously (0.5 ml, 10 days in succession), ten received cultural fluid (0.5 ml each), also 10 days in succession, and ten mice were kept as controls. The experiment lasted for 40 days. Two series of experiments were carried out, giving identical results. No differences could be discovered between the appearance and development of the Crocker sarcoma in the control and experimental mice. Hence, neither the protoplasmic components nor the metabolic products of *Entamoeba moschkowskii* have any influence (at least under these experimental conditions) on the development of the Crocker sarcoma in mice (Levinson, 1948).

EXPERIMENTS ON THE EFFECTS OF LYSATES FROM INFUSORIA

It is not particularly difficult to obtain sufficient numbers of paramaecia from cultures and to prepare lysates from them, or to use simply a suspension of the killed infusoria. Using such preparations from paramaecia, we carried out numerous experiments of varying duration (by various techniques) and with varying dosages on the treatment of mouse

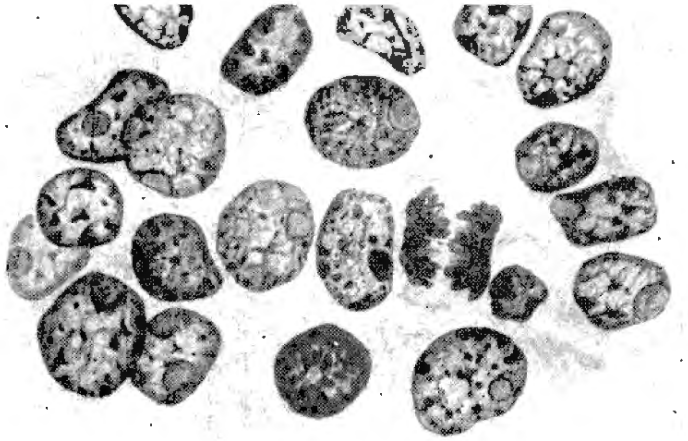


(*a*)

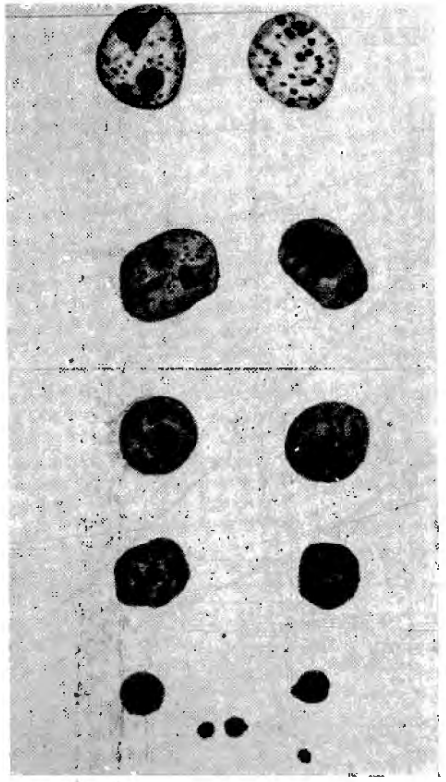


(*b*)

PLATE 126, *a, b*. Spontaneous adenocarcinoma in mouse 5647 before *(a)* and after *(b)* treatment with the trypanosome preparation; *a*—at a magnification of × 400, *b*—at a magnification of × 80.



(*a*)



(*b*)

PLATE 127, *a*, *b*. Structure of the nuclei of spontaneous adenocarcinoma 2986 before treatment (upper drawing) and stages of gradual degeneration of the nuclei under the influence of treatment with the trypanosome preparation (drawings made at a magnification of ×1500).

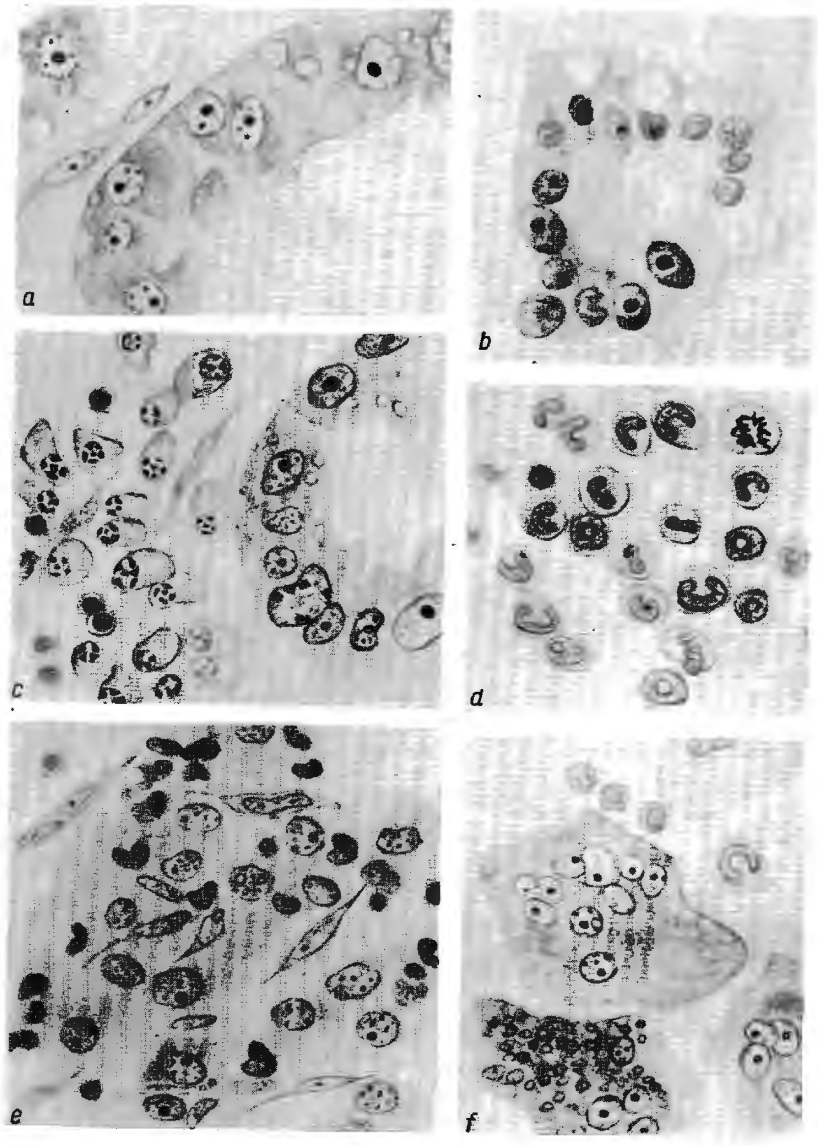
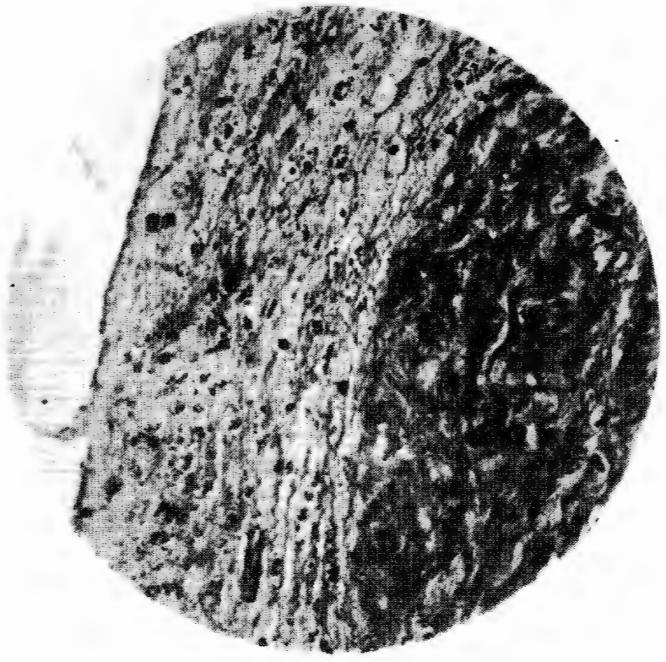


PLATE 127 *c*. Changes in the histological structure of a spontaneous mouse adenocarcinoma under the influence of the trypanosome preparation; *a*—vacuolation of the cancer cell cytoplasm, *b*—formation of multinucleated symplasts, *c*—infiltration of the cancer tissue by lymphocytes, plasma cells and fibroblasts, *d*—an aggregation of histiocytes and leucocytes, *e*—fibroblast proliferation within the degenerating cancer tissue, *f*—the appearance of multinucleated giant cells (Drawing).



(*a*)

(*b*)

PLATE 128, *a, b*. Intensive formation of fibrous connective tissue at the site of the malignant tissue as a result of treatment of a sarcoma.

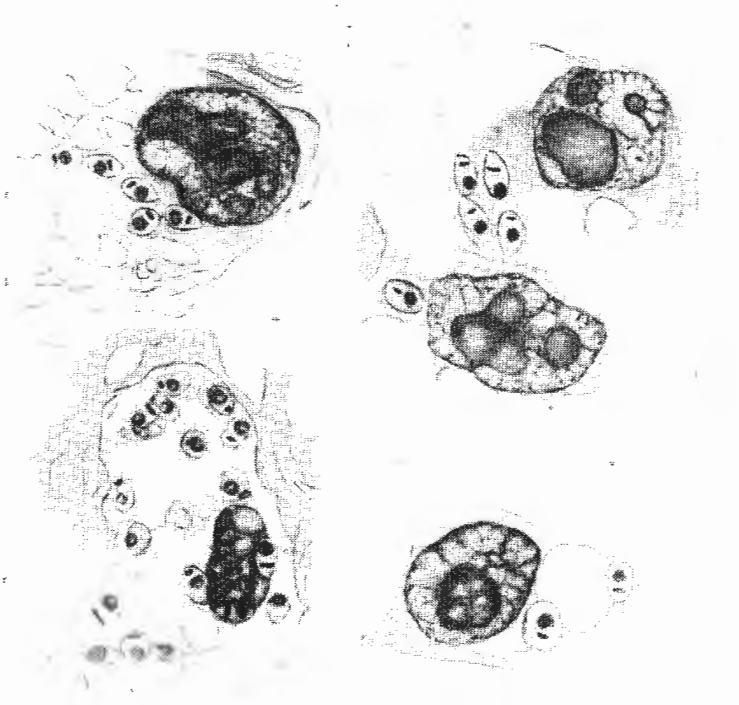


PLATE 129. Cells from the growth zone of a Crocker sarcoma culturable on synthetic nutrient media; signs of degeneration can be seen in sarcoma cells affected by *T. cruzi* (Drawing).

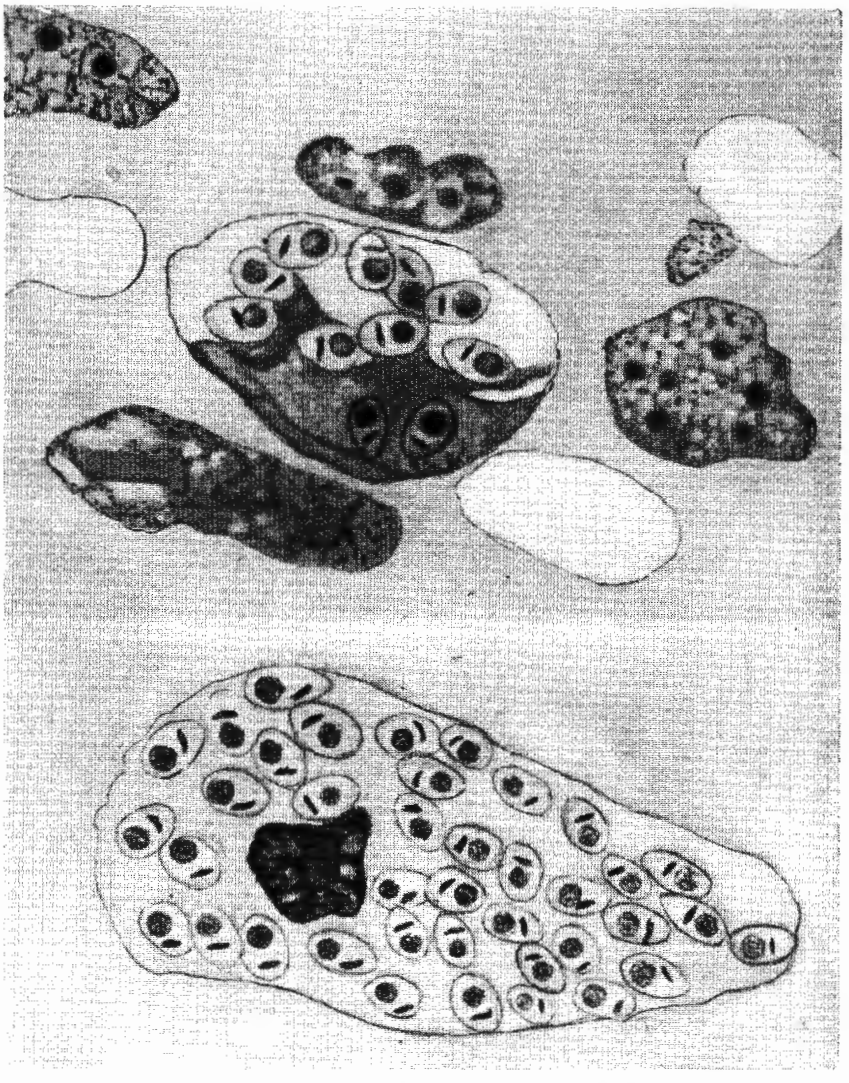
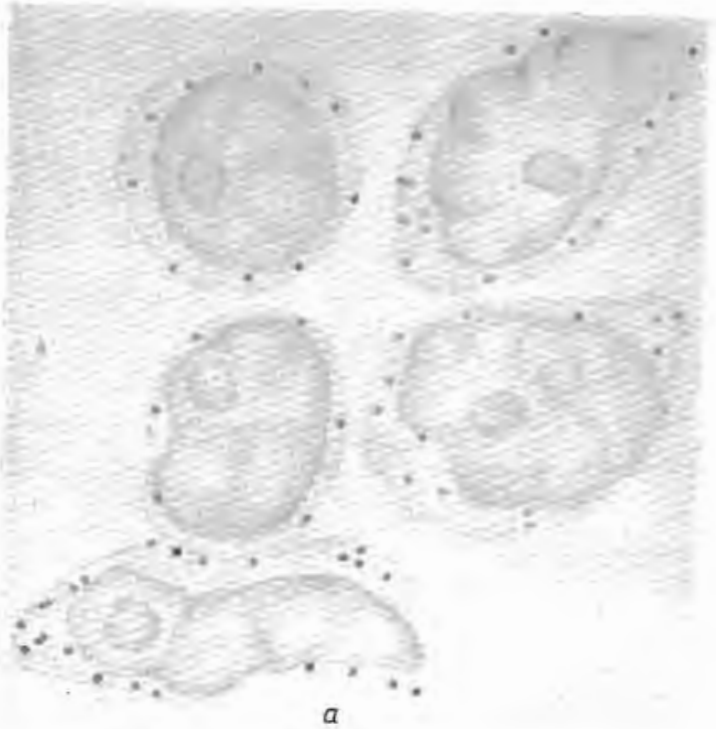
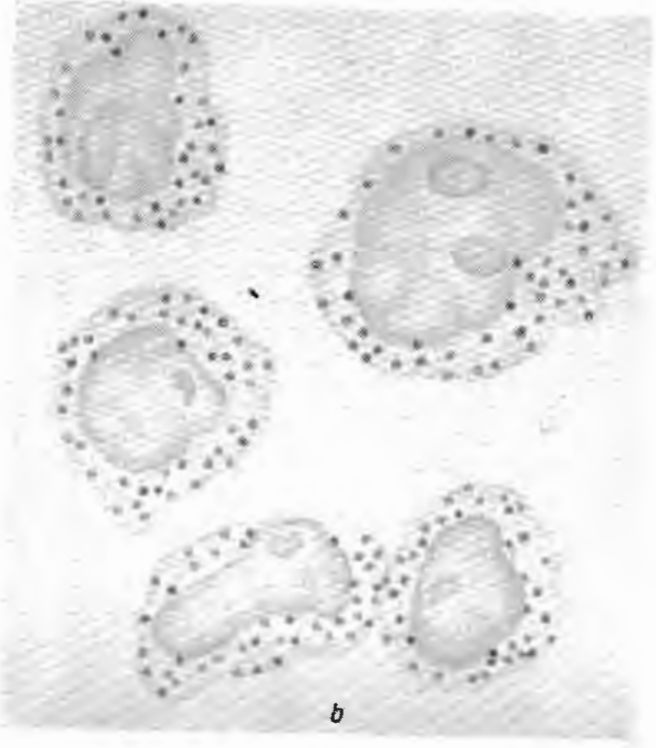


PLATE 130. Lysis of the cytoplasm and degeneration of the nucleus arising in cells of the Crocker sarcoma under the influence of *T. cruzi* (Drawing).



a



b

PLATE 131.

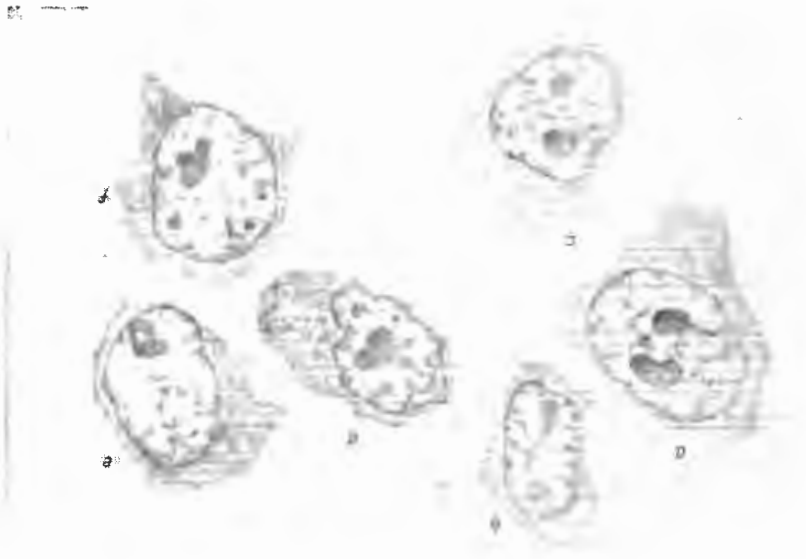


PLATE 132.

sarcomata. All these experiments gave a negative result: we could see no effect at all on the development of the tumours. We were thus bound to accept that in the chosen representatives of the classes Amoebae and Infusoria we were unable to discover any substance of a type inhibiting malignant growth.

THE EFFECTS OF FOWL MALARIA ON THE ROUS SARCOMA

In the course of our search we carried out a series of experiments in collaboration with V. Sidorov on the effects of the fowl malaria organism (*Plasmodium gallinaceum*) on the development of the Rous sarcoma in hens. This is an extremely serious disease of hens, with an enormous degree of red cell destruction, frequently ending in the death of the infected birds.

We carried out experiments on hens with inoculated sarcomata. The experiments showed that infection with *Pl. gallinaceum* has no influence at all on the development of the fowl sarcoma, the tumours growing similarly in both infected and control hens.

It is easy to demonstrate the presence in the large and small vessels of the fowl sarcoma of numerous red cells containing plasmodia. This circumstance, however, has no reflection in the development of the tumours. It must be assumed that the fowl malaria organism does not contain or secrete substances able to suppress the development of the fowl sarcoma.

EXPERIMENTS ON THE INFLUENCE OF TRYPANOSOME INFECTION AND TRYPANOSOME LYSATES

Experiments with trypanosomes are of particular interest, since *T. cruzi* belongs to the family Trypanosomidae. It should first be stated that our early experiments on the effects of a number of trypanosome infections—*T. equiperdum*, *T. gambiense*, *T. lewisi*—on mouse carcinomata gave completely negative results.

In connection with these experiments, it should be remembered that Fenivessi and other investigators have suggested that rats suffering from trypanosomiasis die with clear signs of asphyxiation associated with extreme oxygen insufficiency, since the trypanosomes, in supporting their own life, use all the oxygen needed for the life of the host's tissues and cells. Considering this circumstance, Karczag and his co-workers (1931), having created an oxygen deficiency in the body by means of trypanosome infection, attempted to arrest the development of malignant tumours in white mice. Karczag intended to prevent death of the mice from try-

panosomiasis by the timely use of germanin. However, numerous experiments on the influence of trypanosome infection (*T. equiperdum*) gave no positive results.

Experiments with various trypanosomes were, however, continued. In our laboratory Radionov and Minkovich (1947–1948) studied the effects of *T. equiperdum* lysates on the development of the mouse sarcoma, and with certain doses they were able to note a weak inhibitory effect. Radionov and Minkovich made a more detailed study of the effects of another trypanosome, *T. kohl-jakimow*, and were able not only to establish a certain retarding effect of this infection on tumour growth but also to note that the inhibitory effects of this trypanosomiasis increase as the duration of the infection increases. On injecting sarcoma-bearing mice with lysates prepared by various methods from these trypanosomes, Radionov and Minkovich obtained a definite positive result, expressed as a decrease in the average tumour weight in the experimental animals by $1\frac{1}{2}$–2 times in comparison with the control mice not subjected to the effects of the trypanosome lysates.

EXPERIMENTS WITH LEISHMANIIDAE

A detailed and careful investigation has been made of members of the sub-family Leishmaniidae, i.e. of species closely related to *T. cruzi*. The first experiments concerned *Leishmania tropica*. The choice of this species was dictated both by the known systematic resemblance of these protozoans to *T. cruzi* and, more important, by their physiological similarities, proved so convincingly by M. Lwoff (1940) and A. Lwoff (1944), and also by their clearly expressed organotropism and cytotropism—in short, by a number of properties particularly associating the leishmania with *T. cruzi*. For his study of the effects of leishmania on malignant tumours, L. Levinson used two strains. One strain was isolated from a patient with the ulcerative form of skin leishmaniosis, the other from a patient with the "dry" form.

To study the effects of lysates obtained from leishmania 45 mice were inoculated with the Crocker sarcoma. The lysates were preparad from cultivated forms of the leishmania. They were injected subcutaneously along the back of the mice in doses of 0·5 ml on 5 successive days. The experiments showed that the development of the Crocker sarcoma in mice treated with lysates of the "ulcerative" strain of *Leishmania tropica* lagged behind that in the controls, the differences being:

(a) the first appearance of a tumour in treated mice was 8 days later than in control mice;

(b) the developing tumours were much smaller in treated mice;

(c) death occurred in the treated sarcoma-bearing mice 6–8 days later than in control mice.

Consequently, it may be suggested with a fair degree of probability that the cells of *L. tropica* contain a certain amount of an inhibitory substance retarding the development of the Crocker sarcoma. Similar experiments on the Ehrlich adenocarcinoma gave less clear-cut results.

THE COMPARATIVE EFFECTS OF TWO DIFFERENT STRAINS OF *T. Cruzi* ON THE CROCKER SARCOMA

We were able to obtain two strains of *T. cruzi* from Chile. These strains were given the code numbers "22" and "28". Our first task was to demonstrate the course of infections with these strains in mice. Levinson established that strains 22 and 28 produced typically a *chronic* infection, which distinguished them sharply from the original strain used in our experiments, as this caused death in the infected mice in 9–12 days, with masses of trypanosomes in the blood. Mice infected with the Chilean strains live for 2–3 months or more; trypanosomes appear in the blood for 7–10 days and the infection usually then progresses with a very small number of parasites in the blood (no more than 3–4 per field of vision), and moreover there are remissions—after a flare-up of the infection there is usually a decline, when parasites are not found at all in the peripheral blood for some time. The experimental infection produced by the Chilean strains is therefore typified by: (a) chronic course; (b) the presence of remissions; (c) a low number of parasites in the peripheral circulation; (d) dissimillar course of the disease in different mice.

Experiments were carried out to determine the inhibitory properties of strains 22 and 28 on the Crocker sarcoma. In view of the uniform results obtained, we shall describe only one of them, involving 64 mice inoculated with the Crocker sarcoma. On the following day 23 mice were infected with *T. cruzi* strain 22, and 21 mice with strain 28; 20 mice were left as controls. The experiment lasted for 22 days. As a result, the average tumour weight in mice infected with strain 22 was 2·8 g, in those infected with strain 28 it was 4·08 g and in the control 4·36 g. Levinson's results correspond fully with those obtained by Fradkina, Kats and Skorikova (page 151).

Thus, we see that, while being variable, antiblastic properties are found in various strains of *T. cruzi* and do not appertain to any one particular strain.

The following conclusions may be drawn from the experiments described above:

(1). Arbitrarily chosen protozoan infections have *no noticeable influence* on the development of experimental tumours.

(2). By no means all lysates from arbitrarily chosen free-living or pathogenic protozoans have any noticeable effect on the Crocker sarcoma.

(3). The inhibitory effects of protozoan infections have so far only been established in certain members of the Protozoa; all these protozoans are connected by a close systematic and, in all probability, genetical relationship: they are all included in the family Trypanosomidae.

(4). In cases where experiments show an inhibitory effect on cancer by a given protozoan infection, *a substance may be extracted from the relevant causal agent which has an inhibitory effect on experimental malignant tumours.*

(5). In the light of modern knowledge of the nature of antibiotics obtained from microbes of various systematic categories, it is permissible to assume that antiblastic properties may be a species or type-characteristic of certain micro-organisms. However, within the limits of a single species separate races (strains) may differ in their content of, or ability to produce, an inhibitory anticancer substance, as has been shown by the investigations described in this chapter.

To conclude this chapter, let us consider the position of the trypanosome preparation among other anticancer substances used experimentally and, to some extent, clinically. According to Lettré (1954), these substances may be divided into the following groups:

(1). Cytostatic substances acting on the resting nucleus.

(2). Mitotic poisons acting on various phases of nuclear division.

(3). Toxic substances inhibiting (a) cytoplasmic enzymes, (b) mitochondrial activity.

(4). Antimetabolites. Antimetabolites may involve any of the main components of a cell: nucleus, nucleolus, mitochondria, microsomes, and cytoplasmic enzyme systems.

(5). Substances altering the metabolism of cancer cells.

When we consider all that we know of the properties of the trypanosome preparation, we must relate it to those substances which alter the metabolism of malignant cells. We cannot, however, exclude the idea that it may also be an antimetabolite. In any event, its cancerostatic effect is the result of changes in cell metabolism.

6. A COMPARISON OF THE HISTOLOGICAL AND CYTOLOGICAL CHANGES ARISING IN MALIGNANT TISSUES UNDER THE INFLUENCE OF SYNOESTROL, COLCHICINE, *B. PRODIGIOSUS* POLYSACCHARIDE, SARCOMYCIN AND THE CANCER ANTIBIOTIC FROM *T. CRUZI.*

Are we right in speaking of a definite cyto-histological symptom-complex characterizing the effects of the trypanosome preparation? It may be that we are describing in general symptoms of cell death quite analogous to those seen in dying cells, including cancer cells, which apparently combine their *biological aggressiveness with a relatively short life-span* and a clearly expressed *fragility*, i.e. an *increased sensitivity* to a number of physical and chemical factors. Are we not describing things already long known by pathologists, the so-called fields of spontaneous necrosis, so ordinary that pathologists no longer meditate on their significance? There is no doubt that these fields of spontaneous necrosis may be of very varied origins: (1) as a result of the normal death of short-lived cancer cells; (2) as a result of the effects of the body's natural defensive powers, which may be suppressed or reinforced according to the stage of development of the malignant disease; (3) as a result of local breakdown of the tumour's nutrition or respiration because of the state of its vascular system, etc.

According to the nature of the active factor, the initial changes occurring in the cells may have a *specific character*, but subsequent and particularly the later stages of degeneration as a rule have no specific differences arising from the character of the primary damage.

The specific picture of the later stages may acquire one or another character, depending on the *particular* conditions under which these stages in the cellular changes take place. Of course, the process of production of a dead cell is *less characteristic* and is subject to a *more uniform* pattern than is the primary process. However, the eventual fate of a dead cell may *again vary*, according to the behaviour of the other surrounding tissues, and hence a specific histological picture must be sought in *three stages* of the four mentioned.

We have already pointed out that the changes occurring under the influence of the trypanosome preparation differ both *qualitatively and quantitatively* from necroses of tumour tissue arising spontaneously. In this respect we were particularly interested in a comparison of the changes we have described with the changes taking place in tumour cells under the influence of certain other chemotherapeutic substances and preparations of microbial origin used in experimental oncology.

As examples we shall consider the histological and cytological changes produced by the action of four substances of varying chemical natures used in experimental oncology: the hormone *stilboestrol*, the alkaloid *colchicine*, a bacterial preparation—the polysaccharide from *B. prodigiosus*, and finally a cancer antibiotic—sarcomycin.

CHANGES IN RIBONUCLEIC ACID, NUCLEOTIDES, AND ALSO CYTOCHROME OXIDASE AND SUCCINIC DEHYDROGENASE UNDER THE INFLUENCE OF THE EXTRACT FROM *Schizotrypanum cruzi* *

The following points should be emphasized in the characteristic appearance of malignant cells regressing under the influence of the antibiotic cruzin:

(a) there is a consistent diminution in nuclear size, which is definite evidence of changes in the rate and probably also the character of the metabolism of the malignant cells;

(b) there is a consistent diminution in nucleolar size, i.e. a normalization of the nucleus-nucleolus relationship occurs, leading to morphophysiological changes in the malignant cells;

(c) there is a significant reduction in the ribonucleic acid content of the cells of a treated tumour, and this means the exclusion of one of the essential factors in protein synthesis, which is vital for the rapid growth and multiplication of malignant cells;

(d) there is a fall in the number of mitoses in the malignant cells, together with the changes in nuclear size.

TABLE 3. BIOCHEMICAL OBSERVATIONS SHOW (ACCORDING TO CYTOCHEMICAL FINDINGS) THAT THERE IS A DECREASE IN RIBONUCLEIC ACID IN THE MOUSE SARCOMA UNDER THE INFLUENCE OF CRUZIN

Activity index of antibiotic from *Schizotrypanum* (in arbitary units)	Ribonucleic acid content as % dry weight of sarcoma tissue	
	Before treatment	After treatment
1.5	2.11	1.76
2.1	1.81	1.29
2.12	2.85	2.20
inactive cruzin	2.61	2.61
inactivated cruzin	3.15	3.0

* This section is taken from the paper "Das Problem der Cancerogenese im Lichte von Untersuchungen experimenteller Biotherapie bösartiger Geschwülste", read by Professor Roskin at the Berlin Symposium on Problems of Carcinogenesis, December 11–16, 1959 and published in *Abh. deutsch. Akad. Wiss., Kl. f. Med.* Jahrg. 1960, Nr. 3.

These markedly changed malignant cells and their nuclei, which approach the normal nucleus in form, are capable neither of intensive protein synthesis nor of intensive division, since they have lost a considerable proportion of their ribonucleic acid. Simultaneously with these phenomena there occurs a change in respiratory metabolism: anaerobic respiration is replaced by aerobic.

Parallel experiments on the effects of the antibiotic from *Schizotrypanum* on normal tissue show that in this case no noticeable variation in respiration takes place.

Our own cytophysiological observations (Roskin, Koshukhova, Balicheva) also indicate fundamental cytophysiological changes in malignant cells. The following changes occur in the cells of the Crocker sarcoma under the influence of cruzin injections:

(1) A rise in cytochrome oxidase activity (Plate 131).

(2) Increased nucleotide content (Plate 132).

This latter circumstance is of particular interest, since a low nucleotide content appears to be characteristic of malignant tissue. These findings are supported by results obtained in our laboratory by Trufanov and Palkina, who studied the effect of cruzin on succinic dehydrogenase and cytochrome oxidase in homogenates of the Crocker sarcoma.

It follows from these experiments that in every case cruzin inhibits the activity of succinic dehydrogenase and stimulates that of cytochrome oxidase, and that the inhibition of succinic dehydrogenase and the stimulation of cytochrome oxidase is proportional to the concentration of cruzin given. Control experiments show that cruzin inhibits the activity of succinic dehydrogenase and has no effect on the activity of cytochrome oxidase in homogenates of normal (mouse) tissue, while the inhibition of succinic dehydrogenase is somewhat weaker than the inhibition of this enzyme in an homogenate of the Crocker sarcoma.

The malignant cells markedly changed under the influence of cruzin approach the normal cell in their morphological, cytochemical and physiological properties and enter into a new relationship with the body's defence mechanism. All this indicates that cancer cells can only remain intact at a specific morphophysiological level. Thus aggressive malignant cells, capable of rapid development and multiplication and of infiltrating into the surrounding tissues, under the influence of cruzin themselves become the object of aggression on the part of defensive cells and of the humoral factors of the body. The importance of humoral systems becomes clear from unsuccessful experiments on the treatment of cancer-affected animals when the reticulo-endothelial system is blocked.

TABLE 4. THE EFFECT OF CRUZIN ON THE ACTIVITY OF SUCCINIC DEHYDROGENASE AND CYTOCHROME OXIDASE IN HOMOGENATES OF THE CROCKER MOUSE SARCOMA

Preparation No.	Dose in arbitrary units (CU)	Enzyme activity, expressed in ml CO_2			
		Succinic dehydrogenase		Cytochrome oxidase	
		Control	Experiment	Control	Experiment
1	2	3	4	5	6
132	1	7.85	6.44	—	
	10	7.58	2.06	—	
181	1	8.73	7.46	—	
	5	8.73	6.23	10.42	11.14
	10	8.73	4.78	10.42	12.00
	15	6.22	1.45	8.43	14.74
184	10	12.11	3.99	3.85	5.47
186	5	4.67	2.00	—	—
194	10	7.33	3.56	—	—
195	10	7.33	4.41	—	—
200	10	10.91	5.85	13.67	15.49
204	5	12.34	5.44	—	—
	15	8.74	0.85	9.19	10.59
206	10	3.74	0.42	8.27	13.00
207	10	15.96	4.90	3.63	6.00

Our cytological, experimental and clinical observations on the effects of the antibiotic cruzin have the following significance in the theory of carcinogenesis:

(1) they are evidence against the accepted interpretation of the theory of the autonomy of cancer cells;

(2) they conflict with the hypothesis that the cancer cell is the result of a mutation.

These well-established facts show that by employing the factors contained in microbial extracts such as cruzin or similar substances *one can achieve controlled variations in cancer cells*, since one eliminates the principal characteristics of the cells of malignant neoplasms. These facts cannot be overlooked by exponents of the theory of the viral origin of cancer.

HISTOLOGICAL CHANGES IN CARCINOMATA OF THE PROSTATE UNDER THE INFLUENCE OF STILBOESTROL (SYNOESTROL)

The female sex hormone is able to inhibit both the secretions of the prostate cells and senile hyperplasia of these cells. The use of stilboestrol in malignant neoplasia of the human prostate is based on the fact that

the cells of these tumours retain, in some cases and to various degrees, the ability to be governed by the same regulating mechanism that controls the activities of normal prostate cells.

Professor Rapoport, who has studied in a large amount of material the histology of carcinoma of the prostate gland treated with synoestrol, gives this description of the changes noted in them. "The processes developing in carcinoma of the prostate on treatment with synoestrol are characteristically very complex. One of these processes is atrophy. In this the cancer cells decrease in volume, right down to their complete disappearance. The atrophic processes create an extreme polymorphism of the cancer cells and a wide variation in their measurements. Sometimes they lead to cavitation of extensive areas of the tumour tissue, leaving only a reticular stroma. It should be stressed that in such cases there is no formation of fibrous tissne as takes place during atrophy of parenchymatous organs. The next group of processes developing in carcinomatous prostate tissue on treatment with synoestrol comprises various forms of degeneration, most frequently fatty and particularly hydropic degeneration of the cancer tissues. These processes lead to the rarefaction of wide areas of tumour tissue, its emulsification and absorption. A process of keratinization of the cancer cells is also characteristic of the regression of carcinoma of the prostate under the influence of synoestrol treatment. It involves the conversion of large masses of carcinomatous epithelial cells into keratinized squames with pyknotic nuclei. This process may be termed squamous degeneration. In cases where it involves a considerable part of the cancer nodule or the whole nodule the cellular squames are layered one on another to form compact masses resembling the pearls of a squamous-cell carcinoma. Squamous degeneration is organ-specific to cancer of the prostate. It should be stressed that a picture of necrosis and breakdown of the tumour tissue has never been observed during regression of cancer of the prostate under the influence of synoestrol treatment" (Rapoport, 1948).

Waltgard (1948) describes the changes in the cells of prostatic tumours somewhat differently: under the influence of stilboestrol there is diminution and rounding of tumour cells in the prostate; the cells may become loosened and undergo vacuolar degeneration; groups of squamous epithelial cells are frequently encountered. A comparison of the histological changes seen during the action of stilboestrol with what we know of the cytological and histological moves occurring under the influence of the trypanosome preparation shows that we are dealing here with two distinct processes.

A COMPARISON OF THE EFFECTS OF COLCHICINE AND THE TRYPANOSOME PREPARATION ON EXPERIMENTAL TUMOURS OF ANIMALS AND ON MALIGNANT TUMOURS IN THE HUMAN PATIENT

Sentein, in his book *The Effects of Toxins on Cells During Their Division* (1941) characterizes the effects of colchicine thus:

(1). Colchicine acts directly on the cells, i.e. the role of the body is of no account in this process.

(2). Colchicine arrests nuclear division at the metaphase stage, as a result of suppression of the process of spindle formation, or suppresses all stages of division, if the dose is sufficiently high.

(3). The action of colchicine is more effective, the greater is the number of mitoses occurring in the tissue; this is the source of colchicine's great influence on embryonic and rapidly developing tissues.

The extreme difficulty encountered in the treatment of tumours with colchicine is a result of the insufficient cytological specificity of its action and the general toxicity (particularly for the central nervous system) of the doses required to produce regression of malignant tumours.

More recently, Ludford (1945) has summarized many years of observation on the effects of colchicine on malignant tissues:

(1). Colchicine arrests cell division, *inhibiting the formation of the nuclear spindle*. It is impossible to inhibit mitosis in malignant cells without simultaneously affecting other dividing cells in the body.

(2). Colchicine causes damage to the vessels in rapidly growing tumours, since the endothelial cells of the newly forming capillaries are particularly sensitive to this alkaloid.

(3). Published results show that after treating laboratory animals and humans with colchicine a period of tumour regression is followed by renewed growth.

(4). Complete regression of some tumours of man and animals has been achieved by using doses of the alkaloid approaching the lethal level. Tumour regression is primarily the result of vascular damage.

(5). Newly discovered, less toxic derivatives of colchicine have no advantages, since proportionally greater doses are needed to achieve similar clinical results.

Hence, in distinction from the trypanosome preparation, *which causes no pathological changes in normal tissues, even on prolonged administration*, doses of colchicine active against cancer are *highly toxic;* in distinction from the trypanosome preparation colchicine, as a rule, does not lead even experimentally to complete recovery of the cancer-affected body.

The histological picture of the changes induced by colchicine is completely different from the changes produced in malignant tissue by the trypanosome preparation: with colchicine the predominant phenomenon is that of "*karyoclastic shock*", destruction of the nuclear spindle, the appearance of chromosomal aberrations and similar signs. With active, "anticancer" doses haemorrhages arise in the tumour, and it is quite possible that partial regressions of malignant neoplasms occur as a result of disturbed nutrition of the cancer cells. The whole dynamics of the picture seen during the effects of the trypanosome preparation are different from those seen with colchicine: with colchicine there is an immediate spate of mitoses, followed by rapidly progressive signs of nuclear and particularly chromosomal changes; with the trypanosome preparation all the changes in the nucleus occur as the result of prolonged and gradually developing changes in the cancer cells, beginning with cytoplasmic changes.

A COMPARISON OF THE EFFECTS OF THE TRYPANOSOME PREPARATION AND POLYSACCHARIDE FROM *B. prodigiosus* ON MALIGNANT TISSUE

A large group of American workers has carried out extensive studies on the effects of a polysaccharide from *B. prodigiosus* both on experimental tumours and on malignant tumours in human patients.

Shear and a number of other investigators have shown that under the influence of the polysaccharide from *B. prodigiosus* extensive haemorrhages arise in tumours, there is considerable damage to the tumour capillaries and, as a result of this, disturbances in nutrition and particularly anoxia of the malignant cells lead to destruction of appreciable areas of tumour tissue.

Degenerative changes in tumour tissues of man and laboratory animals induced by the polysaccharide from *B. prodigiosus* were the subject of a detailed investigation by Diller (1947). Her findings showed that the polysaccharide causes direct damage to the cell nuclei of the transplantable mouse sarcoma, as well as the disturbances arising as a result of capillary destruction.

Maximum disturbances are seen within 6 hours, when only cells in the resting stage remain unaffected. *Nuclei in prophase were affected first:* vesicles appeared on their surface, sometimes small and sometimes larger, and in exceptional cases several of them appeared. Next, the nuclei became swollen and pseudopodial projections appeared from time to time. *In most cases, resting nuclei showed no morphological changes.*

Dividing nuclei in anaphase apparently completed division successfully. Consequently, the polysaccharide *does not arrest division by acting on the spindle*, as does colchicine. In many investigations only resting nuclei remained normal 3 hours after the injection. By this time effects on the nuclei had become so considerable that they were undoubtedly irreversible.

After 4 to 5 hours, when considerable haemorrhages began in the tumours, degeneration of the cells first affected had reached its limit: the cell boundaries disappeared and nuclear remnants could be seen among an amorphous mass of degenerate cytoplasm.

The extent of the destruction varied greatly, but in some tumours breakdown took place so intensively that there was almost complete necrosis within 6 hours; nothing was left but amorphous cytoplasmic remnants and nuclear fragments. By this time, the haemorrhagic effect had reached a maximum. At the same time the mice showed a fall in temperature, they did not eat or drink and were in a depressed, immobile state. Some of them died during the next 4 hours, but mice given a dose of 0.01 mg of the polysaccharide usually recovered in 24 hours. Diller first noted newly dividing cells 72 hours after the injection. Some of the divisions were apparently normal, but many were abortive. Division frequently stopped at metaphase and led to pyknosis, but most of the cells degenerated in the telophase stage. Signs of degeneration gradually disappeared, and by 5–6 days after injections of the polysacaccharide the tumours were again growing rapidly. Thus, *the inhibitory influence of this bacterial substance on the processes of division in mouse sarcomata lasted for no more than 3 days.*

All the observations here apply to relatively small tumours. Obviously, older tumours, which contained rather more resting cells, resisted treatment with the polysaccharide and were destroyed only partly, i.e. less intensively than young tumours.

Diller established that in about 25 per cent of all the tumours studied division had not recommenced on the third day after treatment with the polysaccharide, while mitoses were seen in profusion in the other tumours. Obviously, in order to suppress each new wave of mitoses the injections would best be repeated at 3-day intervals. Such an experiment was in fact carried out, using the same doses of the polysaccharide, *but to Diller's disappointment this caused no appreciable cell destruction and brought no real harm to the tumours. "This indicates the acquisition by the tumour or the host of a resistance to the bacterial agent."*

Diller observed similar phenomena in spontaneous tumours of mice and rats after injection of the polysaccharide from *B. prodigiosus*—haemorrhages and karyolytic changes, but these changes were apparently less marked than in the transplantable sarcoma.

Later in her work Diller describes observations on the administration of the polysaccharide from *B. prodigiosus* in 16 cases of sarcoma in human patients. Here too there were considerable haemorrhagic and karyolytic changes, apparently similar to those described for the mouse sarcoma. However, Diller's descriptions are very brief. In preparations from human sarcomata obtained by biopsy the areas of degenerating cells were usually situated in the haemorrhagic regions. Diller states that *in spontaneous tumours, including those of man, the effect was weaker than in transplantable sarcomata of mice.*

It follows from Diller's descriptions that the injection of the polysaccharide from *B. prodigiosus also produces a reaction in the normal tissues of mice.* Although the tumour cells were more sensitive, a reaction *was also seen in other tissues* where there was cell division or rapid synthesis of nucleoproteins. Most damage was found in developing blood vessels of the bone marrow and in the liver. Diller makes particular note of the absence of any sort of reaction by the sex cells to the injection of the polysaccharide, even in extremely high doses. Much more of the polysaccharide was needed for the destruction of normal tissues than for the corresponding degree of degeneration of tumour cells. If we summarize all our earlier knowledge of the effects of the polysaccharide from *B. prodigiosus*, and all that we have learned from the new work by Diller, we are bound to accept that the cytological and histological changes observed after the injection of this polysaccharide and of the trypanosome preparation are *completely different.*

Finally, we must mention the effects of the anti-cancer antibiotic sarcomycin. According to Oboshi and his co-workers (1955), sarcomycin is neither a substance affecting the nucleus during mitosis, nor an antimetabolite, but a specific destroyer of cancer cells both during stages of division and in interphase.

Now, after a comparative review of the histological and cytological changes caused by colchicine, synoestrol, sarcomycin and the polysaccharide from *B. prodigiosus*, we can assert that in malignant tumours under the influence of the trypanosome preparation there occurs a process of regressive development, subject to specific principles, and reflected in peculiar and distinct cyto-histological changes quite different from those seen after the application of the indicated chemotherapeutic and biotherapeutic agents.

7. CONCLUSIONS

The conclusions arising from the clinical observations have been dealt with earlier. Here, we intend to discuss the main conclusions to be derived both from experiments on animals and from an analysis of the mode of action of the trypanosome preparation on the cells of malignant tumours of man and laboratory animals.

(1). To evaluate the changes taking place in treated tumours under the influence of the anticancer antibiotic from *T. cruzi*, it must be borne in mind that the conversion of a normal cell to a malignant cell is associated with profound biological changes in a number of its properties, some of which are relatively easily demonstrated. It has been proved that malignization is reflected in a number of changes:

(a) in increased nuclear size and variability;

(b) in nucleolar hypertrophy and disturbances of the nucleus–nucleolus ratio;

(c) in a sharp rise in the number of mitoses;

(d) in a marked increase in the quantity of ribonucleic acid;

(e) in the appearance of the property of aggressive growth;

(f) in changes in the character of respiratory metabolism.

(2). Under the influence of the trypanosome preparation a complex process arises in malignant tissue, comprising a whole series of factors which in combination not only arrest the growth of the malignant tissue but also, in a considerable number of cases, lead to regression of human and animal tumours.

(3). In order to establish the principles of the diminution or disappearance of tumours under the influence of the trypanosome preparation, this single and complex process—so far as it is expressed in cytological and histological changes—may be broken down into separate characteristic stages:

(a) the stage of regressive development of the malignant cells;

(b) the stage of changes in the stroma and changes in the local and general defensive reactions;

(c) the stage of the monocyte–macrophage reaction;

(d) the stage of the cancerolytic reaction.

The first two stages are preceded by the third, and the fourth completes the whole process arising under the influence of the trypanosome preparation.

(4). Depending on the activity of the preparation, the dose and the duration of its administration, and also on the stage of development of the tumour and the state of the body, the effects of the trypanosome preparation may lead only to the first stage in the changes in the tumour tissue, or they may also induce later stages in the tumour changes, right through to a clearly expressed cancerolytic reaction.

(5). Among the most characteristic signs of the process of regression of malignant tumours under the influence of the trypanosome preparation, the following may be noted:

(a) *A consistent decrease in nuclear size, undoubtedly indicating changes in the level and possibly also the character of the metabolism of the tumour cells.*

(b) *A consistent decrease in nucleolar size*, i.e. normalization of the nucleus–nucleolus ratio—the result of profound morphophysiological changes in the malignant cell.

(c) There is *a reduction in the amount of ribonucleic acid* in the cancer cells of treated tumours, showing very graphically that there are disturbances in the intensive synthesis of the proteins essential for cell growth and multiplication. *An important factor for the rapid growth and multiplication of malignant growth is thus excluded.*

(d) Along with diminution of the nuclei there is a *fall in the number of mitoses in the tumour cells.* This shows *not only that cell metabolism is affected but also that the cells lose their capability of rapid multiplication.*

(6). Malignant cells altered so much by the influence of the trypanosome preparation, with their nuclei and nucleoli reduced in size, their ribonucleic acid lost, incapable either of intensive protein synthesis or of rapid division, and with their respiratory metabolism modified, enter a fresh relationship with the body's defensive forces, and, from aggressive cells capable of rapid growth and multiplication and of infiltrating into and destroying the surrounding normal tissue, *they themselves become the subject of aggression on the part of defensive cells and of humoral factors in the body.*

(7). This moment is associated with the most distinct expression of the second stage. It must be noted that the first two stages may take place *before clinically apparent changes appear.*

(8). The second stage merges imperceptibly into the onset of the third stage.

The appearance of large numbers of monocytes, histiocytes and macrophages in the tumour tissue coincides with the development of the cancerolytic reaction. As the malignant cells become dissolved and destroyed, their places are occupied by fibroblastic cells in a state of enhanced activity, organizing connective tissue at the site of the dead cells. Thus, the

basic process of recovery of human beings or experimental animals affected by malignant tumours takes place *by a combination of the effects of the trypanosome preparation and the natural defensive forces of the body.*

(9). The onset of such a process is based on the injection into the patient's body of *factors* capable of influencing important vital functions of the cancer cell and thus changing the conditions of its development; moreover, it changes their *direction* in such a way that these changes cause the cell to *lose its malignancy*, leading to the onset of a cancerolytic reaction in the body, involving a number of factors which we have already described.

Antiblastic factors are present in a number of species of microbial cells or in the products of their metabolism. The mechanism of their destructive effects varies according to their nature.

The picture of the tumour changes occurring under the influence of these factors may be just as varied and mosaic as the structure of the microbial cells from which the factors derive. The whole thing depends on which of the functions of the cancer cell is not resistant to the active factor.

(10). This complicated process brought about by the trypanosome preparation has its own clearly expressed *specific peculiarities*, different from those relating to certain other substances used in experimental tumour therapy, for example the alkaloid colchicine, the hormone synoestrol, sarcomycin and lastly the bacterial polysaccharide from *B. prodigiosus.*

(11). Numerous observations on the effects of the anticancer antibiotics from *T. cruzi* and on those of a number of other antiblastic factors show that cancer cells, as well as their main property—*aggressiveness*—have a selective lability, *fragility*. The phenomena arising under the influence of the trypanosome preparation are only a *particular case of the expression of this selective fragility of malignant cells.*

(12). The cytological, histological, experimental and clinical findings obtained are of significance in the general theory of cancer:

(a) they form evidence *against too broad an interpretation of studies of the autonomy of malignant tumours and cancer cells;*

(b) they form evidence *against* the hypothesis that *the cancer cell is the result of mutation;*

The facts established enrich the armoury of the oncologist-therapist, since they show that by using factors of microbial origin *cancer cells may be controllably modified* so as to remove their main characteristic properties.

Part VII

AGGRESSIVENESS AND FRAGILITY IN CANCER CELLS

To conclude, we must consider a question arising immediately from our observations and from numerous published findings on the various effects of different products of microbial cells on malignant tumours. We refer here to the problem of reconciling the basic properties of cancer cells—*aggressiveness and autonimity*—with the results of experimental and clinical biotherapy. The logic of our whole investigation has dictated this question, and it would be impossible to avoid it.

There are various interpretations placed upon the concept of the *autonomy* of malignant tumours. Some investigators, such as Petrov (1947), justifiably interpret the idea of autonomy in a very limited way, asserting that autonomy comprises only the particular morphological and physiological principles regulating the life and development of tumours. Other oncologists maintain that there is *complete independence of malignant tissue* both of the life of the whole body and of endogenous and exogenous physiological influences, whatever their origin. As an example of this we would refer to M. F. Glazunov, a typical representative of this group. According to Glazunov (1947): "The unreactive character of a tumour's growth must be considered the most important sign of a true tumour. It is specific to true tumours that they grow by self-expansion, taking this idea not only in the morphogenetic, generally accepted, sense but also in the sense that *the growth-stimulus arises and is maintained in the tumour cells themselves.* Even if in a number of cases some sort of abnormal deviation is needed for a tumour to arise, once arisen *the tumour requires no further outside activators for its continued growth and existence.* How and by what unlimited tumour growth is maintained is unknown, and, in any case, from our aspect *this is not important.*"

If this position is considered from the philosophical point of view, the given definition has a clearly expressed *autogenetic nature:* the growth stimulus is not only maintained but also arises in the cancer cells them-

selves. If our knowledge of the basic properties of tumours is considered from the point of view of our aims in the fight against them, the theory of the autonimity of malignant tumour frustrates the doctor and dooms in advance any attempt to affect malignant growth by means of biological factors. This is why many oncologists and pathologists consider that only the surgeon's knife and radioactive rays are able to destroy malignant cells—we repeat destroy, but not change them. This is why it is asserted that substances influencing cell metabolism or modifying it are incapable of altering malignant cells, arresting their growth and multiplication, and removing their aggressiveness; this position is even more incompatible with the regressive development of tumours under the influence of factors of microbial origin. However, the facts accumulated by science in recent years have necessitated radical reconsideration of the dogmatic approach to the autonimity of malignant tumours. The main stimuli for a reconsideration of this position are: (1) numerous findings regarding the effects of mitotic poisons and cytostatic substances on malignant cells; (2) the results of observations on the influence of certain hormones on malignant tumours; (3) observations on the effects of cancer antibiotics or, more widely, of factors of microbial origin.

While discussing the problem of the autonomy of malignant tumours, it should be added that the main characteristics of malignant cells frequently include the ability of these cells to multiply much more rapidly than normal cells. This, of course, is true in the sense that the cells of a tumour usually multiply much more rapidly than the cells of the surrounding mature tissues. However, by no means all malignant tumours which have been growing for 9 months can equal the size attained by a foetus, which by the time of its birth has developed from a single normal cell during the same period. This property of malignant cells interests us in the sense that it undergoes changes; as we have seen in numerous examples taken from clinical or experimental material, the trypanosome preparation selectively inhibits the multiplication of malignant cells, changing the character of their metabolism. We say *selectively* because in the doses used experimentally and clinically we were unable to discern any suppression of other rapidly multiplying cells, for example those of the haemopoietic organs, as proved by numerous analyses of the blood of patients subjected to prolonged treatment with the trypanosome preparation; besides this, the trypanosome preparation was never seen to inhibit regenerative processes either clinically or experimentally.

Describing the basic properties of a malignant tumour, Petrov (1947) rightly emphasizes that a malignant tumour is characterized by *the destructive nature of its growth*. "The destructive or, to use Gurvich's term "*aggressive*" nature of its growth imparts the most typical feature on the whole essence of both the pathology and the clinical study of a malignant tumour." If we accept this characteristic of cancer cells, fully founded in experiment, we are bound to admit that with the aid of factors of microbial origin the aggressive character of growth may be removed in some cases for more or less prolonged periods and in others completely and permanently. This phenomenon, which we believe to be associated, in the case of administration of the trypanosome preparation, with metabolic changes, inevitably leads to the death of the cancer cell. We associated the particular sensitivity of malignant cells to many substances of chemical or biological origin with *a particular lability, and fragility of the protoplasm* of these cells. If this is so, then the question naturally arises: on what is the fragility of cancer cells based?

To obtain a clearer idea of the biological thought implied in the concept of *the fragility of a cancer cell*, one needs to start from the general and adequately established position that *resistance* to the influence of various factors is one of the basic properties of cells in a state of physiological integrity. We are talking here, of course, of *relative* resistance and *relative* fragility. Every cell is resistant to the extent that it exists in definite, phylogenetically dictated relationship with its medium, with the surrounding cells, with the organ and with the body. Every cell is resistant to the extent that the interrelationships between its various organoids are not broken down, the interrelationships between the biochemical components of its cytoplasm are maintained, etc. Any deviation from this state involves disturbance of the normal vital functions and is reflected, as a rule, in a loss of resistance, characteristic to any given type of cell, to various agents of endogenous or exogenous nature. If we compare normal cells and cancer cells from this point of view, we see in the latter a whole series of profound morphophysiological deviations, each of which, particularly in combination with other changes, may become a cause of loss of normal resistance and thus may bring about fragility of the modified cells.

Increased and selective sensitivity—fragility—of the cancer cell may arise from cytological changes in it which have been established by many investigators. To be more precise: breakdown of nucleus-cytoplasm relationships; breakdown of nucleus-nucleolus relationships; a particular lability of the apparatus of cell division, especially of the chromosomes, reflected in atypical mitoses and in diverse quantitative and qualitative

changes in the chromosomes; qualitative and quantitative changes in the mitochondrial apparatus.

Because of the very important role of the mitochondria in cell metabolism, the changes which they undergo in the cells of malignant tumours must be gone into in more detail.

Relevant observations are given in the table.

Tumour	Mitochondria	Authors
Jensen sarcoma	Finer than in normal mesenchymal cells	Fell & Andrews, 1927
,, ,,	Shorter than in normal fibroblasts	Carrell & Ebeling, 1928
,, ,,	Usually smaller	Ludford, 1934
Walker sarcoma	Smaller than in normal fibroblasts	Lewis, 1927
Guinea-pig hypernephroma	Changes in the shape, number and size of the mitochondria	Roskin, 1930
Rous sarcoma	More numerous, more elongated, less deeply staining than in normal fibroblasts	Zweibaum, 1933
Several types of sarcoma	More numerous and narrower than in normal fibroblasts	Lewis, 1939
Carcinoma of human prostate	More elongated and more numerous than in normal cells	Bothe, Dalton, Hastings & Zillessen, 1950
Rat hepatoma (induced by a carcinogen)	Decreased size and number of mitochondria	Allard, de Lamirande & Cantero, 1953
Mouse hepatoma	Decreased specific (biochemical) activity in comparison with mitochondria in normal liver	Schneider and Hogeboom, 1950

To this should be added observations made on living malignant cells with the aid of the phase-contrast microscope. There is thickening of the mitochondria, breakdown of their fine structure and a random distribution within the cytoplasm (Siering and Aderhold, 1956).

Finally, while discussing cytological changes in malignant cells we must not omit observations by Caspersson (1942, 1950) and later by Ludford (1954) indicating a peculiarity of the structure of these cells in that the nucleolus is closely associated with several chromatin granules lying adjacent to it (nucleolar chromatin).

An increased selective sensitivity—fragility—to certain factors may be brought about by a number of the profound cytophysiological and

biological changes established in malignant cells. To mention the more essential ones:

(i) changes in respiratory metabolism (Warburg, 1926);

(ii) changes in the enzymatic apparatus of malignant cells in comparison with normal cells, which may be reflected in the decreased activity of a number of enzymes—for example, tumour tissues are definitely deficient with regard to cytochrome C and catalase (Mardashev, 1948; Ries and Gersch, 1953; Greenstein, 1951);

(iii) changes in the colloidal properties of the cytoplasm—an increase in its density (Lewis, 1939), viscosity (Chambers and Ludford, 1932) and ultrastructure (Duran-Reynals, 1932);

(iv) changes in the fermentive properties of the nucleus—a marked weakening of the oxidoreductase reaction* (Roskin, 1932; Roskin and Solov'eva, 1936; Roskin and Struve, 1946; Voinov, 1940; Watermann, 1934, 1950; Rauffer, 1935);

(v) a shift in the isoelectric points of cytoplasm and nucleus (Solov'eva, 1936; Roskin, 1946);

(vi) an unstable, colloid-labile state of the cytoplasmic ribonucleic acid (Roskin and Skliar, 1955).

It should be added that Fischer (1935), on the basis of many observations on malignant cells in tissue culture, concluded that such cells have very little resistance to any form of damage, and their life is short in comparison with normal cells. This interesting thought has been confirmed by observations on the duration of the interval between divisions in normal and malignant cells (Widner and co-workers, 1951):

In mice	Duration of division, in minutes	Duration of interphase, in hours
Cells of myeloid series	35.3	155
Cells of testis	29.5	99
Cells of lymphatic nodes	25.2	100
Epidermal cells	30.2	670
Adrenal cells of rats	14.4	1090
Walker carcinoma	24.8	11
Jensen sarcoma	26.6	12

All the changes listed, in various combinations and to different degrees, are shown by the cells of various types of malignant tumours. More-

* Leuco-methylene blue dehydrogenase (Ries and Gersch, 1953).

over, each cancer cell goes through a cycle of development and its properties are not uniform at different stages of this process. However, all the morphophysiological changes in the cancer cell in combination create both increased lability and *selective sensitivity* to certain external factors, in this case to factors of microbial origin.

The sensitivity of a cancer cell to certain factors of microbial origin is only one indication of its particular lability. This may be seen from the following table.

Affecting factor	Normal cells	Malignant cells	Comments
Dehydration (Roffo, 1930)	Chick fibroblasts	Spindle-cell sarcoma of rat	Malignant cells much more sensitive
Effects of temperature (Lambert,1912)	Mouse and rat connective tissue cells	Mouse and rat sarcomata	*ditto*
Light rays of various wavelengths (Roffo, 1934)	Chick fibroblasts	Spindle-cell sarcoma of rat	Malignant cells much more sensitive. Greatest effect by rays in blue portion of spectrum
Intesity of illumination (Roffo,1932)	*ditto*	*ditto*	Malignant cells much more sensitive
X-irradiation (Roffo, 1936)	*ditto*	*ditto*	*ditto*
Various acid and basic dyes (Roffo and Calcagno, 1930-1932)	*ditto*	Spindle-cell sarcoma	*ditto*
Antiseptics: phenol, tricresol etc. (Lustig and Weber, 1936)	Mouse fibroblasts	Mouse carcinoma	*ditto*
Cholesterol salts (Roffo and Calcagno, 1934)	Chick fibroblasts	Spindle-cell sarcoma of rat	*ditto*

All that has been said shows that the conclusions arising from our observations on the regression of malignant tumours under the influence of factors of microbial origin touch upon a number of fundamental theoretical standpoints in the general study of malignant tumours. In the light of the facts presented, our ideas on the aggressiveness of malignant tumours must be altered: the concept of aggressiveness must be supplemented by *the idea of fragility and lability of malignant cells.* Aggressiveness and fragility are *interrelated* properties of cancer cells—this is what our experiments have taught us.

To the question posed earlier — to what extent may we reconcile the idea of fragility of cancer cells with teachings on the autonomy and aggressiveness of malignant tissues—the following answer can therefore be given: the idea of fragility compels us to reconsider the hypothesis of the autonomy of malignant tumours and to supplement and extend the idea of aggressiveness in the sense that aggressive cells have their "weak, injurable points", the existence of which gives real hope for experimental therapy. This position equips and encourages the investigator. That is why the ideas and facts which we tried to defend in our first book (1946) we wish to defend now, supported by the more extensive clinical and experimental observations described in this book. We are dealing not with isolated observations or opinions but with the choice of a *point of view* in a very great problem involving the fate of many, many people, and we are challenging the *outlook* of the investigator, who is bound by his duty as a doctor and a scientist to take action where this appears, at first sight, to be insurmountably difficult.

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