BIOTHERAPY OF MALIGNANT TUMOURS

N. G. KLYUYEVA and G. I. ROSKIN

Translated from the Russian by

J. J. OLIVER

B. Vet. Med., M.R.C.V.S.

Translation Edited by

W. J. P. NEISH, Ph.D.

Cancer Research Unit,
The University of Sheffield

PERGAMON PRESS

OXFORD · LONDON · NEW YORK · PARIS

1963

PERGAMON PRESS LTD.
Headington Hill Hall, Oxford
4 and 5 Fitzroy Square, London, W.1

PERGAMON PRESS INC.
122 East 55th Street, New York 22, N.Y.

GAUTHIER-VILLARS ED.
55 Quai des Grands-Augustins, Paris, 6e

PERGAMON PRESS G.m.b.H.
75 Kaiserstrasse, Frankfurt am Main

Distributed in the Western Hemisphere by
THE MACMILLAN COMPANY · NEW YORK
pursuant to a special arrangement with
Pergamon Press Limited

Library of Congress Card Number: 60-53070

A translation of the original volume
Problema protivorakovykh antibiotikov
(Moscow, 1957)

Printed in Poland to the order of PWN—*Polish Scientific Publishers*
by the Scientific-Technical Printing House (DRP), Warsaw

BIOTHERAPY OF MALIGNANT TUMOURS

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.

N. G. KLYUYEVA and G. I. ROSKIN

Histology Laboratory, Moscow University

CONTENTS

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 diffculties 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 flexneii
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).