Theodor Von Brand

biochemistry and physiology of endoparasites

Elsevier/North-Holland

Biochemistry and Physiology of Endoparasites

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1979

ELSEVIER/NORTH-HOLLAND BIOMEDICAL PRESS
AMSTERDAM · NEW YORK · OXFORD

ISBN 0-444-80073-5

Published by:
Elsevier/North-Holland Biomedical Press
335, Jan van Galenstraat, P.O. Box 211
Amsterdam, The Netherlands

Sole distributors for the U.S.A. and Canada:
Elsevier North-Holland Inc.
52 Vanderbilt Avenue
New York, N.Y. 10017

Library of Congress Cataloging in Publication Data

Von Brand, Theodor, 1899-1978
Biochemistry and physiology of endoparasites.

"An updated version of . . . Parasitenphysiologie."
Includes bibliographies.
1. Parasites—Physiology. I. Title.
QL391.V6613 591.5'24 78-13703
ISBN 0-444-80073-5

Printed in The Netherlands

Preface

The present book is an updated version of my German "Parasitenphysiologie" which was published in 1972 by Gustav Fischer Verlag, Stuttgart and embodied the literature up to the beginning of 1970. In the intervening years a great number of relevant papers have appeared, necessitating a rather profound alteration of the text in many instances. In order not to expand the text too much, some of the older findings had to be omitted. I have tried to present a balanced picture of the present state of our knowledge in the fields covered by the book. Without trying to be encyclopedic, I hope that I have not inadvertently omitted significant new approaches; but I shall be grateful if significant omissions (or errors which can hardly be completely avoided in a work of this magnitude) are brought to my attention.

The title of the book has been changed for two reasons. On the one hand, reviewers of the 1972 book pointed out correctly that it contains at least as many biochemical as physiological data. Secondly the title of the German book has been criticized with some justification because the use of the word "Parasiten" may have misled some readers inasmuch as the purely ectoparasitic arthropods (mosquitos, fleas, lice, ticks, and others) had been excluded. Therefore, I now use the term endoparasites but include also such organisms as monogenetic trematodes or Rhizocephala, since, from a physiological standpoint, they are more closely related to the organisms discussed in the book than to ectoparasitic arthropods.

Finally, I wish to thank the Gustav Fischer Verlag for permission to base the present book on the German text and to my wife without whose patience and understanding the work would not have been completed.

March, 1978 Theodor von Brand

The untimely death of my husband occurred before the final proof of this treatise was received. Fortunately, faithful colleagues who were associated with

my husband's research in the Section of Physiology and Biochemistry, National Institutes of Health, Bethesda, have been very generous with their time in carefully reading the entire proof, in making corrections where it was necessary, in checking the bibliography and in compiling the index. I wish especially to thank Dr. Eugene C. Weinbach, Dr. Teresa I. Mercado and Mr. Elwood C. Claggett from the Laboratory of Parasitic Diseases, National Institutes of Health and Professor J. Eckert of the University of Zurich, Switzerland, for their valuable services. I should also like to express my sincere appreciation to Dr. H. van Hummel for his personal attention and patient cooperation.

Margarethe von Brand

THEODOR von BRAND; A Tribute

With the death of the author of this book on July 18, 1978 in Bethesda, Maryland, parasite physiology has lost its founder and pioneer.

Theodor Curt von Brand was born on September 22, 1899 in Ortenberg, Germany and received his education in that country. In 1922 he was awarded a Ph.D. in Zoology from the University of Munich, and in 1928 earned a doctorate in Medicine at the University of Erlangen where he had begun his studies on the physiology of parasites. In 1933 he left Germany to accept a fellowship in the Laboratory of Zoophysiology at the University of Copenhagen under the direction of August Krogh. During his association with Krogh, Dr. von Brand learned many new microanalytical techniques which were to prove highly useful to him throughout his ensuing career.

His highly productive experimental work in Germany is recorded in 80 published papers, concerned chiefly with the metabolism of helminths and trypanosomes. During these early studies Dr. von Brand first recognized the possibility, which he and others subsequently demonstrated, that anaerobic, rather than aerobic metabolism provides the major, if not exclusive, source of energy for many parasites.

In 1936, he came to the United States at the invitation of W.W. Cort who appointed him as a research fellow at the School of Hygiene and Public Health, The Johns Hopkins University in Baltimore, Md. He then accepted teaching and research posts at Barat College in Lake Forest, Illinois, and the Catholic University in Washington, D.C. While at the latter institution, he worked for one summer at the then National Institute of Health in Bethesda, Maryland. Dr. Willard Wright, Chief of the then Laboratory of Tropical Diseases, Microbiological Institute, N.I.H., invited him to join the Laboratory as a staff member in 1947. Shortly before he moved to N.I.H., Dr. von Brand wrote his first book entitled "Anaerobiosis in Invertebrates." In this monograph, he critically reviewed the role of anaerobic metabolism in lower animals, including his own studies with parasites. Recognizing a great need for a comprehensive review on parasite physiology, he subsequently wrote a book entitled "Chemical Physiology of Endoparasitic Animals". This appeared in 1952, and immediately became an invaluable guide for the slowly growing number of scientists becoming interested in the subject. In 1966 he wrote a greatly enlarged second edition entitled "Biochemistry of Parasites". The change in title reflects a greater emphasis on intermediate metabolism and enzyme systems about which information had been forthcoming during intervening years. Soon after his retirement, Dr. von Brand wrote a book in German on parasite physiology ("Parasitenphysiologie", 1972), and when this was completed, he began to revise his "Biochemistry of Parasites." Fortunately, he was able to complete the present work shortly before his death.

Although an already well-established investigator, Dr. von Brand's contributions to our understanding of the biochemistry of protozoan and metazoan parasites truly burgeoned after he joined the National Institutes of Health. His leadership was not limited to his own research, but also extended to his influence on younger colleagues, many of whom devoted their research exclusively to a field that he had pioneered. In his Presidential Address to the American Society of Parasitology in 1969, Dr. von Brand presented a graph showing that the contributions of physiological papers given at the annual meetings of the Society increased from virtually none, to over 20 percent during the preceding 30 years. This growth in a field for which he was largely responsible must have been a source of great satisfaction to him.

Dr. von Brand was a man of keen intellect and immense scholarship. Although he regarded Professor Weinland of the University of Erlangen as one of the principal founders of parasite physiology, it was Dr. von Brand who, through his prolific research and scholarship, founded and developed this area of parasitology both in Europe and the U.S.A. In fact, for more than 20 years, until the end of World War II, he was the only scientist who consistently engaged in research in this field.

He enjoyed doing experiments with his own hands. Indeed, in 1969, the day before his mandatory retirement at the age of 70, he was working in the laboratory on yet another series of experiments which were completed thereafter. He also continued to write papers; his essay "Glimpses at the Early Days of Parasite Biochemistry" written as the prefatory chapter in "Comparative Biochemistry of Parasites" (H. Van den Bossche, editor, Academic Press, 1972) makes for delightful and informative reading.

Dr. von Brand also was an extremely versatile scientist; he readily adapted to unusual circumstances. When he was associated with the small Barat College in Lake Forest, Illinois, the equipment budget was so low that he conducted metabolic experiments in soft drink bottles. When he told this story, he added, with a twinkle in his eye, that it weally wasn't so bad because the proximity of the Chicago stockyards made it easy for him to obtain an abundant amount of parasites!

Dr. von Brand was an extremely gifted and prolific writer, having published more than 200 papers, aside from being the sole author of six books. He wrote with great facility, and unlike many of his colleagues, he never went through the agonizing process of multiple drafts.

By upbringing and cultural background Dr. von Brand was a perfect gentleman. His courtly manners were a constant source of admiration. Although he spoke softly in private conversation he did so with vigor and clarity from the platform. A kind and gentle man, he never criticized the worker, only the work.

Among the numerous recognitions and honors awarded to Dr. von Brand, one of them should be singled out: In 1969 he was awarded the Superior Service Award of the U.S. Public Health Service "For meritorious research on the chemical composition and metabolism of parasites."

Theodor von Brand's legacy to parasitology will long be appreciated not only by those who had the privilege of knowing him but also by future generations of scientists.

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CHAPTER 1

Peculiarities of parasitic habitats

1.1 INTRODUCTION

Very few, if any animal or plant species exist which are not parasitized at least occasionally by an endoparasitic animal and within a given host parasites can be found in almost any organ and tissue. It is therefore evident that parasites are exposed to quite variable environmental conditions. Furthermore, many have a complicated life cycle. Some spend part of their life as free-living larvae (e.g. hookworms), others as free-living adults (e.g. *Gastrophilus*). Some parasites pass their entire life in warm-blooded hosts (e.g. *Trichinella*) others alternate between warm- and cold-blooded hosts (e.g. many trematodes), or between cold-blooded hosts and plants (e.g. *Phytomonas*). These examples show that a complete characterization of the parasitic habitats would require a discussion of the physiology of plants, cold-blooded and warm-blooded animals, as well as a discussion of the ecological conditions prevailing in aquatic and terrestrial habitats. This, evidently, is impossible within the confines of a book dealing with parasite biochemistry and physiology. Instead, a few environmental factors have been selected for discussion, those important to parasites and subject to significant variations in parasitic habitats. They are oxygen and carbon dioxide, hydrogen ion concentration, and temperature.

1.2 OXYGEN AND CARBON DIOXIDE

1.2.1. General

Most, if not all parasites have at least occasionally access to greater or smaller amounts of oxygen. One parasitic habitat with unusually high oxygen concentrations is the swim bladder of fishes in which both protozoan and helminth parasites live. The oxygen concentration can reach concentrations of up to 95% and in deep sea fishes its tension can surpass 100 atmospheres (see

Wittenberg, 1958) but it is not yet known whether parasites occur under such extreme conditions. A moderate tension (22-58 mm Hg) has been found in the swim bladder of *Wallago attu*, the habitat of the trematode *Isoparorchis hypselobagri* (Siddiqi and Nizami, 1975).

Some parasites (e.g. *Syngamus tracheae*) live in habitats with oxygen tensions approaching that of atmospheric air. But most parasites of internal organs have access to relatively low oxygen tensions only.

The older physiological and biological literature contains many relevant data (O_2 in mm Hg): arterial blood 70-100, venous blood 2-60, urine 14-60, bile 0-30, intestines 0-70 (see von Brand, 1952). Such data are not necessarily characteristic for the environment of a given parasite, because conditions frequently are much more complex than indicated by the overall oxygen tension of a given organ. In the kidney, for example, an oxygen gradient ranging from 9-65 mm in the cortex to 3-17 mm in the medulla exists (Ulfendahl, 1962). Individual variations also occur. The tissue PO_2 in the skeletal muscles of various normal rats averaged from 0.4 to 27 mm (Whalen et al., 1974).

It should be emphazised furthermore that parasites frequently induce pathological processes (e.g. bleedings, inflammations) which can alter the local oxygen tension materially. It can thus decrease from 30-60 mm to 3-6 mm in muscle wounds that have not yet been revascularized (Remensnyder and Majno, 1968). Similarly, the tension is reduced from 35 mm to approximately 4 mm in subcutaneous tissues when artificial bleedings induce hypotonic conditions (Jones et al., 1969). The intraperitoneal injection of *Escherichia coli* endotoxin (Wilson and Matsuzawa, 1968) produces rapid changes in the oxygen concentrations of subcutaneous mouse tissues (Fig. 1). Definite differences in tension are found even in normal tissues when regions only short distances apart are investigated. For instance, a distinctly higher tension prevails over arterial than venous capillaries. In view of such complications caution should be used in trying to draw conclusions in respect to the life style of parasites *in situ*. It would thus be imprudent to assume that equal oxygen tensions must prevail within the *Trichinella* cyst and the surrounding muscle cell.

The oxygen content of the vertebrate intestine will be discussed separately because of its importance in assessing the biological relationships of many intestinal parasites. The composition of intestinal gases has been studied repeatedly, the analyses yielding usually quite low oxygen values, ranging generally between 0 and 5 mm Hg. Distinctly higher values have been reported only from some pigs and were due probably to contamination with swallowed air. It should be realized, however, that the values reported are not necessarily identical with those actually prevailing in the intestinal contents because it is questionable whether the intestinal gases are in complete equilibrium with the surroundings. No such objection can be made to gasanalytical determinations in the fluid intestinal contents which generally yield quite low values (von Brand, 1952). But objections can be raised nevertheless against this procedure

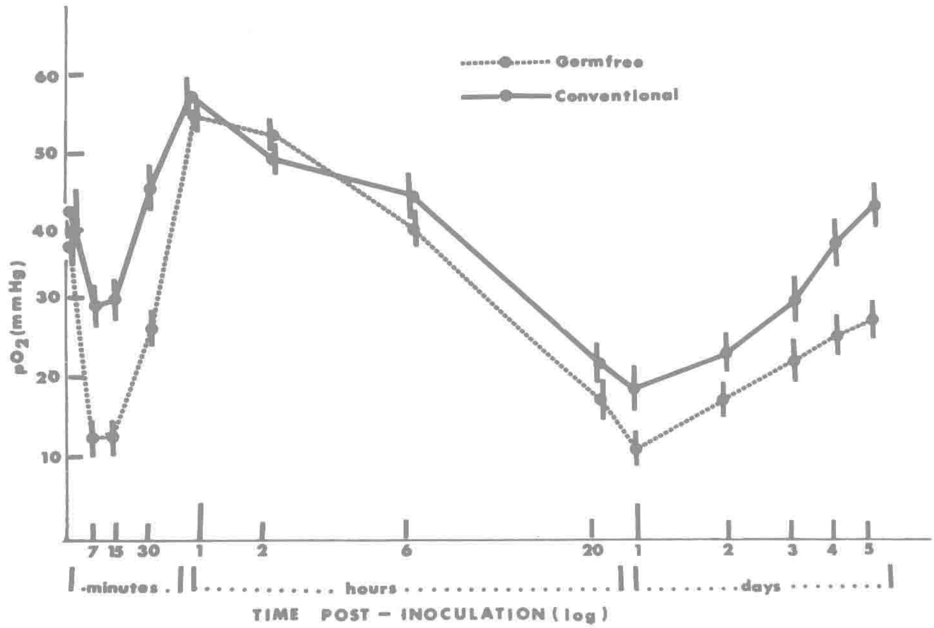

Fig. 1. Change in P_{O_2} in the subcutaneous tissue of germfree and conventional mice after receiving 10 μg *Escherichia coli* endotoxin intraperitoneally. The bars at each point extend 1 standard error above and below the plotted means. (After Wilson and Matsuzawa, 1968).

because the possibility exists that some oxygen may have been lost through the high oxygen demand of the intestinal flora, objections that cannot be discounted completely even if only a short time elapses between taking and analyzing a given sample.

Most convincing are determinations made *in situ* by means of the oxygen electrode. They have been done only rarely sofar and even this procedure has been criticized recently (Podesta and Mettrick, 1974). The first relevant studies are due to Rogers (1949a) who found oxygen tensions of 4-13 and 8-30 mm Hg respectively near the mucosa of the small intestine of sheep and rats respectively. Newer data are due to Crompton et al. (1965). They describe from the duck intestine an oxygen gradient beginning with 25 mm near the mucosa and reaching 0.5 mm in the centre of the lumen of the small intestine. The gradient is due to oxygen diffusing from the intestinal capillaries into the lumen where it is consumed rapidly by the intestinal flora.

In summary, the following conclusions seem justified: The oxygen content of the vertebrate intestine is usually low. Parasites living close to the mucosa probably have access to biologically significant amounts of oxygen. On the contrary, organisms living in the center of the intestinal lumen at least of large animals find themselves in very oxygen-poor surroundings which at least occasionally are truly anaerobic.

Unquestionably, less oxygen occurs in the fermenting contents of the cattle rumen than in the stomach of rats. The oxygen content of the latter's gases varies between 3.0 and 22.5% (Hedin and Adachi, 1962). Whether parasites inhabiting the intestine of cold-blooded vertebrates such as frogs have access to larger amounts of oxygen, although probable, is not yet known with certainty. It is possible furthermore that parasites have a certain influence on the intestinal oxygen tension at least of small animals. Podesta and Mettrick (1974) thus found in the liquid phase of the intestinal contents of rats parasitized by *Hymenolepis diminuta* relatively high oxygen tensions (40-50 mm Hg) which were significantly higher than in non-parasitized specimens. Mettrick (1975) furthermore reports Eh values of —28 to —195 mV for uninfected rat intestines, while the range was +75 to —76 mV in tapeworm infected hosts.

Considerable differences undoubtedly also exist in invertebrates. It is thus reasonable to assume that the intestine of sand- and tsetse flies contains biologically important amounts of oxygen as indicated indirectly by the well known dependency of trypanosomids on oxygen. On the other hand, the oxygen sensitivity of termite flagellates implies that their habitat is essentially anaerobic. This clearly is true in the case of *Cryptocercus*. Ritter (1961) using a sensitive polarographic method, could not detect oxygen in the intestine of this roach. He did find reduced glutathione which evidently determined the degree of anaerobiosis and which, interestingly, was not produced by the flagellates or intestinal bacteria, but by the insect itself.

It can be presumed that plant-parasitizing animals normally have access to ample oxygen. Considerable amounts of this gas occur even in the gases of roots located in practically anaerobic environments. Thus, the root gases of mangroves yield values of usually 10-18% oxygen (Scholander et al., 1955) and Armstrong (1964) showed that oxygen diffuses out from the roots of certain plants (e.g. *Menyanthes trifoliata*) into anaerobic surroundings. However, quite low values have been observed occasionally (e.g. often only 1% oxygen in the root gases of *Juncus effusus* according to Houlihan, 1969). Such observations are important in assessing the biology of nematodes parasitizing the roots of rice plants in fields flooded for extended periods.

Free-living stages of parasites will be able to satisfy in most cases even maximal oxygen needs without difficulties. This can be assumed for instance for cercariae swimming in water or hookworm larvae surrounded by a thin water film or living on the surface of the soil. On the other hand, certain stages (e.g. the cysts of intestinal amebas or the eggs of intestinal helminths) may find themselves often in oxygen-poor or oxygen-free environments, for example septic tanks or similar specialized habitats. However, they often survive such extreme conditions only passively; their development requires normally at least a minimum of oxygen.