Dr. F. R. Lillie in gratitude
from the author.

ANAEROBIOSIS
IN
INVERTEBRATES

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No. 4 of the "Biodynamica Monographs"
edited by B. J. LUYET

Published by
BIODYNAMICA, NORMANDY 21, MISSOURI
1946

Most of the contents of this monograph are reprinted from BIODYNAMICA, No. 92, 1944 and Nos. 100 and 105, 1945.

INTRODUCTION

The review of anaerobiosis in invertebrates presented in the following pages represents an attempt to give a comprehensive picture of a many-sided problem. It is divided into three parts: the first is devoted to a survey of the occurrence of anaerobiosis; in this part attention is given chiefly to ecological problems; the second deals with anaerobic metabolism and the third contains data on the adaptations of invertebrates to anoxic conditions and a discussion of the origin of their anaerobic functions. The monograph is written from the biological standpoint and thus intended primarily for ecologists and comparative physiologists. Biochemists who, until very recently, used almost exclusively vertebrate material or micro-organisms for their work on anaerobiosis, may find this book helpful as a guide in the choice of new experimental animals.

In 1934 we summarized our knowledge concerning the anaerobic life of invertebrates in a review article published in *Ergebnisse der Biologie*. Due to limitation of space, only the subject "anaerobic metabolism" was fully treated there. The present account of that phase of the problem is based in part on this earlier paper, but the many investigations published in the intervening years necessitated a great number of additions and alterations.

In the discussion of anaerobic metabolism, it was found preferable to divide the subject according to the processes involved, such as transition from aerobic to anaerobic metabolism, recovery from anaerobiosis, *etc.*, rather than to arrange the material according to the invertebrate phyla, as was done previously.

The literature on anaerobiosis in invertebrates is extremely scattered; pertinent papers are found in zoological, ecological, limnological, oceanographic, parasitological, physiological, biochemical and bacteriological

journals, as well as in medical and other journals dealing with applied biology. Important data are often hidden in papers having no obvious conection with the anaerobiosis problem. It is manifestly impossible to read the entire scientific output of the last 50 years in the above fields, and consequently some important papers may have been omitted. We shall appreciate it if such cases are brought to our attention. This review covers the literature in the field up to the beginning of 1945.

Our sincere thanks are due to Dr. E. G. Reinhard and Dr. B. J. Luyet, both of whom read the entire manuscript and offered many helpful suggestions. To Dr. Luyet, we are, in addition, deeply indebted for his painstaking editorial work in which he was ably assisted by Dr. P. M. Gehenio. The present work would not have been possible without the cooperation of various libraries; we are especially grateful for the never-failing courtesy of the Army Medical Library, the Library of the Department of Agriculture, the Library of Congress and the Library of the U. S. National Museum.

Washington, D. C., September 25, 1945

THEODOR VON BRAND

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PART I

OCCURRENCE OF ANAEROBIOSIS AMONG INVERTEBRATES

Before surveying the invertebrate phyla for evidence of anaerobic life we shall (1) discuss the methods of investigation used in this field and (2) consider the types of habitats in which anaerobiosis is possible.

CHAPTER I

METHODS OF INVESTIGATION

The study of anaerobic life in invertebrates comprises three essential problems: 1. the presence of animals in habitats in which there is a lack of oxygen; 2. the tolerance of animals for the experimental deprivation of oxygen; 3. the nature of the metabolism under anaerobic conditions. From the standpoint of methodology the first of these problems involves the technique of measuring the oxygen content of various habitats; the second, that of establishing experimental anaerobic conditions; and the third that of determining the type of anaerobic metabolism. These methods, although partially overlapping, will be discussed separately.

1. DETERMINATION OF THE OXYGEN CONTENT OF VARIOUS HABITATS

A. Terrestrial habitats. True terrestrial animals live, in so far as their respiration is concerned, in a gaseous atmosphere. The oxygen content of the latter can usually be determined with the help of standard gas analysis apparatus. Detailed directions for the handling and analysis of gases are given by Peters and van Slyke (1931). In special cases it will be necessary to work with small gas samples; the procedure of micro gas analysis

will then have to be employed. Suitable methods, some of them requiring only a few mm.^{3} of gas, have been developed, especially by Krogh (1926). The new micro gas analyzer described recently by Scholander (1942) should also be mentioned as a very promising apparatus. The taking of samples of soil gases uncontaminated with atmospheric air presents signal difficulties, but suitable methods have been described by Leather (1915).

B. Aquatic habitats. The most widely used method for determining the oxygen content in water is Winkler's (1888) method. Its principle is to absorb the oxygen present in the sample on manganous hydroxide. Upon acidification, in presence of iodide, an amount of iodine equivalent to the absorbed oxygen is liberated and this amount is determined by titration with thiosulfate. The method is rapid and very reliable provided that the water is pure. This limitation is well known, but unfortunately has not been taken into account by the authors of some of the papers to be reviewed in the present survey. Serious errors result if the water is impure, but several modifications have been proposed that may be employed when this is the case. Rideal-Stewart's (1901) procedure is convenient when larger amounts of nitrates or ferrous salts are present; a preliminary treatment eliminates the interfering substances by means of permanganate. For water containing organic matter, hypochlorites, or sulfite wastes, the modification by Theriault and McNamee (1932) may be used in which the impurities are oxidized prior to the actual oxygen determination, through an alkaline solution of sodium hypochlorite. Both the original method and the two modified procedures just mentioned are described in detail in a publication of the American Public Health Association (1936). Another excellent modification for use in the presence of organic contaminants has been developed by Alsterberg (1926). In this case the undesirable constituents of the sample are oxidized with bromide.

The standard Winkler method and its modified forms require rather large samples of water; from 125 to 350 cc. are commonly used for a single analysis. In some types of investigation only smaller samples may be available. One should then employ micro modifications of the original procedure. Krogh (1935) used small water bottles containing but 7 to 15 cc. of water and van Dam (1935) described a method requiring only 1 cc. This latter procedure was further improved by Fox and Wingfield (1938) to whom we refer the reader for details.

Krogh (1935) has pointed out that all forms of the Winkler method have two sources of error in common. Both are of special importance for the problem under consideration here since they become especially noticeable when the oxygen tension of the water is low. "One is the diffusion of oxygen that takes place whenever the sample is not in equilibrium with the atmosphere, and the other is the uncertainty of the correction for oxygen dissolved in the reagents." Krogh (1935) has worked out a micro modification of Winkler's method in which these possibilities of error have been eliminated. He collects the water sample and carries out the reaction in one and the same syringe pipette of 10 cc. capacity. The method gives variations of only $\pm$ 0.007 cc. of oxygen per liter and deserves to be used more widely. It might be possible to modify it further so that it could be applied also to impure waters.

C. Parasitic habitats. Some parasites occur in a gaseous environment, for example, in the intestine of animals, where they are surrounded by the intestinal gases, or in the swim-bladder of fishes or in the lungs of various hosts, etc. The analytical procedures are then obviously the same in principle as those employed in the case of terrestrial habitats. Due to the fact that most of the time only small amounts of gases can be secured, it will often be necessary to use micro gas analysis methods.

In other instances the parasites are present in the semi-fluid intestinal contents. The oxygen tension of this habitat (*cf.* von Brand and Weise, 1932) can well be determined with the van Slyke manometric apparatus (*cf.* Peters and van Slyke, 1931). It is to be noted here, however, that the enormous numbers of bacteria present in intestinal material quite generally exhibit an intensive oxygen consumption which invalidates the results gained from any but the very fresh samples.

The determination of the oxygen content of body fluids like urine, bile, etc., or of tissues—all important parasitic habitats—offers singular difficulties. One method that was much used in former years and which dates back to Ehrlich (1885), consisted in injecting dyes into the tissues: the absence of molecular oxygen was then assumed when the dyes became reduced. It is now generally agreed that this procedure does not give reliable results since, as Krogh (1916) remarked, "the stains employed may very well become reduced even if free molecular oxygen is present."

The more modern procedures are based on entirely different principles. Their classification as given below is taken from Campbell (1931), who has had wide experience with this problem.

(a) Gas extraction method. The gases dissolved in the secretions and excretions—they are in equilibrium with the gases present in surrounding tissues—are extracted by means of gas pumps and then analyzed. Campbell regards the results obtained in this way as doubtful because, on the one hand, oxygen may diffuse out and disappear spontaneously from the fluids during the manipulations and, on the other, small leaks frequently occur in the pumps. This may be a serious drawback since the absolute amounts of gases dissolved in the fluids are very small. In the present writer's view, however, these objections can hardly apply to analyses performed with the van Slyke manometric appa-

ratus, provided that the samples introduced are absolutely fresh. This method is so rapid that the spontaneous disappearances of oxygen ought to be negligible.

(b) T o n o m e t r i c m e t h o d s (*cf.* Krogh, 1908). The tonometric methods consist essentially in 1. introducing a small gas bubble (air, nitrogen, etc.) into the fluid that had been previously withdrawn from the body without coming in contact with the air, 2. shaking this bubble with the fluid to permit the oxygen dissolved in the latter to mix with the introduced gas and come to a pressure equilibrium with it, and 3. analyzing the mixture. Campbell is of the opinion that the oxygen tensions found in this way may be too low since a complete equilibration requires a fairly long time and consequently the spontaneous disappearance of oxygen may vitiate the results.

(c) V e r z á r ' s m e t h o d . (*cf.* Verzár, 1912). This complicated method relies in principle on oxygen determinations carried out on blood flowing to and from an organ while the experimental animal breathes air containing various percentages of oxygen. It has not come into general use and, according to Campbell, it fails in most cases to give reliable results.

(d) G a s i n j e c t i o n m e t h o d . This is the most widely used procedure and seems to give the most dependable results (Campbell). The principle is to inject gas (air, nitrogen or other gases) into the body cavities, the urinary bladder or under the skin, to let it remain long enough (usually a few days) in the body to allow for the establishment of an equilibrium of pressure of the two gases, and then to withdraw the mixture and to analyze it. The amounts of gas injected are usually fairly large because much of it is resorbed during the equilibration period. By resorting to micro analytical procedures smaller volumes may be used. Thus, Meyer (1935) injects only 50 cc. of nitrogen into the subcutaneous tissues in human beings. In invertebrates sim-

ilar methods have been used only rarely, but they seem to give satisfactory results (Adler, 1918; Gaarder, 1918).

2. ESTABLISHMENT OF EXPERIMENTAL ANAEROBIC CONDITIONS

The methods of establishing anaerobic conditions in the laboratory form the basis of all experimental work in the study either of the resistance to lack of oxygen or of anaerobic metabolism. Theoretically there are two ways of inducing anaerobic conditions, one is to inhibit the processes of aerobic oxidation, the other to remove the oxygen from the medium.

A. Inhibition of aerobic reactions. One may inhibit aerobic metabolism in an organism by poisoning the mechanism of aerobic respiration. Obviously, if all the reactions utilizing oxygen could be eliminated by this means and if the animal would still survive, it could do so only by virtue of anaerobic processes. Several substances, like hydrocyanic acid, sodium azide or carbon monoxide, can be used to inhibit aerobic respiration and it is surprising how resistant many invertebrates are to these poisons.

Nevertheless it is not possible in this way to arrive at a purely anaerobic metabolism. The different available poisons interrupt at various points the chain of aerobic processes, but no single substance or combination of various poisons inhibits all the reactions which utilize molecular oxygen. As a rule, a certain, though sometimes quite small, residual oxygen consumption remains.

The use of poisons can consequently not be recommended for a study of the resistance of organisms to the lack of oxygen or of similar problems. Such poisons are nevertheless helpful tools, and when used conjointly with those that act on the anaerobic part of the metabolism (like iodo-acetic acid, iodo-acetamide and others) they give an inside view into the mechanism of anaerobic life that cannot be obtained by other means. So far, in

the study of invertebrates, the former group has been used primarily to elucidate the problems of the depressing action exerted on oxygen consumption (by cyanide in particular), to gain knowledge concerning the aerobic enzymatic chain, to prove the presence of an axial gradient, or to study the importance of the respiratory pigments to the organism. Perhaps with a combination of both groups of poisons one might make a quantitative approach to the question of how far anaerobic can replace aerobic processes, a question that up to now has been primarily attacked by exposing animals to varying oxygen tensions.

B. Removal of oxygen. The second way in which anaerobic conditions can be established consists in removing the oxygen from the environment in which the experimental animals are kept. This can be achieved by the following means: (a) evacuation, (b) absorption of oxygen, (c) replacement of the atmosphere with an inert gas, like nitrogen or hydrogen. A combination of the various procedures is of course possible and in some instances advisable or necessary. We shall consider briefly the advantages and disadvantages of each method.

(a) Evacuation. Small animals are placed in a glass container provided with an opening which can be drawn out to a capillary. By means of a high vacuum pump the atmospheric pressure is then lowered to such an extent (to 10^{-5} mm. Hg for example, in Becquerel's experiments, 1936) that the remaining oxygen will play no significant role after the capillary has been fused. This method can be used effectively with organisms that resist desiccation, *e.g.,* cysts of protozoa, many rotatoria, moss nematodes and others. In active terrestrial animals the evaporation will be so high under these conditions that a lethal effect could hardly be ascribed to lack of oxygen alone. It is moreover questionable whether the lowering of pressure itself may not affect some animals.

(b) Absorption of oxygen. The oxygen present in the medium is absorbed either by living organisms or by chemicals. In the former case the experimental animals are simply put into tightly closed containers and their own oxygen consumption gradually exhausts the oxygen present. The advantage of this method is that anaerobic conditions are established gradually; the main disadvantage is that one can never be quite certain just exactly at what point all the oxygen has disappeared. An approximate idea of the degree of oxygen depletion can, however, be obtained by analyzing, after various time intervals, the oxygen remaining in comparable vessels containing the same number of organisms (*cf.* von Brand, 1927).

Occasionally the oxygen consumption of organisms other than those to be tested has been employed for similar purposes. Fauré-Fremiet, Léon, Mayer and Plantefol (1929) used, for example, germinating bean seedlings kept in the dark to exhaust the oxygen in solutions in which the anaerobic survival of *Paramaccium* was studied. Such a procedure may be more dangerous than the use of the test organisms themselves. If the latter do not consume the last traces of oxygen, they at least remove the amount of oxygen which they are able to utilize and that is the important thing. With another organism, there is always a danger that it may cease its oxygen consumption at tensions that are still of significance to the test organisms. In special cases cultures of aerobic bacteria can be used to exhaust the oxygen. Such a procedure, patterned after the original method of Fortner (1928, 1929) was employed by Levitanskaja (1938), for example, in culturing *Balantidium coli.*

Anaerobic conditions of significant severity can be established without difficulty for mud dwellers. Juday (1908) and Lindeman (1942) simply filled containers with the ooze from the bottom of lakes and tightly closed them after the test organisms had been introduced. The mixed

microorganism flora and fauna of such a medium rapidly exhausts all the molecular oxygen. This method has the advantage of keeping the test organisms in semi-natural environments. The main disadvantage is that the processes occurring in the ooze are beyond control. Often hydrogen sulfide is produced and many organisms seem to succumb to this gas rather than to the lack of oxygen.

Anaerobic conditions can also be produced by absorbing oxygen by chemical methods, *e.g.*, by alkaline solution of pyrogallol. When fluid media are to be deoxygenated in this manner the surface/volume ratio of the culture fluid should always be high enough. Snyder and Meleney (1943), in their work on the anaerobic culture of *Endamoeba histolytica,* inserted alkaline pyrogallate sponges above 1 cc. of culture fluid in tubes of 16 mm. diameter. For animals that can be kept in a gaseous atmosphere any type of container that can be hermetically sealed and in which the organisms can be kept from direct contact with the absorbing fluid can be employed. Anaerobic culture jars as used in bacteriology are often very satisfactory, but in many cases simple improvised containers will be just as good.

The advantage of the chemical absorption of oxygen is that one can judge accurately the onset and the degree of the anoxic conditions. The chief drawback is that at least some organisms seem to be sensitive to an abrupt transition from an aerobic to a strictly anaerobic environment.

(c) Replacement of oxygen with an inert gas. To replace the oxygen by hydrogen or nitrogen is one of the safest and most rapid ways of establishing strictly anaerobic conditions. The advantages and disadvantages are the same as those mentioned above for chemical absorption.

No experimental difficulties are experienced with animals living normally in air. The latter is simply replaced by a stream of inert gas. Naturally, care must be taken

that it is really oxygen-free. This is not the case with the commercially produced gases. Bubbling them through one or two gas wash-bottles containing alkaline pyrogallate, as has sometimes been done, is not sufficient to remove all the oxygen. The gases should be led, before reaching the animal chamber, over copper or platinum that is sufficiently heated. After the chamber has been flushed by a rapid stream of purified gas, one can maintain a slow stream during the rest of the experiment. If the latter is of long duration, however, this is not always convenient. One may then create a higher pressure inside the chamber than prevails outside, so that, after outlet and inlet have been closed, the gas would leak out and no air would enter the container. But even then it is probably safest to pass a fresh stream of gas through the chamber from time to time. In all experiments of this type it should be remembered that rubber is not impermeable to gases. The ideal is to dispense with all rubber connections after the gas has been passed over the heated copper or platinum. If this cannot be done, the rubber connections should at least be reduced to a minimum.

To replace completely the oxygen dissolved in water by hydrogen or nitrogen is more difficult. It is clear that mere boiling of the water is not sufficient to remove all oxygen. Alsterberg (1922) found that even after long boiling and care to insure that during the cooling process only a small surface was exposed to the air, about 0.1 cc. oxygen per liter remained. The oxygen tension can be reduced further by bubbling oxygen-free hydrogen or nitrogen through the water after the latter has been boiled. In this way one can easily arrive at solutions that are sufficiently oxygen-free to be satisfactory for most experiments on larger aquatic animals.

The closing of the experimental vessels should, needless to say, be such that they are absolutely airtight or else a constant stream of gas should be maintained. The

frequently used method of overlaying the water with paraffin oil or similar substances is not very satisfactory. A slow diffusion of oxygen does take place through these substances.

Special precautions are necessary when small aquatic animals, like protozoa, are used, or when processes are studied that go on even at minimal oxygen tension, like the elevation of the fertilization membrane in sea urchin eggs.

Kitching (1939a) devised suitable methods for protozoa; one of them will be described in some detail. It consists in passing oxygen-free gas around a hanging drop containing the organisms. The gas (hydrogen was mostly used), taken from a cylinder, was purified by being bubbled through concentrated sulfuric acid, dilute sulfuric acid, dilute potassium hydroxide and distilled water. It was then made oxygen-free by being passed through an electrically heated quartz-tube containing platinized asbestos. "Finally the gas was carried by pure lead tubing with seals of de Khotinsky cement through a closed glass wash-tube with distilled water in it to the chamber containing the organisms. The organisms were mounted in a hanging drop on a cover glass which was sealed with vaseline or a mixture of vaseline and paraffin wax to the chamber. The hanging drop was not allowed to touch the vaseline. The chamber itself consisted of a glass ring about 2 cm. in diameter and 1 cm. deep, closed underneath by a microscope slide to which it was sealed with de Khotinsky cement, and with glass inlet and outlet tubes. The upper edge of the ring was ground to support a cover glass, and the microscope slide which formed the base of the chamber fitted onto the mechanical stage of the microscope." The outflowing gas was bubbled through a suspension of marine luminous bacteria which luminesce at about 0.005 mm. of oxygen. They did not luminesce, however, in Kitching's

experiments, thus showing that if any oxygen remained, its tension must have been small indeed.

Barron (1932) devised a method for exposing the reproductive cells of marine invertebrates to anoxic conditions; (the method could also be used with larger organisms). He connected with glass tubing three specimen-bottles of 150 cc. capacity, tightly closed with rubber stoppers. The first bottle contained water and was connected with the nitrogen tank. The second bottle contained the eggs in 50 cc. sea water and the last bottle contained a solution of safranin T in Sörensen's phosphate buffer of pH 8.0 and some platinum asbestos. The safranin was first reduced to its leuco form by hydrogen (with platinized asbestos). After this had been done, the nitrogen, purified according to the method of Michaelis and Flexner (1928), was bubbled through the system for the whole duration of the experiment. As long as the safranin remains absolutely colorless, anaerobic conditions persist in the system. Barron (1932) points out that safranin has advantages (because of its low oxidation-reduction potential) over methylene blue or indophenol, reagents that are frequently used in their reduced form to indicate the absence of oxygen.

Concluding remarks. The method to be selected for a particular experiment will depend on the aim of that experiment as well as on the type of material used.

It seems to the present writer that sometimes misconceptions exist as to the term "life without oxygen" which unfairly influence the verdict on a particular work. Many investigations have as their primary purpose the study of the metabolic processes that take place when oxygen is excluded. In these cases—especially with organisms whose oxygen consumption begins to decline already at relatively high oxygen tensions—no serious error results if small traces of oxygen remain in the medium. In other cases, *e.g.,* in work with very small organisms that usually can obtain relatively significant

amounts of oxygen even at low tensions, the strictest precautions are necessary. To put, say, a worm of 5 g. weight into 50 cc. of a solution containing 0.1 cc. of oxygen per liter is one thing, while to put small rotatoria or protozoa (even in large numbers) in the same solution would be quite a different proposition. If an investigation is concerned with the question whether a certain process, for example, the cortical layer response of the echinoderm egg, can go on without oxygen, it will evidently be necessary to eliminate even the smallest traces of oxygen as far as this is technically possible. The same holds true if one investigates the possible influence of very small traces of oxygen on the survival of animals.

To remove even the last molecule of free oxygen that is dissolved in the medium or in the tissues of the experimental animal is an ideal obviously never realized. By selecting the proper methods and applying them correctly, however, one can at least approach this ideal condition.

3. ANAEROBIC METABOLISM.

A complete study of anaerobic metabolism requires the gathering of quantitative data about the changes that occur in the chemical composition of the organism subjected to anoxic conditions and in the medium in which it is kept. The gaseous, liquid and solid substances absorbed or excreted must be determined quantitatively and, as far as possible, identified. This necessitates the use of chemical, physical and physico-chemical methods of a very varied nature, which, however, differ fundamentally in no way from the methods employed in the study of aerobic metabolism. Hence they are not discussed here.

Only one point will be emphasized. Some phases of the anaerobic metabolism of invertebrates are frequently investigated by means of manometric methods in media containing bicarbonate. From the results thus obtained

it has been concluded, even by quite recent investigators, that an animal forms lactic acid when carbon dioxide is liberated. Such a conclusion is in many cases erroneous. The liberation of carbon dioxide from bicarbonate indicates only that an acid stronger than carbonic acid has been formed. The misunderstanding arises from the fact that many investigators simply generalize, applying to all tissues what happens in mammalian tissues. It is true that in the latter lactic acid is the only, or at least by far the most important, organic acid formed. In many invertebrates, however, lower or higher fatty acids, dibasic acids and others are formed instead of or in addition to lactic acid during the anaerobic carbohydrate metabolism. A chemical identification is therefore absolutely necessary if one wishes to make any specific statement as to the nature of the acids formed.

CHAPTER II

ANAEROBIC HABITATS

Most habitats in which, because of lack of oxygen, animals will be forced to gain their energy predominantly or exclusively through fermentations are aquatic or semi-aquatic. (We consider as semi-aquatic such "milieux" as the intestinal contents.) Oxygen-poor or oxygen-free terrestrial habitats are relatively rare.

1. SOILS

The oxygen content of soils seems in most cases to be too high to markedly impair the aerobic functions of the soil fauna. However, nearly anaerobic conditions lasting for several months were recently described by Boynton and Reuther (1938) for two orchard soils.

In swamplands low oxygen contents that may be of biological significance are encountered (Table 1).

TABLE 1

COMPOSITION OF THE GAS MIXTURE IN SOILS UNDER VARIOUS CONDITIONS (ACCORDING TO WAKSMAN, 1932).

The figures are averages of several determinations in per cent.

	Fallow land		Soil near roots of corn	Green manured land	Swamp rice land
	Before rainfall	After rainfall			
Nitrogen	78.05	78.83	80.15	79.18	85.59
Oxygen	20.40	19.26	9.00	7.71	0.54
Carbon dioxide	0.58	0.95	9.00	12.03	4.42
Hydrogen	0	0	0.73	0.07	6.42
Methane	0	0	0	0	2.81

The oxygen tension of a soil may be quite different before and after a rain. A saturation of the upper soil layers with water will impair the exchange between the atmosphere and the soil gases below the water-filled zone. The oxygen demand of the soil fauna and flora, as well as

inorganic oxidations will then rapidly reduce the oxygen content of the deeper soil layers. Ege (1916) found the oxygen tension of the air 30 cm. below the surface of a field to be 153 mm. of mercury; 6 and 11½ hours after a rainfall it had decreased to 64 and 46 mm. respectively; after 36 hours, however, it had risen again to 129 mm.

Rainwater itself entering the soil loses considerable amounts of oxygen in a relatively short time as shown by Merker's (1926) laboratory experiments. This loss is probably due primarily to biological oxidations. The oxygen deficiency so set up, is, according to this author, responsible for large numbers of earthworms coming to the surface after a rainfall.

Other terrestrial habitats where oxygen deficiencies can be expected are large masses of decomposing leaves, compost and similar aggregations of decaying organic material.

It is quite possible that in many soils anaerobic or near-anaerobic conditions prevail locally. This seems to be indicated by the many reports of anaerobic bacteria in soils (for a summary, see Waksman, 1932). To what extent such conditions may influence the soil fauna remains to be investigated.

2. WATER BASINS

A. Bottom of water basins. In nature small and usually shallow ponds and bogs frequently occur that are rich in decaying organic material. An analysis reveals that the bottom mud of these ponds and the water layers in immediate contact with it are frequently oxygen-free and contain hydrogen sulfide (the "Faulschlamm" of Lauterborn, 1908, 1916 and of Wetzel, 1928). The results of a study of one such habitat are presented in Table 2. The prevalence of anaerobic conditions is here largely caused by micro-organisms which develop in the decaying organic matter and which consume oxygen faster than it can be replaced through diffusion from the

surface. These water-basins represent the typical habitat of the sapropelic fauna.

TABLE 2

OXYGEN CONTENT OF THE WATER AT VARIOUS DEPTHS IN A SMALL SHALLOW POND (ACCORDING TO WETZEL, 1928).

(Wetzel's figures for his pond No. 11 have been averaged for each month.)

	Oxygen in cc/l.					
Depth in m.	Jan.	Feb.	March	April	May	June
0	1.6	2.0	4.3	3.2	2.1	0.6
0.05	0.0	0.1	0.9	2.1	1.8	0.5
0.15*	0.0	0.0	0.0	0.0	0.0	0.0

	Oxygen in cc/l.					
Depth in m.	July	August	Sept.	Oct.	Nov.	Dec.
0	0.2	0.3	0.3	0.4	1.2	.....
0.05	0.0	0.0	0.0	0.0	0.0	.....
0.15*	0.0	0.0	0.0	0.0	0.0	.....

*In mud of bottom.

Similar conditions are found occasionally in relatively large and deep fresh water basins. A large artificial lake, heavily polluted by waste material from factories, recently described by Liebmann (1938), represents an interesting case of this sort. To the same class of habitats belong sewage disposal plants. The contents of septic tanks and similar installations are typically anaerobic.

The bottom deposits of the oceans seem to be largely anaerobic, if we disregard the uppermost layers. The deeper strata have repeatedly been reported to be devoid of free oxygen (Moore, 1931; ZoBell and Anderson, 1936; ZoBell, 1939). In some cases oxidation-reduction potentials have been found which indicate that at least some deposits are even markedly reducing. Our knowledge concerning the vertical distribution of bottom in-

vertebrates and their respiratory habits is unfortunately too fragmentary to allow one to make definite statements concerning the biological importance of the oxygen-free bottom layers in the open sea. The wide distribution of anaerobic bacteria in the bottom material of the deep seas (Waksman, 1934; ZoBell and Anderson, 1936) is an indication that anaerobic conditions are perhaps a general feature of the bottom of all major oceans.

It is of interest to note that similar conditions occur near or on the seashore and it is especially significant that ZoBell and Feltham (1942) encountered several species of worms and other burrowing animals as deep as 40 cm., and still deeper in a mud flat, that is, at a depth at which there was no free oxygen and where the E_H was around − 0.2 volts. Severe oxygen deficiencies or complete lack of oxygen have been found in the water permeating the sand of sandy beaches. Such cases have been described in Denmark by Thamdrup (1935) and in North Carolina by Pearse, Hunm and Wharton (1942). Further investigations of similar localities would be promising in view of the rich burrowing fauna occurring on the sea shore.

Fleming and Revelle (1939), in discussing dissolved oxygen in the ocean, state: "The free dissolved oxygen content of the interstitial water in the sediments and immediately above the bottom will have a profound influence upon the conditions in the sediments and will also affect the bottom fauna. Where there is little or no oxygen, there can be little or no life other than bacteria." In view of the facts that will be presented in this monograph, such a generalization can hardly be understood.

B. Bulk of water basins. In many fresh water lakes periodic annual stagnation of the lower water masses occurs. This is primarily caused by a thermal stratification which effectively prevents an exchange between the water masses separated by the so-called thermocline.

There is some biological activity, nevertheless, in the lower strata, and it brings about a more or less pronounced depletion of oxygen. The oxygen content frequently sinks so low that the usual methods of determination indicate a total absence of it for weeks or even months. In winter a thick layer of ice often prevents the diffusion of oxygen from the atmosphere into the water. Ice also inhibits photosynthesis in aquatic plants, hence no oxygen becomes available from this source (*cf.* Birge and Juday, 1911 and Thienemann, 1927).

Occasionally lakes are found which exhibit a permanent stratification (*cf.* Yoshimura, 1937 and Smith, 1940). The lower water strata are then permanently anaerobic. In the case described by Smith (1940) at least some zooplankton organisms were caught in these layers.

Figures 1 and 2 show the annual oxygen variations in different layers of two lakes as illustrations of the transitory and of the permanent oxygen depletion characteristic of the deeper water masses. Many more data will

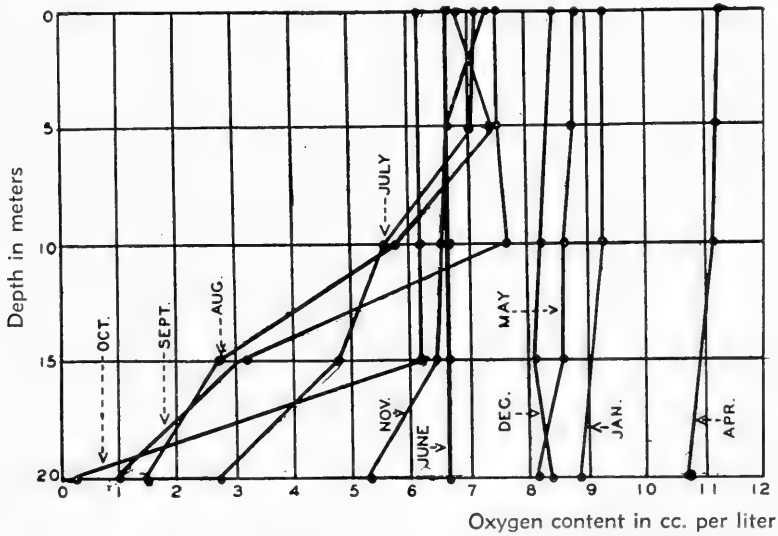

Fig. 1. Seasonal variations in oxygen content of a lake showing temporary oxygen deficiency in the lower strata. (Drawn from Berg's data, 1938.)

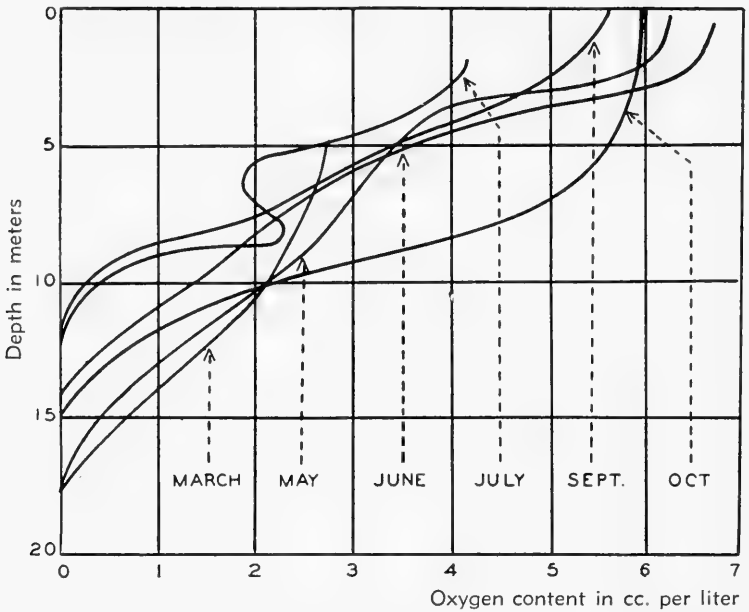

Fig. 2. Seasonal variations in oxygen content of a lake showing permanent oxygen deficiency in the lower strata. (Drawn from Smith's data, 1940.)

be found in the limnological literature and the reader is referred to standard textbooks of limnology (Thienemann, 1925; Welch 1935) for further information.

Fresh water streams will rarely be truly anaerobic. In heavily polluted streams, however, the oxygen may occasionally disappear more or less completely. Examples are given in Whipple's "Microscopy of drinking water" (1927).

The best-known anaerobic or near anaerobic salt water habitats are the lower strata of the Black Sea (below 200 m.), the Sea of Azov and the Caspian Sea. The former are entirely oxygen-free, the two latter are at least very poor in oxygen (Knipowitsch, 1922 and 1925; Nikitin, 1931). Extremely low oxygen values have also been reported from certain parts of the Baltic Sea (Ruppin, 1912) and from oyster basins in Norwegian waters (Helland-Hansen, 1923).

The oceanographic investigations of recent years have clearly demonstrated that in all major oceans a minimum concentration of oxygen occurs at mid-depth, but the reason for this curious phenomenon is still a matter of controversy. The degree of oxygen deficiency varies greatly in various oceans. In the western basin of the North Atlantic, for example, (Seiwell, 1934) the oxygen values are never very low and it seems questionable whether the conditions prevailing there have any influence upon the respiration of the local fauna. In parts of the South Atlantic (Wattenberg, 1929), and especially in the Eastern Pacific (Schmidt, 1925; Moberg, 1930 and 1930a; Ito, 1930; Thomsen, 1931; Thompson, Thomas and Barnes, 1934) extremely low values, frequently only small fractions of a cubic centimeter per liter, have been found. In the Western Pacific the conditions are less extreme. The conclusion that such very low oxygen values must have an influence upon the mode of energy production of the fauna—and it is known that an abundant plankton does occur in these layers (Schmidt, 1925)—seems inescapable. Experiments upon the oxygen requirements and anaerobic abilities of animals secured from these regions have never been performed. They should prove extremely interesting and it would not be too surprising if future investigation would reveal the oxygen-minimum layer as the most important of all anaerobic habitats (because of its large area). One may expect that the results of such experiments fall in line with the observation of Nikitin and Malm (1928) who reported (in a paper which unfortunately was not available) that the zooplankton from the oxygen-poor deeper layers of the Black Sea can be kept alive longer with less oxygen (5-6%) than species from the upper strata.

In Figure 3 are represented vertical sections of two water basins illustrating cases of oxygen minima. In one case (Gulf of California) larger animals should hardly be able to get sufficient oxygen for a purely aerobic

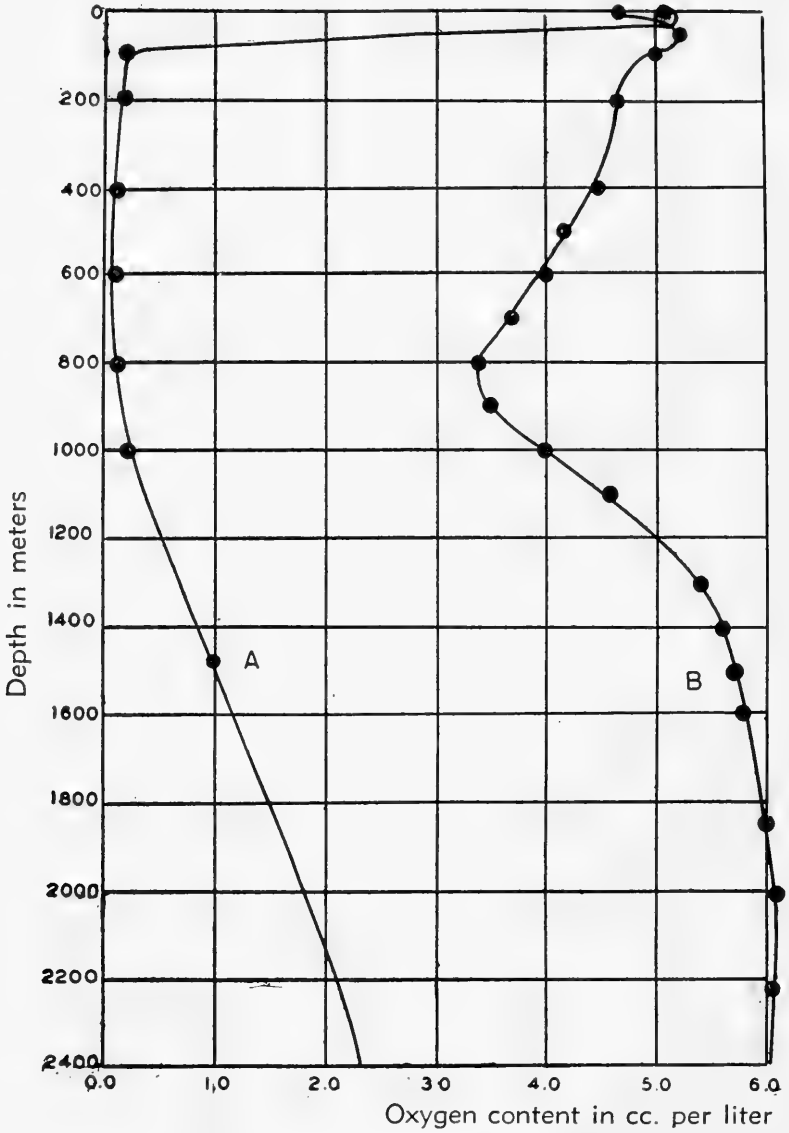

Fig. 3. *A.* Vertical oxygen distribution in the Gulf of California (20° 00′ N., 108° 16′ W.). (Drawn from Sverdrup, Johnson and Fleming's data, 1942.) *B.* Vertical oxygen distribution in the western basin of the North Atlantic (35° 07′ N., 66° 25′ W.) (From Seiwell, 1937.)

life while in the other (Atlantic Ocean) the oxygen demands of planktonic organisms are probably satisfied.

3. THE INTESTINAL TRACT

The intestinal tract of warm-blooded animals is an important habitat for a great variety of parasites. The contents of the intestine consist of more or less liquid masses and of gases. Under ordinary conditions both are quite poor in oxygen, as the analyses summarized in Table 3 and Table 4 show.

TABLE 3

OXYGEN CONTENT OF GAS MIXTURES FOUND IN THE INTESTINAL TRACT OF WARM-BLOODED ANIMALS.

Most figures represent mean values of several determinations.

Animal	Part of alimentary tract	Oxygen in volumes per cent.	Source
Cattle	Rumen	0.2	Tappeiner, 1883
Goat	"	0.03	" "
Sheep	"	0.0	" "
Horse	Stomach	0.1	" "
Rabbit	"	0.0	" "
"	"	0.8	Planer, 1860
Dog	"	0.8	" "
Man	"	9.0	Ewald & Rubstein, *fide* Bardier, 1931
Horse	Small intestine	0.7	Tappeiner, 1883
Cattle	" "	0.0	" "
Pig	" "	5.5	Long & Fenger, 1917
"	" "	4.2	von Brand & Weise, 1932
Dog	" "	0.2	Planer, 1860
Horse	Large intestine	0.04	Tappeiner, 1883
Cattle	" "	0.0	" "
Goat	" "	0.03	" "
Sheep	" "	0.0	" "
Rabbit	" "	0.6	" "
Dog	" "	0.0	Planer, 1860

The source of the oxygen found in the liquid or gaseous intestinal contents seems to be twofold. Some oxygen is always introduced with the air that is swallowed together with the food. Most of it seems, however, to disappear rapidly already in the stomach, partly through absorp-

tion and probably in part through consumption by the bacterial flora of the gastric region. Occasionally larger amounts of oxygen from swallowed air seem to reach the small intestine. Von Brand and Weise (1932) explain in that manner the findings of Long and Fenger (1917), and their own, that oxygen is present, sometimes in appreciable amounts, in the small intestine of slaughtered pigs.

TABLE 4

OXYGEN CONTENT OF THE FLUID INTESTINAL MASSES OF WARM-BLOODED ANIMALS.

Most figures represent mean values of several determinations.

Animal	Part of intestinal tract	Oxygen in volumes per cent.	Source
Horse	Small intestine	0.024	Toryu, 1934
Cattle	" "	0.013	von Brand & Weise, 1932
Sheep	" "	0.012	" " " "
Pig	" "	0.083	" " " "
Dog	" "	0.028	" " " "
Cattle	Large intestine	0.010	" " " "
Pig	" "	0.0	" " " "

The other possibility is that oxygen may diffuse from the intestinal wall into the lumen. McIver, Redfield and Benedict (1926) showed that if oxygen-free nitrogen or hydrogen is introduced into an isolated loop of the small intestine of a cat the gas remaining after periods varying from 1 to 7½ hours contains rather large percentages of oxygen. They found 2.0 to 5.7% after injection of nitrogen, and 2.9 to 15% after injection of hydrogen.

It seems somewhat difficult to reconcile the presence of such large amounts of oxygen with the results of the analyses of naturally formed gases as shown in Table 3. One possible explanation of the difference may be that the animals for which analyses of normal intestinal gases have been recorded either are larger than cats or are

herbivorous animals. In the first case the oxygen has to diffuse over wider distances, whereas in the intestine of herbivores a richer microbial flora may be present. Due to oxygen absorption by the bacteria a larger accumulation of oxygen may be prevented, even if small amounts constantly flow from the wall to the lumen.

In general, the regular presence of hydrogen and methane in the intestinal gases, both typical end products of incomplete oxidations or of fermentations, together with the regular occurrence of reduction processes in the intestine, seem to indicate rather clearly that there can be no abundant supply of oxygen under natural conditions The fact reported by Jahn (1933) that the E_H values in the rat intestine lie well within the anaerobic range is in agreement with this view. According to Campbell (1931) the oxygen tension of the intestinal lumen is practically nil, if food is present. Weighing the entire available evidence, hardly a doubt can remain that the intestinal tract of warm-blooded vertebrates should be regarded as a habitat normally very poor in oxygen, although it is in many cases not purely anaerobic.

Similar conditions will probably be found in the intestine of cold-blooded vertebrates and of large invertebrates, such as large snails, squids and similar organisms although the lack of oxygen might be not quite so pronounced as in warm-blooded vertebrates. Unfortunately no precise data seem to be available.

One would expect that the intestinal tract of smaller invertebrates should be a habitat more favorable to aerobic life than that of larger animals. One element of evidence pointing in this direction is the observation made by most (although not all) investigators, that the developmental stages of the Leishmanias multiply in culture only under aerobic conditions. These organisms are found normally in the intestinal tract of sandflies. But no generalizations are possible, since other evidence points in the opposite direction. Trager (1934) reports

that termite flagellates are rapidly killed when they come in close contact with the atmospheric air. They can be cultured only under reduced oxygen tension or under truly anaerobic conditions. This evidently can be interpreted as an indication that the oxygen tension, at least in the termite intestine, must be low. The definite solution of this problem will have to come from direct determinations, which doubtless could be performed with modern micro methods, at least in some animals.

4. TISSUES AND SECRETIONS

A great variety of parasitic invertebrates have become established in the tissues and secretions (*e.g.*, urine or bile) of host animals. These habitats are seldom truly anaerobic; it is therefore only with a certain reserve that they are mentioned here.

TABLE 5

GAS TENSION IN VARIOUS ORGANS OF WARM-BLOODED ANIMALS.

Animal	Organ	Gas tensions in mm. Hg		Source
		CO_2	O_2	
Fowl	Subcutaneous tissues	46	25	Campbell, 1931
Rabbit	" "	49	23	" "
Rat	" "	52	24	" "
Guinea Pig	" "	52	20	" "
Hedge Hog	" "	51	27	" "
Cat	" "	43	22	" "
Monkey	" "	49	40	" "
Man	" "	40	43	" "
"	" "	45	22	Seevers, 1936
"	" "	41	37	Meyer, 1935
Rabbit	Peritoneal cavity	47	37	Campbell, 1931
Rat	" "	52	33	" "
Guinea Pig	" "	56	28	" "
Cat	" "	41	29	" "
Monkey	" "	49	40	" "
"	Pleural cavity	56–60	30–39	" "
Rabbit and Cat	Mucous membrane of stomach	40–60	10–20	Campbell, 1932
Rabbit	Mucous membrane of small intestine	35–60	20–40	" "
"	Bladder	37–63	33–61	Campbell, 1928
Monkey	"	47	42	" "

TABLE 6

GAS TENSION IN VARIOUS ORGANS OF COLD-BLOODED VERTEBRATES.

Animal	Organ	Temperature	Gas tensions in mm. Hg		Source
		°C	CO_2	O_2	
Toad	Subcutaneous tissues	3	4	100	Campbell, 1931
"	"	17	12	49	" "
"	"	23	14	63	" "
Frog	"	17	14	48	" "
Snake	"	15	21	24	" "
Tortoise	"	15	23	20	" "

Representative figures concerning the oxygen tension prevailing in the tissues of vertebrates have been assembled in Tables 5 and 6. In evaluating the significance of these figures it should be kept in mind that the oxygen tension of a normal atmosphere at sea level is 158 mm. Hg. It is therefore conceivable that the oxygen tension in the tissues of warm-blooded vertebrates, with values ranging from 10 to 45 mm. Hg., is low enough to influence the metabolism of at least some larger parasites.

Campbell (1931) has furthermore pointed out that inflammatory processes lower considerably the oxygen tension of tissues. A similar condition might well prevail in cases of various pathological changes caused by the presence of parasitic protozoa or helminths. This problem would deserve further experimentation.

The data available for cold-blooded vertebrates show that in some organisms (frogs and toads) the oxygen tension of the subcutaneous tissues is somewhat higher than that found in warm-blooded vertebrates but that in other organisms (snakes and tortoises) it is about the same. A complicating factor in the former group is that the oxygen tension of these tissues seems to be dependent on the temperature. At low temperatures the oxygen tension appears to be quite high, so high, in fact,

that almost any parasite should be able to secure all the oxygen it needs.

Little information is available concerning the tissues of invertebrates. Campbell (1931) has collected some data on the oxygen tension under the chitin of various species of insects. The values vary from 2 to 18 per cent of that found in a normal atmosphere.

In bulky animals lacking a circulatory system the oxygen tension of the deeper layers of tissues must be zero or nearly zero. As Krogh (1916) pointed out, the supplying of oxygen to the deeper tissue layers is difficult in this and in similar cases, since the animals depend entirely on the diffusion of oxygen from the surface. Hence only the superficial cell layers receive an ample supply of oxygen, little or none at all reaches the deeper layers. The latter, therefore, must have a more or less pronounced anaerobic metabolism. It is obvious that any parasite that may be found in such tissues will have to lead an anaerobic life.

TABLE 7

GAS TENSION IN NORMAL URINE OF MAN.

Gas tension in mm. Hg		Source
CO_2	O_2	
.....	20–35	Krogh, 1916
40–50	14–35	Campbell, 1928
45	40–60	Buckmaster and Hickman, 1928
58–86	15–43	Sarre, 1937

In so far as body secretions are concerned, little reliable information is available. In urine (Table 7) a moderate amount of oxygen is always present. Less oxygen occurs in concentrated than in dilute urine.

The determination of oxygen in other secretions, like milk or bile, presents difficulties because this gas has a tendency to disappear rapidly in these fluids. An explanation for this phenomenon seems not yet to have been offered. It is obvious that data on specimens that

are not absolutely fresh are not reliable. Von Brand and Weise (1932) carried out a series of analyses on the bile of freshly killed cattle, sheep and dogs. The values were always very low and ranged from 0 to 0.084 cc. in 100 cc. bile. In the case of cattle and sheep, ½ hour elapsed between the securing and the analyzing of the samples. The possibility that some oxygen might have disappeared spontaneously cannot be excluded with certainty. The bile of two dogs, however, was analyzed immediately after death and both samples were found to be oxygen-free. It can therefore be concluded that the bile is, at best, a habitat extremely poor in oxygen.

Occasionally invertebrates are found inside the tissues of living plants. In most cases they probably have access to a sufficient amount of oxygen, but exceptions may occur. Ege (1916) found in the roots and rhizomes of aquatic plants oxygen tensions as low as 15 mm. Hg during the summer and 4 mm. during the winter.

CONCLUDING REMARKS

Only when animals are found in environments absolutely devoid of oxygen and only as long as they remain there can one be reasonably certain that they lead a truly anaerobic life. But even when the environment contains no oxygen aerobic life is sometimes possible. Thus protozoa harboring symbiotic algae may be found in anaerobic surroundings. If sufficient light is present there is no reason to suppose that these protozoa would not be able to use the oxygen produced by their symbionts during photosynthesis.

Most of the habitats mentioned in the preceding paragraphs are not absolutely oxygen-free. They are only poor in oxygen to a more or less marked degree. Animals found in habitats of this type should not *a priori* be assumed to lead a truly anaerobic life. Neither can one assume that fermentations would necessarily have a preponderance over aerobic oxidations. Each case must receive individual attention. Only then can errors and con-

fusion be avoided. A few examples will illustrate this.

Of two species of animals living in the same oxygen-poor habitat, such as the mud of a pond, one, which is equipped with a sufficiently powerful respiratory pigment, may lead an aerobic life since, with the help of the pigment, it can secure sufficient oxygen even at low tensions, while the other, lacking a respiratory pigment, may be forced to a metabolism characterized by the preponderance of anaerobic reactions.

Size, too, may determine whether aerobiosis or anaerobiosis prevails. Von Brand (1938) has pointed out that the long controversy as to whether intestinal worms do or do not live anaerobically is rather pointless. Large helminths, like *Ascaris,* living at the low oxygen tensions prevailing in the intestine, cannot get enough oxygen for a predominantly aerobic life. Small ones, on the other hand, like *Ostertagia* (Davey, 1938) probably can. Similar considerations apply to tissue parasites. *Endamoeba histolytica,* after having penetrated the tissues, can, without question, secure the same amount of oxygen as any of the surrounding cells of the host. On the other hand, a relatively large arthropod, like the maggot of *Cordylobia,* may get only a very limited oxygen supply as long as it is tunnelling under the intact skin of the host. These examples may suffice; they could easily be multiplied.

Conversely, one is not justified in assuming that an organism living normally in surroundings rich in oxygen will always lead a purely aerobic life. Trypanosomes furnish a case in point. At one period of their life cycle they live in the blood stream of vertebrates, a habitat that certainly is rich enough in oxygen. Nevertheless their metabolism is by no means purely aerobic; on the contrary, surprisingly large amounts of sugar are metabolized by them by fermentative processes. Other examples are the malaria parasites where the situation appears to be similar.

CHAPTER III

SURVEY OF INVERTEBRATES FOR ANAEROBIOSIS

I. PROTOZOA

1. NON-PARASITIC PROTOZOA

A. Occurrence in anaerobic habitats. In most cases in which it is claimed that non-parasitic protozoa can live anaerobically, this conclusion was reached after the animals had been found in surroundings that appeared oxygen-free with the currently accepted methods for oxygen analysis. In some instances more refined methods failed to show molecular oxygen. However, even if traces of oxygen have been overlooked, there is hardly a doubt that in most cases the quantity was too small to exert any significant influence on the direction of the metabolism. This applies especially to the organisms mentioned below that are injured by oxygen. So the claim that protozoa occurring in apparently anoxic habitats may be true anaerobes seems justified.

One of the most important anaerobic protozoan faunas is represented by the *sapropelic protozoa* (*cf.* Appendix, Table 1), among which the most characteristic types are ciliates (Lauterborn, 1908, 1916; Noland, 1927; Wetzel, 1928). However only those ciliates should be regarded as truly anaerobic, *i.e.*, dependent on anaerobic metabolism or capable of a permanently anaerobic life, which live actually within the mud or in the thin water layer in immediate contact with it. Here Wetzel (1928) found the following forms: various species of *Metopus, Caenomorpha, Plagiopyla, Epalxis, Discomorpha* and *Saprodinium, Lagynus elegans, Dactylochlamys hystrix, Chaenia binucleata, Pelomyxa bütschlii* and *Ludio parvulus.* He considers, in addition, as regular members of the anaerobic layer the following ciliates observed by Lauterborn (1916): *Chaenia limicola, Legendrea loyesae,*

Spathidium lieberkühni, Perispira ovum, Lacrymaria aquae-dulcis and *Dactylochlamys pisciformis.*

In the upper layers of the shallow sapropelic waters, varying amounts of oxygen do occur and many other protozoa are found there, as shown in the Appendix, Table 1, but it seems likely that these will have to endure from time to time periods of severe or even complete lack of oxygen. The same is probably true of the green forms which, during the night, cannot obtain oxygen through photosynthesis.

It is unfortunate that the culturing of typical sapropelic ciliates has so far not been possible; a real knowledge of the oxygen relationships of these animals can be expected only from experiments under controlled laboratory conditions. We have, at the present, only some indications, from studies by Wetzel (1928) and by Liebmann (1936a) that members of the genera *Metopus, Caenomorpha, Plagiopyla* and *Discomorpha* are injured by oxygen.

A protozoan fauna similar in some respects to the sapropelic fauna is found in *sewage tanks* (Lackey, 1924, 1925, 1926, 1932; Liebmann, 1936, 1936a). But again it should be emphasized that not every protozoon found there is by necessity adapted to a permanently anaerobic life. Some animals are carried into the tanks with the incoming, more dilute and oxygen-containing sewage material. Such organisms will die rather rapidly or form cysts as soon as the oxygen disappears completely. Others are truly anaerobic; they occur regularly in the deeper anaerobic parts of the tanks and thrive there (*cf.* Appendix, Table 2).

Lackey (1932) made an experimental comparative study of the behavior of an *Opercularia* species, which normally is abundant in the aerobic trickling filters but is not found, in the motile form, within the tanks, and of the flagellate *Trepomonas agilis,* which is characteristic of the deeper anaerobic layers of the lat-

ter. He introduced sewage material containing both species into tall cylinders; one cylinder was then aerated by means of compressed air forced through porous plates at the base, while the other remained unaerated. The results of these experiments are shown in Table 8. A striking difference between the two forms is evident. *Trepomonas* disappeared only in the aerated culture and *Opercularia* only in the non-aerated one. Lackey is probably correct in assuming that in these experiments the presence or absence of oxygen was the deciding factor, for it is known that other factors, such as variations in pH, temperature, food, and gases other than oxygen, do not have much influence on the organisms experimented upon.

TABLE 8

ACTIVE *Trepomonas* AND *Opercularia* IN AERATED AND NON-AERATED SEWAGE (ACCORDING TO LACKEY, 1932).

Time after beginning of experiment. (hours)	Species	Number per cc.	
		Aerated	Non-aerated
6	*Trepomonas*	140	No counts
	Opercularia	3200	0
24	*Trepomonas*	0	3300
	Opercularia	12600	0
48	*Trepomonas*	0	4500
	Opercularia	9300	0
120	*Trepomonas*	0	5400
	Opercularia	8300	0
148	*Trepomonas*	0	4000
	Opercularia	4500	0
172	*Trepomonas*	0	2000
	Opercularia	4500	0

Another anaerobic habitat for non-parasitic protozoa is represented by the stagnating strata of *fresh water lakes* (Juday, 1908; Birge and Juday, 1911; Imel, 1915; Moore, 1939; Zhinkin, 1930). Most, but not all protozoa reported from this habitat (*cf.* Appendix, Table 3) are regularly found also in well-aerated surroundings. It seems, therefore, obvious that they live aerobically when oxygen is present, and that, in the absence of oxygen,

they can live anaerobically for several weeks. It has sometimes been assumed that significant amounts of oxygen remained undetected in the above-mentioned strata. But reliable investigators like Birge and Juday are of the opinion that such is not the case. Furthermore, it is especially significant that at least one typically anaerobic ciliate has been found in this kind of habitat. Juday (1919) encountered in Lake Mendota (Wisconsin) a protozoon probably belonging to the genus *Enchelys* which occurred only when the water appeared free of oxygen (with the testing method he used). Later Liebmann (1936a) identified Juday's organism as *Enchelys vermicularis* Smith, a typical anaerobic sewage ciliate.

B. Relationship between availability of oxygen and its utilization. Among protozoa there probably occur all gradations between the anaerobic forms mentioned above and the organisms capable of leading normally only an aerobic existence. *Strombidium* may serve as an example of the latter class. It disappears from cultures, according to the findings of Galadziev and Malm (1929), when the oxygen disappears. It is probably safe to assume that all protozoa living in clear and well-aerated water are, normally, aerobic, though they may exhibit a certain tolerance for experimentally induced anaerobic conditions. Some forms try to escape oxygen deficiencies by wandering away. *Vorticella nebulifera* forms a posterior circle of cilia, separates from its stalk and swims around with the help of the newly formed cilia, until oxygen is restored to the medium. This mechanism will enable such a normally sessile organism to search in nature for a new place of attachment in surroundings having sufficient oxygen.

Loxodes rostrum seems to be a typical intermediate form. The oxygen relationships of this ciliate in its natural habitat were studied by Rylov (1923). He encountered most of the specimens in water strata containing

oxygen in concentrations varying from 9.15 to 45 per cent of saturation. The organisms avoided water containing oxygen in amounts greater than 60 per cent saturation and occurred in small numbers in zones containing no free oxygen at all. This last observation demonstrates definitely that *Loxodes* is able to lead an anaerobic life. The significance of the other figures is not quite clear. One might be tempted to interpret them as indicating a toxicity of oxygen at moderate tensions, but, as will be seen below, such an assumption would be premature without further evidence. As long as the relationship of the oxygen consumption of *Loxodes* to the tension has not been investigated, it is hardly possible to draw any definite conclusions as to the type of metabolism prevailing in these ciliates at those oxygen tensions which they seek by preference. In view of the small size of *Loxodes,* one would *a priori* be inclined to assume that they could get sufficient oxygen for a purely aerobic life even at relatively low tensions. The experiments of Galadziev and Malm (1929) on marine protozoa confirm this view. According to these investigators the oxygen tension has no marked influence upon protozoa. Lund (1918), Amberson (1928) and Adolph (1929) also found the oxygen consumption of *Paramaecium* and *Colpoda* to be independent of the tension. In *Spirostomum,* on the other hand, the situation is different. The respiration of this ciliate is clearly dependent on the oxygen tension, as Specht (1935) has proven.

The interpretation of such observations as those of Rylov requires great caution. Pütter (1904) noted that *Spirostomum* dies when kept in shallow dishes and he concluded that oxygen was toxic for this ciliate. Saunders (1924), however, showed convincingly that this conclusion was faulty and he explains the death of *Spirostomum,* in Pütter's experiments, by a change in the pH of the culture water. When the latter is in contact with air its pH may reach 8.0, a value which is indeed lethal.

In buffer solutions of pH 7.3 the animals can be kept alive in shallow dishes. Specht (1935) even found that they disintegrate in environments completely devoid of oxygen. He is of the opinion that they survive at low oxygen tensions, because their normal rate of oxygen consumption is low.

C. Resistance in experimentally induced anaerobic conditions. Many data are available on the survival of protozoa under experimental anaerobic conditions. They have been collected in Table 9. In the following paragraphs observations concerning the reactions to lack of oxygen of normally aerobic protozoa will be discussed separately for flagellates, rhizopods, ciliates and the cysts of these organisms.

(a) Flagellates. Little is known about flagellates in this connection. Alexander (1931) found that *Euglena gracilis* became motionless within 24 hours if kept anaerobically in the dark. To experiment in darkness is of course necessary with forms capable of producing oxygen by photosynthesis. Von Dach (1940), on the other hand, succeeded in culturing a colorless euglenoid, *Astasia klebsii,* under nearly anaerobic conditions. The organisms grew well, although more slowly than in control cultures. A correct evaluation of these experiments is impossible, since the data at hand do not allow one to decide whether the absence of oxygen was complete enough throughout the course of the experiments to inhibit aerobic metabolism. At low temperatures various flagellates seem to survive quite well under anaerobic conditions (Lindeman, 1942).

(b) Rhizopods. Rhizopods have been used more extensively than flagellates for studies on anaerobiosis. Ishikawa (1912), Pantin (1930, 1930a), Hulpieu (1930) and Kitching (1939, 1939b) have experimented with various species of amoebae. Regardless of whether the large *Amoeba proteus* or small amoebae of the *limax* type were used, the amoeboid movements continued un-

der strictly anaerobic conditions for several hours. Eventually, however, the animals became motionless, but they recovered if oxygen was readmitted. The time required for recovery depended upon the duration of the preceding anaerobic period. Variations in sensitivity towards lack of oxygen seem to occur in different species. *Flabellula,* for instance, became motionless in a very short time and the activity of its digestive vacuoles ceased rapidly (this phenomenon, too, was reversible). On the contrary, the ability of *Pelomyxa* to live anaerobically is quite pronounced. Lindeman (1942) found that this organism survived for at least 60 days in anaerobic cultures.

Regarding other free-living rhizopods only a few data, obtained by Harvey (1926) in his experiments with radiolarians, are available. He reports that *Thalassicola* and *Colozoun* can be kept for 45 minutes under strictly anaerobic conditions and that these organisms will still luminesce whenever the culture is shaken.

(c) C i l i a t e s . A considerable number of experiments (Table 9) were made with a variety of ciliates, like *Paramaecium, Colpoda, Vorticella,* etc., which, under ordinary circumstances, lead a clearly aerobic life (Loeb and Hardesty, 1895; Pütter, 1905; Löhner, 1913; Fortner, 1924; Kalmus, 1928; Fauré-Fremiet, Léon, Mayer and Plantefol, 1929 and 1929a; Galadziev and Malm, 1929; Nikitinsky and Mudrezowa-Wyss, 1930; Lwoff, 1932; Gersch, 1937; Kitching, 1939, 1939a, 1939b).

The experimental evidence accumulated by these investigators is unfortunately somewhat contradictory. Gersch (1937) found that anaerobic conditions could be tolerated for only a few seconds, an observation which led him to a sharp and, it would seem, somewhat unjustified criticism of the work of his predecessors. A conclusion at the other extreme was reached by Pütter (1905), Nikitinsky and Mudrezowa-Wyss (1930) and Lindeman (1942). Pütter kept *Paramaecium* up to 10 days and

Colpidium up to 16 days under anaerobic conditions. Lindeman found that *Frontonia* and *Colcps* could tolerate an anaerobic medium for 30 days at 10°C. According to most other authors the survival periods range from several hours to several days.

It is quite certain that many of the variations observed are correlated with the conditions under which the experiments were conducted, but it is difficult to explain all differences in a satisfactory manner. It seems doubtful whether, in all cases, the oxygen was excluded sufficiently, in particular in Pütter's (1905) and Nikitinsky and Mudrezowa-Wyss' (1930) experiments. Lindeman's (1942) procedure appears more satisfactory. As to the reasons for Gersch's failure to keep his animals for any length of time in the absence of free oxygen, they remain obscure.

Fauré-Fremiet, Léon, Mayer and Plantefol (1929) and Lindeman (1942) point out that *Paramaecium* and other ciliates survive anaerobic conditions at 0 to 5°C better than at higher temperatures—obviously on account of the lowered metabolic rate. Pütter (1905) and Barbarine (1938) found that nutritional conditions are very important, starving animals dying much more rapidly than well-fed ones. This, too, is not surprising since during anaerobiosis larger amounts of reserve substances have to be mobilized than in the presence of oxygen, in order to furnish sufficient energy through fermentations.

Pütter (1905) observed also that Paramaecia suspended in a large amount of fluid survive anaerobic conditions better than if they are confined in a small amount of medium, an observation suggesting that toxic products may accumulate in the medium. It would be worthwhile to repeat these experiments with bacteriologically sterile cultures of ciliates in order to decide whether such toxic products result from the anaerobic processes of the infusoria themselves or whether they must be attributed to contaminants of bacterial origin. This question is

important since the formation of toxic products can easily simulate a sensitivity to the lack of oxygen. Kalmus (1928) explains the rapid death of Paramaecia which he had described in a previous paper (Kalmus, 1927) by this mechanism. The animals had been confined in the absence of oxygen in a minute quantity of fluid, in a capillary tube.

(d) C y s t s . It seems likely that cysts of protozoa would be less sensitive to lack of oxygen than the active forms because of their reduced rate of metabolism, but this is again a point where but little information is available. Alexander's (1930) observation that anaerobically kept *Euglena gracilis* encyst but nevertheless die quickly cannot be considered a proof of the sensitivity of the cysts. The organisms may have already been injured fatally before the cysts were fully formed. Lindeman (1942) is of the opinion that protozoa are not able to withstand anaerobic conditions in the cyst stage. There seems, however, to be no certainty that he was actually dealing with cysts in his experiments. His account might just as well be interpreted as meaning that the species used by him were not able to form cysts under anaerobic conditions.

Van Rooyen (1932), on the contrary, was probably dealing with cysts when he kept plate cultures of *Hartmanella castellani* under anaerobic conditions for a week. During this time no multiplication took place, but multiplication was resumed after the restoration of aerobic conditions (this is why one can assume that the amoebae were encysted).

Becquerel (1936) subjected dried earth containing what must have been the resting stages of various amoebae, of *Paramaecium,* of *Euglena viridis* and of *Actinophrys,* for 3 months to a high vacuum (10^{-5} mm. of mercury at 35°C) and found—even after exposure to extremely low temperatures—that the organisms survived. Similar ex-

TABLE 9

MAXIMAL SURVIVAL TIME OF NON-PARASITIC PROTOZOA UNDER ANAEROBIC CONDITIONS[1].

Species	Temp. °C	Strictly anaerobic conditions?	Survival time in days	Source
Sarcodines				
Acanthocystis sp.	0 and 5	Yes	>30 <60	Lindeman, 1942
" "	10	"	<30	" "
Actinophrys sol (dry cysts)	35	"	>90	Becquerel, 1936
Amoeba dactylifera (cysts)	35	"	>90	" "
" *geminata*	Room temp.	?	2	Nikitinsky & Mudrezowa-Wyss, 1930
" *limax* (dry cysts)	35	Yes	>90	Becquerel, 1936
" *proteus* (dry cysts)	35	"	>90	" "
Arcella vulgaris	0 and 5	"	>30 <60	Lindeman, 1942
" "	10	"	>30	" "
Centropyxis sp.	0 and 5	"	>30 <60	" "
" "	10	"	<30	" "
Dinamoeba sp.	0 and 5	"	>30 <60	" "
" "	10	"	<30	" "
Hartmanella castellani (cysts?)	Room temp.	"	> 8	Van Rooyen, 1932
Pelomyxa palustris	0 and 5	"	>60	Lindeman, 1942
" "	10	"	<30	" "
Flagellates				
Astasia klebsii	Room temp.	No	< 8	Von Dach, 1940
Ceratium hirudinella	" "	?	18 hrs.	Nikitinsky & Mudrezowa-Wyss, 1930
Cryptomonas erosa	" "	?	11	" " " "
Eudorina elegans	" "	?	5	" " " "

1) In this and the following tables of similar character the organisms belonging to larger taxonomic groups have been arranged in alphabetical order in order to enable the reader to find out rapidly whether an organism has been tested.

TABLE 9 — (Continued)

Species	Temp. °C	Strictly anaerobic conditions?	Survival time in days	Source
Euglena deces	0 and 5	Yes	>30 <60	Lindeman, 1942
" *gracilis*	Room temp.	?	1	Alexander, 1931
" *gracilis* (dry cysts)	35	Yes	<90	Becquerel, 1936
" *viridis*	Room temp.	?	7	Nikitinsky & Mudrezowa-Wyss, 1930
Glenodinium cinctum	0 and 5	Yes	>30 <60	Lindeman, 1942
Heteronema acus	0 and 5	"	>30 <60	" "
" sp.	0 and 5	"	>30 <60	" "
" "	10	"	>30	" "
Phacus pyrum	0 and 5	"	>30 <60	" "
Polytoma uvella	Room temp.	?	4	Nikitinsky & Mudrezowa-Wyss, 1930
Synura uvella	" "	?	1	" " " "
Trachelomonas euchora	0 and 5	Yes	>30 <60	Lindeman, 1942
Volvox globator	0 and 5	?	3	Nikitinsky & Mudrezowa-Wyss, 1930
Ciliates				
Amphileptus claparedei	Room temp.	?	10	Nikitinsky & Mudrezowa-Wyss, 1930
Aspidisca lynceus	" "	?	17	" " " "
Bursaria truncatella	0 and 5	Yes	>30	Lindeman, 1942
" "	10	"	<30	" "
" "	Room temp.	"	4.5 hrs.	Liebmann, 1936
Caenomorpha medusula	" "	"	3	Wetzel, 1928
Carchesium lachmanni	" "	?	3 hrs.	Nikitinsky & Mudrezowa-Wyss, 1930
Chilodon cucullus	" "	?	13	" " " "
" *uncinatus*	" "	?	13	" " " "
Coleps hirtus	" "	?	16	" " " "
" "	0 and 5	Yes	>30	Lindeman, 1942
" "	10	"	>30	" "

TABLE 9 — (Continued)

Species	Temp. °C	Strictly anaerobic conditions?	Survival time in days	Source
Colpidium colpoda	Room temp.	?	4	Nikitinsky & Mudrezowa-Wyss, 1930
" "	" "	?	16	Pütter, 1905
" "	" "	Yes	15	Liebmann, 1936
Cyclidium glaucoma	" "	?	>30	Nikitinsky & Mudrezowa-Wyss, 1930
Didinium nasutum	" "	?	> 2	" " " "
Discomorpha lauterborni	" "	Yes	3	Wetzel, 1928
Enchelys pupa	" "	?	5	Nikitinsky & Mudrezowa-Wyss, 1930
Euplotes charon	" "	?	12	" " " "
Frontonia leucas	0 and 5	Yes	>30	Lindeman, 1942
" "	10	"	>30	" "
Glaucoma pyriformis	Room temp.	"	3	Liebmann, 1936
" *scintillans*	" "	?	8	Nikitinsky & Mudrezowa-Wyss, 1930
Halteria grandinella	" "	?	6	" " " "
Loxocephalus granulosus	0 and 5	Yes	>30	Lindeman, 1942
" "	Room temp.	?	8	Nikitinsky & Mudrezowa-Wyss, 1930
" "	10	Yes	<30	Lindeman, 1942
Loxodes rostrum	0 and 5	"	>30	" "
" "	10	"	<30	" "
Loxophyllum meleagris	Room temp.	?	> 3	Nikitinsky & Mudrezowa-Wyss, 1930
Metopus contortus	" "	Yes	3	Wetzel, 1928
" *sigmoides*	" "	"	3	" "
" *spiralis*	0 and 5	"	>30	Lindeman, 1942
" "	10	"	<30	" "
Oxytricha pelionella	Room temp.	?	12	Nikitinsky & Mudrezowa-Wyss, 1930
Paramaecium aurelia	" "	?	4	" " " "
" *bursaria*	" "	?	16	" " " "
" " (cysts)	35	Yes	>90	Becquerel, 1936
" *caudatum* (starving)	Room temp.	?	1 hr.	Barbarine, 1938
" " (fed)	" "	?	> 2	" "

TABLE 9 (Continued)

Species	Temp. °C	Strictly anaerobic conditions?	Survival time	Source
Paramaecium caudatum	Room temp.	Yes	10 sec.	Gersch, 1937
" "	24–28	"	12 hrs.	Kitching, 1939a
" "	Room temp.	"	3	Liebmann, 1936
" "	0 and 5	"	>30	Lindeman, 1942
" "	10	"	<30	" "
" "	Room temp.	?	18	Nikitinsky & Mudrezowa-Wyss, 1930
" "	" "	?	10	Pütter, 1905
" *multimicronucleatum*	24–28	Yes	12 hrs.	Kitching, 1939a
" sp.	4	?	4	Fauré-Frémiet *et al.*, 1929
" "	15	?	2	" " "
" "	25	?	1	" " "
" "	Room temp.	?	> 1	Loeb & Hardesty, 1895
Plagiopyla nasuta	" "	Yes	3	Wetzel, 1928
Spirostomum ambiguum	" "	?	2	Pütter, 1905
" "	0 and 5	Yes	>30	Lindeman, 1942
" "	10	"	<30	" "
" "	Room temp.	?	13	Nikitinsky & Mudrezowa-Wyss, 1930
" *teres*	" "	?	12	" " " "
" "	0 and 5	Yes	>30	Lindeman, 1942
" "	10	"	<30	" "
Stentor coeruleus	0 and 5	"	>30	" "
" "	10	"	<30	" "
" "	Room temp.	?	> 2	Nikitinsky & Mudrezowa-Wyss, 1930
" *polymorphus*	" "	?	1	" " " "
Stylonichia mytilus	" "	?	12	" " " "
Uroleptus musculus	" "	?	> 2	" " " "
Uronema marina	" "	Yes	3	Liebmann, 1936
Vorticella microstoma	" "	?	6	Nikitinsky & Mudrezowa-Wyss, 1930

periments with the cysts of *Colpoda* were performed for a shorter period by Taylor and Strickland (1936).

Concerning the influence of low oxygen tensions on encystment the results reported vary. According to Brown (1939) *Colpoda* does not encyst if the oxygen pressure is below 15 mm. of mercury. Cysts, however, survived the treatment, since excystment proceeded normally after oxygen was readmitted to the medium. Johnson and Evans (1941), on the other hand, found that low oxygen tensions induced the formation of unstable cysts in *Woodruffia,* but no data on the survival of these are given.

Concluding remarks. One fact is brought forcibly to mind if one weighs the evidence presented so far, and that is the striking difference in the resistance of normally aerobic protozoa to the lack of oxygen under *experimental* and under *natural* conditions. In the former case the periods of survival are, in general, relatively short and the organisms rapidly exhibit abnormal phenomena (*e.g.,* cessation of amoeboid and ciliary movement, of vacuolar contraction and of cyclosis). In nature, on the other hand, a fauna rich in "aerobic" protozoa which appear perfectly normal, is often found in habitats that, like the stagnating strata of lakes, are devoid of oxygen for weeks or even months at a time. According to Juday (1908) and von Brand (1934) the explanation for this curious discrepancy may lie in the fact that in a natural habitat a gradual decrease of the oxygen tension occurs, while under experimental conditions an abrupt change to anaerobiosis takes place. The slow transition in nature may allow a gradual adaptation, while the sudden changes in the laboratory may be injurious. It is well known that protozoa withstand much higher concentrations of poisons and abnormal osmotic concentrations if the changes are brought about step by step than if the extreme conditions are established at once. An experimental analysis of the anaerobiosis problem from this angle should prove fruitful.

2. PARASITIČ PROTOZOA

1. PROTOZOA OF THE ALIMENTARY TRACT OF VERTEBRATES AND OF RELATED HABITATS

A. Culture experiments. Culture experiments have demonstrated that protozoa which live in the alimentary tract or in physiologically related habitats in vertebrates (uterus, vagina) have well-developed anaerobic functions. The following forms have been cultured under more or less strictly anaerobic conditions: intestinal Trichomonads (Andrews, 1926; Cleveland, 1928 and 1928a), *Trichomonas foetus* (Witte, 1933), *Trichomonas vaginalis* (Johnson, 1941), *Endamoeba histolytica* (Dobell and Laidlaw, 1926; Snyder and Meleney, 1941, 1942, 1943), *Balantidium* (Barret and Yarbrough, 1921; Scott, 1927; Pritze, 1928; Schumaker, 1931; Tanabe and Komada, 1932; Nagahana, 1932; Levitanskaja, 1938), ciliates parasitizing the stomach of ruminants (Knoth, 1928; Westphal, 1934; Hungate, 1941, 1942, 1943), *Nyctotherus* and opalinids from the frog (Pütter, 1905; Konsuloff, 1922).

It is a well-established fact that these species, with the exception of the rumen ciliates and perhaps the opalinids, can also be cultured when the surface of the medium is freely exposed to the atmospheric air. *Balantidium coli* can, as Schumaker (1931) has shown, even withstand an oxygen pressure of 18 lbs. per square inch for 32 hours. It is difficult in many cases to know with certainty, however, whether appreciable amounts of oxygen are present in those layers of the media in which the parasites predominantly occur, *i.e.,* whether diffusion from the surface is sufficient to bring about an equilibrium with the atmosphere. With the exception of some *Trichomonas,* the protozoa were grown in mixed cultures with bacteria. This, of course, complicates the situation considerably. The bacteria usually have a high rate of oxygen consumption, and since the depth of the media commonly employed in the culture of parasitic protozoa is rather considerable, it seems likely that the oxygen tension will be

appreciably lowered, at least in the deeper strata, even if the surface of the fluid is exposed to air. This view is substantiated by Jacobs' (1941) observation that in tubes in which *Endamoeba histolytica* is grown together with bacteria, low oxidation reduction potentials are established. Analyses of the gases accumulating in flask cultures of the same parasite likewise support this idea (von Brand, Rees, Jacobs and Reardon, 1943).

A few observations have been reported indicating that at least some of the protozoa dealt with in this section prefer low to high oxygen tensions. Snyder and Meleney (1941) produced the excystment of *Endamoeba histolytica* in bacteriologically sterile surroundings only when the conditions were anaerobic. The same investigators recently pointed out (1943) that culture forms of this amoeba which had been grown anaerobically were quite sensitive to molecular oxygen. Birch-Hirschfeld (1936), on the other hand, seems to have cultured *Endamoeba histolytica* under clearly aerobic conditions. A different behavior of various strains or a gradual adaptation to various conditions may explain these differences.

Cleveland's (1928) experiments are very suggestive in this connection. He showed that *Trichomonas fecalis* could be grown with dead bacteria as food only if the oxygen tension of the medium was maintained low by a vaseline seal. This precaution was not necessary if living, oxygen-consuming bacteria were present in the culture. Chatton (1918 and 1918a), reported that a *Trichomastix* species from a gecko and grown in a bacteriologically sterile medium was injured by an excess of oxygen. The necessity of small amounts of oxygen seems to be well established for this species.

B. Type of life in normal habitat. It would be premature to draw general conclusions as to the mode of life of the intestinal protozoa within the intestine, from the scattered observations available. The oxygen relationships are probably different in various species.

Obviously, the protozoa inhabiting the *intestinal lumen* and having no direct contact with the wall of the intestine, will gain their energy purely from anaerobic processes when the intestinal contents are found to be entirely devoid of molecular oxygen. But when small amounts of oxygen are found in analyses, one is hardly justified in concluding the existence of an aerobic life. There will be a competition between the bacterial flora and the intestinal fauna for this oxygen. One can surmise that the bacterial activity might cause a complete local depletion of oxygen. The "microatmosphere" in which the protozoa live might thus be frequently anaerobic, even if the "macroatmosphere" of the intestine is otherwise. In this respect the situation is quite different for the small protozoa and the much larger helminths. It is interesting in this connection to note that Hinshaw (1927) regards *Trichomonas buccalis* as an obligate anaerobe, despite the fact that it lives in the mouth of vertebrates, which is a large, open cavity. If this is correct, this parasite could survive in its normal habitat only if the oxygen consumption of the local bacterial flora is potent enough to change the microatmosphere from aerobic to anaerobic. If, on the other hand, oxygen is at times present in the immediate surroundings, the protozoa will be able to get a maximum amount of oxygen more easily than can the worms since their relative surface is incomparably greater.

Doubtless, most of the intestinal protozoa (and those occurring in related habitats) actually use oxygen when they can get it. Some, like *Balantidium* and *Trichomonas foetus* consume rather large amounts of this gas, at least when its tension is high, as Daniel (1931) and Riedmueller (1936) have demonstrated. Protozoa living close to the intestinal wall may get oxygen directly from the host cells. This is illustrated in the case of *Giardia* which has a habit of fixing itself to the epithelial cells of the intestine. Von Brand (1934) is of the opinion that this or-

ganism may require more oxygen than other intestinal protozoa.

A different situation seems to prevail in the *rumen ciliates,* organisms which are very sensitive to oxygen (Westphal, 1934; Hungate, 1941 and 1942) and appear to be truly anaerobic. This is remarkable since large amounts of oxygen will doubtless reach the stomach of ruminants from time to time, although this oxygen disappears rapidly, due to the pronounced bacterial activity in the rumen. The above-outlined possibility of locally prevailing anaerobic conditions in the microatmosphere may allow these parasites to survive until the excess oxygen has disappeared from the macroatmosphere.

When certain protozoa, like *Balantidium coli* or *Endamoeba histolytica* migrate from the intestinal lumen into the tissues, the whole situation changes. It is probable that, in their new habitat, they have regular access to the same amounts of oxygen as the surrounding cells of the host. Whether this oxygen is sufficient to cover their maximum consumption is another question, the answer to which will require more knowledge concerning the oxygen relationships of these organisms.

2. PROTOZOA OF THE ALIMENTARY TRACT OF INVERTEBRATES

The only protozoa inhabiting the alimentary tract of invertebrates for which a pronounced ability to live anaerobically has so far been demonstrated are the *flagellates* occurring in the *intestine of the termites.* They are, *in vitro,* rapidly injured and killed by oxygen (Trager, 1934; Hungate, 1939) and the same holds true in nature, as Cleveland's (1925) well-known defaunation method by oxygenation indicates. Trager (1934) furthermore showed that the following termite flagellates can be cultured for various lengths of time if the culture medium is overlaid with a vaseline seal, *i.e.,* if the oxygen supply is reduced: *Trichomonas* sp., *Trichomonas termopsidis, Tricercomitus termopsidis* and *Trichonympha*

sphaerica. Most of his experiments were performed with *Trichomonas termopsidis* and he could show that this organism develops very well even in the complete absence of oxygen. But, curiously enough, Gilmour (1940) observed that the termite parasites were harmed when their hosts were exposed to nitrogen. The population of *Trichonympha,* in particular, decreased after 5 hours or more. This may, he thinks, be readily explained by assuming an accumulation of abnormal endproducts of the anaerobic metabolism of the host, causing injury to the parasites. The experiences gathered so far seem, on the whole, to give credence to the idea that the termite intestinal parasites have but little, if any oxygen available in their normal habitat.

Many *blood parasites* of vertebrates undergo part of their life cycle in the intestine of invertebrates. The first stage of development in this habitat is in many cases the formation of gametes from gametocytes. Marchoux and Chorine (1932) have shown that the formation of both macro- and microgametes of *Haemoproteus columbae* is possible in an atmosphere of hydrogen.

Since, in culture, blood parasites usually assume the forms characteristic of the stages present in the intestine of the intermediate host rather than the form found in the blood stream of the definitive host, we shall discuss in this section their behavior as observed in culture. It has been shown that the developmental stages of *Trypanosomidae* are fairly resistant to lack of oxygen but no unanimity of opinion on this point exists among the different investigators. Soule (1925), Adler and Theodor (1931) and Ray (1932) reported that *Trypanosoma lewisi* and various *Leishmania* species, in cultures, withstand anaerobic conditions for periods up to 8 to 14 days, but that they do not multiply. Senekji (1941), on the contrary, found a good growth under anaerobic conditions in cultures of five *Leishmania* species. In view of these contradictory results it is impossible, at the pres-

ent time, to reach any definite conclusion concerning the oxygen relationships of *Trypanosomidae* inside the insect intestine.

Regarding the intestinal stages of *gregarines*, only a single observation is available. Von Brand (1943) showed that the trophozoites of *Gregarina steini, G. cuneata* and *G. polymorpha*—all three parasitizing the intestine of *Tenebrio molitor* larvae—stayed alive for 16 hours when the hosts were exposed to strictly anaerobic conditions. Longer observation was unfortunately not possible since the mealworms died after 24 hours. However, after 16 hours the gregarines were perfectly normal, and although counts were not made, no noticeable decrease in numbers seemed to have occurred. This is of some interest in view of the different behavior of *Trichonympha* mentioned above and would seem to convey a hint that the gregarines are quite well adapted to anaerobic life. Since these organisms cannot be cultured and since the hosts are not very resistant to lack of oxygen the progress of research on this point will probably be slow and difficult.

3. BLOOD AND TISSUE PROTOZOA

Our knowledge of the anaerobic functions of the protozoa inhabiting the blood stream is extremely rudimentary. These protozoa are probably not well equipped for withstanding complete lack of oxygen since their normal surroundings are obviously rich in oxygen.

Von Brand (1933) found that the blood-forms of *trypanosomes* survived only a few hours in anaerobic surroundings and Wendel (1943) was able to maintain *malaria* parasites alive under analogous conditions for only an hour or two. It is a curious fact, to be discussed in detail later, that, despite the easy availability of oxygen in their normal surroundings, the normal metabolism of trypanosomes and malaria parasites is characterized by incomplete oxidations.

That protozoa parasitizing *tissues* proper should, as a rule be able to obtain at least some oxygen from their surroundings appears probable but whether it will suffice to allow a purely aerobic metabolism is at the present stage of our knowledge a matter of speculation.

The only data available concerning the oxygen relationships of tissue parasites were obtained with *Coccidia.* They indicate very definitely that oxygen is available to the tissue-stages of the parasites. The immature oöcysts of many species eventually reach the intestinal lumen, the lumen of enlarged bile ducts, or the gall bladder of the host—places where they can get only insignificant amounts of oxygen. It is characteristic that the oöcysts of these species do not mature inside the host but only in the outside world where an adequate oxygen supply becomes available (Balbiani, 1884; Pfeiffer, 1892; Metzner, 1903). On the other hand, these oöcysts are quite resistant under experimentally induced anaerobic conditions (Metzner, 1903). In other species the oöcysts remain in the tissues of the host, those of *Eimeria subepithelialis,* for example, in the subepithelial tissues. Moroff and Fiebiger (1905), while studying this species, observed that its oöcysts mature *in situ,* and they assumed that maturation would not require oxygen. Later on, however, Fiebiger (1913) reached the tentative conclusion that the oxygen present in the host tissues might be sufficient to allow the maturation process to take place. The idea that the maturation or the non-maturation of the coccidian oöcysts inside the host depends solely on the different amounts of oxygen available to the different species has been expressed by Reichenow (1929).

II. COELENTERATES

In nature, coelenterates do not seem to occur in anoxic habitats. They exhibit, nevertheless, a certain tolerance for experimental deprivation of oxygen.

It has been shown that *hydras* may survive several hours in anaerobic conditions (Welch and Loomis, 1924; Beutler, 1933). Normally, however, these animals lead an aerobic life and even prefer water rich in oxygen. After several days in water of very low oxygen content they are irreparably damaged.

Beutler (1933) has demonstrated, furthermore, that brown species of *Hydra* are actually attracted by oxygen, *i.e.,* they exhibit an oxygeno-taxis. In green species this phenomenon takes place also but only in the dark, apparently because, in the light, the symbiotic algae are able to provide sufficient oxygen to render such a reaction unnecessary.

Whether or not *actinians* can be kept for any length of time under strictly anaerobic conditions is as yet unknown. There is no reason to assume that in nature they are apt to support long-lasting anoxic conditions. We do know, however, that they are able to survive in water containing but little oxygen. Their behavior under such conditions has led to a controversy between Piéron (1908, 1908a,b,c,d) and Bohn (1908, 1908a). The former maintains that the animals contract in water poor in oxygen, while the latter asserts that they expand. The problem is still unsolved and should be reinvestigated. The actinians have been recognized long ago (Henze, 1910) as being among the best examples of animals whose oxygen consumption depends on the oxygen tension. On the other hand, their oxygen consumption is far below maximal even when the water is saturated with air. It appears probable therefore that the deeper lying tissues depend at least in part on anaerobic processes for the supply of energy required by the cells. This problem will be discussed later.

Corals can be kept alive for some time in water poor in oxygen (Yonge, Yonge and Nicholls, 1932). The most resistant genus so far known is *Porites*. Representatives of this genus, in the experiments of the authors just mentioned, lived for 6 days when confined in a closed container. The water in the container had an initial oxygen content of 0.51 cc. per liter, while at the end of the experiment it was completely free of molecular oxygen.

The resistance of *jellyfish* to lack of oxygen varies apparently with the genus, but is certainly not very pronounced in any of them. *Rhizostoma* seems to become asphyxiated quite rapidly (Winterstein, 1905; Baglioni, 1905), whereas *Cassiopea xamachana* could be kept for 7 hours (McClendon, 1917), and *Eleutheria dichotoma* for 12 hours (Drzewina and Bohn, 1911) under anaerobic conditions. In this last case, curious developmental changes were observed. While normally young daughter medusae originate from buds formed in the angles between the tentacles, under the influence of a lack of oxygen supernumerary tentacles originated from the buds and the formation of the daughter medusae was completely inhibited.

Certain functions of the *polyps* of coelenterates are much more dependent upon an adequate supply of oxygen than is their general metabolism. According to Miller (1937), Barth (1938, 1940) and Schechter (1941) oxygen appears to be the controlling factor in the regeneration of the hydranths of *Tubularia* and *Corymorpha*. In the latter, a considerable inhibition became noticeable even when the oxygen content of the water was lowered only 20 per cent below the normal value. Under nearly anaerobic conditions no regeneration whatever took place. While these experiments seem to indicate a close correlation between oxygen consumption and regeneration, further information is desirable. Barth (1940) writes in conclusion to the experiments reported: "Thus the rate of regeneration and the rate of O_2 con-

sumption may be dependent upon two different processes which thus far are affected by the same treatment and conceivably some treatment might be found where either could be changed independently of the other."

Other special functions of the coelenterates do not depend on the presence of oxygen. Harvey (1926) and Harvey and Korr (1938) demonstrated that the luminescence of *ctenophores* and of the *medusa Pelagia noctiluca* does not require molecular oxygen in the surroundings.

III. WORMS AND WORM-LIKE ORGANISMS[1]

1. NON-PARASITIC SPECIES

A. Occurrence in anaerobic habitats. There is no definite evidence that any non-parasitic worms or worm-like organisms are adapted to a permanently anaerobic life, but many are known that live temporarily in the absence of oxygen.

(a) Fresh-water forms. Lauterborn (1916) describes many gastrotricha and rotifers, some oligochaetes, nematodes and flatworms as members of the sapropelic fauna (see Appendix, Table 1). It should be remembered, however, that Wetzel (1928) has demonstrated that only the mud itself and the water in immediate contact with it are truly oxygen-free in typical sapropelic habitats. To what extent the animals are confined to these zones has not yet been sufficiently established.

Many of these organisms are much larger than the anaerobic sapropelic protozoa. This is an important point to keep in mind, for it is conceivable that larger animals, being able to wander over farther distances than smaller ones, may from time to time reach layers containing molecular oxygen and thus they will only tempo-

[1] Under "worm-like organisms" we include animals like gastrotricha, rotifers and chaetognatha.

rarily be confined to the real anaerobic zone. It is quite likely, however, that they will occasionally be subjected to complete anaerobiosis. On the whole, the sapropelic worm fauna should show pronounced adaptation for life at quite low oxygen tensions and future investigation may even reveal that some species can live and reproduce in the complete absence of oxygen. An experimental approach to this problem is much desired.

Another anaerobic or nearly anaerobic habitat in which worms are also found is the bottom of polluted lakes or streams, or that of lakes in which the circulation has ceased. *Tubificidae* are especially characteristic of such environments. They are frequently present in greatest numbers just in the most heavily polluted areas (Hentschel, 1917; Richardson, 1925) where only minute amounts of oxygen are present. In uncontaminated and non-circulating bodies of water where the oxygen disappears completely during summer- or winter stagnation they must undergo total anaerobiosis.

The Tubificidae are nevertheless not animals that can live indefinitely in the complete absence of oxygen. They, like many other organisms in this type of habitat, endure anaerobic conditions, but do not select them (Eggleton, 1931). Alsterberg (1922) described a very interesting respiratory mechanism in these worms, in particular, in *Tubifex* and *Limnodrilus*. While they may actually be buried in oxygen-free mud, their tails usually project into the water above it. If there is much oxygen in the water, the tail is kept motionless, but as soon as the oxygen content of the water in contact with the mud becomes insufficient, the worms begin to wag their tails and to thrust them the farther out of the mud the lower the oxygen content of the water becomes. In this manner oxygen is drawn into the "anaerobic" zone and becomes available to the worms. At very low tensions feeding ceases; *Limnodrilus* then becomes quiescent, while *Tubifex* tries to escape asphyxia by wandering away.

Field observations indicate that in nature the Tubificidae survive relatively long periods under truly anaerobic conditions. They are still found, although in reduced numbers, in the bottom material of lakes of which the water has been found free of oxygen for several weeks (Juday, 1908, 1922; Lindeman, 1942). In this connection the quantitative figures presented by Lindeman (1942) are of special interest. He found in mud samples of Cedar Bog Lake, Minnesota, the following numbers of *Tubifex* per Birge-Ekman sample of 225 cm^2 bottom mud:

January 31, 1940 (7 days before onset of anaerobiosis):	6.0 ± 1.8
March 3, 1940 (24 days after onset of anaerobiosis):	5.2 ± 0.5
March 30, 1940 (51 days after onset of anaerobiosis):	1.0 ± 0.7

Rotatoria seem to occur occasionally in relatively large numbers in the sand of beaches of fresh water lakes where the interstitial water has a very low oxygen content or is even completely free of oxygen. Pennak (1940) found in such habitats from 79 to 130 rotatoria per 80 cc. of sand. The predominant species were apparently *Lecane inquieta, L. paraclosterocerca* and *L. scutata.* Moore (1939) encountered *Rotaria rotatoria* and *R. tridens* in the oxygen-free bottom ooze of Douglas Lake, Michigan, where also the *nematode Trilobus bastian* and a species of *Chaetonotus* occurred. *Gastrotrichs,* in general, seem to live rather frequently in similar localities (Juday, 1908; Imel, 1915).

Sewage plants might well be inhabited by worms able to live in the absence of oxygen. But no conclusive evidence from the literature that such is the case has come to the writer's attention. Reynoldson (1939), on the other hand, has pointed out that Enchytraeid worms which do occur in sewage plants must be regarded as living aerobically.

(b) Marine forms. It has been shown previously that the *bottom material of the sea* is frequently free of oxygen. Let us consider the possibilities which

this habitat offers for anaerobic worms. ZoBell and Feltham (1942) found several undetermined species at depths exceeding 40 cm. in a marine mudflat where completely anaerobic conditions prevailed. The observations of Moore (1931) are also very significant in this connection. He investigated the bottom fauna of the mud in the Clyde Sea area (Scotland), in a region where the interstitial water was completely free of oxygen at the very surface. He found large numbers of unidentified nematodes in the upper 4 cm. and a few down to a depth of 7 cm. Unspecified polychaetes, most of them immature forms, were encountered only in the uppermost centimeter, and only a few went down as far as 5 cm. Moore (1931) thinks it possible that the nematodes in question might live permanently without oxygen. He exposed these worms to an experimental anaerobic medium for a period of 35 days after which they still appeared normal and active.

Another question of interest is whether worms living on the *sea shore* are normally exposed to anaerobic conditions at *low tide*. Many of these worms live in tubes and have but little chance to renew rapidly the water that fills these tubes, water on which they depend for their oxygen supply. If any is renewed, the replacement will not be with water rich in oxygen but with the oxygen-poor water that permeates the ground. This does not necessarily imply that the worms must live in a predominantly anaerobic manner. It is possible that they stir up the water present in the tubes, causing it to circulate and thus giving it a chance to absorb oxygen directly from the atmosphere. This seems to happen in *Arenicola*, but whether it occurs in worms like *Scoloplos, Heteromastus* or *Nephthys* has not yet been definitely decided (Thamdrup, 1935).

The case of *Arenicola* has been studied thoroughly by Borden (1931). She found that the haemoglobin-containing blood of this animal plays an important role in

permitting its metabolic activities to be carried on during the period of low tide. The *Arenicola* haemoglobin has a steep dissociation curve, thus enabling the worm to secure oxygen even at rather low tensions. The amount of oxygen present in the blood, when the latter is saturated, was found to last over a period of 71 minutes. These factors combined may suffice to prevent the necessity of a predominantly anaerobic life during low tide.

The same conditions may be assumed in the case of the gephyrean *Urechis caupo*. Redfield and Florkin (1931) calculated that the oxygen present in the blood of this animal would be sufficient to last for about 3 hours. The bottom in which the worms build up their tubes may frequently be out of water for periods extending to 6 hours, occasionally to 18 hours. The oxygen content of the water present in the tubes, however, never decreases to less than 0.6 cc. per liter and, if the ground becomes drier, somewhat higher values are found. At these tensions the blood is still saturated to 60 per cent with oxygen which should allow a sufficient supply for the tissues. MacGinitie (1935) observed furthermore that burrowing animals of this habitat (amongst them *Urechis*) cease all activity at low tide. This, naturally, lowers their oxygen requirements. If they are placed in oxygen-free water, they become entirely quiet and "scarcely can be prodded with a glass rod sufficiently to make them show activity."

Whether *planktonic marine worms* must occasionally supplement their aerobic metabolism through anaerobic processes is not yet clear, although it is known that some of them penetrate readily into strata quite poor in oxygen. According to Nikitin (1931) some unspecified polychaete larvae, and less frequently *Sagitta euxina,* are found in the deeper layers of the Black Sea where the oxygen concentration reaches only 2 to 5 per cent of the saturation value.

(c) Terrestrial forms. To what extent terrestrial worms, under natural conditions, live anaerobically is not known. There is good reason to believe that they often find themselves in very difficult respiratory conditions after rains. Normally, earthworms get their oxygen directly from the atmosphere, but they are also able to extract oxygen from water. Nagano (1934) kept *Eisenia foetida* alive up to 94 days, and *Pheretima communissima* up to 41 days, in running water, at 14° to 25°C and Raffy (1930) showed that earthworms immersed in water saturated with oxygen consume the same amounts of oxygen as controls kept in the air. When the oxygen content in either case was lowered, the oxygen consumption decreased, the decrease being more pronounced in water than in air. The curves obtained by various authors (Jordan and Schwarz, 1920; Dolk and van der Paauw, 1929; Thomas, 1935) for the oxygen consumption of earthworms in relation to the oxygen tension of the atmosphere are not very uniform, but they all show that, despite the presence of haemoglobin, the oxygen consumption is considerably lower than normal at low tensions.

Since rain water upon entering the ground rapidly loses its oxygen (Merker, 1926), it seems quite certain that it is actually the danger of asphyxiation that drives the worms to the surface after heavy rains (Merker, 1931). To what extent the metabolism of these animals becomes anaerobic before they leave the ground is difficult to ascertain. But not all the earthworms come to the surface or die in the ground after a rain, even after a long-lasting rainy period; the mere fact of the survival of many suggests a quite marked ability for at least partial transition to anaerobiosis.

B. Resistance under experimental anaerobic conditions. The data concerning the resistance of non-parasitic worms experimentally deprived of oxygen have been summarized in Table 10. It is quite apparent, on the whole,

that a parallelism exists between this resistance and the occurrence of anaerobic conditions in the normal surroundings of the worms. Thus we see a remarkable endurance exhibited by *Tubifex* and its allies, by mud-dwelling nematodes and mesosaprobic rotifers like *Colurella, Rotifer* or *Philodina;* while others, such as the oligosaprobic rotatorians *Synchaeta, Schizocerka, etc.,* die rapidly in the absence of molecular oxygen.

The minor differences in survival times of different worms do not appear to be really significant. Very likely the figures given in Table 10 do not, in many cases at least, represent the true resistance in anaerobic conditions. In some of the anaerobic cultures, notably those of Hecht (1932) and Nikitinsky and Mudrezowa-Wyss (1930) rather considerable amounts of hydrogen sulfide developed; since this gas is toxic for many organisms, its development may well mask the real anaerobic functions. It seems obvious that small differences in anaerobic survival cannot be used with advantage for ecological speculations. Rode's (1925) attempt to do so with planarians has been convincingly repudiated by Legendre (1925).

One can state, however, that, whereas some worms, like *Tubifex,* exhibit very great resistance, other groups, notably many polychaetes, support only a slight lack of oxygen. The latter succumb to asphyxiation in closed containers while varying amounts of oxygen still remain in the water. Data concerning this point are presented by Bounhiol (1902) and von Brand (1927). The forms involved are probably seldom, if ever, subjected to strictly anaerobic conditions in nature.

It is clear that the resistance to lack of oxygen depends in many cases on the *physiological state of the worms.* Any condition lowering the energy requirements will tend to increase the anaerobic resistance. The less active the anaerobic metabolism that is necessary to sustain life, the less non-oxidized substances will accumulate in the

body. The latter are quite generally toxic and the level they reach is probably often the factor limiting the anaerobic resistance.

The most extreme cases of reduced metabolism are those of completely dried organisms. In them, life processes are obviously reduced to a minimum, if indeed they do not cease completely. That animals in a state of suspended animation should be more resistant to the lack of oxygen than in the active stages is so obvious, that it needs no further comment. Experiments proving this have been performed by Rahm (1929) with moss-inhabiting nematodes and rotatoria and by Becquerel (1936) with three species of rotatoria.

The nutritional stage is another factor often involved in determining the energy requirements. It is of interest, in this connection, that engorged specimens of *Hirudo medicinalis* die more rapidly under anoxic conditions than leeches which have been starved for several months (Pütter, 1908). On the other hand, it is a well-established fact that leeches starving under aerobic conditions have lower energy requirements than fed ones. This is evidenced by their smaller oxygen consumption. It seems logical to assume that an identical situation prevails under anaerobic conditions and that the relatively low rate of anaerobic metabolism explains the increased resistance in anoxic conditions observed in unfed specimens.

Not all worms, however, have a lowered rate of metabolism during starvation. According to Hyman (1919), after a high oxygen consumption which is due to the presence of food and which lasts only a few hours in planarians, the rate decreases, reaching a minimum within the first two weeks of starvation and, after that time, a marked and progressive increase is observed. Starving planarians should, if the above assumption of an inverse correlation between rate of metabolism and resistance in anaerobic medium is correct, be more affected by lack of oxygen than specimens which had been fed prior

TABLE 10

MAXIMAL SURVIVAL TIME OF NON-PARASITIC WORMS AND WORM-LIKE ORGANISMS UNDER ANAEROBIC CONDITIONS.

Species	Temp. °C	Strictly anaerobic conditions?	Survival time in days	Source
		Flatworms.		
Turbellarians				
Dendrocoelum lacteum	Room temp.	?	8	Rode, 1925
" "	" "	Yes	2	Bunge, 1889
Planaria subtenticulata	" "	?	7	Rode, 1925
" *torva*	" "	Yes	2	Bunge, 1888
Polycelis cornuta	" "	?	4	Rode, 1925
" *nigra*	" "	?	9	" "
Nemertineans				
Eunemertes	12 - 17	?	<1	Jacubowa & Malm, 1931
Nemertineae	" "	?	>5	" " "
		Nematodes.		
Nematodes				
Anguillula aceti	Room temp.	Yes	14	Heuneberg, 1900
" "	" "	"	7	Bunge, 1889
" "	" "	"	10	Peters, 1928
Gordius sp.	" "	"	>1	Bunge, 1889
Undeterm. fresh water nematodes	0 and 5	"	>90 <120	Lindeman, 1942
Undeterm. marine nematodes	Room temp.	"	>35	Moore, 1931
		Rotatoria.		
Rotifers				
Adineta gracilis (dry)	35	Yes	>90	Becquerel, 1936
Anurea aculeata	Room temp.	?	>1	Nikitinsky & Mudrezowa-Wyss, 1930
" *cochlearis*	" "	?	>1	" " " "
Asplanchna priodonta	" "	?	6 hrs.	" " " "
" *sieboldi*	" "	?	3	" " " "
Brachionus angularis	" "	?	5	" " " "
" *pala*	" "	?	4	" " " "
Callidina angusticollis (dry)	35	?	>90	Becquerel, 1936

TABLE 10 — Continued

Species	Temp. °C	Strictly anaerobic conditions?	Survival time in days	Source
Cathypna luna	Room temp.	?	6	Nikitinsky & Mudrezowa-Wyss, 1930
Colurella bicuspidata	" "	?	17	" " " "
Diurella stylata	" "	?	1 hr.	" " " "
Euchlanis dilatata	" "	?	18 hrs.	" " " "
Metopidia lepadella	" "	?	5	" " " "
Monostyla bulla	" "	?	6	" " " "
" *lunaris*	" "	?	3	" " " "
Philodina roseola	" "	?	12	" " " "
Polyarthra platyptera	" "	?	1	" " " "
Rattulus cylindricus	" "	?	1 hr.	" " " "
Rotifer vulgaris	" "	?	5	" " " "
" " (dry)	35	Yes	>90	Becquerel, 1936
Schizocerka diversicornis	Room temp.	?	2 hrs.	Nikitinsky & Mudrezowa-Wyss, 1930
Synchaeta pectinata	" "	?	3 hrs.	" " " "
" sp.	" "	?	6 hrs.	" " " "
Triarthra longiseta	" "	?	7 hrs.	" " " "
		Gastrotrichs.		
Gastrotrichs				
Chaetonotus maximus	0 and 5	Yes	>60 <120	Lindeman, 1942
" sp.	" " "	"	>60 <120	" "
Lepidoderma rhomboides	" " "	"	>60 <120	" "
		Annelids.		
Polychaetes.				
Amphitrite sp.	Room temp.	Yes	1	Packard, 1905
Arenicola marina	" "	"	several days	Hecht, 1932
Capitella capitata	12 - 17	?	8	Jacubowa & Malm, 1931
Capitomastus minimus	" "	?	8	" " "
Eteone picta	" "	?	6	" " "

TABLE 10 — Continued

Species	Temp. °C	Strictly anaerobic conditions?	Survival time in days	Source
Eulalia viridis	12 - 17	?	<1	Jacubowa & Malm, 1931
Exogone gemmifera	" "	?	<1	" " "
Fabricia sabella	" "	?	<1	" " "
Glycera convoluta	" "	?	10	" " "
Harmothoe incerta	" "	?	5	" " "
" *reticulata*	" "	?	<1	" " "
Lysidice ninetta	" "	?	8	" " "
Nereis diversicolor	" "	?	3	" " "
" sp.	Room temp.	Yes	1½	Packard, 1905
" sp.	" "	"	>8	Hecht, 1932
" *zonata*	12 - 17	?	<1	Jacubowa & Malm, 1931
Owenia fusiformis	Room temp.	Yes	>21	von Brand, 1927
Pectinaria neapolitana	12 - 17	?	8	Jacubowa & Malm, 1931
Perinereis cultrifera	" "	?	6	" " "
Pholoe synophthalmica	" "	?	<1	" " "
Phyllodoce tuberculata	" "	?	<1	" " "
Platinereis dumerilii	" "	?	<1	" " "
Staurocephalus rubrovitatus	" "	?	<1	" " "
" *rudolphii*	" "	?	3	" " "
Sthenelais boa	" "	?	2	" " "
Oligochaetes				
Allolobophora sp.	17 - 23	?	3	Nikitinsky & Mudrezowa-Wyss, 1930
Eisenia foetida	Room temp.	No	15	Nagano, 1934
" sp.	17 - 23	?	7	Nikitinsky & Mudrezowa-Wyss, 1930
Lumbriculus sp.	Room temp.	Yes	1	Bunge, 1888
" "	15 - 16	"	10	Alsterberg, 1922
" *variegatus*	14 - 16	?	48	Jatzenko, 1928

TABLE 10 — (Continued)

Source	Temp. °C	Strictly anaerobic conditions?	Survival time in days	Species
Lumbricus rubellus	3 - 5	Yes	4	Konopacki, 1907
" "	19	"	1½	" "
" *terrestris*	3	"	1	" "
" "	18	"	6 hrs.	" "
Pheretima communissima	Room temp.	No	2	Nagano, 1934
Tubifex sp.	0 and 5	Yes	>120	Lindeman, 1942
" "	10	"	>90	" "
" "	15 - 16	"	25	Alsterberg, 1922
" "	?	"	>90	Eggleton, 1931
" *tubifex*	17 - 23	?	9	Nikitinsky & Mudrezowa-Wyss, 1930
Hirudineans				
Clepsine sp.	Room temp.	?	6	Bunge, 1888
Glossosiphonia complanata	" "	Yes	5	Alsterberg, 1922
" "	" "	?	4	Nikitinsky & Mudrezowa-Wyss, 1930
" "	14 - 16	?	16	Jatzenko, 1928
Haemopis sanguisuga	Room temp.	Yes	5	Alsterberg, 1922
" " sp.	" "	?	2	Bunge, 1888
Helobdella stagnalis	" "	Yes	5	Alsterberg, 1922
Herpobdella atomaria	" "	"	5	" "
" "	" "	?	3	Nikitinsky & Mudrezowa-Wyss, 1930
Hirudo medicinalis	" "	"	3	Bunge, 1888
" " (fed)	" "	?	4	Pütter, 1908
" " (starved)	" "	?	>10	" "
Nephelis sp.	" "	?	2	Bunge, 1888
" *vulgaris*	" "	?	4	Bunge, 1889
Gephyreans.				
Dendrostoma zostericola	Room temp.	Yes	7	Peebles & Fox, 1933
Sipunculus nudus	" "	No	several days	Henze, 1910
" "	" "	?	2	Baglioni, 1915

to the experiments. It is very suggestive that Child (1919) observed that the susceptibility of *Planaria dorotocephala* to anaerobic conditions does indeed increase with progressive starvation at least up to an inanition period of four months. Child (1919a), observed also that exposure to cyanide decreases the resistance of planarians to lack of oxygen. He assumes that the two factors are additive in causing injury, but a real understanding of these last observations will require further study.

Our knowledge concerning the anaerobic resistance of *developmental stages of worms* is rather scanty. Barron (1932) found that the eggs of *Nereis* could be kept for 5 hours, and their sperm for 3 hours and 15 minutes, in the complete absence of oxygen, without being damaged, but the limit of endurance was not determined. After restoration of aerobic conditions the eggs thus treated could be fertilized by normal sperm, and the experimental sperm could fertilize normal eggs. If the aerobic conditions were not restored only the first stages of fertilization took place, *i.e.,* only the fertilization cone was formed, but no further changes occurred during the entire 3-hour period of observation. Nuclear changes did not take place.

2. PARASITIC SPECIES

A. Evidence from experiments in vitro. (a) Introductory remarks. Endo-parasitic worms live in environments that show great variations in their oxygen relationships. Many helminths are found in the lumen of the intestine of vertebrates, which, as discussed previously, is extremely poor in oxygen. Others are tissue parasites; their environment is characterized by a somewhat higher, but still fairly low oxygen tension. Still others occur in the blood, in the trachea, in the swim-bladder of fishes or similar habitats in which an abundant oxygen supply is available.

The situation is complicated still further by the fact that different stages in the life cycle of one and the same worm may occur under conditions differing widely with respect to oxygen content. For instance the parasitic generation of *Strongyloides* lives in the intestine, whereas the larvae normally undergo their further development in the outside world. In the case of so-called heterogenetic development an entire free-living generation intervenes before the offspring returns to parasitic life.

It can be expected that such differences will be reflected in the metabolic processes, but unfortunately relatively few species have been investigated thus far. The available information concerns chiefly intestinal parasites, fewer data are at hand for tissue helminths, and practically nothing is known about the metabolism of parasitic worms that live in environments rich in oxygen.

The behaviour and metabolism of parasitic helminths can be studied under controlled conditions only if they are maintained outside their hosts. A great difficulty, quite generally encountered in these investigations, is that most helminths remain alive only for very limited periods when removed from their normal habitat. However, in recent years promising results have been obtained in lengthening the survival period *in vitro,* or even in rearing the worms in true culture. Glaser and Stoll (1938) succeeded in obtaining a sterile culture of the free-living stages of *Haemonchus contortus.* Glaser (1940, 1940a) carried *Neoaplectana glaseri,* a nematode parasite of the Japanese beetle, through its entire life cycle in culture. Ferguson (1940, 1943) obtained the development of the metacercariae of *Posthodiplostomum minimum* into adults and of the cercariae of *Diplostomum flexicaudum* into metacercariae *in vitro,* Hoeppli and Chu (1937) maintained *Clonorchis sinensis* alive up to 5 months, Lee and Chu (1935) *Schistosoma japonicum* up to 2½ months, von Brand and Simpson (1942) the larva

of *Eustrongylides ignotus* up to almost a year in laboratory conditions.

In none of these experiments was oxygen excluded, although in some cases, for example, in Hoeppli and Chu's (1937) investigations, the oxygen supply was rather limited. It is quite apparent that the above helminths and others that will be mentioned later are not injured by oxygen. Since these experiments were not performed to gain an insight into the anaerobic functions of the worms, no comparative study of aerobic and anaerobic survival was made.

(b) Experimental data. Some data on anaerobic survival of *helminths* are available. They have been gathered in Table 11, and when experiments were performed under both anoxic and aerobic conditions two figures are given. It is evident that small differences in technique play a great role in the determination of the survival times. Only data gathered with one and the same procedure can be rightly compared. For this reason the data of workers who studied only the aerobic survival have not been included.

In almost all the experiments summarized in Table 11 the worms were kept in non-nutrient solutions, and bacterial contaminants were invariably present except probably in the case of *Trichinella* larvae. These two factors may largely account for the short survival periods observed under both anaerobic and aerobic conditions. The presence of bacteria is an especially important factor that occupies more and more the attention of the investigator. It seems that even the bacteria which develop in non-nutrient solutions, and which find perhaps a suitable medium in the excreta of the worms, shorten the life of the latter considerably. The case of the larva of *Eustrongylides ignotus* illustrates this point. Its maximal aerobic survival is listed in Table 11 as 19 days. Recently, however, the present author, in collaboration with W. F. Simpson, maintained a series of these lar-

TABLE 11

SURVIVAL OF PARASITIC WORMS OUTSIDE THE HOST UNDER ANAEROBIC CONDITIONS AND COMPARISON OF ANAEROBIC AND AEROBIC SURVIVAL.

Species	Medium	Temp. °C	Survival time in days		Source
			Anaerobic	Aerobic	
Trematodes					
Cryptocotyle lingua (metacercariae)	Modified Ringer & Glucose	Room temp.	>4	12	Stunkard, 1930
Fasciola hepatica	Blood	38–39	1½	1½	Weinland & von Brand, 1926
Opisthorchis felineus	Ringer	37	18	18	Erhardt, 1939
Sphaerostoma bramae (Distomum globiporum)	1% NaCl	Room temp.	5	4	Hausmann, 1897
Cestodes					
Bothriocephalus bipunctatus	1% NaCl	Room temp.	>1	several	Harnisch, 1937a
Moniezia expansa	Ringer	38–39	½	—	von Brand, 1933a
Nematodes					
Ancylostoma caninum (larvae)	Tapwater	Room temp.	>4, <12	—	McCoy, 1930
" *duodenale* (larvae)	"	17	21	—	Boycott, 1904
Ascaris lumbricoides	1% NaCl	35–38	7	—	Bunge, 1889
" "	"	37	9	6	Weinland, 1901
" *mystax*	1% NaCl + 0.1% Na_2CO_3	38	6	15	Bunge, 1883
Cooperia curticei	Ringer	37	<1	4–12	Davey, 1938
" *oncophora*	"	"	<2	"	" "
Eustrongylides ignotus (larvae)	1% NaCl	"	3	19	von Brand, 1938a
Nematodirus filicollis	Ringer	"	<2	4–12	Davey, 1938
Ostertagia circumcincta	"	"	<1	"	" "
Parascaris equorum	"	38	5	2	Toryu, 1935
" "	"	35–38	2	—	Bunge, 1889
Raphidascaris acus	1% NaCl	Room temp.	6	—	" "
Trichinella spiralis (larvae)	Tyrode	37	7 or more	—	Stannard, McCoy & Latchford, 1938
Trichostrongylus colubriformis	Ringer	"	<1	4–12	Davey, 1938
" *vitrinus*	"	"	<1	"	" "

vae in sterile saline with small losses for a period of 6 weeks, after which time the animals were sacrificed for chemical analysis. In sterile nutrient solutions the larvae stayed alive, as mentioned above, for many months.

In the light of such experiences, the data of Table 11 ought to be used only with great caution. Different microbial floras will develop depending on whether the worms are kept aerobically or anaerobically and the influence of various bacteria on the worms may vary widely. One of the problems for future investigation is, therefore, that of the survival of helminths under varying oxygen tensions in sterile nutrient media. The experiments summarized in Table 11 are sufficient in the writer's opinion to demonstrate that most of the worms investigated show a decided resistance to lack of oxygen. Further conclusions can be drawn only with reserve and only if corroborated by other evidence.

Besides the above data some further facts are known concerning the *anaerobic relationships of helminth eggs and larvae*. Many observations indicate conclusively that the eggs of a number of nematodes withstand anaerobic conditions exceedingly well. The eggs of *Ancylostoma, Parascaris, Ascaris, Trichocephalus* and *Nematodirus* are not easily damaged by lack of oxygen. They can be kept, without losing their viability, for days or even weeks under anoxic conditions (Looss, 1911; Bataillon, 1910; Zawadowski, 1916; Fauré-Fremiet, 1913; Zawadowski and Orlow, 1927; Zviaginzev, 1934; Dinnik and Dinnik, 1937; Cram and Hicks, 1944). In all cases, however, the development of the eggs ceases sooner or later, to be resumed only when oxygen again becomes available.

The details seem to vary with different species. In *Parascaris,* only maturation, fertilization and probably the first cleavage stages are possible under anaerobic conditions (Fauré-Fremiet, 1913; Szweikowska, 1929; Dyrdowska, 1931). In *Oxyuris equi* development con-

tinues up to the gastrula stage and in *Enterobius vermicularis* even up to the tadpole stage (Zawadowski and Schalimow, 1939; Schalimow, 1931; Wendt, 1936).

To decide whether certain helminth larvae can complete their development in anaerobic environments is important in view of the question of "auto-invasion". Nishigori (1928) claims to have observed the transformation in the absence of oxygen of the rhabditiform larvae of *Strongyloides* into the infective filariform larvae. Lee (1930), however, was unable to confirm these results conclusively since the establishment of truly anaerobic conditions is exceedingly difficult in the culture medium required by these animals. There can be no doubt that auto-invasion occurs in *Strongyloides* infections, *i.e.*, under certain conditions, the rhabditiform larvae change into the filariform stage inside the intestinal tract of the host. These larvae are, however, very small and it is quite possible that in view of their relatively large surface the amount of oxygen that they can get is not negligible even at the low tensions prevailing in the intestine.

In the case of the hookworm larvae, on the other hand, the normal oxygen content of the intestine of the host seems to be clearly insufficient to insure development. According to McCoy (1930) these larvae will still hatch in water containing only 0.4 cc. oxygen per liter, *i.e.*, about one fifteenth of the saturation value. Below this figure the development of the eggs is greatly retarded.

The eggs of *Ascaris* seem to need a still higher oxygen tension for their development. According to Brown (1928) the development is retarded about 30 per cent in media containing 1.3 to 1.8 cc. of oxygen per liter and about 50 per cent if the oxygen content is 1.1 cc. per liter (23°C). Kosmin (1928) showed that the eggs of *Parascaris* do not develop at all at tensions of 5 mm. of mercury, but between 10 and 80 mm. the development is possible, although retarded. Above 80 mm. development takes place normally.

One would expect that in the natural, free-living developmental stages the helminths will not be injured by oxygen of normal tension. On the other hand certain life activities of parasitic stages may be inhibited if the animals are exposed to much more oxygen than is normally present in their surroundings. The experiments of Stoll (1940) are especially significant in this regard. He found that the first parasitic ecdysis of *Haemonchus contortus,* which occurs at the end of the third larval stage, is distinctly favored, in bacteria-free cultures, if the oxygen supply of the medium is limited.

B. Type of life in normal habitat. Only worms occurring in the intestinal tract (inclusive of bile ducts) and the tissue parasites will be discussed here; helminths living in the lungs, in the trachea, in the swim-bladder or similar habitats will, as a rule, have access to an abundant supply of oxygen and will live aerobically.

(a) Intestinal worms. It has been regarded as axiomatic for many years that intestinal worms are truly anaerobic animals. It was assumed that they would not make use of oxygen even if they had access to it (*e.g.,* Weinland, 1901, 1913; Weinland and von Brand, 1926); occasionally the opinion has even been expressed that oxygen would be toxic to them (Jordan and Hirsch, 1927). This view can no longer be held in its old form; all recent investigations (Alt and Tischer, 1931; Adam, 1932; Harnisch, 1933a; 1937a; von Brand, 1934a; Krüger, 1936, 1937) have shown that intestinal helminths do utilize oxygen when it is offered to them.

Some investigators (Slater, 1925; Mueller, 1929; Adam, 1932; Davey, 1937, 1938) maintain that the animals live aerobically in the intestine, but they are not too specific in answering the question as to where the necessary oxygen for an aerobic metabolism comes from.

The present author discussed the metabolic relationships of intestinal worms in some detail in a previous

paper (von Brand, 1938) and the account of this problem as presented here is patterned along the lines developed in his earlier work. Several large parasites (*Ascaris, Parascaris, Moniezia, Fasciola*) are quite resistant in experimentally induced anaerobic conditions, as was explained in a preceding section. Their oxygen consumption is strictly dependent on the tension (Harnisch, 1933a, 1937a; Krüger, 1936) and since the tension in the intestinal lumen is low, the oxygen consumption must fall far short of the maximum that they are capable of consuming at high tensions. Furthermore, even in presence of an abundant supply of oxygen, a large part of the metabolism is anaerobic (*cf.* Alt and Tischer, 1931; von Brand, 1934a). The conclusion thus seems inescapable that when the worms are in their normal habitat anaerobic respiration markedly predominates. One is therefore justified in considering these animals as predominantly anaerobic.

There are some clear indications, however, that, at least in *Ascaris,* even the small amount of oxygen present in the intestine may take part in metabolism. The ascarids have some haemoglobin (Flury, 1912; Krüger, 1936), which will assist them in gathering oxygen at low tensions. Another important observation is that made by Slater (1925) according to which death was more rapid in electrically stimulated specimens under anaerobic than under aerobic conditions. Finally, von Brand (1937) demonstrated that *Ascaris* is able to resynthesize some glycogen in a period of aerobic metabolism following one of anaerobic metabolism. The occurrence of such a clearly aerobic process could hardly be understood unless it existed also in nature.

Other helminths are probably capable of leading a more aerobic life in the intestine. They may either get significant amounts of oxygen from the blood which they withdraw from the host, as happens in the case of hook-

worms (Well, 1931), or they may be so small and possess a surface/volume ratio high enough to permit a relatively large amount of oxygen to enter the body even at low tensions. Von Brand (1938) explains on this basis Davey's (1937, 1938) observation that small nematodes from the sheep survive much better *in vitro* when oxygen is present than when it is absent. Davey himself postulated an aerobic existence also in the intestine.

To sum up, then, we are of the opinion (see von Brand, 1938) that the old controversy "aerobiosis or anaerobiosis," with respect to intestinal worms, should be abandoned, and that it should be recognized that one animal may, depending on its organization, live a predominantly anaerobic life in the same surroundings in which another worm is capable of leading a chiefly aerobic life.

(b) Tissue helminths. The same variability in metabolic relationships probably applies also to helminths parasitizing tissues but this topic does not seem to have been discussed anywhere in detail. The reason is evidently that most of the facts tending to clarify the issue have come to light only in comparatively recent years.

It can be stated with some degree of certainty that some worms lead inside the host tissues a predominantly aerobic life. Stannard, McCoy and Latchford (1938) studied the metabolism of *Trichinella larvae*. They found a well developed aerobic metabolism though some anaerobic processes persist even at high oxygen tensions. Under anaerobic conditions the animals became more or less motionless, but survived well. The cellular respiratory system, as evidenced by the reactions to a variety of respiratory poisons, suggests an aerobic rather than an anaerobic existence. It should be remembered, however, that the larvae are practically motionless once the host tissues have formed the cyst wall around them. It is possible that the procuring of oxygen might be difficult

and that, in nature, the anaerobic phase of the metabolism is of greater importance than appears from the experiments of Stannard, McCoy and Latchford.

An aerobic existence within the intermediate host can definitely be expected in the case of the larval *Eustrongylides ignotus*. The animals are blood-red due to the presence of haemoglobin in their body fluid (von Brand, 1937a). If they are exposed experimentally to anaerobic conditions they accumulate a considerable oxygen debt which is repaid if the animals are kept subsequently in an oxygen-rich atmosphere. Worms, however, that have been isolated freshly from fish exhibit only a trace of this phenomenon. Such a behavior indicates that they had lived aerobically within the fish for if they would have lived anaerobically one would expect them to have to repay a maximal oxygen debt once they have access to large amounts of oxygen (von Brand, 1942).

An aerobic or primarily aerobic existence can probably also be assumed for many tissue helminths parasitizing invertebrates. The clearest evidence that this assumption is well-founded—evidence, however, which does not yet constitute real proof—has been found by Vogel and von Brand (1933) in the case of the developmental stages of the liver fluke *Fasciola hepatica*. The metabolism of the adult fluke is anaerobic, one of the endproducts being higher fatty acids demonstrable in the excretory system (von Brand and Weinland, 1924). Vogel and von Brand showed that this excretory fat begins to appear only when the parasites reach the vertebrate host, probably because they there encounter oxygen deficiencies. In the stages parasitizing the snail no trace of such fat could be found, a condition which apparently indicates other, perhaps aerobic metabolic processes.

On the other hand, there are indications of an anaerobic existence for some tissue helminths. Coutelen (1931) observed fat in the excretory system of *Echinococcus* sco-

lices within the hydatid cyst. These morphological observations gave probability to the assumption that anaerobic processes occur of which the endproduct is fat. The hydatid fluid itself contains succinic, valeric, propionic, acetic and lactic acids (Flössner, 1924, 1925). Lactic acid probably occurs also in the cysts of *Cysticercus tenuicollis* according to Schopfer (1932). All these acids are common endproducts of anaerobic processes in invertebrates. One cannot, however, be quite specific as to their origin here. The properties of the internal fluid of the vesicular stages of cestodes liken it to a transudate in equilibrium with the blood plasma of the host (Schopfer, 1932; Wardle, 1937). It is obvious therefore that any substance found in the fluid may have had its origin in the host and is not necessarily derived from the metabolic activities of the parasite. It is regrettable that the gas contents of the hydatid fluid have so far never been studied; it would be of interest to know whether larger amounts of oxygen enter the cysts by diffusion.

IV. ECHINODERMS

No echinoderm has so far been reported from oxygen-free surroundings. This might be due to poorly developed anaerobic functions in all members of this phylum, or possibly to the lack of investigation in that direction. It is common knowledge, however, that many sea urchins and holothurians bury themselves in the sand and mud of the sea bottom. If it is true, as was pointed out previously, that the bottom material of the sea is frequently devoid of molecular oxygen, the echinoderms which penetrate deeper into the bottom should be exposed to severe anaerobic conditions. Whether they have means, in all cases, of securing oxygen from the overlying water layers remains to be seen.

Echinoderms have not been subjected to much experimental study, at least not in the adult stage. We know only the work of Baglioni (1905) who showed that *Echinus*

microtuberculatus and *Ophioderma longicauda* hardly survive a 24-hour exposure to anaerobic conditions.

Thus, while no definite statement is possible at present as to whether any adult echinoderm is able to lead a completely anaerobic life for any length of time, it appears safe to assume that a partial transition to anaerobic metabolism is possible in many cases. This is indicated by the fact that the oxygen consumption of various species is dependent on the tension, as has been shown for the holothurians *Caudina chilensis* (Nomura, 1926) and *Thyone briareus* (Hiestand, 1940), as well as for the starfish *Patiria miniata* (Hyman, 1929), *Asterias rubens* (Meyer, 1935) and *Asterias forbesii* (Maloeuf, 1937).

Much more work has been done with the eggs and sperms of echinoderms, especially of sea-urchins. Unfertilized eggs of *Arbacia* were kept for periods of five to eight hours in the complete absence of oxygen (Harvey, 1930; Barron, 1932). This treatment did not deprive them of their ability of being fertilized after the restoration of aerobic conditions. However, after 24 hours without oxygen many eggs were damaged and the fertilization rate had decreased to 15 to 20 per cent.

On the other hand, earlier observers, as for example, Loeb and Lewis, 1902, Loeb, 1915 (*c.f.* also Lyon, 1902), had reported that the fertilization rate of the *Arbacia* egg was not affected at all after a 24-hour exposure to hydrogen, and that some eggs could be fertilized even after 64 hours. It is probable, as Barron (*l.c.*) points out, that in these older experiments the oxygen was not completely eliminated.

The spermatozoa of the sea-urchins proved to be more sensitive than the eggs, both in Barron's and in Harvey's experiments. They became rapidly immobilized and lost the power of fertilizing normal, untreated eggs when they had been kept for 3 hours under hydrogen.

The reproductive cells of starfish behave somewhat differently (Barron, *l.c.*). Both unfertilized eggs and sperm were dead after being deprived of oxygen for 4 hours (the species is not indicated).

The fertilization of *Arbacia* eggs does not take place if both eggs and sperm are kept under anoxic conditions. When care is taken to eliminate every trace of oxygen (Barron, *l.c.*) not even the stage of the elevation of the fertilization membrane is reached, although this process seems to require only minimal traces of oxygen (Harvey, *l.c.*).

Under normal conditions the first response of the egg to insemination consists in the breakdown of a thin layer of cortical granules. This phenomenon, known as the cortical layer response, can be elicited under experimental conditions by a variety of stimuli (Moser, 1939). It is indeed interesting that it takes place in unfertilized *Arbacia* eggs maintained under rigid anaerobic conditions. Kitching and Moser (1940) observed it when the eggs were treated in the absence of oxygen with ¼ per cent saponin in sea water, with isotonic urea or sucrose solutions, or when they were punctured with a microneedle. If unfertilized eggs of the sea-urchin were first treated with urea and this substance was then removed by repeated washings with sea water—strictly anoxic conditions being maintained during all these manipulations —the eggs began to undergo cleavage after oxygen was admitted. Obviously, then, the stimulus that prepares the eggs for cleavage does not require molecular oxygen.

The cleavage process itself, on the other hand, is entirely dependent on the presence of oxygen. It does not take place in the complete absence of oxygen, neither at very low tensions, *e.g.*, below 4 mm. of mercury (Loeb, 1895; Harvey, 1927; Amberson, 1928; Tang and Gerard, 1932). The fact that the oxygen consumption of both fertilized and unfertilized *Arbacia* eggs is somewhat de-

pendent on the tension (Tang, 1931; Tang and Gerard, 1932) is of interest, for it suggests the possibility that part of the required energy may be derived from anaerobic processes at low tensions.

A curious observation, the significance of which is not yet fully apparent, is that anaerobiosis definitely decreases the permeability of the *Arbacia* egg to water; it has no influence, however, on the equilibrium volume of the eggs when the latter are exposed to 66 or 80 per cent sea water (Kekwick and Harvey, 1934).

Another remarkable fact is that the prevention of oxygen consumption, either by the use of cyanide or by immersion in oxygen-free sea water, increases by several hours the period during which unfertilized starfish eggs may be fertilized by sperm, or activated artificially. The period necessary to induce activation by heat or acid also becomes shorter with increasing length of the anoxic conditions (Lillie, 1931).

V. MOLLUSCS

Among the molluscs, the anaerobic functions are most pronounced in the lamellibranchs, they are little developed in gastropods and seem practically non-existent in cephalopods. These differences are clearly related to ecological conditions as will become apparent from the following discussion.

1. LAMELLIBRANCHS

A. Occurrence in anaerobic habitats and tolerance of experimental anaerobic conditions. Lamellibranchs, in general, show little locomotion; many are even truly sessile organisms. Consequently they cannot readily escape asphyxiation by wandering away if the oxygen disappears in their surroundings. Many clams live buried in the mud, where, as shown previously, severe oxygen

deficiencies or complete lack of oxygen often occur. But one cannot, of course, conclude that the metabolism is purely anaerobic in all these cases.

Alsterberg (1930) has shown that *Sphaerium corneum* reacts to the micro-stratification of oxygen by extending its siphon, and that the poorer the surroundings become in oxygen the farther out the siphon is projected, in other words, this mollusk behaves, in principle, like *Tubifex.*

Such a behavior, which may be widespread among lamellibranchs, seems to be correlated with the resistance in experimentally induced anaerobic conditions. Thus, according to Moore (1931), the clam *Syndesmya alba,* which normally keeps its siphon in contact with the water overlying the bottom mud, never survived longer than 3½ days in the absence of oxygen, while *Nucula tenuis,* which feeds at a depth of several inches below the surface of oxygen-free mud, keeping no direct communication with the overlying water, remained alive and active during anaerobic periods of 5 to 17 days; Moore even attributed their death after that time to poisoning of the water by the animals' excreta rather than to asphyxiation.

Certain fresh-water clams, especially *Pisidium idahoense,* are not killed by periodic stagnation of the overlying water masses (Juday, 1908). They also show remarkable endurance under experimental conditions (Juday, 1908; Cole, 1921; compare Table 12). They reduce their energy requirements to a minimum by keeping their shells closed and performing no movements. The survival of lamellibranchs over periods of winter anaerobiosis which are often of long duration, is probably due to the fact that in them, as in many other animals, the length of the survival time under anaerobic conditions is inversely related to temperature. This is shown by Collip's (1921), Pieri's (1895) and Dotterweich and Elssner's (1935) data recorded in Table 12.

Besides living in the above-mentioned anaerobic habitats, clams also frequently occur in localities where the oxygen supply is scanty. Cole (1926) found *Anadontoides ferrussacianus* buried from two inches to a foot deep in soft muck consisting mainly of decomposing plant material and fine silt. The oxygen content of the water permeating the mud was only about 6 per cent of that of the stream water. The animals were nevertheless active and most of them had their foot extended. Another similar case is that of *Anodonta mutabilis* which was found alive by Fehlmann (1917) in water containing only 0.081 cc. of oxygen per liter.

It seems very unlikely that animals found under such conditions could have maintained a purely aerobic metabolism. While the oxygen consumption of these clams seems not yet to have been studied in relation to the tension, it is to be noticed that Maloeuf (1937) found a clear case of dependency in *Mytilus edulis* and Nozawa (1929) a less marked one in *Ostrea circumpicta.*

Unlike *Pisidium, Anodonta* extends its foot and begins to wander around when the oxygen of the water is almost depleted (Alsterberg, 1930). *Sphaerium corneum* behaves still differently. Jatzenko (1928) immersed some of these clams in tightly closed containers which had been filled with water rich in oxygen. During the first day they had their siphons extended and their shells open. After they had consumed some oxygen and thus lowered the oxygen tension of the water, they closed their shells and kept them closed for 18 days. Later on, however, they began again to crawl around and their locomotion became increasingly active until the experiment was discontinued at the end of 46 days (from the start). This observation might be interpreted as indicating an adaptation to the anoxic conditions, but the present writer would prefer to accept such an explanation only after Jatzenko's results have been confirmed.

TABLE 12

MAXIMAL SURVIVAL TIME OF MOLLUSCS UNDER ANAEROBIC CONDITIONS.

Species	Temp. °C	Strictly anaerobic conditions?	Survival time in days	Source
		1. Lamelli-branchs		
Anodonta cygnea	Room temp.	?	7	Koch, 1917
" "	15	No	>8	Dotterweich & Elssner, 1935
" "	0	"	>30	" " "
" "	Room temp.	?	>1	van Dam, 1937
Anodontoides ferrussacianus	" "	?	14	Cole, 1926
Cardium edule	" "	Yes	5	Jacubowa & Malm, 1931
Mya arenaria	31	"	1	Collip, 1921
" "	14	"	8	" "
" "	"very low"	"	"weeks"	" "
" "	0 to 10	"	>7	Berkeley, 1921
Mytilus galloprovencialis	Room temp.	"	5	Jacubowa & Malm, 1931
Nucula tenuis	" "	"	17	Moore, 1931
Ostrea sp.	" "	?	7	Mitchell, 1912
" *taurica*	" "	Yes	5	Jacubowa & Malm, 1931
Paphia (Tapes) decussata	Summer temp.	"	4	Piéri, 1895
" " "	Winter temp.	"	8	" "
" " *rugata*	Room temp.	"	10	Jacubowa & Malm, 1931
" *staminea*	0 to 10	"	>21	Berkeley, 1921
Pecten ponticus	Room temp.	"	<1	Jacubowa & Malm, 1931
Pisidium idahoense	" "	"	>14	Juday, 1908
" "	" "	"	90	Cole, 1921
" sp.	15 to 16	"	3	Alsterberg, 1922
Saxidomus giganteus	0 to 10	"	>14	Berkeley, 1921

TABLE 12 — (Continued)

Species	Temp. °C	Strictly anaerobic conditions?	Survival time in days	Source
Sphaerium corneum	14 to 16	Yes	46	Jatzenko, 1928
Syndesmia alba	Room temp.	"	3½	Moore, 1931
" *ovata*		"	10	Jacubowa & Malm, 1931
		2. Gastropods		
Prosobranchia				
Bithynia sp.	20	?	5	Alsterberg, 1930
Cerithiolum reticulatus	Room temp.	Yes	1	Jacubowa & Malm, 1931
Rissoa venusta	" "	"	1	" " "
Vivipara sp.	20	?	5	Alsterberg, 1930
Opisthobranchia				
Aplysia sp.	Room temp.	Yes	several hrs.	Henze, 1910
Pulmonata				
Helix aspersa	Room temp.	Yes	3	Fischer, 1931
" *hortensis*	20	?	2½	Alsterberg, 1930
" *pomatia*	"	?	"	" "
" "	Room temp.	No	5	Yung, 1888
Limax agrestis	" "	Yes	7 hrs.	Thunberg, 1905
Limnaea ovata	20	?	2½	Alsterberg, 1930
" *stagnalis*	"	?	2½	" "
" "	0 and 8 to 10	?	>7	" "
" *truncatula*	20	?	2½	" "
Planorbis corneus	"	?	3	" "
" sp.	14 to 16	?	25	Jatzenko, 1928

Clams living in the tidal zone usually close their shells tightly when the ground is uncovered. Mitchell (1912) showed that the oxygen consumption of oysters that have shut their valves is not greater than that of empty shells, that is, than the oxygen consumption of microorganisms growing on the shells. This means that the oxygen uptake of the living tissues has ceased. In other species, *Mya arenaria* and *Venus mercenaria,* the oxygen consumption stopped only if the valves were closed artificially. *Venus* especially continued to consume oxygen when the valves were closed by the animal itself. Nevertheless, even in this case, the oxygen intake seems to be insufficient to support a purely aerobic metabolism. The recent work of Dugal (1939), which led to the conclusion that there is an anaerobic metabolism in this species, was performed with *Venus* specimens kept in air. This, of course, caused them to keep their shells closed as tightly as possible.

Altogether considered, it seems very likely that during low tide many lamellibranchs have a more or less anaerobic metabolism. This is an interesting difference between lamellibranchs and the previously discussed worms occurring in the same habitat.

B. Type of life of lamellibranchs in the absence of oxygen. Whether clams found in media where there is no molecular oxygen, or such as are brought experimentally under anoxic conditions, actually lead an anaerobic existence, in the true sense of the word, has been questioned. It has sometimes been assumed that they have some special sources of oxygen.

Cole (1921) found that mud containing decaying plant tissues was able to oxidize guaiacum in the darkness and in the absence of molecular oxygen. He is of the opinion that the oxidizing agent may be continually formed in the bottom layers. There were some indications that this oxidant might be atomic oxygen which then would be avail-

able to the bottom organisms. But the amounts of oxygen that could be obtained in this manner are obviously extremely small and would likely be used up by the rich bacterial flora of the mud before the much bulkier bottom metazoa could obtain any significant quantity.

Another possibility was that the lamellibranchs "have a store of available oxygen in their tissue which suffices to supply the necessary oxygen when the outside supply is cut off " (Collip, 1921). Searching for such a source, Berkeley (1923) was the first to point out that in the crystalline style of *Saxidomus giganteus* a compound occurs that oxidizes guaiacum, but not paraphenylenediamine or pyrogallol, in the absence of hydrogen peroxide or air, and that is not destroyed by boiling. Berkeley (*l.c.*) expressed the opinion "that the substance consists of a complex of an oxidizing agent and an enzyme, the enzyme being capable of conveying oxygen from the oxidizing agent to the body oxidized. It would thus resemble an oxidase except that its activity would be limited by the amount of oxygen available for transfer from the non-enzyme component." Similar enzyme complexes have been found in the crystalline style of many lamellibranchs, but also in other organs, for example, the gills (Cole, 1921; Berkeley, 1933a, 1935; Yonge, 1926; Graham, 1931). A detailed discussion of the various enzymes involved is presented by Berkeley (1933). One of the main arguments brought forward by him (1923) to support his view of the importance of this system for anaerobic life as a "store of fixed oxygen" was that the crystalline style disappears rapidly if the animals are kept under anaerobic conditions, an observation confirmed by Nozawa (1929).

There can be no question about the reality of this enzyme complex, but it is doubtful whether it has the significance attributed to it by Berkeley. There is, first of all, the curious point that, though the crystalline style seems to disappear very rapidly in the absence of oxygen,

the animals have been reported to survive anaerobically for long periods both under natural and under experimental conditions. If they would obtain their oxygen from the crystalline style, such a discrepancy would be hard to explain. Secondly, it has been emphasized, especially by Maloeuf (1937), that the crystalline style is such a small structure that one cannot easily conceive how significant amounts of oxygen could be liberated from it. Thirdly, according to Nelson (1925) and Yonge (1926a), there is no correlation between the size of the lamellibranchs' crystalline style and the degree of aeration of their surroundings. Finally, recent studies (*e.g.,* Dugal, 1939) have directly demonstrated that even in clams that are not kept under very strict anaerobic conditions true anaerobiosis takes place. (This will be reviewed in detail later.)

Weighing the available evidence, the present writer is convinced that the clams behave like other invertebrates and that they do not store oxygen in amounts which are large enough to exert a significant influence on the metabolism during longer periods of oxygen want.

2. GASTROPODS

The gastropods seem to be the first invertebrates that were exposed to anoxic conditions. In his posthumous work "*Memorie su la respirazione*" Spallanzani (1802) states that he confined snails in vessels in which the air had been replaced either by carbon dioxide or by hydrogen. In the former case he found a survival of less than 40 hours, in the latter, a survival of at least 18 hours. He noticed also that snails kept in closed vessels were able to consume all the oxygen present. He finally gives some data on the carbon dioxide produced under these circumstances. Before Spallanzani's work appeared, it was generally assumed, on the basis of Vauquelin's experiments (1792) that snails placed in closed vessels die rapidly after all the oxygen has disappeared.

The more recent findings about the anaerobic survival of gastropods are summarized in Table 12. They indicate that, contrary to what has been found in lamellibranchs, there is no direct connection between the ability to respire anaerobically and the possible lack of oxygen in the surroundings. Alsterberg (1930) pointed out that, at given temperatures, terrestrial gastropods, like *Helix,* survive about as long as the aquatic snails *Limnaea* or *Planorbis,* and almost as long as *Vivipara* or *Bithynia.* He concludes that the gastropods show a certain resistance to lack of oxygen regardless of their ecological relationships.

Alsterberg might even underestimate the anaerobic functions of *Helix.* He immersed his specimens in water containing little oxygen and determined the death time in this medium. It is well known that *Helix* and other terrestrial snails die rather rapidly if submersed in water, even when it is well aerated. This has been shown by Raffy and Fischer (1931, 1934) for *Helix* and *Succinea* and by Colosi (1932) for *Helix* and *Limax.* But death seems to be due primarily to an osmotic disturbance and thus the true resistance of the animals to lack of oxygen might have been masked in Alsterberg's experiments.

There are no indications that, under natural conditions, *terrestrial gastropods* may have to respire anaerobically to any marked extent. The possibility, however, that some anaerobic processes may go on, even at high oxygen tensions, cannot be excluded, since snails, in general, seem to belong to the class of animals whose oxygen consumption depends to a marked degree on the tension (Thunberg, 1905; Harnisch, 1932).

Whether or not hibernating terrestrial snails ever find themselves in oxygen-free surroundings has, to the present writer's knowledge, never been investigated. It seems likely, however, that this may happen from time to time. Many species burrow rather deeply into the ground and,

during rainy periods, their oxygen supply may be considerably depleted. It should be emphasized, however, that normally hibernating terrestrial snails do have access to oxygen and it has been shown that they are able to consume it. Even in such species as *Helix,* which close their shells with a rather heavy calcareous epiphragm, the persistence of an oxygen consumption has been demonstrated. The epiphragm itself, and even—though to a much lesser degree—the shell proper are permeable to gases (Fischer, 1931). Both Schurmans-Stekhoven (1920) and Fischer (1931) found that the oxygen consumption of hibernating snails is increased when the epiphragm is removed. Of course, this does not necessarily indicate the replacement of anaerobic by aerobic respiration; the increase in evaporation, or the small muscular movements that may take place under these circumstances may increase the need for energy.

It is not uncommon for *aquatic snails,* on the contrary, to find themselves in surroundings very poor in oxygen or even entirely deprived of oxygen. An important difference between snails and lamellibranchs is the fact that the former are able to move rather rapidly from place to place. When oxygen disappears during the summer stagnation period, the snails simply wander to places that are still aerated, a thing which clams cannot usually do. From the standpoint of the survival of the species, therefore, it matters little that the resistance of aquatic gastropods to the complete lack of oxygen is not so well developed.

Furthermore, some aquatic snails have a sufficiently powerful respiratory pigment in their blood to enable them to secure significant amounts of oxygen even at low tensions. This question has been investigated thoroughly only in the case of *Planorbis* (Leitch, 1916; Borden, 1931; Wolvekamp, 1932) whose respiratory pigment is haemoglobin. This snail, it is true, is not purely aquatic in its respiratory habits. It comes to the surface and

breathes air through its lung when the oxygen tension of the water drops below a certain level.

During the winter, escaping to a new habitat is obviously impossible if the water is covered with ice, as was noted by Alsterberg (1930). But, according to this investigator's experiments on *Limnaea stagnalis,* the aquatic snails support anaerobic conditions much better at low temperatures. At 20°C none of the organisms survived longer than 2½ days, while they all were still alive after seven days when kept anaerobically at 8 to 10° and 0°C.

Worms and clams living in the *tidal zone* have been shown to owe their survival to different mechanisms. Worms, on the one hand, with the help of their respiratory pigments, store some oxygen in their blood and are also able to extract oxygen from the water surrounding them even if it contains only very little of this gas. Clams, on the other hand, seem to switch over more readily to a partially anaerobic metabolism.

Of the numerous snails which live in the regions covered by the tide only *Littorina neritoides* and *L. rudis* seem to have been investigated. Fischer, Duval and Raffy (1933) state that these animals consume oxygen when exposed to air, although they consume 5 or 6 times less than when they are submerged in water. Tidal animals, in general, reduce muscular movements to a minimum when exposed to air and this may largely explain the decreased oxygen consumption observed in *Littorina.* We have, at the present stage of our knowledge, no reason to assume a transfer to partial anaerobic life during the periodic exposure to air that occurs in the life of these animals.

The less well developed anaerobic functions of snails as compared to those of clams may also become apparent in the *reaction of certain organs* to the lack of oxygen. Yung (1888) found that the frequency of the heart beat of *Helix* is reduced under anoxic conditions while Koch

(1917) observed no marked change in the case of *Anodonta*. There seems to be some doubt, however, whether the slowing of the heart beat of *Helix* is directly due to lack of oxygen. Ysseling (1930), in experiments of much shorter duration than those of Yung, found that the frequency of the heart beat depends only indirectly on the oxygen tension. The decisive point, according to him, is whether the lung orifice is kept open or closed. If it is open, the heart beat is almost normal in a nitrogen atmosphere containing 2 per cent oxygen; if it is closed, the frequency of the heart pulsations decreases and the organ may cease to beat entirely for varying lengths of time. If *Helix* is transferred from air to an atmosphere poor in oxygen (below about 9 per cent), its respiratory movements become gasping, *i.e.*, the lung orifice is alternately closed and opened in rapid sequence (Ysseling, *l.c.*).

Earlier observations on the respiratory movements of snails in oxygen-poor or oxygen-free surroundings were reported by Schurmans-Stekhoven (1920). He found a tendency on the part of the animals under these conditions to keep the lung orifice closed. This tendency, according to him, leads to a preservation of the oxygen that was originally present in the lungs, since it then does not become diluted with the inert gas as it would be if an open communication existed between the lungs and the surroundings.

3. CEPHALOPODS

Cephalopods have very poorly developed anaerobic functions and it is one of their characteristic ecological features that they are never found in environments which are really poor in oxygen.

Redfield and Goodkind (1929) introduced squids (*Loligo pealii*) into aquaria of 5 to 8 liters capacity filled with water rich in oxygen and overlayed with ½ inch of par-

affin oil. The temperature was 18 to 22°C. The squids ceased to respire in 40 to 60 minutes under these conditions, and in 10 to 15 minutes when carbon dioxide was dissolved in water. The shortened life in water containing larger quantities of carbon dioxide was not due, however, to a toxic action of this gas but to its influence in hindering the oxygenation of the blood. Redfield and Goodkind found that death by asphyxiation took place at all those combinations of oxygen and carbon dioxide pressures that prevented the arterial blood from absorbing more than 0.5 to 1.5 volumes per cent of oxygen.

The nervous system of cephalopods is quite sensitive to the lack of oxygen. The ganglia generally succumb earlier than the nerves, and the various ganglia are not alike in their resistance to asphyxiation, according to the investigations of Baglioni (1905) and Fröhlich (1910).

VI. ARTHROPODS

1. CRUSTACEANS

Planktonic crustaceans are reported rather frequently from surroundings poor in oxygen or completely devoid of it; copepods especially, and to a lesser degree cladocerans, seem to tolerate well a reduced oxygen supply.

Fresh water species have been observed in water quite poor in oxygen by Birge and Juday (1908), Huss (1913), Fehlmann (1917), Alsterberg (1922), Thienemann (1925), and Lönnerblad and Naumann (1934). According to Lauterborn (1916), *Cyclops* spp., *Canthocamptus* sp. and *Lathonoura rectirostris* occur in oxygen-free sapropelic habitats. Ziegelmayer (1923) found *Cyclops albidus* in oxygen-free marshy waters, deep in mines, and *Cyclops serrulatus* in sulfur springs of the Appenine Mountains which were also devoid of oxygen (1924). Ward (1940) encountered *Cyclops albidus,* as well as the cladocerans *Daphnia pulex* and *Simocephalus exspinosus,* in the oxygen-free waters of a pond; *Cyclops bi-*

cuspidatus was reported from a similar locality by Imel (1915).

Several *Cyclops* species proved to be quite resistant also in experimentally induced anaerobic conditions (Table 13), as shown by the figures of Huss (1913), Nikitinsky and Mudrezowa-Wyss (1930) and Lindeman (1942). It appears very likely, therefore, that some, but certainly not all, species of *Cyclops,* and perhaps other copepods, as well as some cladocerans, are able to live for relatively long periods in the absence of oxygen in nature. Before coming to a definite conclusion, however, it is desirable to study more thoroughly the oxygen relationships of the organisms involved. Several factors that may prove serious objections to the view of a marked ability of such animals to live anaerobically will therefore be considered in the following paragraphs.

One is whether the animals really stay for any length of time in the truly anaerobic layers. They are good swimmers and might from time to time come to the surface strata which are never entirely devoid of molecular oxygen.

Another objection that might render doubtful the significance of the long survivals reported by Huss (1913) and Lindeman (1942) is that it is not sufficiently clear whether the animals found motile at the end of the experiments did not pass at least part of the time in resting stages. This possibility should be considered seriously; it was given some attention by Huss but the problem was not adequately solved. Birge and Juday (1908) and Moore (1939) observed that in nature *Cyclops bicuspidatus* regularly forms a cocoon in which immature specimens lead a latent life for several months.

It is important to note that these animals were able to hatch even when the lake water was still oxygen-free. But since neither the hatching nor the formation of the cocoons can be correlated with any definite change in the

composition of the water, these observations are difficult to evaluate in regard to the anaerobic survival during the experimental periods.

Comparable cyst formations have been observed also in other copepods, especially in Harpacticoids (Lauterborn and Wolf, 1909; Kessler, 1912; Donner, 1928; Moore, 1939; Deevey, 1941). The last-named investigator found no hatching from cysts of *Canthocamptus staphylinoides* during experimental anaerobic periods lasting 5 to 15 days at temperatures between 10 and 20.5°C. He is of the opinion "that encystment is a constant feature of the life cycle of *Canthocamptus,* but that the time of onset and of emergence is under environmental control, so that encystment is an efficient form of anaerobiosis."

Planktonic marine crustaceans may prove to have well-developed anaerobic functions; a better knowledge of the fauna in the layer of minimum oxygen concentration in the Pacific might reveal interesting adaptations. *Pseudocalanus elongatus* and *Calanus finmarchicus,* as Nikitin (1931) has observed, penetrate into layers of the Black Sea where the oxygen concentration is well below 1 cc. per liter. Furthermore, Marshall, Nicholls and Orr (1935) reported that the oxygen consumption of *Calanus finmarchicus* begins to decline when the concentration of oxygen sinks below 3 cc. per liter. The conclusion that, in the Black Sea, this animal should be forced to supplement the failing aerobic metabolism by anaerobic processes seems almost inescapable.

Another interesting, though as yet less analyzed case is that of *Mysis relicta.* This animal occurs both in fresh and in sea water. The minimum oxygen concentration for its well-being is 4 cc. per liter in fresh water, while in the Baltic Sea the oxygen concentration of the deep layers in which the animal is mostly found is only around 1.6 cc. per liter (Thienemann, 1928). These observations give rise to the following questions: (a) Is

the oxygen needed by the tissues supplied so much more easily in salt water than in fresh water (*cf.* Thienemann, *l. c.*)? (b) Is the oxygen requirement greater in fresh water than in salt water (*cf.* Schlieper, 1929)? (c) Does a partial transition to anaerobiosis take place in the sea? Only experimentation will bring an answer.

Small crustaceans that burrow in the *bottom* material must also sometimes find themselves in very difficult respiratory conditions. Moore (1931), who studied the distribution of Harpacticoids, especially *Danielssenia typica* and *Cletodus longicaudatus*, and of ostracods in the mud of the Clyde Sea in areas where the interstitial water was free from oxygen, found these two copepods and several ostracods in the surface layer mostly; very few were found at depths below 1 cm. It is doubtful whether the uppermost layers were completely free of oxygen and since the copepods, according to Moore, showed only little resistance in experimental anaerobic conditions, one cannot assume that they normally live in the complete absence of oxygen. But there is no reason to doubt that they must be adapted to life at low oxygen tensions.

Fresh-water *ostracods* seem to be even more resistant. Juday (1908) found living *Candona* in lakes during the stagnation periods; Moore (1939) reported *Candona exilis, Candona reflexa, Cypria exsculpta* and *Cypria lacustris* from a similar habitat and Ward (1940) found *Cypria elegantula* in the oxygen-free water of a pond. Experimentally, ostracods proved to be very resistant, as Lindeman (1942) has shown.

Little is known about *isopods*. *Asellus aquaticus* has been found in oxygen-free water during the winter (Alsterberg, 1930); and the occurrence of *Glyptonotus entomon* in layers of the Baltic Sea poor in oxygen has been assumed to be linked with well-developed anaerobic functions (von Buddenbrock, 1939). But the last-named or-

ganism has not, so far, been studied experimentally in that respect.

Interesting observations have been made on *cirripeds.* The barnacle *Chthalamus stellatus,* according to Monterosso (1932, 1932a) withstands without difficulty strictly anaerobic conditions for periods of more than 14 days. Naturally, the question arises whether this surprising resistance is of ecological significance. It is well known that barnacles frequently establish themselves in localities where they may not be submerged in water for rather long periods. Moore (1935) remarks that *Balanus balanoides* may be exposed to air as long as a week in its normal habitat. He states: "when exposed to air, the barnacle's shell is closed, and the barnacle is extremely resistant to adverse outside conditions." As shown in a previous section, clams kept in a similar fashion close their valves so tightly that an anaerobic milieu becomes definitely established inside the shell. Barnacles do not seem to behave exactly in the same manner. Some observations by Monterosso (1927) indicate that in *Chthalamus* a minimal opening of the shell persists, and Borsuk and Kreps (1929) have demonstrated that *Balanus balanoides* and *Balanus crenatus* are actually able to consume oxygen, though at a decreased rate, when kept outside the water. The figures given by the last-mentioned authors are unfortunately insufficient to decide whether the decrease in oxygen consumption is so marked as to lead one to assume that there is a partial transition to anaerobic life. The question is very interesting and should be investigated further.

An indication that transition to a partially anaerobic metabolism is possible in cirripeds can be found in observations by Kreps (1929). He demonstrated that the oxygen consumption of *Balanus crenatus,* in water, depends to a marked degree on the salinity. In water with a salt content of less than 0.6 per cent it is minimal, the animals being anabiotic. The interesting point, how-

ever, is that the decline of the oxygen consumption goes farther than can be explained merely by lowered energy-needs due to cessation of all muscular movements. When such animals are brought back into water of normal salinity, their oxygen consumption is markedly higher than normal. In other words, they have contracted an oxygen debt—an indication that partially oxidized substances have accumulated in their bodies.

Decapod crustaceans, such as crabs or crayfish, show little resistance in experimentally induced anaerobic conditions (Table 13). It is true that crayfish are sometimes found in highly polluted waters where only traces of oxygen are present (Jewell, 1918) but they probably do not stay long in such environment. It should further be remembered that crayfish are able to regulate their respiratory movements (*cf.* Peters, 1938; Jordan and Guittart, 1938) which become proportionately more frequent as the oxygen supply of the water becomes poorer. It is possible, however, that at very low tensions this mechanism becomes inadequate and that the animal is then forced to resort to incomplete oxidations.

It should be realized, in this connection, that the oxygen consumption of most decapods is independent of the tension over a wide range (some exceptions are known). Data concerning this point are presented by Henze (1910), Amberson, Mayerson and Scott (1924), Helff (1928), Hiestand (1931), and Maloeuf (1937). Hiestand has collected some evidence indicating that an oxygen debt may be built up in crayfish, and Maloeuf (1936) has demonstrated that the respiratory quotient of these animals rises at low tensions.

A few other *isolated observations* concerning the influence of a lack of oxygen on crustaceans may finally be mentioned here. Fröhlich (1938) reports that asphyxiation of *Daphnia pulex* proceeds more slowly in the water of the hot springs of Gastein than in ordinary water

and that the radium emanation present in the thermal water is only partially responsible for this effect.

Levin (1933) found that the asphyxiation of *Crangon vulgaris* is retarded if certain vital dyes are added to the sea water (*e.g.*, neutral red, 1: 50,000; Bismarck brown, 1: 100,000; or methylene blue, 1: 50,000). In a second paper (1933a) he reported that the survival period of this shrimp in oxygen-poor water was shorter if small amounts of arsenious acid plus neutral red were added than if the water contained either of these substances alone. Under conditions of good oxygenation, on the other hand, an opposite effect was observed, the survival being better when both substances were present together.

2. AQUATIC INSECTS

I. LARVAE

Larvae of insects leading an aquatic life are found in several habitats which are poor in oxygen or without oxygen. Lauterborn (1916) reports the larvae of *Eristalis tenax* in sapropelic ponds. One may doubt, however, whether this actually implies an anaerobic life. It seems quite possible that these larvae regularly get oxygen from the surface by means of their long prehensile tail which represents a respiratory syphon.

As to *Donacia* larvae, they occasionally get only small amounts of oxygen from their surroundings. Their normal oxygen supply is derived from the airspaces of submerged aquatic plants (Boeving, 1910; Deibel, 1911). The oxygen concentration in this air can at times be very low as has been pointed out previously. But recent experiments (Hoffman, 1940) have demonstrated that these larvae are also capable of utilizing directly the oxygen dissolved in the water. Nevertheless, most investigators agree (see Table 14) that the *Donacia* larvae are very resistant in experimentally induced anoxic conditions. The seemingly contradictory observation of Nikitinsky

TABLE 13

MAXIMAL SURVIVAL TIME OF CRUSTACEANS UNDER ANAEROBIC CONDITIONS.

Species	Temp. °C	Strictly anaerobic conditions?	Survival time	Source
Entomostraca				
Alona quadrangularis	Room temp.	?	4 hrs.	Nikitinsky & Mudrezowa-Wyss, 1930
Artemia franciscana (dry cysts)	16–22	Yes	180 days	Whitaker, 1940
" *salina*	Room temp.	?	2 hrs.	Nikitinsky & Mudrezowa-Wyss, 1930
Bosmina longirostris	" "	?	"	" " " "
Chthamalus stellatus	" "	Yes	>14 days	Monterosso, 1932
Chydorus sphaericus	" "	?	18 hrs.	Nikitinsky & Mudrezowa-Wyss, 1930
Copepods (Harpacticoid)	" "	Yes	14 hrs.	Moore, 1931
Cyclops insignis	" "	?	18 hrs.	Nikitinsky & Mudrezowa-Wyss, 1930
" *leuckarti*	" "	?	2 hrs.	" " " "
" *serrulatus*	" "	?	2 hrs.	" " " "
" *strenuus*	" "	?	5 hrs.	" " " "
" sp.	0 and 5	Yes	>90, <120 days	Lindeman, 1942
" "	Room temp.	?	55 days	Huss, 1913
Daphnia longispina	"	?	3 hrs.	Nikitinsky & Mudrezowa-Wyss, 1930
Diaptomus caeruleus (female)	4	?	24 min.	Hasegawa, 1911
" " (male)	"	?	18 min.	" "
" " (male)	25	?	5 min.	" "
Ostracods (small)	0 and 5	Yes	>90, <120 days	Lindeman, 1942
Scapholebris mucronata	Room temp.	?	4 hrs.	Nikitinsky & Mudrezowa-Wyss, 1930
Simocephalus expinosus	"	?	1 hr.	" " " "
" *vetulus*	"	?	7 hrs.	" " " "
" "	4	?	8 min.	Hasegawa, 1911
" "	19	?	9 min.	" "
" "	25	?	6 min.	" "

TABLE 13 — (Continued)

Species	Temp. °C	Strictly anaerobic conditons?	Survival time	Source
Malacostraca				
Asellus aquaticus	14–16	No	<3 days	Jatzenko, 1928
" "	Room temp.	?	2 days	Nikitinsky & Mudrezowa-Wyss, 1930
	"	?	7 hrs.	" " " "
Astacus leptodactylus	21	Yes	several hrs.	Maloeuf, 1936
Cambarus bartoni	?	?	"assez long sejour."	Bazikalowa, 1941
Echinogammarus cyaneus				
Eupagurus sp.	Room temp.	Yes	15 hrs.	Packard, 1905
Gelasimus sp.	" "	"	1 day	" "
Palaemonetes sp.	" "	"	"	" "
Panopaeus sp.	" "	"	"	" "
Porcellio scaber	2–3	No	4 days	Reinders, 1933
" "	13	"	>1, <2 days	" "
" "	20	"	>4, <7 hrs.	" "
" "	Room temp.	"	5 hrs.	Popovici, 1932
Talorchestia sp.	" "	Yes	2 days	Packard, 1905

TABLE 14

MAXIMAL SURVIVAL TIME OF AQUATIC INSECT LARVAE UNDER ANAEROBIC CONDITIONS.

Species	Temp. °C	Strictly anaerobic conditions?	Survival time	Source
Caenis sp.	Room temp.	?	5 hrs.	Nikitinsky & Mudrezowa-Wyss, 1930
Chaoborus plumosus	" "	?	5 days	" " " "
" sp.	0, 5, 10	Yes	>120 days	Lindeman, 1942
Chironomus decorus	0, 5	"	"	" "
" "	10	"	>90, <120 days	" "

TABLE 14

Species	Temp. °C	Strictly anaerobic conditions?	Survival time	Source
Chironomus gregarius (1st inst.)	Room temp.	Yes	2 hrs.	Pause, 1918
" *gregarius* (2nd inst.)	" "	"	1 day	" "
" *gregarius* (3rd inst.)	" "	"	2 days	" "
" *lobiferus*	0, 5	"	>120 days	Lindeman, 1942
" "	10	"	>90, <120 days	" "
" *plumosus*	0, 5, 10	"	>120 days	" "
" "	Room temp.	?	several days	Comas, 1927
" "	" "	?	12 days	Nikitinsky & Mudrezowa-Wyss, 1930
" sp.	" "	?	5 days	Miall, 1891
" sp.	" "	Yes	3 days	Alsterberg, 1921
" *tentans*	?	"	50 days	Cole, 1921
" *thummi*	Room temp.	"	1 day	Harnisch, 1937
Cloeon dipterum	" "	"	4½ hrs.	" 1939
Culex sp.	" "	"	95 min.	Babak, 1912
" " (pupae)	" "	"	55 min.	" "
Donacia semicuprea	" "	"	10 days	Boeving, 1910
" sp.	" "	"	17 days	Deibel, 1911
" "	" "	"	1½ hrs.	Nikitinsky & Mudrezowa-Wyss, 1930
" "	20	"	7 days	Hoffman, 1940
Ephemera vulgata	Room temp.	Yes	<15 hrs.	Harnisch, 1939
Ephemeridae	14–16	No	<1 day	Jatzenko, 1928
Eutanytarsus inermipes	Room temp.	Yes	15 hrs.	Harnisch, 1937
Helophilus pendulus	" "	"	1 hr.	Weise, 1938
Libellulidae	14–16	No	<1 day	Jatzenko, 1928
Limnophilus sp.	Room temp.	?	6 hrs.	Nikitinsky & Mudrezowa-Wyss, 1930
Palpomyia	5, 10	Yes	>120 days	Lindeman, 1942
Tanypus sp.	Room temp.	"	12 hrs.	Alsterberg, 1921
Tanytarsus sp.	" "	"	3 days	" "
Trichoptera	14–16	No	<3 days	Jatzenko, 1928

and Mudrezowa-Wyss (1930) can be disregarded, since the early death of the animals in their experiments may have been due to a toxic influence of hydrogen sulfide rather than to a lack of oxygen. The metabolism of the larvae has unfortunately never been studied at low oxygen tensions. It is therefore at the present time impossible to decide whether or not the anaerobic functions which they unquestionably possess play a significant role in their natural surroundings.

It has been pointed out (Varley, 1937; Krogh, 1941) that a number of other aquatic insect larvae—those of the syrphid fly *Chrysogaster hirtella,* of the rice water weevil *Lissorhaptus,* and of the mosquitoes of the genera *Taeniorhynchus, Ficalbia* and *Mansonia*—normally obtain their oxygen in the same way as the *Donacia* larvae. It would be of great interest to study experimentally their reactions to low oxygen tensions or to complete lack of oxygen. Krogh (1941) alone has performed some experiments on the *Mansonia* larvae. He states that their viability was not impaired in an atmosphere containing only 3.4 per cent oxygen but that they became paralyzed in pure nitrogen.

The mosquito larvae that normally obtain their oxygen directly from the atmosphere by means of their breathing trumpets, like the *Culex* or *Anopheles* larvae, seem unable to lead an anaerobic life for long periods of time. One of the best known means of combating malaria is the asphyxiation of the vectors by covering the water surface with a layer of oil. This prevents the larvae from getting oxygen from the air but obviously does not prevent them from utilizing the oxygen dissolved in the water. Thus Fraenkel and Herford (1938) found that *Culex* larvae submerged in oxygenated water, were still able to consume about half as much oxygen as they normally use when they have access to the surface. It would seem that not even a partial transition to anaerobic processes is possible, at least not for long periods. Ba-

bak (1912) also observed that *Culex* larvae become asphyctic in a few hours when completely immersed in water, but if truly anaerobic conditions are established, they are asphyxiated already in 95 minutes. The larvae of *Theobaldia annulata,* submerged in water apparently saturated with oxygen, usually die on the fourth day, although a few are still alive on the fifth day (Sautet, 1936). It would be of interest to study the *Aedes* larvae at low oxygen concentrations, since Gjullin, Hegarty and Bollen (1941) found that a low oxygen concentration (about 3 mg. per liter) greatly favors their hatching from the eggs, while this is not the case with *Culex, Anopheles* or *Theobaldia.*

The fact that living mosquito larvae are occasionally found in water covered by ice, *i.e.,* severed from direct connection with the atmosphere, does not contradict the above view. Not only will their metabolism be low at these temperatures, but gas bubbles will also frequently be found below the ice covering and from these the larvae can get oxygen in the normal manner. It is obvious that as the oxygen is withdrawn from the bubbles by the larvae, it will be replenished from the surrounding water, since a gradient will be established as a result of the oxygen consumption.

Much more resistant to lack of oxygen than the forms hitherto named are some larvae that occur rather regularly in the *deep zone of lakes,* especially the *chironomids* and *Chaoborus* (*Corethra*) larvae.

The fact that the chironomids are not exacting with respect to the amounts of oxygen present in their surroundings seems to have been emphasized first by Miall (1891). Since his time many observations have come to light indicating that a number of species survive relatively well both the summer- and winter stagnation periods in eutrophic lakes (Juday, 1908; Muttkowski, 1918; Thienemann, 1918; Eggleton, 1931; Lindeman, 1942). Not

all species, however, are equally well adapted to these periodically recurrent anaerobic periods. Indeed, according to European workers (especially Thienemann, 1913, 1915, 1916, 1925) the various kinds of midge larvae can be used to differentiate the lakes according to the oxygen concentration: the *plumosus* group occurs predominantly in eutrophic lakes, the *Tanytarsus* group in oligotrophic lakes.

The non-aerated zone of lakes is not the only anaerobic habitat in which chironomid larvae are found. Larvae and pupae of *Chironomus interruptus* were observed even in heavily polluted waters in the complete absence of oxygen (Thienemann, 1909). There seems little doubt, at least in this instance, that considerable, perhaps complete development took place under truly anaerobic conditions. This, however, may be an exceptional case. Philipp (1938) could rear only few specimens of *Chironomus thummi* to the adult stage in water poor in oxygen, and he obtained only few imagines from water containing 0.3 mg. oxygen per liter. Some chironomid larvae have apparently developed an escape mechanism that tends to remove them from surroundings too poor in oxygen. Lloyd and Turner (1936) state that at least the second and third instar larvae of *Metriocnemus longitarsus*—which live in sewage bacteria beds—have a tendency to leave this medium by climbing or moving towards the light and that this tropism is greatly intensified if the larvae are subjected to a lack of oxygen by immersion in water very poor in oxygen.

The chironomid fauna of eutrophic lakes cannot withstand anoxic conditions indefinitely; after a certain time the populations decrease. The figures presented by Lindeman (1942) are especially instructive in this connection. He found that during the winter stagnation of Cedar Bog Lake (Minnesota) the numbers of *Chironomus* per 225 square centimeters of bottom remained practically stationary for the first 24 days of anaerobiosis,

while after 51 days a considerable decrease had taken place (there were 44.0 ± 9.0 at the beginning, 38.5 ± 2.8 after 24 days, and 17.2 ± 3.4 after 51 days).

The fact that numerous authors could maintain midge larvae for long periods under experimentally induced anoxic conditions (Table 14) is in good agreement with field observations. The experiments of Cole (1921) and Lindeman (1942) are especially remarkable in this respect. There seems to be no clear connection, however, between resistance to lack of oxygen under laboratory conditions and the oxygen content in the normal habitat of the various species.

The question of the factors which enable some species of chironomids to maintain their population in waters poor in oxygen, while other species are incapable of doing so, has received considerable attention but is still far from a satisfactory solution, as the following account will show.

Many of the midge larvae are blood-red, due to the presence in them of rather considerable amounts of haemoglobin. Other species have only traces of the respiratory pigment and still others none at all. Since the haemoglobin of chironomids has a steep dissociation curve (Leitch, 1916), it is well-suited to supply the tissues with oxygen even at low tensions. But it is obvious that even the most potent respiratory pigment will be useless if the oxygen disappears completely, and thus it must be emphasized that the presence or absence of haemoglobin can hardly be the decisive factor in determining the ecological distribution of the various species. Ssinitza (1936), working with *Chironomus plumosus, Glyptotendipes polytomus, Stictochironomus* sp., *Protenthes kraatzi, Psectrotanypus brevicalcar* and *Culicoides* sp., has pointed out that, in water containing oxygen corresponding to a tension of 0.2 mm. of Hg., the haemoglobin-containing species die much earlier than those lacking this pigment.

Harnisch (1929) measured the oxygen consumption of some chironomids at various tensions and determined the point at which the oxygen consumption begins to drop. He found that the animals should be classified as follows, if one considers the order of decreasing oxygen tension that they require to maintain the normal oxidative level: *Prodiamesa* - *Microtendipes* - *Eutanytarsus* - *Chironomus*. Of these species only *Prodiamesa* is without haemoglobin. But, as Harnisch points out, if one considers the normal distribution in waters of decreasing oxygen content, the order is the following: *Eutanytarsus* - *Microtendipes* - *Prodiamesa* - *Chironomus*. Obviously there is no connection between the ecological distribution and the possibility or impossibility of maintaining a maximal oxygen consumption at low tensions.

This negative result led Harnisch (1933) to investigate whether it might not be possible for various chironomids to supplement the failing aerobic oxidations by anaerobic processes. He measured the amount of carbon dioxide produced and the amount of oxygen consumed at low tensions, and since any excess of carbon dioxide over that which was formed from the consumed oxygen, could not have originated from aerobic oxidations, he considered the ratio, excess carbon dioxide to oxygen, as a measure of the extent of the anaerobic processes that substitute for aerobic oxidations. The higher the quotient was, the better the mechanism for anaerobic respiration was developed. He found the following figures: *Eutanytarsus*: 22.5, *Prodiamesa*: 30.8 and *Chironomus*: 36.1. It is evident that this series agrees well with the one given above for the ecological oxygen demand and this agreement is certainly suggestive.

However, Harnisch in a later publication (1937) emphasized that a great many experimental errors accumulate in these figures and render them rather unreliable. He seems inclined to seek another explanation for the ecological observations. Aerobic animals subjected to

lack of oxygen accumulate within their bodies endproducts of incomplete oxidations or fermentations, which sooner or later will prove toxic unless removed. The removal, however, can be accomplished in this group of animals only by aerobic processes that take place when oxygen is again available, and apparently not by excretion. These products accordingly occasion an excess oxygen consumption, the repayment of the incurred oxygen debt. Studying then the post-anaerobic oxygen consumption of *Chironomus thummi* and *Eutanytarsus inermipes,* Harnisch found that in both species characteristic curves were obtained if the preceding anaerobic period had not been too long. If it was too long, the repair mechanism was damaged and the animals did not fully recover. But a significant point is that the recovery mechanism failed earlier in *Eutanytarsus* than in *Chironomus, i.e.,* in the species that normally does not invade habitats poor in oxygen. Harnisch's tentative conclusion is that the potency of the recovery mechanism might be more decisive than anything else in determining to what extent oxygen may be the limiting factor in the distribution of the midge larvae.

In a still later publication the same author (1939) describes yet another mechanism which he studied, so far, only in *Chironomus thummi.* He observed that during short periods of anaerobiosis lower fatty acids accumulate in the body in greater amounts than during long-lasting periods. Harnisch believes that some of these acids are removed little by little through oxidations, despite the lack of oxygen in the immediate surroundings. The oxygen necessary would originate from the transformation of glycogen into fat, a process occurring in the later stages of anaerobiosis. It is obvious that the significance of this mechanism for the ecological distribution can be estimated correctly only after further investigation will have shown whether or not its potency is markedly different in various species. One wonders

whether these findings might not somehow tie up with earlier observations of Cole (1921) who had found in the body of *Chironomus tentans* an enzymatic complex capable of building up peroxide from which, under certain conditions, oxygen was split off.

Ssinitza (1936) observed another difference between the chironomid larvae which contain haemoglobin and those which do not. While the oxidation-reduction potential was about the same in the two groups if the animals were kept in oxygenated surroundings, it was considerably lowered in the haemoglobin-containing species, and was maintained at about its original level in the species without that pigment, when the animals were kept in oxygen-free water. The implications, however, of this difference do not seem to the present writer to be sufficiently clear to allow speculations on ecological problems.

The case of the *Chaoborus* larvae has been much less analyzed than that of the chironomids. It is well known that these larvae are recovered quite often from the oxygen-free deep zones of eutrophic lakes (Appendix, Table 3) and Lindeman's (1942) experiments have demonstrated that they possess an extremely well-developed tolerance for experimentally induced anaerobic conditions (Table 14). There is some doubt as to the extent to which they will actually stay confined in nature to the truly anaerobic layers. They possess a well-developed hydrostatic apparatus which enables them to rise to the surface. According to Juday (1921)—compare also Eggleton, 1932—*Chaoborus punctipennis* stays in the deep zone only during the day, during the night it rises to the surface strata. Obviously, it can frequently get oxygen there; but in the winter this mechanism is of no avail if the oxygen disappears from the whole water column under the ice. The anaerobic metabolism of these animals has unfortunately never been investigated; it would provide interesting problems.

Very little is known about the anaerobic functions of other aquatic insect larvae. Those of *Palpomyia* seem to withstand fairly well both natural and experimental oxygen deficiency (Lindeman, 1942). Whether the same applies to *Trichoptera* larvae has not yet been investigated. Milne (1938) is of the opinion that the cases built by the caddis flies represent an adaptation to life in an environment poor in oxygen. Her contention is that the current of water forced through the case sets up a circulation in the surrounding water over a considerable distance from the case and thus always brings in fresh water with at least some oxygen. If a larva without a case simply undulates its abdomen in the water, the water movements are confined more or less to the immediate vicinity of the animal. The only gas exchange therefore would be a diffusion and this would be insufficient to replace all the oxygen consumed. Whether this view of the caddis cases as an adaptation to life at low oxygen tensions will stand the test of experimental analysis remains to be seen.

Other developmental stages of insects that withstand quite considerable oxygen deficiencies in nature are found among the *ephemerids*. Sing-Pruthi (1927) states that the larva of *Cloeon dipterum* is not injured until the oxygen concentration drops to 0.20 to 0.30 cc. per liter and those of *Hexagenia recurvata* survive in water containing 0.50 cc. of oxygen per liter. Fox and Simmonds (1933) and Fox, Simmonds and Washbourn (1935) have shown that the oxygen consumption of nymphs of various species of may-flies taken from swift streams that are rich in oxygen is greater than that of species occurring in ponds where the oxygen content is often abnormally low. Whether this merely indicates that the energy need of various species is different or whether it means that the latter have some anaerobic processes going on even at relatively high tensions is not yet clear. It is, in any event, interesting that *Cloeon dipterum* from

a pond survived for more than 4 hours but less than 19 hours in water containing 2.9 cc. of oxygen per liter, while *Baetis rhodani* from a stream died in as short a time as 3 minutes in water containing 2.6 cc. of oxygen per liter. The interpretation of the ecological findings, however, presents difficulties similar to those outlined above for the midge larvae. Fox, Wingfield and Simmonds (1937) investigated the oxygen consumption of some nymphs in its dependency on the tension and found that the animals could be classified as follows in the order of decreasing dependency: *Baetis* sp. - *Ephemera vulgata* - *Leptophlebia vespertina* - *Baetis scambrus* - *Cloeon dipterum*. Both *Baetis* sp. and *Baetis scambrus* lived in a swift stream while *Cloeon* and *Ephemera* were taken from a pond. There exists, therefore, no connection between habitat and dependency of oxygen consumption on tension.

II. ADULTS

The resistance of adult aquatic insects to asphyxiation is not very pronounced (*cf.* Table 15). Plateau's (1872) much quoted figures for the time during which these animals can be forced to stay under water vary from 3 hours in the case of *Notonecta* to 65 hours in that of *Dytiscus marginalis*. But Plateau neglected to remove the air which the insects carry down with them when they dive. He assumed that the oxygen present in these air bubbles would last only a very short time. His reasoning was faulty; he did not realize that the oxygen lost from the bubble will be replaced from the surrounding water.

This point was first assumed to be of importance by Comstock (1887) and later by Hagemann (1910); it was then studied experimentally in a thorough manner by Ege (1915) who showed that the oxygen which diffuses from the water into the bubble is sufficient at winter temperatures to cover the oxygen needs of diving insects

TABLE 15

MAXIMAL SURVIVAL TIME OF AQUATIC INSECT IMAGINES UNDER ANAEROBIC CONDITIONS.

Species	Temp. °C	Strictly anaerobic conditions?	Survival time	Source
Agabus bipustulatus	Room temp.	No	6 hrs.	Plateau, 1872
" sp.	22	?	2 hrs.	Hasegawa, 1911
" *sturmii*	Room temp.	No	7 hrs.	Plateau, 1872
Acilius sulcatus	" "	"	1 day	" "
Corixa sp.	" "	Yes	2½ hrs.	Hagemann, 1910
Dytiscus dimidiatus	" "	No	2 days	Plateau, 1872
" *marginalis*	" "	"	2½ days	" "
" "	" "	?	5 hrs.	Hasegawa, 1911
" sp.	15–20	Yes	1½ days	Ege, 1915
Gyrinus natator	Room temp.	No	3 hrs.	Plateau, 1872
Haliplidae	4–8	"	7 days	Hickmann, 1930
"	12–15	"	4 days	" "
"	20	"	2 days	" "
Haliplus elevator	Room temp.	"	11 hrs.	Plateau, 1872
Hydaticus transversalis	"	"	20 hrs.	" "
Hydrophilus piceus	"	"	2 days	" "
Hydroporus palustris	"	"	10 hrs.	" "
Hyphydrus ovatus	"	"	21 hrs.	" "
Naucoris cimicoides	"	Yes	1, 2 days	Wrede & Kramer, 1926
Nepa cinerea	?	"	30 min.	Dogs, 1908
" "	Room temp.	No	1½ days	Plateau, 1872
Notonecta glauca	"	"	3 hrs.	" "
" "	20	?	20 min.	Hasegawa, 1911
" sp.	17	Yes	5 min.	Ege, 1915

even during periods of activity, and that, at summer temperatures, it was adequate for periods of rest, in most species. As to the smaller species of *Corixa* and *Hyphydrus* they can, even in the summer, get all the oxygen needed for their movements. Thus, the only reason why aquatic insects cannot stay indefinitely under water at low temperatures is that in time—and this time is not too long—the air bubble disappears due to a nitrogen gradient established between bubble and water, which leads to a gradual diffusion of nitrogen into the water.

The unreliability of Plateau's (*l. c.*) figures becomes apparent if one compares his results with those of Ege (*l. c.*), which were obtained under truly anaerobic conditions. As we said above, Plateau reported a survival of 65 hours for *Dytiscus* and of 3 hours for *Notonecta*. Ege's figures for the same animals are 36 hours and 5 minutes respectively.

That some species of aquatic beetles are able to remain active in winter even if they are prevented from renewing their stores of air at the surface because of the ice is explained by the fact that they catch bubbles rising from plants or from the mud. "Even if these latter contain very little oxygen they may be essential for keeping up the quantity of gas on which the uptake of oxygen from the water depends" (Krogh, 1941).

The resistance of diving *Hemiptera* in experimental anaerobic conditions seems to be about the same as that of beetles. Among the most reliable figures we may mention those of Wrede and Kramer (1926). These authors found that *Naucoris cimicoides* survives 24 hours, but not 45 hours, in strictly anoxic conditions.

3. TERRESTRIAL INSECTS

A. Anaerobiosis under natural conditions. There is little reason to assume that terrestrial insects will often find themselves under strictly anaerobic conditions in nature but it is conceivable that they may, from time to

time, encounter situations in which their oxygen consumption will be greatly reduced.

After a heavy rainfall many terrestrial insects may be immersed temporarily in water. They then close their spiracles and must get along with the little oxygen they can obtain by cutaneous respiration. This change will frequently imply that at least part of the metabolism will have to become anaerobic. Similar conditions ought to be especially frequent along beaches.

That the larvae of *bees* within the cells of the hive may live under sharply reduced oxygen tensions seems probable but has not yet been investigated. The fact that the respiratory quotient of larval queens and workers is higher than 1, even when investigated at the oxygen tension of the atmospheric air (Melampy and Willis, 1939) is perhaps significant in that connection. On the other hand, it is certain that the air enclosed in the cocoons of silk worms has, nearly always, a high oxygen content (Regnard, 1888; Portier and Rorthays, 1926).

But even a sufficient oxygen supply does not necessarily indicate that all processes must be aerobic at all times. Kozancikov (1935) has shown that the larval diapause of some *Pyralidae* "is characterized by anoxybiotic rearrangements." The nature of these processes will be discussed later.

B. Experimentally induced anaerobiosis. Some early investigators (Vauquelin, 1792; Emery, 1869; Bert, 1878) confined insects in closed vessels and found that they became immotile after a certain length of time. Analyses of the remaining air, performed in some cases, showed that the oxygen had more or less completely disappeared; upon readmission of air the animals usually revived.

Many other investigators simply immersed insects in ordinary water, assuming that this would cut them off from their source of oxygen. This method which was

used at an early date (Lyonet, 1762; Treviranus, 1814; Straus-Durkheim, 1818) has been followed until recently (Plateau, 1872; Devaux 1891; Gauckler, 1897; Fielde, 1904, 1904a; Bruneteau, 1931). The figures found in such experiments are open to serious objections. In many cases no attention was paid to the question whether or not the animals were thoroughly wetted when immersed, *i.e.,* whether they were not surrounded by a sheet of air that would set up a mechanism similar to that outlined above for aquatic insects.

Furthermore, the metabolism could still have been aerobic in part, even if the animals had no air bubbles close to their body. Fraenkel and Herford (1938) showed that at least some insect larvae, amongst them the rather heavily chitinized *Tenebrio* larva, have a quite marked cutaneous respiration when immersed in water, a respiration which amounted, in the latter case, to about 20 per cent of the normal. It is likely that the extent of the anaerobiosis induced by immersion in oxygen-containing water will vary greatly with the species employed. Bodine (1928) states that he observed, with grasshoppers, the same survival irrespective of whether he immersed them in water or whether he established strictly anaerobic conditions by employing atmospheres of hydrogen, carbon dioxide or nitrogen.

Some other investigators immersed insects into water from which oxygen had been partly removed by boiling (Nigmann, 1908; Deibel, 1911). That, of course, reduced the possible cutaneous respiration almost to the vanishing point. Nonetheless, one can still object that the results obtained in that manner, as in all immersion procedures, are complicated by secondary effects which the water itself may have on the animals.

A last group of investigators either introduced the organisms into an atmosphere of inert gases, a procedure much less open to objection (Hausmann, 1803;

Luciani and Piutti, 1888; Regen, 1906; Walling, 1906; Schwartz, 1908; Cook, 1932; Gilmour, 1940; Harnisch, 1939, 1941) or removed the oxygen by evacuation or absorption (Kalmus, 1935; von Brand, 1943).

The more important results have been summarized in Table 16. It will be seen from this table that the anaerobic periods tolerated by terrestrial insects are rather short. If we disregard the exceptional survival periods reported for ants in submersion experiments (Devaux, 1891; Fielde, 1904, 1904a), survivals which, as shown above, may be due to faulty technique, it becomes apparent that the insects are not well equipped for anaerobic life.

This becomes all the more evident if one studies the behavior of insects in the complete absence of oxygen. Practically all investigators agree that the animals then become motionless in a very short time, a fact which indicates definitely that they are unable to maintain normal life processes. The paralysis thus established seems to be preceded in some instances by a brief period of excitation. This has been described for various *coleoptera* by Babak and Foustka (1907), for *Carabus* by Schwartz (1908) and for grasshoppers by Lee (1925).

Since, in most cases, the paralysis comes about very rapidly, it is questionable whether its onset is actually due to an accumulation of non-oxidized metabolic endproducts. It seems more likely that an acute oxygen shortage, affecting first the more sensitive nerve centers is responsible for the phenomenon. There is, however, no doubt that when anaerobic conditions are maintained for a long time an accumulation of such endproducts does occur and that it is then linked with the persistence of the paralysis. This is clearly evidenced by the two following facts. First, it has been generally recognized that the time required for full recovery, as judged by the resumption of normal motility, is directly de-

pendent on the duration of the preceding anoxic period (Plateau, 1872; Bruneteau, 1931; Kalmus, 1935). Second, in all cases investigated, the repayment of an oxygen debt, indicating the removal of oxidizable substances, has been observed (Gaarder, 1918; Davis and Slater, 1927; Bodine, 1928; Gilmour, 1940, 1940a, 1941, 1941a; Harnisch 1941).

C. Influence of low oxygen tensions. The metabolism of terrestrial insects seems, on the whole, to be independent of the oxygen tension within wide limits, but if the tension is considerably lowered (in the case of grasshopper eggs, for example, below 25 mm. of mercury, according to Bodine, 1934) a gradual replacement of aerobic by anaerobic processes may be expected, as in any other group. This will be discussed in greater detail later.

A number of interesting experiments concerning the influence of low oxygen tensions have been carried out with *Drosophila.* Csik (1939) studied the sensitivity of six fruit fly species, determining the minimal amounts of oxygen required for maintaining motility of the imagines at the temperatures optimal for each species. He arrived at values ranging from 1.6 per cent in *Drosophila melanogaster* to 2.8 per cent in *Drosophila obscura.* Kalmus (1937) found that about the same oxygen concentration (2 to 3 per cent) was required to allow the pupae to develop into adults. If the pupae were exposed for a period of 8 hours (Kalmus, 1935a) to a vacuum of 18 mm. Hg, their subsequent hatching in a normal atmosphere was retarded for almost 8 hours, which seems to indicate that the development had stopped more or less completely during the period of oxygen deficiency. In a later paper (1937), however, Kalmus stated that in longer-lasting experiments and with even stricter anaerobic conditions, indications were found that at least some developmental processes went on in the absence of oxygen. He compared the time necessary for completion

TABLE 16

MAXIMAL ANAEROBIC SURVIVAL TIME OF TERRESTRIAL INSECTS (IMAGINES UNLESS OTHERWISE SPECIFIED).

Species	Temp. °C	Strictly anaerobic conditions?	Survival time	Source
Orthoptera				
Cryptocercus punctulatus	Room temp.	Yes	6 hrs.	Gilmour, 1940a
Grasshoppers	" "	"	>1 day	Lee, 1925
Gryllus campestris	" "	"	1 hr.	Regen, 1906
Melanoplus differentialis	" "	"	<1 day	Willis, 1925
" "	" "	"	>7 hrs.	Bodine, 1928
Isoptera				
Zootermopsis nevadensis	" "	"	2 days	Cook, 1932
" "	20 to 25	"	7 hrs.	Gilmour, 1940
Homoptera				
Macrosiphum tulipae	?	?	>2½ hrs.	Kalmus, 1935
Lepidoptera				
Acentropus niveus	Room temp.	?	12 min.	Nigmann, 1908
Bombyx mori (eggs)	0 and 9 to 12 12 to 14	Yes	<54 days	Luciani & Piutti, 1888
Crateronyx (caterpillar)	?	No	>1 day	Gauckler, 1897
Diptera				
Calliphora vomitoria (larva)	Room temp.	Yes	4 hrs.	Komárek, 1936
Chrysomyia megacephala (larva)	" "	No	2 days	Hoeppli & Watt, 1933
Cordylobia anthropophaga (larva)	" "	Yes	>6 hrs., <10 hrs.	Blacklock, Gordon & Fine, 1930
Drosophila melanogaster	20	?	>1, <1½ days	Kalmus, 1935
" "	8	?	>1½ days	" "
" (pupae)	?	Yes	4 days	" 1937
Musca domestica (larva)	Room temp.	"	4 hrs.	Komárek, 1936
Sarcophaga carnaria (larva)	" "	"	"	" "
Coleoptera				
Agelastica alni	Room temp.	No	>3, <4 days	Plateau, 1872
Agonum (Anchomenus) assimile	" "	"	>1½, <2 days	" "
Aphodius distinctus (inguinatus)	" "	"	1 day	" "
" *fimetarius*	" "	"	>2 days	" "

TABLE 16 — (Continued)

Species	Temp. °C	Strictly anaerobic conditions?	Survival time	Source
Attagenus undulatus	40	Yes	<13 hrs.	Barnes & Grove, 1916
" "	35	"	<30 hrs.	" " "
" "	30	"	<55 hrs.	" " "
Carabus auratus	Room temp.	No	>3 days	Plateau, 1872
Coccinella septem-punctata	" "	"	>12 hrs.	" "
Donacia sp.	" "	Yes	4 hrs.	Deibel, 1911
Dromius agilis (maculatus)	" "	No	>1½ days	Plateau, 1872
Geotrupes stercorarius	" "	"	>4, <5 days	" "
Hylobius abietis	" "	"	>4 days	" "
Leptinotarsa decemlineata	" "	"	>1 day	Bruneteau, 1931
Loricera caerulescens (pilicornis)	" "	"	>1½ days	Plateau, 1872
Macroplea (Haemonia) sp.	" "	Yes	1 day	Deibel, 1911
Melolontha vulgaris	" "	No	>2 days	Plateau, 1872
Oryctes nasicornis	" "	"	>4 days	" "
Rhizopertha dominica	40	Yes	<6 hrs.	Barnes & Grove, 1916
" "	35	"	<16 hrs.	" " "
" "	30	"	<31 hrs.	" " "
Silpha opaca	Room temp.	No	>1 day	Plateau, 1872
Sitophilus (Calandra) oryza	40	Yes	<4 hrs.	Barnes & Grove, 1916
" " "	35	"	<11 hrs.	" " "
" " "	30	"	<28 hrs.	" " "
Tenebrio molitor (larva)	Room temp.	Yes	>1 day	Harnisch, 1941
" " "	22	"	16 hrs.	von Brand, 1943
Hymenoptera				
Ants	?	No	>2, < 5 days	Devaux, 1891
Aphaenogaster (Stenamma) fulva	10	"	>8 days	Fielde, 1904a
Apis mellifica, worker	?	?	>1 hr.	Kalmus, 1935
Bumble bee	?	?	>17 hrs.	" "
Camponotus pennsylvanicus	10	No	>8 days	Fielde, 1904a
Lasius latipes	?	"	>1 day	" 1904

of development in air of pupae that lacked oxygen for varying periods with that required by pupae kept during the whole time in air and found that after 3 days in the absence of oxygen the total time required for development in air was shorter than in the controls. After 4 days without oxygen, however, a retardation became apparent and the few emerging adults appeared damaged. Finally, after 5 days no adults emerged; the pupae had succumbed to the lack of oxygen.

According to Lobaschow (1934) periods of asphyxiation in the larval and pupal stages of *Drosophila* increased the numbers of lethal and semi-lethal mutations. Margolis (1939) kept bar-eyed fruit flies for varying lengths of time (2 to 5 days), during the egg-larval period, at a reduced pressure (half an atmosphere) and found a small decrease in the number of facets on the eye. Although the decrease was in most cases not statistically significant, he is inclined to regard it as real, since an increase in the partial pressure of oxygen had the opposite effect.

Schlottke (1934) studied the influence of various oxygen concentrations upon the larvae and pupae of *Habrobracon juglandis*. He found that the larvae did not develop unless the atmosphere contained more than 4 per cent of oxygen, a figure somewhat higher than that mentioned above for *Drosophila*. When the atmosphere contained 8 or 12 per cent oxygen there resulted an increased pigmentation of the animals. This result, however, is not specific since an abnormally high oxygen tension had the same effect.

Finally, Snell's (1932) investigations on the luminescence of *Photuris pennsylvanica* may be mentioned. He observed that the duration of the light flash depends on the oxygen tension, a lowering of the tension below 20 mm. of Hg resulting in flashes of longer duration. At tensions below 4 mm. of Hg flashes were not observed

but, instead, a continuous glow occurred, and at oxygen concentrations nearing zero the luminescence ceased entirely. That insects do not luminesce under conditions of severe lack of oxygen has been known a long time (Boyle, 1667; von Grotthuss, 1807; Owsiannikow, 1864; de Bellesme, 1880; Kastle and McDermott, 1910).

4. ARACHNOIDS, TARDIGRADES, CHILOPODS AND DIPLOPODS

The only marine *arachnoid* that has been used for experiments on anaerobiosis is *Limulus*. Newman (1906) immersed 3 young specimens in oxygen-free sea water. They became entirely paralyzed after 45 hours and only one showed some signs of recovery when brought into aerobic surroundings. The hearts of all three animals, however, were still beating. This observation, and experiments on excised hearts demonstrated that the cardiac ganglion of *Limulus* is fairly resistant to lack of oxygen, much more so, in fact, than the central nervous system.

No field observations have come to the author's attention that would indicate a predominantly anaerobic life of *Limulus* in nature. Due to its habit of burrowing into the bottom material it ought to encounter, at least occasionally, rather difficult respiratory conditions. This, coupled with the observation that its oxygen consumption depends to a marked degree on the oxygen tension (Amberson, Mayerson and Scott, 1925; Maloeuf, 1937a) leads to the supposition that anaerobic processes may constitute an important part of its metabolism, but future investigations alone will decide whether this assumption is correct.

Tardigrades and their eggs have occasionally been found in oxygen-free surroundings (Lauterborn, 1916; Pennak, 1940). On the whole, they do not seem to be very resistant in experimentally induced anaerobic conditions when they are kept moist. That moss tardigrades become asphyctic when immersed in oxygen-poor or oxygen-free

water is a well-known fact (see *e.g.,* Basse, 1905; Marcus, 1928; Baumann, 1929) which has been much used in morphological studies since the animals then become extended and are well suited for observation. It seems that during the asphyxiation process the epithelial cells lose the ability to regulate their water content and swell up; this phenomenon seems to be without parallel in other animals. The time necessary for a tardigrade to reach the asphyctic state depends upon the temperature; at 17°C. it takes about 2 days. At 27°, however, this stage is never attained, the animals die in a few hours due to lack of oxygen. The asphyctic stage, once reached, is endured for relatively short periods only, by *Milnesium,* for example, for about 3 days (Baumann, 1929).

The behavior of dry specimens is in marked contrast with that of moist ones. They exhibit an extremely high resistance to lack of oxygen. Rahm (1929) and Becquerel (1936) kept them in this condition for weeks or even months and found that the animals revived upon restoration of suitable conditions. The explanation is, of course, the same as that given in a previous chapter for dry nematodes. Dry tardigrades are in a stage of *vita minima* or even in suspended animation, and therefore need only infinitesimal amounts of oxygen, or none.

Little information is available for *chilopods* and *diplopods.* In a few experiments of immersion in water by Plateau (1890) and Rossi (1901) they survived a remarkably long time; *Geophilus longicornis,* for example, survived 15 days (Plateau). Rossi, however, pointed out that these experiments do not prove much since the animals are capable of a considerable aquatic respiration. He himself carried out some experiments with inert gases. *Lithobius forficatus* survived only a few hours; *Julus terrestris* was much more resistant, it could be kept alive for more than five days in nitrogen.

5. PARASITIC ARTHROPODS

Only a few parasitic arthropods have been studied with respect to their ability to withstand lack of oxygen. The best-known case is that of *Gasterophilus intestinalis* (= *G. equi*). The larvae of this fly live in the stomach of horses and other animals where they probably encounter from time to time quite low oxygen tensions (Weinland, 1915). Normally, they are animals with an aerobic metabolism (von Kemnitz, 1916). They have in their abdomen an organ rich in tracheae, the so-called red organ, which consists of very large cells containing haemoglobin (Prenant, 1900; Vaney, 1902; von Kemnitz, 1916). It seems likely that this organ functions as an oxygen store (Weinland, 1915; Krogh, 1941) that will tide the animals over periods of adverse conditions.

The *Gasterophilus* larvae withstand a complete absence of oygen for periods of several days to several weeks as the experiments of Schwab (1858), von Kemnitz (1916), Dinulescu (1932), and Blanchard and Dinulescu (1932) have conclusively demonstrated. It seems that the ability to live anaerobically is well developed in the entire family. Dinulescu (*l.c.*) found that the larvae of *Gasterophilus pecorum*, of *G. intestinalis* and of *G. inermis* survive at 38°C., under anaerobic conditions, for at least 25 days, those of *G. haemorrhoidalis* for more than 17 days and finally those of *G. nasalis* for 17 days.

Many insects and insect larvae which have the habit of tunnelling in the skin of higher animals and of man will probably also experience some difficulty in getting their normal oxygen supply when no direct communication with the outside world is maintained. The study of the resistance of animals leading this type of life has not yet, to the present writer's knowledge, been undertaken on a larger scale. The only form investigated is the larva of *Cordylobia anthropophaga*. Its behavior has been studied by Blacklock, Gordon and Fine (1930) who have shown that the animal normally keeps the aperture in the host's

skin open. Its posterior end on which the large stigmata are located, alternately retracts and protrudes through the opening. Thus there is no doubt that this animal can get as much oxygen as it wants. The situation is probably different in immune or partly immune hosts, since here a scab occludes the opening and presumably interferes with the normal oxygen supply of the parasite. The latter survives nevertheless.

Experimentally, it has been shown that at least the third instar larva of *Cordylobia* is quite resistant to lack of oxygen. It survived in nitrogen for 27 hours and—what is especially remarkable in view of the different behavior of the majority of other insects—it remained motile for 22 hours. The first instar larva is more sensitive; it survived more than 2½ hours but less than 19 hours. Pupation was also found to be possible in the absence of oxygen. A larva that had been kept in nitrogen for 48 hours and had pupated during this time developed into a normal fly upon readmission of air. Another pupa, however, did not survive a 3-day period without oxygen.

Non-parasitic arthropods are occasionally found in parasitic habitats in which they must presumably lead a more or less anaerobic life. The best-known instances are those encountered in the so-called *urinary* and *intestinal myiasis*. Numerous cases have been described in which arthropods (acarines, myriapods, larvae of *coleoptera* and especially *diptera*) were recovered from human urine or feces. It has been claimed that the animals had passed through the alimentary canal alive, or even that they had become established for long periods in the stomach, the intestine or the urinary bladder.

It is obvious that the possibilities of error in such cases are many. Hysterical patients may be too imaginative or the receptacles that they used may not be clean. Chevrel (1908) who reviewed the cases of urinary myiasis

reported in the medical literature considers 7 of them as genuine. Newer trustworthy cases were later reported by King (1914) and Leon (1921). Intestinal myiasis was reviewed by Seifert (1926) and Herms (1939); some new instances have been described by Chagnon (1940), Swartzwelder and Cali (1942) and Chandler (1943). A survey of the literature seems to indicate that at least a number of the cases reported are not a result of imagination or faulty observation.

The question then naturally arises as to how the myiasis-producing animals can resist the variety of adverse conditions that they encounter, for example, in the intestinal tract. Experimental studies, such as those of Desoil and Delhaye (1922), Hoeppli and Watt (1933), Komárek (1936) and Causey (1938) are not too enlightening in so far as the explanation of myiasis is concerned. In no case was it possible to establish insect larvae within experimental animals; as a matter of fact, such larvae always died rather rapidly. The evidence concerning the point of primary interest in this discussion, namely, how the animals survive at the low oxygen tensions prevailing in the intestine, is also rather negative. Komárek's experiments certainly fail to indicate a sufficient resistance of dipterous larvae to oxygen lack. It is regrettable that no one has tried to rear under low oxygen tensions insect larvae recovered from myiasis cases. Could it not be possible that in the genuine myiasis cases the animals belonged to strains (or were mutants) especially resistant to oxygen deficiencies? There is certainly no valid ground to assume that a normal dipterous larva should be able to live for weeks or even months at those oxygen tensions that usually prevail in the intestine of vertebrates.

SUMMARY

Methods of investigation.

1. The methods used in the study of anaerobic life in invertebrates involve: (a) The determination of the oxygen content in the normal surroundings of the animals, that is, in terrestrial, aquatic and parasitic habitats; (b) The establishment of experimental anaerobic conditions, the chief procedures being evacuation of air, absorption of oxygen and replacement of oxygen by inert gases; (c) The determination of the type of metabolism (whether and to what degree it is aerobic or anaerobic).

Anaerobic habitats.

1. A severe lack of oxygen is rare in soils.

2. The bottom material of both fresh-water and salt-water basins is often without molecular oxygen and represents an important anaerobic habitat.

3. Circulation ceases periodically in many lakes, with the result that the animals living in the stagnant strata frequently cannot get any oxygen for long periods.

4. In the oceans, there is, at a certain depth, a layer of minimum oxygen concentration, which seems quite important for anaerobic life.

5. The intestinal tract of vertebrates represents a parasitic habitat in which only very small amounts of oxygen are available; the conditions seem to be more variable in the intestines of invertebrates.

6. The tissues and secretions of the body of host animals constitute, in some cases, a partly anaerobic habitat for their parasites.

Protozoa.

1. Non-parasitic protozoa leading an exclusively or predominantly anaerobic life occur in the sapropelic habitat, in sewage tanks and in the stagnating strata of lakes.

2. Most of these animals are facultative, not obligatory anaerobes.

3. Many free-living protozoa which normally lead an aerobic life are able to withstand experimental anaerobic conditions for quite long periods of time.

4. Parasitic protozoa living in the alimentary tract of vertebrates, or in related habitats, can usually be cultured in the complete or nearly complete absence of oxygen. These protozoa, however, are not injured by oxygen (except a few). It seems likely, therefore, that in their natural habitat they undergo changes from anaerobic to aerobic life.

5. The conditions to which protozoa living in the intestine of invertebrates are exposed seem to depend largely on the host species. Thus, termite flagellates appear to live primarily anaerobic lives, while the developmental stages of *Trypanosomidae* seem to require oxygen.

6. Blood protozoa and tissue protozoa, which have probably always access to some oxygen in their normal habitat, do not seem to be very resistant in completely anaerobic conditions. However, the metabolism of some forms, *e.g.*, trypanosomes and malaria parasites, is partly anaerobic even in aerobic environments.

Coelenterates.

1. Coelenterates do not seem to occur in truly anaerobic habitats in nature, but some species survive rather well in water poor in oxygen.

2. The regeneration of polyps is inhibited in anoxic media, but the luminescence of some forms does not require molecular oxygen.

Worms and worm-like organisms.

1. Non-parasitic worms and worm-like organisms are found rather frequently in oxygen-free or oxygen-poor environments, mostly in the sapropelic habitat, at the bottom of lakes and at the bottom of the sea.

2. There is no definite indication, so far, that animals found in these or similar habitats can live indefinitely without oxygen, though it is a possibility.

3. Worms living in the tidal zone are probably not dependent primarily on anaerobic metabolism for their survival during low tide.

4. A partial transition to anaerobiosis may be of great importance for the survival of earthworms in rain-soaked soil.

5. A high resistance of non-parasitic worms to the experimental deprivation of oxygen is observed, in general, in those organisms which are found normally in environments lacking oxygen.

6. The resistance to oxygen deficiency varies with environmental factors (temperature) and internal factors (hydration, nutrition).

7. Most intestinal and tissue helminths show a considerable resistance to the experimental deprivation of oxygen.

8. The eggs of helminths are quite resistant to the lack of oxygen but they require aerobic conditions for their development.

9. The controversy over aerobiosis versus anaerobiosis of intestinal worms is based on a misunderstanding, since the type of metabolism of various helminths may be different in identical environments depending on the organization of the animals. The same is true of tissue helminths.

Echinoderms.

1. Adult echinoderms seem not to occur in anaerobic surroundings and they are quite sensitive to complete lack of oxygen.

2. Eggs and sperm of echinoderms exhibit a greater resistance to experimental anaerobic conditions than do adults.

3. The initial stimulation of the eggs preparatory to cleavage does not require molecular oxygen (in *Arbacia*), but the cleavage process itself does.

Molluscs.

1. Many lamellibranchs are found in surroundings very poor in oxygen and are able to withstand for long periods an experimental deprivation of oxygen.

2. Lamellibranchs of the tidal zone lead a predominantly anaerobic life during low tide, but gastropods occurring in the same habitats do not.

3. It seems quite certain that lamellibranchs kept in anaerobic conditions have no special source of oxygen of their own.

4. A certain ability to live anaerobically is developed both in terrestrial and in aquatic gastropods, but there is no direct parallelism between that ability and the occurrence of anoxic conditions in the normal habitat of these animals.

5. The anaerobic functions of cephalopods are very poorly developed; these organisms never penetrate anaerobic habitats.

Arthropods.

1. Copepods and, to a lesser degree, cladocerans are found quite often in oxygen-free surroundings. Some species also survive the experimental deprivation of oxygen.

2. Other resistant forms are found among ostracods and cirripedians, while decapods are in general more sensitive.

3. Some aquatic insect larvae (*Donacia,* chironomids, *Chaoborus* and others) have extremely well developed anaerobic functions.

4. The question of the factors enabling some chironomids to establish themselves in habitats very poor in

oxygen, while other chironomids are not able to do so, is still unanswered.

5. Adult aquatic insects are not very resistant to the lack of oxygen.

6. Terrestrial insects are only rarely exposed to severe oxygen deficiencies in nature; their resistance to experimental anaerobic conditions is not well developed either.

7. A low oxygen tension influences certain functions such as the rate of mutations in some insects; in *Drosophila,* for example, the rate of mutations is increased following periods of asphyxiation during the pupal stage.

8. *Limulus* seems to survive relatively long periods without oxygen; anaerobic metabolism may play an important role in the survival of this animal in nature.

9. The resistance of tardigrades to oxygen deficiencies depends on their water content; desiccated specimens are, of course, extremely resistant.

10. *Gasterophilus* larvae are better adapted to anaerobic life than the other parasitic arthropods so far studied, but even they do not survive for indefinite periods without oxygen.

11. The cases of urinary and intestinal myiasis offer interesting, though still unsolved problems on the mode of survival of various insects and larvae under low oxygen tensions.

PART II

THE ANAEROBIC METABOLISM OF INVERTEBRATES

CHAPTER I

PARTIAL TRANSITION FROM AEROBIC TO ANAEROBIC METABOLISM

A partial transition from aerobic to anaerobic metabolism seems to occur when the oxygen consumption of an organism declines for one reason or another, and the energy requirements do not decrease in the same proportion. The most common cause of a diminished rate of oxygen consumption in nature is an abnormally low oxygen concentration in the respiratory medium.

In our survey of anaerobiosis in invertebrates,[1] numerous instances were cited in which the oxygen consumption depended on the tension and it was pointed out that this might imply the presence of fermentative processes at low tensions. A more positive stand was avoided in most cases, because the mere fact of a lowered oxygen intake, without other evidence, does not justify more definite conclusions. It is well to remember that many organisms, such as, worms, crustaceans or insects, become motionless in surroundings very poor in oxygen. A cessation, or even a mere decrease of muscular movements will diminish the oxygen needs; the rate of oxidation may therefore simply decrease and the animal may be able to satisfy its reduced energy needs without resorting to anaerobic processes.

Further evidence of a transition to anaerobic metabolism will be obtained if the following points can be

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established: (A) That the end products characteristic of anaerobic processes or incomplete oxidations, such as, organic acids, have accumulated in the body, or have been excreted into the medium. (B) That the rate of carbohydrate consumption has increased beyond that prevailing at normal tensions. A considerable increase in the consumption of carbohydrates, the common mother-substance for fermentations, must be expected because fermentations, as a rule, yield very small amounts of energy. (C) That the respiratory quotient is higher than normal. When values above 1.0 are reached, it is evident that some carbon dioxide has been evolved which cannot be accounted for by the oxygen consumed. (D) That an oxygen debt has been incurred during the period of stay in the oxygen-poor surroundings, as evidenced by an oxygen intake greater than usual after restoration of a normal oxygen tension. The surplus oxygen is used to eliminate non-oxidized or partially oxidized products formed during the preceding period of partial asphyxiation.

Anaerobic oxidations may become necessary to an organism if the distribution of oxygen to the various parts of the body is impaired or if the mechanisms of aerobic oxidations are otherwise impeded: (A) through the action of poisons like carbon monoxide, cyanide, *etc.*, or (B) by the concentration of salts in the medium. Accordingly, two methods of experimentation were developed which, in some cases, contributed valuable evidence of anaerobiosis and will be treated separately below.

But before considering these various points of evidence for a partial transition from aerobic to anaerobic processes we shall review the more general problem of the influence of oxygen tension on oxygen consumption.

1. INFLUENCE OF LOWERED OXYGEN TENSION ON OXYGEN CONSUMPTION

The invertebrates are usually divided into two groups according to the degree of dependence of their oxygen consumption on the oxygen tension. In the first group, the consumption remains constant over a wide range of tensions (*cf.* Table 17 and Fig. 9), while in the second group, when the tension is gradually lowered, the consumption soon drops, and in some cases it begins to drop while the tension is still far above the highest met with in nature (*cf.* Tables 18 and 19).

To the first group belong the following representatives of the various phyla: PROTOZOA: almost all (Bledowski and Zweibaum, 1915; Lund, 1918; Amberson, 1928; Adolph, 1928; Baker and Baumberger, 1941); COELENTERATES: *Pelmatohydra* (Palmhert, 1933) and several jellyfish (Henze, 1910); WORMS: planarians (Lund, 1921; Hyman, 1929; Buchanan, 1931); *Tubifex* (Harnisch, 1935a), *Branchiura* (Kawaguti, 1936), *Hirudo* (Lindeman, 1932), the larvae of *Trichinella* (Stannard, McCoy and Latchford, 1938), perhaps *Nereis* (Hyman, 1932) and the earthworm (Konopacki, 1907; Dolk and van der Paauw, 1929; Thomas, 1935; Krüger, 1940; Krüger and Becker, 1940); ECHINODERMS: the eggs of various forms, (Henze, 1910; Tang, 1931; Tang and Gerard, 1932) and the holothurian *Thyone* (Hiestand, 1940); MOLLUSCS: some marine snails and some fresh-water and marine lamellibranchs (Henze, 1910; Moore, Edie, Whitley and Dakin, 1912; Dakin and Catherine, 1925; Raffy, 1933; Thamdrup, 1935), as well as the cephalopods (Henze, 1910; Amberson, Mayerson and Scott, 1925); ARTHROPODS: many crustaceans (Henze, 1910; Marshall, Nicholls and Orr, 1935; Helff, 1928; Kalmus, 1930; Maloeuf, 1937; Weymouth, Crimson, Hall, Belding and Field, 1944), the developmental stages and adults of insects (Gaarder, 1918; von Buddenbrock and von Rohr, 1922; Hiestand, 1931;

TABLE 17

EXAMPLES OF OXYGEN CONSUMPTION AT VARIOUS TENSIONS IN ORGANISMS WHOSE RESPIRATION IS INDEPENDENT OF THE TENSION OVER A WIDE RANGE.

Tubifex tubifex (According to Harnisch, 1935a)		*Trichinella spiralis* larvae (According to Stannard, McCoy and Latchford, 1938)	
Oxygen tension in mm. Hg	Oxygen consumption in % of consumption at the tension of atmospheric air	Oxygen tension in mm. Hg	Oxygen consumption in % of consumption at the tension of atmospheric air
760	100	760	89
160	100	160	100
53	98	38	101
23	87	15	103
6	31	7.6	92

Unfertilized *Arbacia* eggs (According to Tang, 1931)		*Zootermopsis nevadensis* (According to Cook, 1932)	
Oxygen tension in mm. Hg	Oxygen consumption in % of consumption at the tension of atmospheric air	Oxygen tension in mm. Hg	Oxygen consumption in % of consumption at the tension of atmospheric air
760	100	760	93
160	100	160	100
86	101	76	91
42	92	38	85
20.5	92	15	69
9.9	69	6	28
6.1	54		
2.0	43		

Harnisch, 1929 and 1939; Cook, 1932; Bodine, 1934; Fox, Wingfield and Simmonds, 1937; Morgan and Wilder, 1936).

The reasons advanced to explain how these animals can maintain a more or less uniform oxygen consumption over a wide range of tensions are fundamentally all concerned with the facility with which oxygen enters into the body and with the ease of its distribution to the various tissues. Some of these reasons may be mention-

ed briefly. In echinoderm eggs, or in small animals, like protozoa, planarians, *etc.*, the surface/volume ratio is high enough to allow a rapid diffusion of oxygen to those parts of the body that are not in direct contact with the atmosphere. In other cases, like that of the jellyfish, the animal may be fairly large, but, since it contains very little organic material (its body consisting of such

TABLE 18

EXAMPLES OF OXYGEN CONSUMPTION AT VARIOUS OXYGEN TENSIONS IN ORGANISMS WHOSE RESPIRATION IS DEPENDENT ON THE TENSION.

Spirostomum ambiguum (According to Specht, 1935)		*Urechis caupo* (According to Hall, 1931)	
Oxygen tension in mm. Hg	Oxygen consumption in % of consumption at the tension of atmospheric air	Oxygen tension in mm. Hg	Oxygen consumption in % of consumption at the tension of atmospheric air
760	151	116.8 [1]	100
158	100	62.6	43
3.8	71	28.2	41
Very low (Almost pure nitrogen)	3	14.4	10

Plerocercoids of *Diphyllobothrium latum* (According to Friedheim and Baer, 1933)		*Limax agrestis* (According to Thunberg, 1905)	
Oxygen tension in mm. Hg	Oxygen consumption in % of consumption at the tension of atmospheric air	Oxygen tension in mm. Hg	Oxygen consumption in % of consumption at the tension of atmospheric air
760	130	730	122
132	100	380	117
38	72	160	100
		120	87
		80	73
		40	46

1) No values at higher tensions are available; the oxygen consumption at 116.8 mm. is therefore taken as 100%.

a high percentage of water), the metabolic level will be low and diffusion will suffice to distribute oxygen to all living cells even at relatively low tensions. In still other animals there is an efficient circulatory system, often coupled with the presence of respiratory pigments (as in cephalopods, crustaceans, and some worms), or the air is brought in more or less direct contact with the cells by means of tracheae (insects).

The second group includes the following: PROTOZOA: *Spirostomum* (Specht, 1935); SPONGES (Moore, Edie, Whitley and Dakin, 1912; Raffy, 1933); COELENTERATES: the actinians (Trendelenburg, 1909; Henze, 1910; Harnisch, 1932; Raffy, 1933), *Cassiopea* (McClendon, 1917) and, to some extent, the corals (Yonge, Yonge and Nicholls, 1932; Kawaguti, 1937); WORMS: many parasitic nematodes, trematodes and cestodes (Harnisch, 1932a and 1933a; Friedheim and Baer, 1933; Krüger, 1936; Laser, 1944), nemerteans (Raffy, 1933), some leeches (Raffy, 1933), *Sipunculus* (Henze, 1910; Raffy, 1933) and, to a lesser degree, *Urechis* (Hall, 1931); ECHINODERMS: adult starfish, sea-urchins and some holothurians (Hyman, 1929; Nomura, 1927; Meyer, 1935; Maloeuf, 1937) and gastropods (Thunberg, 1905; Dahr, 1927; Harnisch, 1932; Fischer, 1931); ARTHROPODS: *Limulus* (Amberson, Mayerson and Scott, 1924; Maloeuf, 1937a) and some crustaceans (Moore, Edie, Whitley and Dakin, 1912; Amberson, Mayerson and Scott, 1924; Chen, 1932).

An explanation for the failure of some of these organisms to secure a maximal amount of oxygen even at high tensions was first attempted for the actinians by Henze (1910) and Krogh (1916). According to these investigators, the oxygen demand of the external layers of the body of these relatively large animals is so high that most, if not all the oxygen that enters the surface by diffusion is consumed before it reaches the inner parts. These latter receive either an insufficient amount or none

at all. This condition is aggravated by the fact that no circulatory system is present that would distribute the oxygen to the tissues. The inner layers must therefore have an oxygen tension of zero or nearly zero and their cells obviously must gain their energy more or less completely through anaerobic processes (Krogh, *l. c.*). If the oxygen tension in the surroundings is increased or decreased, a thicker or thinner layer of tissues, respectively, will receive an adequate oxygen supply and, as a consequence, the over-all oxygen consumption will rise or fall.

If the assumption is correct that the rate at which oxygen diffuses into the tissues is the limiting factor in the oxygen consumption, it should obviously be possible to eliminate or at least reduce the influence of the oxygen tension by reducing the distance through which the gas has to diffuse in order to reach the cells where it is used. Experiments of that type were undertaken by Harnisch (1932). He determined the oxygen consumption of sections of tissues from the body wall of actinians in presence of varying percentages of oxygen, and also of pieces of tissues that had been either simply minced with scissors or minced and then forced through silk gauze. His results, given in relative values only, are summarized in Table 19. It is obvious that in whole sections the oxygen consumption changed with the tension throughout the entire range, as was found by Henze (1910) for whole animals. The pieces forced through silk gauze, on the other hand, exhibited a fairly uniform aerobic respiration at least at tensions extending from 11 to 60 per cent oxygen. However, the enormous increase in oxygen consumption in pure oxygen seems to indicate that other factors than mere diffusion are also involved. Except for this one point, the experiments of Harnisch, as a whole, lend support to the explanation proposed by Henze and Krogh.

TABLE 19

RELATIVE OXYGEN CONSUMPTION OF WHOLE SECTIONS THROUGH THE BODY WALL OF ACTINIANS, AND OF MINCED PIECES.

Mean values calculated from the data presented by Harnisch, 1932.

Per cent oxygen in the atmosphere used	Oxygen consumption in per cent of the value obtained at the oxygen tension of atmospheric air (21 per cent)		
	WHOLE PIECES	MINCED PIECES	
		Minced with scissors alone	Minced with scissors and forced through silk gauze
100	328		182
60	134		99
21	100	100	100
11	83	94	92
6	75	74	87
3	51		62
0.8	24		37

But the following observation by Petrik (1931) raises another difficulty. He found that the oxygen consumption of fed actinians is considerably higher than that of starving ones, and that it stays higher for several days. Since the oxygen consumption of unfed specimens is supposed to be already at its maximum, being limited only by diffusion, the increased rate can only be understood if, by a regulatory mechanism, feeding increases the surface through which oxygen can enter the body. There seems indeed to be such a regulatory mechanism: fed actinians swell up, due to water intake, and in this way increase their body surface to a noticeable degree (the ratio of the surface of absorption to the amount of *organic material* thus becoming higher).

A situation similar to that prevailing in actinians probably exists also in other bulky animals lacking a circulatory system, like the large worm, *Sipunculus*, and perhaps also in sea-urchins, starfish and other organisms that possess neither a true circulatory system nor a powerful respiratory pigment. Furthermore, a sea-ur-

chin or a starfish has no known mechanisms for regulating the respiratory surface. It would be of considerable interest to compare the rates of oxygen consumption of such animals when fed and when unfed, or to test whether substances, like dinitrophenol, which increase the rate of oxidations in many animals, have any effect on them. The results obtained might elucidate the theory of the limiting action of diffusion.

But why the circulatory system of *Helix*, for example, or that of *Callinectes* and *Homarus* should not be efficient enough to provide all the tissues with oxygen at moderate tensions is by no means obvious. More baffling still is the question why the oxygen consumption of the small *Spirostomum* should be dependent on the tension. It is a case that would merit reinvestigation from various angles.

Gerard (1931), who presents a mathematical treatment of the oxygen diffusion into unicellular organisms at various tensions, points out that the assumption of a uniform diffusion throughout the cell is probably an oversimplification. The phenomenon may be complicated by different diffusion constants and the respiratory rate may not be uniform in all zones of a given cell.

One should also take into account the observations of Kempner (1937) who showed that, for a variety of biological objects, the effect of oxygen tension on cellular respiration is definitely influenced by factors like pH, carbon dioxide tension, salt content or temperature.

Thus it is questionable whether the above division into two groups is justified. This has been emphasized recently by Maloeuf (1937) whose views have also been accepted by von Buddenbrock (1939). Their argumentation is that, if one lowers the tension gradually, a point will be reached where, in *all* animals, the oxygen consumption will decline. In other words, the difference between various animals consists only in the position of

the critical point along the scale of oxygen tensions, and the seemingly qualitative difference between the two groups is reduced to a merely quantitative one.

It should be emphasized, furthermore, that the critical point is by no means constant in a given species but is influenced by external conditions (*e.g.,* temperature) and perhaps also by internal conditions. It is higher at higher temperatures, as has been repeatedly observed (Gaarder, 1918; von Buddenbrock and von Rohr, 1922; Lindeman, 1935). In fact, one may expect that all the factors increasing or decreasing the rate of metabolism will affect the position of the critical point (von Buddenbrock, 1939).

The tension at which the oxygen consumption of some organisms begins to decline varies considerably in the experiments of various authors, especially in experiments with worms: *Tubifex* (Dausend, 1931; Harnisch, 1935), the earthworm (Konopacki, 1907; Dolk and van der Paauw, 1929; Thomas, 1935; Krüger, 1938), the planarians (Lund, 1921; Hyman, 1929; Fraps, 1930; Buchanan, 1931) and *Nereis* (Amberson, Mayerson and Scott, 1924; Hyman, 1932). An investigation on metazoa of the factors mentioned by Kempner (1937, *cf.* above) might clear up some of these discrepancies. So far the only observation which may account for such differences is that of Harnisch (1935a) who found that the oxygen consumption of *Tubifex* (and of other organisms as well) showed a greater dependence on the tension when the animals were kept, prior to the determinations, in surroundings poor in oxygen than when they were kept in well-oxygenated water. However, this observation could hardly explain all the other cases mentioned above.

One other point should receive more attention in future investigations. Some of the data that seem to indicate an increase in oxygen consumption at tensions exceeding that found in the atmosphere may not be quite trust-

worthy due to the fact that the oxygen which is merely in solution within the animal tissues and body fluids and does not take part in the oxidations has been neglected. Von Buddenbrock (1939) found that the oxygen consumption of the sea-urchin *Sphaerechinus granularis* rises from 2.28 cc. to 7.2 cc. per hour if the animal is transferred for one hour from normal sea water to water having an abnormally high oxygen content. This, however, does not actually indicate an increased rate of oxidation since, during that same time, the oxygen content of the body fluid rose from 1 or 2 cc. to 10.1 cc. per liter. It is clear that the experiments should be conducted over longer periods in order to eliminate this source of error. Longer observations on the above-mentioned sea-urchin (also on *Asterias,* according to Meyer, 1935, and on leeches, according to Raffy, 1933) show that the oxygen consumption becomes lower again later on. Where a true dependency on the tension does exist, even at high oxygen concentrations, the increased rate of respiration will be maintained for long periods. Such is the case with actinians as von Buddenbrock's (1939) experiments on *Anemonia sulcata* have shown.

2. EVIDENCE OF PARTIAL TRANSITION TO ANAEROBIC METABOLISM

1. TRANSITION CAUSED BY A LOWERING OF THE OXYGEN TENSION

Let us now consider separately the features mentioned above as furnishing a definite evidence for the existence of anaerobic metabolism.

A. Excretion or accumulation of end products of anaerobic metabolism. As will be shown in a subsequent chapter, many investigations have been made on this point for completely anaerobic conditions, but hardly any for low oxygen tensions. The only observation to be mentioned here is that of Krüger (1936) who found that *Ascaris* excretes more organic acid at low than at

high tensions. This case is not, however, unequivocal, because the metabolism of this worm is characterized by the persistence of anaerobic processes even in surroundings very rich in oxygen as will be seen in more detail later on. It is highly desirable that more investigations of the nature of the end products of respiration at reduced oxygen tensions be undertaken.

B. Increase in the rate of carbohydrate consumption. Again very little work has been done along this line. Von Brand (1927) investigated the rates of glycogen consumption of the polychaetes *Spirographis spallanzanii* and *Halla parthenopeia* in well-aerated water and during asphyxiation (which in these forms takes place while there is still some oxygen left in the water). Glycogen disappeared at a rate of 0.15 and 0.86 g. per 100 g. *Spirographis* in 24 hours under these two sets of conditions respectively; the corresponding figures for *Halla* were 0.13 and 0.71 g. It was moreover observed that 0.25 g. polysaccharide was consumed daily by the latter worm when kept in an aquarium which was insufficiently aerated but in which the oxygen consumption remained higher than in the asphyxiation experiments. There is hardly any doubt, therefore, that these polychaetes are capable of undergoing a partial transition to anaerobic metabolism and of maintaining it for a limited time.

Dausend (1931) also observed that the rate of glycogen consumption increases in *Tubifex* if the oxygen tension of the medium is lowered (Fig. 4).

C. Increase in the respiratory quotient. A significant increase in the respiratory quotient has been observed in many cases when invertebrates were transferred to surroundings deficient in oxygen (Table 20). The figures obtained with marine organisms by earlier investigators, like Vernon (1895), are not too reliable, owing to the difficulty of determining precisely small quantities of carbon dioxide in sea water by the methods then avail-

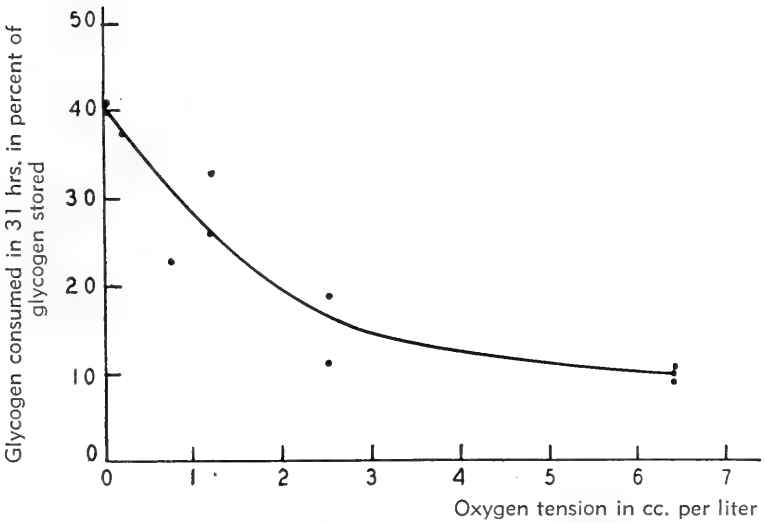


Fig. 4. Dependence of glycogen consumption on oxygen tension in *Tubifex*. (According to Dausend, 1931.)

able. Whether technical errors are responsible for the exceptional cases (Table 20) in which the respiratory quotients did not increase at lowered oxygen tension, as in *Rhizostoma, Carmarina* and *Cestus,* or whether these animals are incapable of anaerobic metabolism is a matter of conjecture.

Maloeuf (1937) expressed the dependence of the respiratory quotient RQ on the oxygen tension P by the relation RQ =K/P, where K is a constant (P is the product: 22.4 $\times$ molar concentration $\times$ T/273 atmospheres, T being the absolute temperature). The value of the constant K is 25 $\times$ 10^{-4} for *Cambarus bartoni* and *Cambarus clarkii,* 44 $\times$ 10^{-4} for *Asterias forbesii,* and 77 $\times$ 10^{-4} for *Mytilus edulis.* This formula, however, can be applied only within a limited range of oxygen pressures: in the crayfish between 0.00025 and 0.0045, in the starfish between 0.0020 and 0.0056 and in the mussel between 0.0016 and 0.0064. Above these ranges the respiratory quotient, of course, remains more or less constant since the maximal rate of oxidation is reached.

TABLE 20

RESPIRATORY QUOTIENTS OF VARIOUS INVERTEBRATES AT HIGH AND AT LOW OXYGEN TENSIONS.

Species	High oxygen content in surroundings		Low oxygen content in surroundings		Source
	Oxygen content	R. Q.	Oxygen content	R. Q.	
Protozoa					
Collozoum inerme	Saturation	1.06	(During asphyxiat.)	1.59	Vernon, 1895
Spirostomum ambiguum	21%	0.84	0.5%	1.36	Specht, 1935
Coelenterates					
Beroe ovata	Saturation	0.84	(During asphyxiat.)	2.24	Vernon, 1895
Carmarina hastata	"	1.10	"	0.97	" "
Cestus veneris	"	0.79	"	0.88	" "
Rhizostoma pulmo	"	0.90	"	1.00	" "
Salpa pinnata	"	1.12	"	1.64	" "
" *tilesii*	"	0.62	"	2.46	" "
Worms					
Ascaris lumbricoides	21%	1.07	0.8%	10.2	Harnisch, 1933a
Fasciola hepatica	21%	0.90	1.0%	3.0	" 1932a
Lumbricus communis	20%	1.00	2.5%	1.89	Konopacki, 1907
" "	21%	0.75	5%	0.99	Krüger, 1940
Sipunculus nudus	5.4 cc/l.	1.48	0.81 cc/l.	10.0	Henze, 1910
Tubifex tubifex	6 cc/l.	1.20	0.64 cc/l.	4.48	Dausend, 1931
" "	21%	0.70	0.8%	2.75	Harnisch, 1935a

TABLE 20 (Continued)

Species	High oxygen content in surroundings		Low oxygen content in surroundings		Source
	Oxygen content	R. Q.	Oxygen content	R. Q.	
Molluscs					
Cepaea hortensis	21%	0.57	2.6%	>1.3	Liebsch, 1928
Chilotrema lapicida	21%	0.65	1.3%	>1.3	" "
Helix pomatia	21%	0.65	2.5%	>1.3	" "
Octopus vulgaris	Saturation	0.95	(During asphyxiat.)	1.18	Vernon, 1895
Ostrea circumpicta	6.5 cc/l.	0.86	0.5 cc/l.	7.5	Nozawa, 1929
Arthropods					
Cambarus immunis	5.45 cc/l.	0.65	0.39 cc/l.	12.7	Maloeuf, 1936
Chironomus plumosus	21%	0.70	0.8%	1.15	Harnisch, 1933
" *thummi* (sections)	21%	0.7	0.8%	2.8	" 1930
Cloeon dipterum	21%	0.77	0.8%	1.13	" 1939
Dixippus morosus	21%	0.87	1.25%	1.1	von Buddenbrock, 1939
Eutanytarsus inermipes	21%	0.62	0.8%	1.31	Harnisch, 1933
Prodiamesa praecox	21%	0.62	0.8%	5.68	" "

It is clear that a respiratory quotient in excess of 1 demonstrates the production of carbon dioxide that is not derived from aerobic processes. But the mere fact of a surplus carbon dioxide production does evidently not warrant any further conclusion as to the chemical nature of the anaerobic processes going on. The carbon dioxide may be derived directly from the metabolized substances, or it may be liberated from bicarbonate or other inorganic substances through acids formed during the fermentative metabolism. It will frequently be of mixed origin and its attribution to a definite reaction is a problem which has apparently not yet been studied experimentally in cases in which fermentations induced by a partial lack of oxygen proceed simultaneously with aerobic oxidations. We shall return to this problem later in discussing purely anaerobic metabolism.

D. Accumulation of an oxygen debt. Lund (1921) investigated this question, using *Planaria agilis*. He found as mean values of one of his series, that the oxygen consumption in well-aerated water (5.25 cc. of oxygen per liter) was 282 cc. per 100 g. per 24 hrs.; in water containing only 0.25 cc. of oxygen it was 37 cc. per 100 g. per 24 hours, and finally, during recovery in water with an oxygen content of 5.40 cc. per liter it amounted to 492 cc. per 100 g. per 24 hrs. Considerable variations were observed in individual experiments, the increase varying from 2 to 85% over the normal rate. Curiously enough it was not proportional to the time of exposure to partial asphyxiation, but was rather related to the physiological state of the worms: the increase being more pronounced in starved than in fed animals. Whether planarians are able to excrete some of the non-oxidized substances—this might explain some of Lund's contradictory results—has not yet been investigated.

It is quite obvious that no oxygen debt will accumulate if an organism eliminates all waste products through

excretion. A study of *Nereis* in this respect may explain why Hyman (1932) observed no increased oxygen consumption in these animals when they were transferred to oxygen-saturated water after having first been kept for one or more hours in oxygen-poor water. Not only was there no increase, but the oxygen consumption remained at an even lower level during the recovery period than had been found during the initial period in oxygen-rich water preceding the partial deprivation of oxygen.

Hall (1931) also found very little indication that an oxygen debt accumulated in *Urechis* when the animals were kept in water which sharply reduced their oxygen intake.

2. TRANSITION CAUSED BY RESPIRATORY POISONS OR BY THE SALINITY OF THE MEDIUM

A. Respiratory poisons. The oxygen-carrying function of haemoglobin can be blocked by suitable concentrations of carbon monoxide without considerable interference with the ability of the tissues to perform aerobic oxidations, that is, to utilize the oxygen that reaches them. One finds then that the dependency of the oxygen consumption on the tension begins at higher tensions than normally. The reason is that, at reduced tensions, only the respiratory pigment could insure a sufficient oxygen supply; if the pigment action is blocked, the animals must get along with what oxygen reaches them by diffusion.

In the case of animals which, in normal conditions, shift partially to fermentations in surroundings very poor in oxygen, the transition will take place earlier, *i.e.*, at higher oxygen tensions, when the specimens are poisoned. Experiments along that line have been performed with the earthworm (Jordan and Schwarz, 1920; Dolk and van der Paauw, 1929; Thomas, 1935; Krüger,

1938 and 1940; Johnson, 1942), *Tubifex* (Dausend, 1931; Krüger and Becker, 1940), *Nereis* (Jürgens, 1935), *Planorbis* (Leitch, 1916; Probst, 1933) and *Chironomus* larvae (Leitch, 1916; Harnisch, 1936; Ewer, 1942). In several instances results indicative of anaerobic metabolism were recorded, in particular in the case of the earthworm where the respiratory quotient of carbon-monoxide poisoned animals rose, at an oxygen tension equal to that of air, from 0.88 to 1.04 and at a tension of 5 per cent oxygen from 0.99 to 1.24 (Krüger, 1940).

Fewer experiments have been carried out with poisons that interfere directly with the oxidation mechanisms. Buchanan (1926) found that, during recovery from the influence of a dilute cyanide solution, planarians consume oxygen at a higher rate than usual for several hours. There was, however, no clear correlation between the amount of excess oxygen consumed and the length of exposure to the poison. Buchanan therefore doubts that the excess oxygen consumption is due to an accumulation of non-oxidized substances, but he did not consider the possibility that such substances may have been excreted, at least in part (compare the above mentioned experiments of Lund, 1921). Harnisch (1937b), on the contrary, stated that *Tubifex* repays almost fully the oxygen debt incurred during a period of exposure to dilute potassium cyanide.

Fink (1926) observed that the respiratory quotient of insects which had been fed arsenicals showed, in general, a significant increase. However, since it remained always below unity, the experiments are not quite conclusive in regard to the question under consideration.

The case of the mite *Tyroglyphus farinae,* investigated by Hughes (1943), is very definite. Its respiratory quotient rose under the influence of cyanide from 0.94 to 1.32.

B. Salinity of the medium. Kreps (1929) found that the oxygen consumption of *Balanus crenatus* decreases

to a minimum when the animal is kept in water of very low salinity. His supposition that during this time non-oxidized substances accumulate in the body, *i.e.*, that the metabolism has become partially anaerobic, seems well founded since there was a marked increase in oxygen consumption (as compared to the normal rate) for two days, when the animals were again transferred to sea water of normal salinity.

In general, it is a well-established fact that many animals show considerable variations in the intensity of oxygen intake depending upon the salt concentration of the medium, but there seems to be no regularity in either the direction or the extent of the change. Data concerning *Planaria, Nereis* and *Carcinus,* to give only a few examples, will be found in the papers of Schlieper (1929) and Buchanan (1931). Whether, in any of these cases, fermentations take place is unknown, but it is a problem which deserves attention.

CHAPTER II

AEROBIC FERMENTATIONS

Oxidations may be incomplete even in presence of an abundant supply of oxygen. When this is the case, one may find in one and the same animal some incomplete oxidations that do not utilize any molecular oxygen and, proceeding at the same time, but more or less independently, the ordinary, complete aerobic oxidations. In other organisms the metabolism may be only partially aerobic, that is, molecular oxygen enters into the reactions but the oxidations remain incomplete.

Some of these reactions are referred to in the literature as *aerobic glycolysis* or as *aerobic fermentations*. This latter expression will be used throughout the present review.

Several criteria may be used to determine the presence of aerobic fermentations. A respiratory quotient above 1 in well oxygenated surroundings proves that the oxidations are incomplete—as, for example, in the case of freshly isolated specimens of *Ascaris* for which von Brand (1934a) reported an R.Q. of about 4 — but a low R.Q. does not prove that they are complete. Thus, in cases in which incomplete oxidations in presence of abundant oxygen were observed by other criteria, the respiratory quotient was either abnormally low, for example, 0.16 in *Trypanosoma rhodesiense* (Christophers and Fulton, 1938) or it was in the neighborhood of 1, for example, 0.95 in *Leishmania tropica* (Soule, 1925) or exactly 1.0 as in *Strigomonas fasciculata* and *Strigomonas oncopelti* (Lwoff, 1934).

A fairly reliable indication of aerobic fermentations may be obtained by a comparison of the rates of carbohydrate consumption under aerobic and under anaerobic conditions. In purely aerobic animals the two rates us-

ually differ greatly (in the earthworm, for example, their ratio is 1:5, according to Lesser, 1910), while they differ much less if fermentative processes persist in the presence of oxygen (in *Ascaris,* von Brand, 1934a, found a ratio of 1:1.32).

The best evidence for aerobic fermentations is, of course, the direct demonstration that non-oxidized or partially oxidized substances have been formed under conditions of good oxygenation. If one finds, for instance, that an animal, like *Moniezia,* excretes exactly the same amounts of non-oxidized substances in an atmosphere of 95 per cent oxygen and in the absence of oxygen and if its oxygen consumption is identical in 21 per cent and in 100 per cent oxygen (Alt and Tischer, 1931), then the existence of true aerobic fermentations is certain.

The distinction between partial transition to anaerobic processes when oxygen is lacking, as outlined in the preceding chapter, and aerobic fermentations is based on the fact that, when more oxygen becomes available, the cells which were forced to resort to anaerobic processes because of lack of oxygen, revert again to complete oxidations, while those which carry on aerobic fermentations continue to form non-oxidized or partially oxidized substances.

It is usually found that the end products of incomplete oxidations are excreted. An accumulation in the body will take place rarely, probably because it would become injurious. The two following cases might possibly represent exceptions to this rule. The proglottids of tapeworms, which are destined to live only a short while, may suffer a certain accumulation of waste products (von Brand, 1933a). During the larval diapause of some insects, in particular in the case of the *Pyralidae* (Kozancikov, 1935) there is some evidence that end products of incomplete oxidations accumulate (though the

information that we obtained from Kozancikov's paper is rather fragmentary). That no harm results may be due to the fact that the diapause is only a transitory stage in the life of an insect. Some of the end products may even be utilized for subsequent synthetic processes.

1. OCCURRENCE OF AEROBIC FERMENTATIONS

A survey of the invertebrate phyla shows that the occurrence of aerobic fermentations is somewhat erratic.

It was reported in all major groups of *protozoa,* both free-living forms and parasites, with the exception of the rhizopods. In general, it has been revealed by the fact that the pH, or the alkaline reserve of the medium in which the organisms were kept, decreased or that carbon dioxide was liberated from bicarbonate. A direct identification of the end products was undertaken in only very few cases. It is clear that, with indirect methods, many cases may have escaped detection. Loefer (1936) points out that this failure to find, by pH determination, an acid fermentation of carbohydrates in *Paramaecium bursaria* may be due to a neutralization of acids by concomitantly formed bases, perhaps ammonia. The occurrence of aerobic fermentation has been definitely proven in the following cases: in many species of *Trypanosoma* (von Fenyvessy, 1926; von Fenyvessy and Reiner, 1928; Kligler and Geiger, 1928; Kligler, Geiger and Comaroff, 1929; Geiger, Kligler and Comaroff, 1930; von Brand, Regendanz and Weise, 1932; Reiner and Smythe, 1934; Reiner, Smythe and Pedlow, 1936), in various species of *Leishmania* (Colas-Belcour and Lwoff, 1925; Noguchi, 1926; Salle and Schmidt, 1928; Salle, 1931), *in Leptomonas* and some species of *Strigomonas* (Colas-Belcour and Lwoff, 1925; Lwoff, 1934), in *Eutrichomastix* (Cailleau, 1937), in some species of *Trichomonas* (Witte, 1933; Cailleau, 1934, 1936, 1937; Riedmüller, 1936; Andrews and von Brand, 1938; Trussell and Johnson, 1941; Plastridge,

1943), in *Plasmodium knowlesi* (Wendel and Kimball, 1942; Wendel, 1943), in some species of *Glaucoma* (Colas-Belcour and Lwoff, 1925; Johnson, 1935), in some species of *Colpidium* (Elliott, 1935; Loefer, 1938), in *Paramaecium* (Cunningham and Kirk, 1941), and in other free-living flagellates and ciliates (Glaser and Coria, 1935; Jay, 1938; Loefer, 1938). Data on the oxygen consumption of most of these protozoa will be found in the papers quoted; for further information the reader is referred to the reviews of von Brand (1935) and Jahn (1941).

The cysts of *Iodamoeba* might be an exception to the above statement that no aerobic fermentation occurs in rhizopods. The large glycogen vacuole characteristic of these cysts disappears at the same rate whether they are kept under anaerobic or aerobic conditions. According to von Brand (1932) this may indicate identical degradation processes and obviously they would have to be of the type of incomplete oxidations. But the observations are too inadequate to provide more than a suggestion.[1]

In *coelenterates* the persistence of anaerobic processes at high oxygen tensions is due, in general, as explained in the preceding chapter, to the fact that even then oxygen does not reach all the cells. Whether in some cases oxidations are incomplete even when oxygen reaches the internal cells is uncertain. Petrik (1931) observed that the oxygen consumption of contracted actinians is very different from that of expanded ones, while no such

1. The mycetozoa, though usually considered as plants, are classified by some authors as rhizopods. If this classification is accepted, the above statement that no aerobic fermentations occur in this group needs, perhaps, to be modified. According to Seifriz and Urbach (1944) two types of metabolic processes can be distinguished in *Physarum polycephalum*. One is responsible for locomotion and spreading and depends on the availability of much oxygen, while the other, which is responsible for intraplasmatic streaming, requires but very little oxygen. It is possible that the latter is primarily of the fermentation type; in the presence of air, the two processes would then go on side by side.

variations were found in the carbon dioxide output (Parker, 1922). No conclusion as to whether this is only a special case of partial transition to anaerobic processes caused by a local insufficiency of oxygen or if it happens even when sufficient oxygen is available can be arrived at on the basis of the data at hand. This would, however, be an interesting topic for further research.

In *free-living worms,* the only case in which, under clearly aerobic conditions, organic acids were excreted is that of *Hirudo* (Lafargue and Fayemendy, 1932; Braconnier-Fayemendy, 1933).

But aerobic fermentations are very characteristic of the metabolism of *parasitic helminths* in oxygenated media. As a matter of fact the metabolism of every parasitic worm investigated so far in the presence of air either definitely showed incomplete oxidations or at least gave clear indications of them. The tendency is especially well developed in those helminths which lead a predominantly anaerobic life in nature. In *Ascaris* and *Parascaris* the formation of organic acids has been demonstrated during experimental exposures to aerobic conditions by Weinland (1901), Fischer (1924), von Brand (1934a), Krüger (1935, 1936), Oesterlin (1937) and Toryu (1936); in *Fasciola* and *Dicrocoelium* by Weinland and von Brand (1926) and Flury and Leeb (1926); in *Moniezia* and *Diphyllobothrium* by Alt and Tischer (1931) and Friedheim and Baer (1933). Whether similar processes occur in the *acanthocephala* is not yet definitely known; their presence has been suspected in the case of *Macracanthorhynchus,* since this worm consumes nearly the same amount of glycogen under aerobic and under anaerobic conditions (von Brand, 1940). That the afore-mentioned parasitic nematodes, trematodes and cestodes actually consume oxygen is a well-established fact; in addition to the data published in some of the

above papers the reviews of McCoy (1935), Wardle (1937) and von Brand and Jahn (1942) may be consulted.

Aerobic fermentations are less conspicuous in parasitic worms capable of leading a predominantly aerobic life inside their hosts. Non-oxidized or partially oxidized end products have not yet been isolated from them. In the larvae of *Trichinella* and *Eustrongylides* respiratory quotients above 1 have been observed at the oxygen tension of atmospheric air (Stannard, McCoy and Latchford, 1938; von Brand, 1942). This probably must be interpreted as an indication that not all oxidations are carried to completion. That some of the processes actually fall into the class of aerobic fermentations is definitely indicated by the fact that the oxygen consumption of at least the *Trichinella* larva remains constant down to a tension of 1 per cent oxygen. This obviously proves that the maximum amount of oxygen required is capable of entering the body at very low tensions.

No processes that could be classified as aerobic fermentations have so far been described for *echinoderms* and *molluscs,* with the exception of snails, where fat synthesis from carbohydrates has been observed (Biedermann, 1911).

In *arthropods* we mentioned above the case reported by Kozancikov (1935). This author summarizes his investigation on the role of anaerobic processes during the larval diapause of some *Pyralidae* in the statement: "The larval diapause represents a compensation period to the development in unfavorable conditions and is characterized by anoxybiotic rearrangements." Since there can hardly be a doubt that these organisms are capable of consuming oxygen during this period, their metabolism is probably of the type of fermentations in the presence of air.

Clear-cut cases of aerobic fermentations occur amongst the chironomids. The larvae of both *Chironomus batho-*

philus and *Chironomus thummi,* when kept under aerobic conditions, liberate considerable amounts of carbon dioxide from bicarbonate (Harnisch, 1943). Good evidence is also available for the larva of *Gasterophilus intestinalis.* Von Kemnitz (1916) showed that this insect produces much less carbon dioxide than would correspond to the glycogen disappearing from the body if one assumes that this glycogen is completely oxidized. It follows that some of the polysaccharide is only partially oxidized.

Melampy and Willis (1939) found respiratory quotients of 1.14 to 1.29 and 1.03 to 1.42 in larval queens of the honeybee and larval workers, respectively, at the oxygen tension of atmospheric air. They interpret this finding as an indication of the synthesis of fats from carbohydrates, a process that, from a biochemical standpoint, must be classified with fermentations in an excess of oxygen.

2. THE SIGNIFICANCE OF AEROBIC FERMENTATIONS AND THEIR RELATIONSHIPS WITH OTHER TYPES OF OXIDATIONS

Two questions of special interest arise here: (A) Why are some oxidations incomplete in some animals in the presence of oxygen? and (B) What are the relationships between incomplete and complete oxidations? These points will be discussed separately.

A. Reasons for the occurrence of incomplete oxidations in the presence of oxygen. Incomplete oxidations, as was said above, take place in some animals which experience no difficulty in securing oxygen in such amounts that all the energy needed could be gained by complete oxidations. One must further remember that the additional amounts of oxygen that would be required to furnish a quantity of energy comparable to that gained by the fermentations must be relatively small, since fermentations yield only little energy while much more is

released through complete oxidations. This holds true even in a parasite like *Ascaris* where the reactions of the incomplete type are very pronounced. Von Brand (1934a) determined the oxygen consumption of 100 g. of *Ascaris* at the oxygen tension of atmospheric air and found that 0.21 g. were consumed in 24 hours; he likewise showed that during this time 0.86 g. of glycogen were decomposed through fermentative processes. The energy yield of the latter is estimated to lie between 6 and 12 per cent of that liberated by the complete oxidation of the carbohydrate (von Brand and Jahn, 1942). Taking a mean value of 9 per cent, 0.077 g. of glycogen would have to be decomposed through complete oxidations to yield the same amount of energy. This would require 0.092 g. of oxygen, or an additional intake of 44 per cent over the amount actually consumed. It is likely that in free-living organisms, like the ciliates mentioned in the preceding section, the percentage increase would be much smaller, but the available data do not allow detailed calculations. Thus, the sum total of the evidence indicates rather strongly that it is not the inability of the animals to secure sufficient oxygen which compels them to leave their oxidations incomplete.

Incomplete oxidations are very uneconomical because the energy bound up in the excreted end products is simply lost. Free-living animals have rarely an over-supply of food; the occurrence of these processes in such organisms is therefore especially surprising. It is easier to see, from the nutritional viewpoint, why wasteful processes are no handicap to parasites; these animals normally live in surroundings in which an abundance of food is always available.

As was stated previously, many intestinal parasites are forced to resort to a predominantly anaerobic metabolism in their normal habitat because of the low oxygen content of the latter. One may assume that they

have become adapted to this type of metabolism to such a degree that a complete transition to purely aerobic oxidations has become incompatible with their organization (an assumption which, of course, is no real explanation). It is of interest to note, in this connection, that at least in intestinal worms the fermentations in the presence of air lead to the same end products as the fermentations under purely anoxic conditions. Experiments proving this have been performed with *Ascaris* by Weinland (1901 and 1904) for anaerobic conditions, and by Oesterlin (1937) for aerobic conditions, with *Parascaris* by Toryu (1936), with *Moniezia* by von Brand (1929 and 1933a) for anaerobic conditions and by Alt and Tischer (1931) for aerobic conditions.

An adaptation to incomplete oxidations due to lack of oxygen in the normal surroundings cannot be assumed for blood parasites like the trypanosomes. It is true that they live in the blood plasma which contains only relatively small amounts of oxygen, but they are able to draw on the vast store of oxygen present in the erythrocytes of the host. It can easily be shown that blood containing trypanosomes rapidly assumes the color of reduced haemoglobin when removed from the host. This was demonstrated as early as 1911 by Nauss and Yorke.

The situation in malaria parasites is directly comparable to that found in trypanosomes. That they are also able to consume the oxygen bound to the haemoglobin of the host has been shown experimentally by Christophers and Fulton (1938). The reasons why incomplete oxidations occur in blood parasites are entirely unknown.

A comparison of two processes which are quite similar chemically but are of different physiological significance in two different groups of animals may furnish a tentative explanation of at least one type of incomplete oxidation. The synthesis of fat from carbohydrates in animals living aerobically is primarily intend-

ed to supply a storage material which can subsequently be utilized in times of need by means of aerobic oxidations; though the fat synthesis releases some energy, that energy production is of secondary importance to the animal. On the contrary, in the aerobic or anaerobic fermentations which occur in *Fasciola, Moniezia* or *Gasterophilus* and which have higher fatty acids as end products, energy production is the essential feature and the same higher fatty acids that, in the other cases, represent such important storage products are here generally only waste products. The ability to form reserve fat from carbohydrates is probably widespread amongst invertebrates. There is good evidence that the process occurs in protozoa (Nirenstein, 1910; Doflein, 1918; Pringsheim, 1928; Zingher, 1933), in snails (Biedermann, 1911) and in insects (Melampy and Willis, 1939; Wigglesworth, 1942). It seems likely that many more cases will be discovered. Now, Zhinkin (1930) and Barbarine (1938) reported that various free-living protozoa transform glycogen into fat when oxygen is lacking and that this fat, in turn, disappears, *i.e.*, is used for energy production when the medium again receives a sufficient supply of oxygen. Thus, under one set of conditions, the fat is a metabolic end product which is not utilized further as long as these conditions prevail, and which is directly comparable to the fat excreted by *Moniezia* or *Fasciola,* where it seems never to be used for the production of energy. Under another set of conditions (good oxygenation) this same fat represents for the protozoa an energy reserve and has exactly the same importance as fat synthesized from carbohydrate and deposited in the tissues of aerobic animals as a reserve against future needs.

B. Relations of aerobic fermentations with other metabolic processes. In this section only those incomplete oxidations will be considered in which energy production

is the salient feature. The aerobic formation of reserve fat from carbohydrate will not be discussed further, since, as mentioned above, the energy production, there, is only incidental and seems to have no direct connection with other energy-producing metabolic processes. The point that has aroused most interest—and controversy—is the relationship between aerobic oxidations and aerobic fermentations in the case of animals which, in nature, gain their energy predominantly by fermentations. The discussion has centered chiefly around the parasitic worms.

Weinland (1901), working with *Ascaris,* expressed the opinion that the metabolism of this helminth was purely anaerobic and that any aerobic process that might occur in oxygenated surroundings did not have its seat in the worm proper; he attributed it either to an aerobic bacterial flora in the medium, or to developing eggs. This view has today only a historical interest since it has been shown—first by Alt and Tischer (1931) on *Moniezia* and then by Adam (1932) on *Ascaris*—that the tissues of all parasitic worms tested are able to utilize oxygen.

Harnisch (1932a, 1933a, 1935, 1937a) developed the idea that parasitic nematodes, trematodes and cestodes gain their energy, even in presence of oxygen, exclusively through fermentations, and that the processes utilizing oxygen would serve only for the removal of the end products of the anaerobic metabolism. He originally based his view on his observation that the carbon dioxide production of these animals remained identical in oxygenated and in oxygen-free surroundings. This had also been observed by Weinland and von Brand (1926) in the case of *Fasciola hepatica.* Harnisch reasoned that it would be a curious coincidence if the sum total of carbon dioxide originating from both the aerobic and the anaerobic processes would be the same as that produced under

anaerobic conditions by fermentations alone. He assumed, therefore, that the origin of the carbon dioxide must, under either condition, be sought in fermentations, *i.e.*, no carbon dioxide production would be connected with the aerobic processes; these latter would have a respiratory quotient of zero. He sees no reason why parasitic worms kept under aerobic conditions should have higher energy requirements than those kept under anaerobic conditions, and he concludes that the aerobic processes, in these cases, produce no energy. This would, of course, imply that aerobic oxidations in helminths are fundamentally different from those met with in normally aerobic animals; they would resemble rather the processes occurring in the latter during recovery from lack of oxygen.

More recent investigations, however, have shown that the amount of carbon dioxide excreted by aerobically kept ascarids is greater than that produced under anaerobic conditions (von Brand, 1934a; Krüger, 1936 and 1937), a fact already observed by Weinland (1901). Further information is necessary before it can be stated definitely whether this holds true also for trematodes and cestodes. In *Ascaris* it was shown that the excretion of organic acids is more pronounced under complete lack of oxygen or under low oxygen tensions than if much oxygen is available (von Brand, 1934a; Krüger, 1936). It has also been definitely established that one of the end products of the aerobic processes is carbon dioxide, and the respiratory quotient was found to be almost unity, instead of zero, as postulated by Harnisch. One is, therefore, forced to conclude that some energy is released by the aerobic processes.

For what purposes this energy is used is another question. Harnisch (1937a) still believes that it plays no part in the normal energy supply of these worms, a view somewhat similar to that of Krüger (1937) who thinks that

this energy is wasted and is not connected directly with any physiological function.

Krüger (1937) assumes that the aerobic processes in *Ascaris* are similar to those in any free-living animal, while according to Harnisch (1937a), as was said above, they would correspond only to the post-anaerobic processes of the latter. Harnisch, in his later work, gives two new reasons for thus placing in a special class the aerobic processes in helminths and abandons his previous line of reasoning. First, he points out the fact that the dependency of the oxygen consumption of parasitic worms (and the post-anaerobic oxygen consumption of free-living animals) on the oxygen tension cannot be eliminated by mincing the material, which is equivalent to lessening the distance through which the oxygen would normally have to diffuse before reaching the inner cells. This is in marked contrast with the data on normal oxygen consumption of many free-living animals, in which, as was pointed out previously, a shortening of the diffusion distance reduces the influence of the oxygen tension to a considerable degree. Secondly, Harnisch showed that both the post-anaerobic excess oxygen consumption of free-living animals and the entire oxygen consumption of parasitic worms are regulated by enzyme complexes which apparently have another location than those responsible for the normal oxygen consumption of free-living animals. When the latter were carefully minced and the cellular suspension washed, the oxygen consumption remained, within reasonable limits of error, the same as it was after mincing, but before washing. This is interpreted as indicating that the enzymes responsible for this type of oxygen consumption (Harnisch's primary aerobic processes) are located within the cells and as showing that the procedure used did not break up the cells to such an extent as to allow the removal of the enzymes by washing. If, on the con-

trary, parasitic worms or free-living animals which had undergone a period of oxygen deprivation were treated in the same manner, the entire oxygen consumption (parasitic worms) or the post-anaerobic excess oxygen consumption (free-living animals) was largely eliminated by washing, but could be restored by the addition of cell-free body fluid to the cellular suspension. These observations indicate, then, that this type of oxygen consumption (Harnisch's secondary aerobic processes) is due to enzymes located not within the cells but in the body fluids surrounding them.

These differences are indeed very interesting and it would be desirable to extend such investigations to organisms that, in respect to their normal oxygen supply and their ability to live anaerobically, occupy intermediate positions between *Ascaris* and typically aerobic animals. *Trichinella* and *Eustrongylides* larvae might be favorable objects. Harnisch (1937a) himself considers it possible that at least some parasites (for example, *Bothriocephalus bipunctatus*) may still possess vestiges of primary aerobic processes. In this connection, it would be of great interest to test whether a change from secondary to primary aerobic processes is possible in parasitic worms after they have been kept for a long time in aerobic surroundings. In view of the encouraging progress that has been made lately with the problem of maintaining parasitic helminths *in vitro*, promising results may be expected.

Finally, a word is necessary concerning the difficulty of distinguishing, in some cases, between incomplete oxidations in the presence of oxygen and incomplete oxidations induced by a lack of oxygen as described in the preceding chapter. At high oxygen tensions—in the range in which the oxygen consumption is independent of the tension—no confusion is possible; the second phenomenon does not occur, there being no lack of oxygen.

The situation may become confusing when animals that show the phenomenon of aerobic fermentations are partially deprived of oxygen. The excretion of non-oxidized end products will then become accentuated. This happens, for example, in *Ascaris* (Krüger, 1936). It is then, however, not possible to tell what portion of the end products is due to processes induced by local oxygen deficiencies of certain cells or tissues and what portion to aerobic fermentations since in this worm the end products of both processes are identical. In animals like the trypanosomes in which the end products of the two processes are at least partially different, a distinction would probably be possible even at low oxygen tensions. But, so far, experiments have been carried out with these animals only under fully aerobic conditions and under complete lack of oxygen, not with intermediate, low oxygen tensions.

CHAPTER III

THE SOURCES OF ENERGY IN ANAEROBIOSIS

Vertebrates can derive energy from the aerobic oxidation of carbohydrates, fats and proteins, substances which can usually be used interchangeably. In invertebrates the situation is the same in principle, although under aerobic conditions one of these substances is often used predominantly. A few examples will illustrate the various possibilities.

A starving earthworm consumes chiefly protein and glycogen, during the first days of starvation, while later more fat is used (Lesser, 1908). The snail *Helix,* during hibernation, oxidizes chiefly carbohydrate and some protein; its fat content remains entirely unchanged (von Brand, 1931). The actinians metabolize chiefly protein (Pütter, 1911), *Hydra,* protein and some fat (Kolodziejski, 1923; Beutler, 1924). A predominance of protein metabolism occurs also in leeches (Pütter, 1907, 1908; Bialasczewicz, 1919; Lafargue and Fayemendy, 1932). A relatively high rate of fat utilization has been found in developing sea-urchin eggs (Hayes, 1938), in flies during their metamorphosis, (Evans, 1932) and in starving insects (Mellanby, 1932).

The metabolized substances are not so diverse when oxygen is lacking. It is quite generally observed that under such conditions the carbohydrate metabolism becomes predominant, protein utilization decreases considerably and fats are used only in exceptional cases, if at all. The same is observed in organisms that live in nature under anaerobic or nearly anaerobic conditions.

We shall now consider in detail the evidence for the utilization of these substances as sources of energy in anaerobic metabolism.

1. CARBOHYDRATES

Both lower and higher carbohydrates, either occurring in the media or stored in the body, may be utilized.

A. Lower carbohydrates. Protozoa. Though no determination of the amount of simple sugars used by protozoa during periods of anaerobiosis has been made, there is considerable evidence that such sugars are consumed. Lwoff (1932) found that *Glaucoma pyriformis* withstands lack of oxygen better in media containing glucose than in those that are sugar-free. Hall (1933) reported that *Colpidium,* which grows slowly under practically anaerobic conditions if no sugar is present, grows even better than under aerobic conditions, if the medium contains glucose. Cook (1943) demonstrated that the termite protozoa, inside the oxygen-poor intestine of their host, are capable of utilizing glucose, fructose, galactose, maltose and sucrose.

Simple sugars serve frequently as mother-substances for aerobic fermentations. A sugar fermentation, under aerobic conditions, leading to organic acids as end products has been demonstrated for trichomonads (Cailleau, 1934, 1937; Andrews and von Brand, 1938; Plastridge, 1943; Trussell and Johnson, 1941), and for many members of the family *Trypanosomatidae* (Colas-Belcour and Lwoff, 1925; Kligler, 1926; Noguchi, 1926; Salle and Schmidt, 1928; Yorke, Adams and Murgatroyd, 1929; Regendanz, 1930; Geiger, Kligler and Comaroff, 1930; von Fenyvessy and Scheff, 1930; Salle, 1931; von Issekutz, 1933; von Brand, 1933; Reiner and Smythe, 1934; Reiner, Smythe and Pedlow, 1936; Christophers and Fulton, 1938). Similar processes occur also in malaria parasites (Maier and Coggeshall, 1941), in some ciliates like *Glaucoma, Colpidium* and *Paramaecium,* and others (Colas-Belcour and Lwoff, 1925; Johnson, 1935; Elliott, 1935; Glaser and Coria, 1935; Loefer, 1938; Cunningham

and Kirk, 1941) and in *Euglena proxima* (Glaser and Coria, 1935).

The sugar that is usually at the disposal of the above-mentioned organisms under natural conditions is glucose, and this sugar is also the one quite generally used under experimental conditions. But, as the data assembled in Table 21 demonstrate, a variety of other simple sugars may also be utilized by many protozoa in fermentations in the presence of air. Of the common hexoses, glucose, mannose and fructose are the ones most commonly used, and galactose the least. The disaccharides maltose and saccharose are frequently fermented, lactose only rarely, but the trisaccharide raffinose seems to be easily metabolized. Pentoses are the least used of sugars. (Some data on glucose consumption are gathered in Table 22.)

M e t a z o a . Very little information is available concerning the use of simple carbohydrates by metazoa in anaerobic metabolism, either in the absence or the presence of air. Such substances are usually not stored in large amounts in the body. Satisfactory feeding experiments under conditions of severe lack of oxygen are in most cases not possible, either because the animals then do not take food readily, or because the presence of bacteria in the surroundings renders it impossible to gather reliable data on the disappearance of sugar from the medium.

Kramer (1937) kept isolated tentacles of *Anemonia sulcata* under anaerobic conditions in a medium containing 0.1 per cent glucose and ascertained that considerable amounts of carbon dioxide and organic acids were formed, but he did not study to what extent the sugar was actually consumed.

Meyerhof and Schulz (1929) state that the anaerobic acid production of isolated nerves of *Maja squinado* persists longer in a sugar-containing than in a sugar-free medium.

TABLE 21

UTILIZATION OF VARIOUS COMPOUNDS AS MOTHER SUBSTANCES FOR THE AEROBIC FERMENTATIONS OF PROTOZOA.

The sign + indicates that the compound is utilized, — that it is not utilized, ? that the utilization is questionable, and 0 that no test was made.

	Species	Pentoses			Hexoses				Disaccharides			Trisaccharide
		Arabinose	Rhamnose	Xylose	Fructose	Galactose	Glucose	Mannose	Lactose	Maltose	Saccharose	Raffinose
	Flagellates											
1	*Euglena proxima*	0	0	0	0	0	+	0	—	—	—	0
2	*Eutrichomastix colubrorum*	—	—	—	+	+	+	0	+	+	+	+
3	*Herpetomonas culicidarum*	+	—	+	+	+	+	+	—	+	+	+
4	" *lygaeorum*	—	—	—	+	—	+	+	—	—	—	—
5	" *media*	—	—	—	+	+	+	+	—	—	+	+
6	" *muscidarum*	+	—	+	+	+	+	+	+	+	+	+
7	" *oncopelti*	+	—	+	+	+	+	+	—	+	+	+
8	" *parva*	—	—	—	+	+	+	+	—	—	+	+
9	*Leishmania brasiliensis*	—	—	—	+	—	+	+	—	—	+	+
10	" "	0	0	0	0	0	+	0	—	—	—	0
11	" *donovani*	—	—	—	+	—	+	+	—	—	+	+
12	" *infantum*	—	—	—	+	—	+	+	—	—	+	+
13	" "	—	0	—	+	—	+	0	—	—	—	0
14	" "	0	0	0	0	0	+	0	—	—	+	0
15	" *tropica*	0	0	0	0	0	+	0	+	+	+	0
16	" "	—	0	—	+	—	+	0	—	—	—	0
17	" "	—	—	—	+	—	+	+	—	—	+	+
18	*Leptomonas ctenocephali*	—	—	—	—	—	—	—	—	—	—	—
19	" "	—	0	—	+	—	+	0	—	—	—	0
20	*Trichomonas columbae*	—	—	—	+	+	+	0	+	+	+	—
21	" *foetus*	—	—	—	+	+	+	0	+	+	+	+
22	" "	?	—	?	+	+	+	+	—	—	—	—
23	" *vaginalis*	—	—	—	+	+	+	—	—	+	—	—
24	*Trypanosoma brucei*[1]	—	0	—	+	+	+	+	—	+	0	0
25	" *rotatorium*	—	—	—	—	—	—	—	—	—	—	—
	Sporozoa											
26	*Plasmodium Knowlesi*[1]	—	—	—	+	—	+	+	—	—	—	—
	Ciliates											
27	*Colpidium campylum*	—	—	—	+	—	+	+	—	+	—	—
28	" *striatum*	—	—	—	+	—	+	+	—	+	—	—
29	*Glaucoma ficaria*[2]					+	+			+		
30	" *pyriformis*[2]					+	+			+		
31	" "	—	0	—	+	+	+	0	—	+	—	0
32	*Saprophilus oviformis*	0	0	0	0	0	+	0	—	+	—	0
33	*Trichoda pura*	0	0	0	0	0	+	0	—	—	—	0

[1]Only the consumption of the sugars was demonstrated, not the acid formation. Since it is known, however, that these organisms oxidize glucose only partially, the above data have been included on the assumption that other sugars are utilized in a similar manner.

[2]The author states that he tested 16 carbohydrates and that only five—those we mark in the table with a + sign—were fermented. He does not list the other substances employed.

TABLE 21 (Continued)

UTILIZATION OF VARIOUS COMPOUNDS AS MOTHER SUBSTANCES FOR THE AEROBIC FERMENTATIONS OF PROTOZOA.

The sign + indicates that the compound is utilized, — that it is not utilized, ? that the utilization is questionable, and 0 that no test was made.

	Polysaccharides			Alcohols					Glucoside	Source
	Dextrin	Inulin	Soluble Starch	Dulcite	Erythrite	Glycerol	Mannite	Sorbite	Amygdalin	
1	0	0	0	0	0	0	0	0	0	Glaser & Coria, 1935
2	—	—	0	—	—	—	—	—	0	Cailleau, 1937
3	+	+	0	—	0	0	+	0	+	Noguchi, 1926
4	—	—	0	—	0	0	—	0	—	" "
5	—	+	0	—	0	0	—	0	—	" "
6	+	+	0	—	0	0	+	0	—	" "
7	+	+	0	—	0	0	+	0	—	" "
8	—	—	0	—	0	0	—	0	—	" "
9	—	—	0	—	0	0	—	0	—	" "
10	0	0	0	0	0	0	—	0	0	Kligler, 1926
11	—	—	0	—	0	0	—	0	—	Noguchi, 1926
12	—	—	0	—	0	0	—	0	—	" "
13	0	—	—	0	0	0	—	0	0	Colas-Belcour & Lwoff, 1925
14	0	0	0	0	0	0	—	0	0	Kligler, 1926
15	0	0	0	0	0	0	—	0	0	" "
16	0	—	—	0	0	0	—	0	0	Colas-Belcour & Lwoff, 1925
17	—	?	0	—	0	0	—	0	—	Noguchi, 1926
18	—	—	0	—	0	0	—	0	—	" "
19	0	—	—	0	0	0	—	0	0	Colas-Belcour & Lwoff, 1925
20	+	+	+	—	—	—	—	—	0	Cailleau, 1937
21	+	+	+	—	—	—	—	—	0	" "
22	0	—	—	—	0	—	—	0	0	Plastridge, 1943
23	+	—	+	0	0	—	0	0	0	Trussell & Johnson, 1941
24	0	0	0	0	0	0	0	0	0	von Brand, 1933a
25	—	—	0	—	0	0	—	0	—	Noguchi, 1926
26	0	—	0	0	0	+	0	0	0	Maier & Coggeshall, 1941
27	+	0	+	0	0	0	—	0	0	Elliott, 1935
28	+	0	+	0	0	0	—	0	0	" "
29	+		+							Johnson, 1935
30	+		+							" "
31	0	—	—	0	0	0	—	0	0	Colas-Belcour & Lwoff, 1925
32	0	0	0	0	0	0	0	0	0	Glaser & Coria, 1935
33	0	0	0	0	0	0	0	0	0	" " " "

TABLE 22

GLUCOSE CONSUMPTION OF PROTOZOA CAPABLE OF AEROBIC FERMENTATIONS.

The amount of sugar consumed is expressed in milligrams per hour per billion organisms.

Species	Temp. °C	Sugar consumed	Source
Flagellates			
Trichomonas foetus	37	16	Andrews & von Brand, 1938
Trypanosoma brucei	"	8.0	von Brand, 1933a
" *congolense*	"	7.8	" " "
" *evansi*	"	10	Geiger, Kligler & Comaroff, 1930
" *gambiense*	"	6	Yorke, Adams & Murgatroyd, 1929
" "	"	8.3	von Brand, 1933a
" *lewisi*	"	1.0	Regendanz, 1930
" "	"	1.4	von Brand, 1933a
" *rhodesiense*	"	8.3	" " "
Sporozoa			
Plasmodium knowlesi	"	2.2	Christophers and Fulton, 1938
Ciliates			
Colpidium campylum	28	287	Loefer, 1938
Glaucoma piriformis	"	166	" "

Feeding and injection experiments performed by Weinland and Ritter (1902) with *Ascaris* showed a glycogen-sparing action of glucose, fructose, maltose, and perhaps galactose, but not of lactose. They concluded that the former sugars were probably utilized. Hoffmann (1934) and Krüger (1936) demonstrated with the same worm that the heat production and the oxygen consumption increase if the animals have the opportunity of absorbing glucose and other sugars from the medium. Whether, however, under these conditions, the fermentations in the presence of oxygen increase correspondingly, has not been sufficiently studied.

Weinland (1901) stated that ascarids starving under anaerobic conditions consume from their body reserves 0.1 g. glucose per 100 g. body weight per 24 hours, besides a much larger amount of glycogen. According to him the glucose content of the worms is 1.6 per cent by

weight while, according to Schulte (1917) it is only 0.9 per cent. It seems probable, however, that both these authors overestimate the sugar content of the ascarids; their relatively high figures seem due to a partial hydrolysis of glycogen during the analyses. It should be noted in this connection that Foster (1865) and von Brand (1934a) found only traces of reducing sugars in these helminths.

Recent investigations have shown that sugar occurs to the extent of 1.26 per cent in the larvae of *Tenebrio molitor* and that it disappears at the rate of 50 mg. per 100 g. per hour if the animals are kept in pure nitrogen (Gilmour, 1941).

Many more similar cases will probably come to light if more attention is paid to the occurrence of simple sugars in metazoa than has been done hitherto.

B. Higher carbohydrates. Glycogen, its close relative paraglycogen, and starch, are the polysaccharides most commonly utilized during periods of deprivation of oxygen and the most studied. Much less is known concerning cellulose, dextrin and inulin, and nothing about paramylon and galactogen.

(*a*) *GLYCOGEN AND PARAGLYCOGEN.* These two polysaccharides differ only in minor characters and can therefore be treated here together. Glycogen is stored in all invertebrate phyla, paraglycogen only in some protozoan groups (gregarines and coccidia and probably some ciliates). The importance of these carbohydrates for energy production in anaerobic metabolism will be reviewed here briefly for the major phyla.

P r o t o z o a . It has repeatedly been reported that in free-living ciliates, like *Paramaecium, Stentor* and others, the polysaccharides stored in the body disappear under anaerobic conditions (Pütter, 1905; Galadziev and Malm, 1929; Zhinkin, 1930; Liebmann, 1936; Barbarine, 1938). The rate of consumption is then evidently much higher

than if the animals are starved under aerobic conditions. All the available data are, however, at best only semi-quantitative. They were gained in the study of the carbohydrate content with the help of specific stains. More adequate methods of chemical analysis have not yet been used. The increased interest in bacteria-free mass cultures of free-living protozoa in recent years will, it is hoped, lead to such investigations in the near future.

Not even semi-quantitative experiments have so far been carried out with non-parasitic flagellates and rhizopods. It would be of great value to study along such lines organisms like *Pelomyxa* or *Difflugia*, the resistance of which in anaerobic conditions is well known. These animals store considerable amounts of polysaccharides as evidenced by the findings of Stole (1900), Zülzer (1904) and Leiner (1924). But no analogous accumulation of glycogen has been reported for the *Amoeba* species that have been used in experiments on anaerobiosis. Unpublished observations by the present writer on various *Hartmanella* species and amoebae of the *limax* type failed to show any significant amount of polysaccharide.

Glycogen and paraglycogen are unquestionably important reserve substances for the anaerobic metabolism of *parasitic protozoa*, although usually only the storage of these substances and not their actual consumption has been demonstrated. It has thus been shown that the flagellates *Giardia* and various trichomonads accumulate considerable glycogen reserves (Deschiens, 1924; Giovannola, 1934; Stewart, 1938; Stewart-Lyford, 1941) and that glycogen or paraglycogen occurs in parasitic ciliates like *Balantidium, Opalina* and *Nyctotherus* (Bütschli, 1887; Barfurth, 1885; Jirovec, 1926; Kedrowsky, 1931; Armer, 1944).

A remarkably intensive glycogen metabolism is found in the ciliates parasitizing the stomach of ruminants and it may also occur in the related species found in the

caecum of horses. These parasites have been known for a long time to store very large amounts of glycogen (Certes, 1889; Schulze, 1924, 1927; Trier, 1926; Usuelli, 1930; Weineck, 1931; MacLennan, 1934; Westphal, 1934; Hungate, 1943). Both Westphal and Hungate found that this reserve disappears very rapidly in starvation experiments under anaerobic conditions. A comparison between aerobic and anaerobic metabolism is not possible in this case, since the animals are injured rapidly by oxygen.

Our knowledge concerning the importance of glycogen as an energy reserve in *parasitic rhizopods* is rather scanty. A large glycogen vacuole is regularly found in the cysts of *Iodamoeba.* Dobell (1919) was the first to point out that this represented a food reserve since the polysaccharide disappeared slowly from cysts kept outside the host. Von Brand (1932) observed that the rate at which this vacuole decreased in size and finally disappeared was identical under aerobic and anaerobic conditions. He concluded that the polysaccharide was probably fermented in both cases.

Concerning the glycogen content of human *intestinal amoebae* the following points have been reported: Kuenen and Swellengrebel (1913, 1917) showed that the vegetative stages of these animals store some glycogen. According to the present writer's unpublished observations, glycogen occurs in larger amounts and more regularly in vegetative stages of *Endamoeba coli* than in any other amoeba of the same habitat. It is also known that culture forms of *Endamoeba histolytica* have a much higher glycogen content than specimens taken directly from the intestine (Morita, 1938). Starvation experiments have not yet been performed with any intestinal amoeba. Real progress in the study of the metabolism of these organisms will only be possible when they can be cultured under sterile conditions.

In endamoebae taken from their natural habitat glycogen appears abundantly only in the immature cysts. This is somewhat surprising since the precystic forms seem not to feed. It is possible that large amounts of carbohydrate have previously been stored in a form not demonstrable with the conventional staining methods for polysaccharides. Equally surprising and as yet unexplained is the rapid disappearance of these glycogen vacuoles in mature cysts. Future investigations may reveal a high rate of carbohydrate fermentation during the maturation process.

A considerable polysaccharide storage has been reported for *Coccidia* and for *Gregarines* (Brault and Loeper, 1904b; Giovannola, 1934; Joyet-Lavergne, 1926; Edgar, Herrik and Fraser, 1944), but it has not yet been demonstrated that these carbohydrates are consumed under anaerobic conditions.

M e s o z o a . These curious parasites accumulate rather considerable glycogen reserves, as evidenced by the morphological observations of Nouvel (1929, 1929a, 1931, 1933, 1935). This applies both to the dicyemids and the orthonectids, parasites of cephalopods and of various invertebrates respectively. Nouvel assumes that, in nature, glycogen is used up in the anaerobic metabolism of these organisms, or at least of some of their stages. Under experimentally induced anoxic conditions the infusoriform stages of dicyemids are able to swim around only as long as their glycogen reserve lasts. They cannot withstand an experimental deprivation of oxygen for more than a few hours. As to the vermiform stages, they are still alive after 3 days, but no glycogen consumption could be demonstrated in them by staining methods. Nouvel thinks that during that time a glycogen synthesis even took place, though he assumes that at the same time a glycogen-lactic acid fermentation occurred. He offers no proof from chemical analysis in support of either of

his assumptions. If they are correct, the power of glycogen synthesis of these organisms must be remarkably well developed.

C o e l e n t e r a t e s . Whether coelenterates metabolize polysaccharide when deprived of oxygen, has never been investigated. The occurrence of glycogen in *Hydra,* in actinians and in corals has been established by Beutler (1924, 1929) and Hosoi (1932), but the amount stored never appears to be high.

W o r m s . Glycogen is by far the most important energy reserve for the anaerobic life of both free-living and parasitic worms. The quantitative data available on the glycogen consumption of worms have been assembled in Table 23. Wherever possible figures for the glycogen degradation in the presence of air have been included. Thus the quotient of the consumption in anaerobic *versus* aerobic conditions can be computed. It is quite obvious that in worms, like the earthworm or planarians, which in nature are adapted to an aerobic life, this quotient is high. Two reasons can be found for this fact: (1) Under conditions of good oxygenation the carbohydrate is completely oxidized and much more energy is produced than in the fermentative decomposition of a corresponding amount of polysaccharide under anaerobic conditions. So, even if the energy requirements of an organism deprived of oxygen are materially reduced due to a decrease in muscular activity, large amounts of carbohydrate must be metabolized in order to produce just the minimum quantity of energy required. (2) Under anaerobic conditions, relatively more glycogen but less fat or protein is decomposed, *i.e.,* a larger percentage of the total energy is derived from carbohydrate.

In worms like *Ascaris,* on the contrary, which, in nature, lead a predominantly anaerobic life and whose metabolism in air is characterized by the fermentation of carbohydrates, the quotient is low. The reason is ob-

TABLE 23

GLYCOGEN CONSUMPTION OF WORMS UNDER ANAEROBIC AND UNDER AEROBIC CONDITIONS.

The values are calculated in grams per 100 grams of worms per 24 hours, unless otherwise specified.

Species	Temp. °C	Glycogen consumption		Quotient Anaer./Aer.	Source
		Anaerobic	Aerobic		
Free-living worms					
Lumbricus sp.[1]	Room temp.	3.33	0.51	6.5	Lesser, 1910
Owenia fusiformis	" "	0.23	0.09	2.6	von Brand, 1927
Planaria torva	25	1.40	0.26	5.4	" " 1936 (partly unpublished)
Tubifex tubifex[2]	Room temp.	.....	.....	about 4	Dausend, 1931
Parasitic worms					
Ascaridia galli[3]	41	3.28	.....	.....	Reid, 1944
" " [3]	"	2.24	.....	.....	" "
Ascaris lumbricoides	37	0.70	.....	.....	Weinland, 1901
" "	"	1.1	.....	.....	Schulte, 1917
" "	"	1.39	1.18	1.2	von Brand, 1934a
Eustrongylides ignotus larva	"	0.9	0.3	3.0	" " 1938a
Macracanthorhynchus hirudinaceus	"	0.95	0.80	1.2	Rudolph[4] (anaer.); von Brand, 1940 (aer.)
Moniezia expansa[5]	"	1.00			von Brand, 1933a
Parascaris equorum	"	1.39	1.58	0.9	Toryu, 1936
Railletina cesticilla[3]	41	4.8	.....	.....	Reid, 1942

1) Values per 100 animals, not 100 gm.

2) The author gives no definite numerical values.

3) Reid's experiments were carried out by letting the worms starve within the intestine of the host (chicken). It is assumed that the conditions are predominantly anaerobic.

4) Rudolph's experiments seem not to have been published *in extenso*. They are quoted by Weinland (1910).

5) Wardle (1937a) performed experiments on aerobic glycogen consumption. His results were inconclusive and his data are not included here since his technique allows no comparison with the quoted anaerobic experiments.

vious: the glycogen metabolism predominates even under aerobic conditions and the low yield of such fermentation processes calls for a high rate of polysaccharide decomposition, a rate which may be almost as high as in the absence of oxygen.

With free-living worms the quotients seem never to be as low as with parasites. The lowest value reported is 2.6, found in the polychaete *Owenia fusiformis* (von Brand, 1927). Whether this indicates an exceptionally great reduction of metabolic rate under anaerobic conditions, or a more pronounced participation of fat or protein in the anaerobic degradation processes, or whether it is due to fermentative processes in aerobic conditions has not yet been established.

Some points concerning the anaerobic glycogen consumption of parasitic worms need further comment. (1) The intensity of the polysaccharide metabolism of *Ascaris lumbricoides* varies within rather wide limits in the experiments of various authors (Table 23). This is due to the fact that they used experimental periods of various lengths. The longest experiments (up to 6 days) were those of Weinland (1901) and these gave the lowest values. The shortest experiments (24 hours) were performed by von Brand (1934a) and they yielded the highest rate of consumption. Schulte (1917) used an intermediate experimental period of 48 hours and obtained an intermediate value. Thus the initially high rate of polysaccharide degradation seems to decrease with increasing time of starvation. This has definitely been proven by von Brand (1937a) who investigated uniform material during inanition periods ranging from 1 to 3 days and always found a lower average glycogen consumption in longer experiments. (He also observed a more pronounced decline in metabolic rate in females than in males.)

(2) Another interesting point is the marked variation in intensity of polysaccharide consumption with different species (Table 23). This may indicate a different energy yield of the fermentations characterizing the metabolism of the various worms. Such differences would not be surprising in organisms as far apart in the animal kingdom as flat worms and round worms. But there is an alternate explanation which is perhaps more plausible at least for animals belonging to the same taxonomic group and which is well illustrated in the case of the cestodes. Reid (1942) determined that the daily glycogen consumption of *Railletina cesticillus* inside the host intestine (chicken) amounted to 4.8 g. per 100 g. body weight as compared to 1.0 g. in the case of *Moniezia expansa,* investigated *in vitro* by von Brand (1933a). Reid points out that the slightly higher temperature prevailing in his experiments and the smaller size of the chicken tapeworms may help to explain the difference. The latter point might in itself offer a satisfactory explanation, since in many cases the rate of metabolism depends on the surface area and the smaller an organism is the larger its relative surface will be. A *Railletina* weighing 0.100 g. consumes in 24 hours 4.8 mg. glycogen, a *Moniezia* of 5 g. consumes 50 mg. The ratio of the figures for the polysaccharide degradation is about 1:10. The surface areas of the organisms, calculated according to the expression Area= $\text{Weight}^{2/3}$ which is quite satisfactory when organisms of the same general body form are compared, are 21.5 and 292 respectively. Their ratio is 1:14, a value approximating that found for the glycogen consumption.

Probably most, if indeed not all parasitic worms living in environments with limited oxygen supply or totally devoid of oxygen have a well-developed carbohydrate metabolism. The fact that all of them store large amounts of glycogen in their bodies—from about 30 to

50 per cent of the dry weight in some tapeworms and roundworms—is suggestive. Further quantitative data on the polysaccharide content of a variety of nematodes, trematodes, cestodes and acanthocephala will be found in the papers of Weinland (1900), Schimmelpfennig (1903), Flury (1912), Flössner (1924, 1925), Flury and Leeb (1926), Toryu (1933), Smorodincev and Bebesin (1936), Wardle (1937a), von Brand and Otto (1938), Salisbury and Anderson (1939), Markov (1939), von Brand (1940), von Brand and Simpson (1944). Qualitative data gathered by staining methods are presented by Brault and Loeper (1904, 1904a), Busch (1905), von Kemnitz (1912), Ortner-Schönbach (1913), Martini (1916), Coutelen (1931), Giovannola (1935, 1936) and Miller (1943).

One can expect that the predominance of the glycogen metabolism will be less pronounced in stages that have free access to oxygen than, for example, in intestinal worms. Micro-filariae which live in the blood stream seem not to store any glycogen (Brault and Loeper, 1904a). In the free-living larval stages of various nematodes small to moderate amounts of this polysaccharide are encountered (Stepanow-Grigoriew and Hoeppli, 1926; Giovannola, 1936). A marked glycogen accumulation is reported in the *Parascaris* egg (Fauré-Fremiet, 1912 and 1913; Szwejkowska, 1929; Wottge, 1937) and it seems quite certain that at least in the period from fertilization till after the formation of the second polar body relatively large amounts of carbohydrate are used for energy production; but it is doubtful whether anaerobic processes occur when the eggs are kept in oxygenated surroundings. Dyrdowska (1931) kept the eggs under anaerobic conditions and found, by means of staining methods, only a slight glycogen diminution.

That free-living worms, like leeches, which normally have a predominant protein metabolism, should shift to

a typical carbohydrate metabolism under anaerobic conditions, is probable, but has not yet been definitely established. It is known from the work of Pütter (1907 and 1908), Lafargue and Fayemendy (1932a) and Braconnier-Fayemendy (1933) that the carbon metabolism of these worms increases in the absence of oxygen, as is evidenced by the increased excretion of organic acids, and it is also a well-established fact that glycogen is present (Vieweger, 1923; Vialli, 1927). It should not be difficult to ascertain experimentally whether there is some connection between these two sets of observations.

Concerning free-living worms other than those mentioned in Table 23, it is known that a considerable amount of glycogen occurs in *Sipunculus* and many sessile polychaetes (von Brand, 1927; Chaigne, 1934). It has also been shown qualitatively that some rotatoria occurring in the sapropelic habitat store this polysaccharide (Lauterborn, 1916). It would be especially interesting to test whether this is true also of the non-parasitic nematodes of anaerobic habitats, since in their parasitic relatives the carbohydrate metabolism is so markedly developed.

E c h i n o d e r m s . Small amounts of glycogen have been found in *Holothuria tubulosa* (Chaigne, 1934), and in the muscles of *Holothuria nigra.* In the latter case it has been shown that glycogen is transformed into lactic acid during periods of activity (Boyland, 1928). An annual cycle in glycogen content has been demonstrated in *Paracentrotus lividus* and *Asterias rubens,* varying quantities of the polysaccharide being deposited in the pyloric caeca and in the genital organs of these echinoderms. The maximum is reached when the genital organs are fully developed (Chaigne, 1933a; *cf.* also Moore, Whitley and Adams, 1913; Kilborn and Macleod, 1920). Only traces of glycogen, however, have been reported in the unfertilized eggs of *Arbacia punctulata* (Perlzweig and Barron, 1928).

M o l l u s c s . A considerable accumulation of glycogen is regularly encountered in lamellibranchs (Bizio, 1866; Chittenden, 1875; Mitchell, 1915; McDowell, 1927; Kobayashi, 1929; Okazaki and Kobayashi, 1929; Boyland, 1928; Semichon, 1932; Masumoto, Masumoto and Hibino, 1934; Chaigne, 1934) and in gastropods (Hammarsten, 1885; Barfurth, 1885; Bellion, 1909; Schöndorff, 1912; Boyland, 1928; Petree and Alsberg, 1929; von Brand, 1931; May, 1934; Chaigne, 1934; Baldwin and Bell, 1940); while cephalopods store only insignificant amounts (Starkenstein and Henze, 1912; Boyland, 1928; Chaigne, 1933). It should be remembered in this connection that only members of the two former groups tolerate well the lack of oxygen.

Berkeley (1921) is the only investigator who compared the glycogen consumption of lamellibranchs under aerobic and anaerobic conditions, but his results are rather inconclusive. *Saxidomus giganteus* lost, during periods of equal length in the absence and in the presence of air, amounts of glycogen equal to 0.99 and 0.28 per cent of the body weight respectively. The ratio of the two figures, 3.5, approximates that found in free-living worms. No similar difference in glycogen degradation was encountered with *Mya arenaria* and *Paphia staminea*. A survey of Berkeley's figures indicates, however, that his material was probably not uniform. This is suggested by the fact that at least in one series of experiments the anaerobically starving animals had a higher glycogen content than the controls analyzed at the beginning of the experiment.

Dugal reported recently (1940), in a preliminary note, that an anaerobic metabolism can be demonstrated in *Venus mercenaria* kept under anaerobic conditions as long as the hepato-pancreas contains glycogen, but further details on his experiments are needed for the evaluation of the results.

Arthropods. Stott (1932) found that the blood sugar of crabs increased considerably when they were deprived of oxygen. Since it is unlikely that large stores of simple sugars exist anywhere in the body of these animals, the sugar observed was probably derived from a polysaccharide reserve. Boyland (1928) demonstrated that the abdominal muscles of decapods convert glycogen into lactic acid during fatigue, in the state of rigor and when they are placed in buffer solutions.

It seems, on the whole, that decapod crustaceans never accumulate large quantities of glycogen and there are some indications that in many instances the substance serves merely as a source of the carbohydrate component required for chitin formation (Bernard, 1879; Vitzou, 1882; von Schönborn, 1912; Verne, 1924; Dorman, 1928; Baumberger and Dill, 1928; Chaigne, 1934; Regan, 1944), rather than as an energy reserve. This does not, however, exclude the possibility that it may take on the latter role when the oxygen becomes scarce or is lacking entirely.

The crustaceans that exhibit the most marked resistance in anaerobic conditions (*Cyclops* species, *Cirripedia*) have, as far as the present writer is aware, not yet been studied for their glycogen content.

Glycogen storage is common amongst insects though large amounts of it are found only in rare cases. One of these rare cases is that of the larvae of the honey bee. Data (some of them quantitative) for a variety of insects are presented by Bataillon and Couvreur (1892), Straus (1911), Kaneko (1924), Dinulescu (1932), de Boissezon (1932), Ditman and Weiland (1938), Paillot (1938), Yeager and Munson (1941), Babers (1941), Wigglesworth (1942) and others.

It has been shown conclusively in a number of instances that glycogen is utilized by insects for anaerobic processes. The parasitic larvae of *Gasterophilus intestinalis* consume 1.33 g. glycogen per 100 g. body weight per 24

hours if they have access to oxygen, but only 0.72 g. in the absence of air (von Kemnitz, 1916). This, and the already mentioned case of *Parascaris* (Table 23) are the only instances known to the present author in which less polysaccharide is decomposed under anaerobic than under aerobic conditions. The reasons for this aberrant behavior are not obvious, but the following may be suggested. Von Kemnitz assumes with good ground (*cf.* preceding chapter) that a large part of the glycogen used in the presence of oxygen is not completely oxidized, but that it is decomposed in fermentation processes and with the same energy yield that characterizes the fermentations in the complete absence of oxygen. If, during anaerobiosis, less energy is needed due, for example, to a restriction of muscular movements, the low glycogen consumption becomes understandable. It should also be kept in mind that von Kemnitz' figure may be too low. Blanchard and Dinulescu (1932, 1932a) report that during the first days of anaerobiosis relatively small amounts of glycogen are metabolized, but that from the tenth day on larger amounts are used, as much as 1.0 g. per 100 g. animal per 24 hours. Whether sufficient oxygen is stored in the "red body" (Weinland, 1915; Krogh, 1941) to allow an aerobic metabolism during the first days of deprivation of oxygen, or whether the food present in the intestine of the larvae (Dinulescu, 1932) is sufficient to provide most of the energy needed, will have to be decided by further work.

Other insects follow the regular pattern, *i.e.*, consume more glycogen in the absence than in the presence of oxygen. The figures given by Harnisch (1938) for the larva of *Chironomus thummi* are 0.63 to 0.79 per cent of the body weight in the former case and 0.065 to 0.085 per cent in the latter. The larva of *Tenebrio molitor* metabolizes 40 mg. glycogen per 100 g. per hour under anaerobic conditions and 4 mg. under aerobic conditions (Gil-

mour, 1941). Harnisch (1941), working independently of Gilmour, also found a pronounced glycogen consumption of the mealworm larvae deprived of oxygen. His results, however, were too variable to allow the calculation of an average value.

The papers quoted above are the only ones in which it was proved by direct chemical analysis that a glycogen consumption occurs in insects under anaerobic conditions. The same is probably true also of cockroaches according to the observations of Slater (1927) and of Davis and Slater (1926, 1928). These authors made no glycogen determinations but studied the respiratory metabolism and lactic acid content of aerobically- and anaerobically-kept roaches and came to the conclusion that a glycogen-lactic acid reaction takes place when the animals are deprived of oxygen.

(b) *STARCH.* It was first shown by Dobell and Laidlaw (1926), and later confirmed by numerous authors, that *Endamoeba histolytica* grows best in culture if it is fed rice starch which it engulfs and digests readily. According to Snyder and Meleney (1943) the addition of starch is absolutely necessary for a successful anaerobic culture of this parasite while some strains can grow without it if oxygen is not excluded from the medium. Westphal (1934) demonstrated that starch is also indispensable for the culture of *Eudiplodinium medium*, a ciliate parasite of the stomach of cattle, which requires anaerobic conditions when cultured *in vitro*.

Many parasitic protozoa, in addition to those mentioned above, avidly engulf starch granules in culture. They will not be enumerated here since the correlation between the use of starch food and the existence of anaerobiosis has not yet been established experimentally.

It is interesting in this connection to note that, in general, a diet rich in starch, or in other carbohydrates, administered to the host, whether vertebrate or inverte-

brate, facilitates the development of intestinal protozoa (*cf.* Mowry and Becker, 1930; Sassuchin, 1931; Hegner and Eskridge, 1937; Armer, 1944). Armer is the first to have attempted to explain this phenomenon on the assumption that the parasites then have access to suitable mother-substances for an anaerobic carbohydrate metabolism.

One may expect that starch—or more likely its degradation products—will also favor the development of many metazoan parasites that normally lead a predominantly anaerobic existence. Von Brand (1933a), showed that the glycogen content of the dog tapeworm, *Taenia hydatigena,* is higher when the host receives a diet high in carbohydrates than if it is kept on a normal diet. Chandler (1943a) found that the growth of *Hymenolepis diminuta* is markedly inhibited if the host (chicken) is kept on a carbohydrate-free diet.

It should finally be emphasized that soluble starch is quite a good mother-substance for the fermentative metabolism of various protozoa in aerobic conditions (*cf.* Table 21).

(c) *CELLULOSE.* Ingested cellulose is the substrate for anaerobiosis in two groups of protozoa, the intestinal parasites of termites and of the roach *Cryptocercus punctulatus,* and some ciliates which live in the stomach of ruminants. It will be recalled that these organisms thrive only in surroundings that are largely anaerobic.

It is a well-established fact that many termite protozoa ingest particles of wood (Cleveland, 1924, 1925a; Swezy, 1923) and that they digest it by means of a cellulase and cellobiase (Trager, 1932; Cleveland, Hall, Sanders and Collier, 1934; Hungate, 1938, 1943a).

The question whether cellulose plays a role in the nutrition of rumen ciliates has long been a controversial one. Some investigators (Dogiel, 1925; Margolin, 1930; Usuelli, 1930; Westphal, 1934) doubt that these animals

digest cellulose; others (Braune, 1914; Schulze, 1924, 1927; Trier, 1926; Weineck, 1934) maintain that they do. The truth seems to be that different species behave differently. Hungate (1942, 1943) demonstrated conclusively that all *Diplodinium* species utilize cellulose; while *Entodinium, Isotricha, Dasytricha* and *Bütschlia* do not. Tests designed to prove the presence of a cellulase were positive in the case of *Diplodinium maggii.*

(d) *VARIOUS POLYSACCHARIDES AND SUGAR-ALCOHOLS.* Dextrin and inulin are used by some protozoa in their aerobic fermentative reactions in the presence of oxygen (Table 21) and seem to represent, at least in some cases, valuable mother-substances for these processes. Sugar-alcohols have been little studied and seem to be used for fermentations by only a few protozoa (Table 21). It would be of great interest to investigate whether snails kept in the absence of air are capable of metabolizing galactogen. Young eggs of *Helix* would be an especially suitable material, since they store large amounts of this polysaccharide and no glycogen (May, 1932).

2. FATS

The evidence that invertebrates are capable of utilizing fat under anaerobic conditions is rather meager.

Brault and Loeper (1904b) described a decrease in the number of microscopically visible fat globules in the oocysts of *Eimeria stiedae* in their normal habitat (enlarged bile ducts of rabbits) which is very poor in oxygen. The observations of these authors, however, do not carry much weight because of the unreliability of staining methods. It is a well-known fact that the disappearance of morphologically demonstrable fat globules does not necessarily indicate that some of the fat has actually been used up. The same criticism applies to Dyrdowska's (1931) statement that considerable amounts of lipids which can be demonstrated with the help of dyes

disappear from the *Parascaris* egg when it is kept in an atmosphere free from oxygen. In adults of *Ascaris lumbricoides,* in any event, no fat is consumed during anaerobic periods (Weinland, 1901; von Brand, 1934a) and Fauré-Fremiet (1913) even thinks that the fat content of anaerobically kept *Parascaris* eggs *increases.*

Barnes and Grove (1916) exposed larvae of the insect *Attagenus undulatus* to an atmosphere of reduced oxygen content by enclosing them for a period of 5 days in very small containers where the oxygen supply was clearly insufficient to allow a purely aerobic life. The amount of fat decreased from 28.7 to 26.3 per cent of the dry weight and the authors concluded that the fat had been utilized for energy production through anaerobic processes. This conclusion is obviously not convincing. The fat could have been used up during the initial period when oxygen was still present in larger quantities, and substances, such as glycogen, may have been metabolized later.

Kozancikov (1935) found that the fat content of the larvae of *Pyrausta nubilalis,* during diapause, decreased markedly when the animals were immersed in water at 25° C for 3 days. But this environment can certainly not be regarded as wholly free from oxygen.

Gilmour (1941) states that, in *Tenebrio molitor* larvae, an anaerobic fat consumption of 4.5 mg. per g. fresh weight per hour was observed in one series of his experiments as compared to 0.18 mg. under aerobic conditions. He writes further ". . . in another set of experiments (four in this case) variation was such that it was not possible to demonstrate a significant difference in fat content between a control series of insects and a similar series analyzed after anaerobiosis and recovery. If exact quantitative data on the anaerobic processes are to be obtained it will be necessary to have a stock of insects of

more uniform composition." Harnisch (1941) likewise reports only inconclusive results concerning possible changes in the fat content of the same animal during anaerobiosis.

The best authenticated case of an anaerobic fat consumption is probably that reported by Weinland (1906). He observed the disappearance of relatively large amounts of fat in minced material of *Calliphora* pupae, when oxygen was excluded. He is convinced that no bacterial contamination was responsible for these changes. But, lacking further evidence, one cannot decide whether the same process would take place in intact animals.

3. PROTEINS

The protein metabolism of an aerobic animal serves a dual purpose: the synthesis of new protoplasm and the production of energy. It is obvious that the formation of new protein material must take place also in those animals that in nature occur only in oxygen-free surroundings and multiply there. As especially clear-cut examples of such an anaerobic protein metabolism resulting in synthesis, we may mention the sapropelic ciliates and the ciliates inhabiting the stomach of ruminants, but a similar situation will prevail also in the case of many parasitic helminths and possibly in many other organisms. Whether the same holds true also of animals that, in nature, are exposed for only relatively short periods to low oxygen pressures is not known. This important problem would merit a thorough investigation. A certain synthetic anaerobic protein metabolism exists in parasitic worms even under conditions of starvation. This is clearly indicated by the fact that, when deprived of oxygen, they release large quantities of reproductive cells. In *Ascaris* the nitrogen bound up in the released eggs amounts to about one-fourth of the total nitrogen eliminated (von Brand, 1934a).

In most cases, the evidence presented to show that proteins serve as a source of energy under anoxic conditions is not too reliable. Pütter (1905) pointed out that specimens of *Paramaecium* that had stored but little glycogen survived for long periods in the absence of oxygen, and Lauterborn (1916) observed that the ctenostomids, typical sapropelic ciliates, do not store any glycogen at all. Both these authors assume that proteins may furnish the necessary energy to sustain life. This problem needs to be reinvestigated, however, since Liebmann (1936) pointed out that a quite different mechanism may be involved. According to him the anaerobic survival of *Colpidium colpoda* depends upon the ability of this ciliate to engulf green sulfur bacteria. A temporary symbiosis is then established and the ciliates participate in the energy produced by the assimilative function of the bacteria.

A similar mechanism may be widespread among certain groups of ciliates. Liebmann (1937, 1938) found some undetermined bacteria regularly associated with sapropelic ciliates and his experiments indicate strongly that it is only these bacteria that enable the ciliates to survive in their normally oxygen-free habitat. They live at first as symbionts in vacuoles or they are embedded directly in the protoplasm. From time to time they gather in food vacuoles and are digested. If the supply of these specific bacteria fail the ciliates die, despite the abundance of other food. Liebmann's (1937, 1938) tentative assumption that the bacteria liberate oxygen from some undetermined source and supply the ciliates with it lacks experimental proof as yet. To this assumption one may also object that the ciliates should be able to survive without bacteria in media containing oxygen, if only the hypothetically produced oxygen maintains them in otherwise anaerobic media. As mentioned previously,

however, the sapropelic ciliates seem to be truly anaerobic animals, *i.e.*, they are injured by molecular oxygen.

A well-developed anaerobic protein metabolism is encountered in *Hirudo medicinalis* (Lafargue and Fayemendy, 1932a; Braconnier-Fayemendy, 1933). One hundred grams of leeches excrete, according to Braconnier-Fayemendy, from 7 to 9 milligrams of non-protein nitrogen in 24 hours under anaerobic conditions. The author speaks of an enormous drop in the total nitrogenous excretion ("une baisse énorme") under anaerobic as compared to aerobic conditions, and she lists this excretion as amounting to 17 to 19 mg. A study of her tables, however, indicates that this figure is obtained in 48-hour periods. Either, therefore, the caption of the table is erroneous, or her above statement is unwarranted. A clarification of this point through further experiments is desirable, since so little is known about the anaerobic protein metabolism of any invertebrate and since leeches are probably one of the best objects for such a study.

There seems to be a certain difference in the significance of protein metabolism in *Hirudo* and in parasitic worms. In *Hirudo* the nitrogenous end products result, in all probability, from a typical energy-liberating mechanism. In the parasitic worms, on the contrary, in which, as was mentioned above, large quantities of reproductive cells are released during anaerobiosis, a large part of the soluble nitrogenous excreta probably originate from processes which lead to the formation of these cells, and which would then have no connection with energy production.—For quantitative data the reader is referred to the papers of Weinland (1904a), Weinland and von Brand (1926) and von Brand (1933a; 1934a).—It is possible, however, that *Ascaris* derives some energy from an anaerobic protein metabolism. According to Schulte's (1917) observations only 80 per cent of the heat produced by the worms can be ascribed to carbohydrate

breakdown. Since these animals do not metabolize fat (von Brand, 1934a, 1941), one can hardly escape the conclusion that proteins play a role in energy production.

The very curious findings of Harnisch (1938) deserve mention here. He exposed larvae of *Chironomus thummi* to starvation under anaerobic conditions and found that the nitrogen content of their bodies, as determined by Kjeldahl's method, increased. Since a nitrogen fixation is very unlikely, Harnisch assumes that nitrogenous substances which, before deprivation of oxygen, could not be determined by Kjeldahlization were transformed during the period of oxygen deprivation so as to become demonstrable by that procedure. He also suggests that these processes might liberate oxygen which then, of course, could be used for energy-producing aerobic oxidations. If this is so, the anaerobic protein metabolism would serve, in an indirect manner, to produce energy. It should be emphasized, however, that Harnisch performed only two experiments. A reinvestigation based on more material is desirable before these findings, which Harnisch himself designates as only preliminary in nature, can be accepted as facts.

CHAPTER IV

ANAEROBIC PROCESSES

In organisms that are adapted to permanent anaerobiosis anaerobic processes have to fulfill two requirements, the production of energy and the synthesis of new protoplasm. In organisms which are only temporarily anaerobic the production of energy might be the only requirement. At all events, the synthetic phase of anaerobic metabolism has, so far, received practically no consideration in invertebrates; the following discussion will therefore deal almost exclusively with the energy-yielding processes.

We shall study these processes separately for carbohydrates, fats and proteins, and shall then turn our attention to the anaerobic gas metabolism and to the question of anaerobic enzymes. Most of the work performed with invertebrates has been concerned with the identification of metabolic end products and the rate at which these are formed; intermediate processes have hardly been touched upon. We shall, therefore, treat this latter phase very briefly in this review, though we do not, by any means, minimize the importance of the knowledge of intermediate metabolism; it is hoped that research may soon be initiated in that direction.

1. CARBOHYDRATES

The anaerobic processes liberating energy from carbohydrates are essentially oxidation-reduction reactions or molecular rearrangements. The sugar molecule is broken down into smaller units, for example, into two lactic acid molecules, and the energy content of the end products is less than that of the mother-substance. It was suggested (*e.g.*, Rahn, 1932) that it is the shifting of oxygen from the inner to the outer carbon atoms and the massing of oxygen on one carbon atom that liberates energy.

Szent-György (1939), however, pointed out that this transfer of oxygen is only a final result which cannot be achieved directly. It is attained, as he expresses it, by "taking off and putting on H, taking off water and putting it on again" in a complicated series of steps which, in the case of the lactic acid fermentation, involves triose —glyceric acid—enol pyruvic acid—pyruvic acid and the participation of cozymase. Szent-György suggests that these shifts of the hydrogen atoms are of paramount importance in the actual liberation of energy.

The main reactions in which carbohydrates are utilized anaerobically by invertebrates are: (A) lactic acid fermentations, (B) fatty acid fermentations and (C) mixed fermentations in which lactic, fatty and dibasic acids as well as alcohol may be formed and in which at least two of these substances appear. These possibilities will be discussed separately in the following sections.

A. Lactic acid fermentation. Lactic acid fermentation is one of the best-known anaerobic processes occurring in animals. It has been studied intensively in vertebrates where it is, in fact, the only definitely known way of carbohydrate utilization under anaerobic conditions. It is usually designated as glycolysis and the present writer feels that this term should be reserved only for this one process and should not be applied, as is done by some authors, to other types of carbohydrate fermentations.

It was pointed out previously that investigators sometimes assume the formation of lactic acid in invertebrates without actually having demonstrated it. They take for granted that there is only one type of anaerobic carbohydrate utilization, namely, true glycolysis. Such an assumption may lead to erroneous, or at least insecure conclusions, as a brief review of some cases will show.

Von Fenyvessy and Reiner (1924, 1928) as well as Geiger, Kligler and Comaroff (1930) believe that a true glycolysis occurs in the metabolism of trypanosomes. Von Fenyvessy and Reiner draw this conclusion from the ob-

servation that an acidification takes place in the sugar-containing solution in which trypanosomes are kept; Geiger, Kligler and Comaroff from the decrease of the alkaline reserve of the same culture media. That these observations are inconclusive has been shown by von Brand, Regendanz and Weise (1932) and by Reiner, Smythe and Pedlow (1936) who demonstrated, by direct determination, that lactic acid does not occur in significant amounts among the end products of the anaerobic metabolism of pathogenic trypanosomes. The small traces of lactic acid that were found by Reiner, Smythe and Pedlow were possibly not due to the trypanosomes but to leucocytes which can hardly be separated completely from the flagellates when the latter are being prepared for the experiments.

Lwoff (1934) observed that some trypanosomidae liberate carbon dioxide from bicarbonate under anaerobic conditions. With *Strigomonas oncopelti,* 88 c.mm. of carbon dioxide were liberated per milligram dry weight per hour; with *Strigomonas fasciculata,* 65 c.mm. and with *Leptomonas ctenocephali,* 5 c.mm. He concludes from his manometric determinations that the acid which unquestionably was formed is lactic acid. Though there is no evidence directly contradicting this view, a confirmation by direct chemical analysis is very desirable, since, as has been explained above, lactic acid is certainly no common end product in this group of organisms.

Dausend (1931) follows a different line of reasoning in the case of *Tubifex.* This worm metabolizes large amounts of glycogen during anaerobiosis; and if, after having been kept for 23 hours under anoxic conditions, it is transferred for 4 to 6 hours to oxygenated surroundings, about 50 per cent of the lost glycogen is resynthesized. It is a well-established fact that, under similar conditions, an isolated vertebrate muscle also reconverts 4/5 of the lactic acid produced into glycogen. Dausend

is of the opinion that the analogy between the two cases warrants the assumption of identical processes; he therefore assumes that in *Tubifex*, as in the muscle, the source for the newly-formed polysaccharide is lactic acid. He must then further assume that the worm is able to excrete part of the acid formed during the anoxic period, in order to account for the reduced quantity of glycogen resynthesized. Pending a direct lactic acid determination, one can just as well assume (von Brand, 1934) that glycogen is reformed from other substances, *e.g.*, from fatty acids, and it might well be that such a process is less effective than the resynthesis from lactic acid. The whole question should be reinvestigated.

These examples may suffice to show how the assumption of lactic acid fermentation is often unwarranted.

We now turn to those cases in which the occurrence of glycolysis has actually been proven. One has to distinguish here between two possibilities. In some organisms lactic acid is the only organic acid produced in anaerobic carbohydrate metabolism; such organisms form an exact parallel to vertebrates. In many other organisms, however, this acid is but one of several end products and sometimes it plays, from a quantitative standpoint, only a minor role. These latter cases will be discussed in the section dealing with mixed fermentations. Here we shall consider only *pure glycolysis* as it occurs in malaria parasites, in echinoderms and in molluscs.

Silverman, Ceithaml, Taliaferro and Evans (1944) demonstrated in quantitative experiments that lactic acid accounts for practically all the glucose disappearing from the medium when *Plasmodium gallinaceum* is kept under anaerobic conditions. Previous to this work only a negative observation on the metabolism of malaria parasites had been recorded, namely, that pyruvic acid is not produced by *Plasmodium knowlesi* in the absence of oxygen (Wendel and Kimball, 1942).

Perlzweig and Barron (1928) found, in unfertilized eggs of *Arbacia punctulata,* 3.14 mg. of lactic acid per gram of egg protein if the eggs were kept in normal sea water, but 5.68 mg. if the medium contained 0.02 per cent potassium cyanide, *i.e.,* if aerobic processes were largely eliminated. Similar experiments, giving identical results, were performed with fertilized *Paracentrotus* eggs by Rapkine (1931). (Ashbel, 1929 and 1929a, had demonstrated, by a manometric method, that anaerobic glycolysis is much more pronounced in fertilized than in unfertilized sea urchin eggs). Glycolysis may also prevail in the anaerobic metabolism of adult echinoderms, but this has not been proven definitely by experiments under anaerobic conditions. Boyland (1928), however, demonstrated that the lactic acid content of fatigued muscles of *Holothuria nigra* is greater than that of normal muscles. In fatigue, as is well known, products of incomplete oxidations accumulate, due to a shortage of oxygen, and one is therefore justified in assuming that the same processes take place under anoxic conditions.

Lactic acid seems also to be the only end product of anaerobic carbohydrate metabolism in some lamellibranchs and perhaps in other molluscs. Boyland (1928) performed, with various species of *Pecten, Buccinum, Eledone* and *Sepia* experiments similar to those mentioned above with echinoderms. Some lactic acid, though not very much, was formed in all cases. Kobayashi (1929) also found an increase in the lactic acid content of fatigued muscles of *Ostrea circumpicta.*

Experiments with isolated tissues of oysters were carried out by Chapheau (1932, 1932a). He demonstrated first by chemical analysis that lactic acid was actually produced, then he determined manometrically its quantity. The gills and the hepato-pancreas had the highest rate of lactic acid formation, the muscle the lowest. Three-year-old oysters formed only about half as much

as very young animals did. Curiously enough, Dugal and Fortier (1941) insist that the organic acid produced by oysters is not lactic acid, but another as yet unidentified acid. In *Venus,* on the other hand, rather large amounts of lactic acid are formed under analogous conditions (Dugal, 1939, 1939a).

A few other cases have been described in which lactic acid is the only identified organic end product resulting from anaerobic metabolism, but they are all open to question. These instances will be reviewed briefly here although in the future they will probably have to be classified among mixed fermentations.

Slater (1927), working with cockroaches kept under anaerobic conditions, found a much smaller accumulation of lactic acid than was to be expected on the assumption that the gas metabolism during recovery involved the removal of lactic acid. In one series of experiments, for example, the amount measured was 80 mg. per 100 g., the expected one 540 mg. Gilmour (1941) found also that whereas *Tenebrio* larvae consumed 0.9 mg. of carbohydrate per gram of body tissue per hour during anaerobiosis, only 0.15 to 0.40 mg. of lactic acid was formed, *i.e.,* maximally, less than 50 per cent of the expected amount.

Finally, a few words may be said about the intermediate processes occurring during lactic acid fermentation. The chain of reactions leading from carbohydrate to lactic acid has been thoroughly studied for micro-organisms and vertebrate muscles (*cf.* summaries by Rahn, 1932; Parnas, 1937; Meyerhof, 1937 and Stephenson, 1939). So far, not a single study has been made with invertebrates that exhibit pure glycolysis and only very few with animals in which lactic acid is formed along with other acids, that is, in which the fermentations are of the mixed type. Thomas (1942) assumes that lactic acid formation in *Tetrahymena* follows the same pattern as in vertebrates but he does not adduce direct evidence (iso-

lation of intermediate products) for his view. As to phosphorylization, which plays an important role in the intermediate reactions of higher organisms, no sign of it was found in the protozoa *Glaucoma, Bodo, Polytoma* and *Plasmodium* (Needham, Robertson, Needham and Baldwin, 1932; Fulton, 1939). On the other hand, relatively large amounts of phosphoric acid are liberated in the aerobic fermentation of minced material of *Parascaris*, where lactic acid is the predominant organic end product (Fischer, 1924).

B. Fatty acid fermentations. Pure fatty acid fermentations have been described in protozoa, in *Fasciola,* in *Hirudo* and in *Chironomus.* But no search for lactic acid has been made in any of these cases. It is possible that lactic acid is a universal intermediate product of all animal fermentations and that it is transformed into the less toxic fatty acids in a secondary reaction.

Zhinkin (1930) and Barbarine (1938) observed that the disappearance of glycogen in *Stentor, Paramaecium* and other ciliates under anaerobic conditions is accompanied by the formation of microscopically demonstrable fat droplets. This fat appears, thus, as an end product of the anaerobic carbohydrate metabolism. Whether a similar type of fermentation occurs in parasitic protozoa cannot yet be stated definitely. Von Brand (1935) expressed the opinion that the observations of Erdmann (1917) concerning the glycogen-fat relationships of*Chloromyxum leydigi* can be interpreted in this manner.

In *Fasciola hepatica* the anaerobic fermentations lead to both higher and lower fatty acids. Weinland and von Brand (1926) found that 100 g. living tissue form, in 24 hours, 0.91 to 1.36 g. higher fatty acids and 0.30 g. volatile acid. The latter may be mostly butyric acid which was identified by Flury and Leeb (1926) in some mixed material of *Fasciola* and *Dicrocoelium.* The excretion of fat by *Fasciola* is of special interest. With the help

of staining methods fat can be demonstrated within the excretory system (Prenant, 1922; von Brand and Weinland, 1924; Vogel and von Brand, 1933; *cf.* Fig. 5). Its identification there is a definite proof that its appearance amongst the excreta cannot be due to bacterial contaminants.

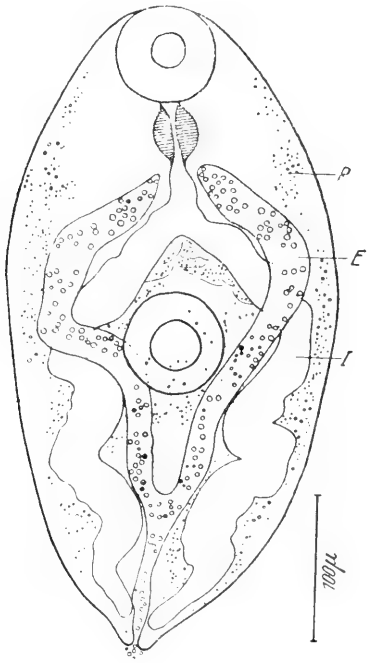


Fig. 5. Young *Fasciola hepatica* from body cavity of guinea-pig, 24 hours after infection, showing the excretion of fat. The fat droplets were stained by means of Sudan III and are represented in the drawing by circles and dots. *I*, intestine; *E*, excretory bladder containing fat droplets; *P*, fat deposited in the parenchyma. (From Vogel and von Brand, 1933.)

The excretion of organic acids by *Hirudo medicinalis* under anaerobic conditions was studied by Braconnier-Fayemendy (1933). She states that 98 per cent of the excreted acids are volatile and belong to the fatty acid series. Butyric acid was found to be the predominant one. The other acids were not definitely identified. They

seem to be a mixture of lower and higher members of the fatty acid series.

Harnisch (1939) identified as end products of the anaerobic carbohydrate metabolism of the larvae of *Chironomus thummi* butyric and caproic acid. The formation of these acids was more rapid in experiments lasting 8 to 10 hours than in those lasting from 23 to 24 hours. There was no such difference in the rate of glycogen degradation which was rather more rapid during the later stages of the experiments, and then "fat" (*i.e.*, essentially higher fatty acids) accumulated in the body of the animals. Harnisch assumes that during this fat formation some oxygen is liberated in an especially active form which would then serve to eliminate a part of the lower fatty acids by oxidation. An alternate assumption is that during longer periods of anaerobiosis the lower fatty acids are transformed into higher acids. Such a process would be advantageous to the animals, since "fat" is definitely less toxic than large amounts of lower fatty acids.

C. Mixed fermentations. Mixed fermentations, in which lactic acid, pyruvic acid, lower or higher fatty acids, dibasic acids or alcohol, appear amongst the end products in various combinations or proportions, occur in protozoa, worms, possibly coelenterates, and in arthropods. We shall now review briefly the evidence available for each of these groups.

P r o t o z o a . Among protozoa the best-known fermentations of this type occur in trypanosomes, termite flagellates and *Tetrahymena.*

Two species of trypanosomes, *Trypanosoma equiperdum* and *Trypanosoma lewisi,* were studied by Reiner and Smythe (1934), Reiner, Smythe and Pedlow (1936) and Searle and Reiner (1940, 1941). These investigators showed that anaerobically kept *Trypanosoma equiperdum* transform one molecule of sugar into one molecule of

glycerol and one molecule of pyruvic acid. This same reaction takes place also as the first step in the decomposition of sugar under aerobic conditions, but it is followed by the oxidative degradation of the glycerol molecule to one molecule of pyruvic acid and water. It is clear that this scheme leaves no room for lactic acid and carbon dioxide. But, in fact, small amounts of both these substances were found, and instead of a theoretical R.Q. of zero, a value of 0.062 was obtained. The appearance of lactic acid and carbon dioxide may, however, not be related to the metabolism of the organisms in question, but may have been due to the metabolism of leucocytes which can hardly be eliminated completely when the trypanosomes are isolated prior to the determinations.

The metabolic processes in *Trypanosoma lewisi* are quite different. Under anaerobic conditions one molecule of glucose is broken down into one molecule of succinic acid and probably one molecule of glycol. The latter is further transformed into acetic acid and ethyl alcohol[1], a process in which acetaldehyde is probably an intermediary. It should be noted that this anaerobic glucose decomposition proceeds only if the medium contains bicarbonate (Searle and Reiner, 1940, 1941). It appears that under anaerobic conditions carbon dioxide is consumed and that the amount consumed (from 0.25 to 1.0 mole per mole of glucose) is about equivalent to the amount of succinic acid produced. Instead of carbon dioxide, pyruvic acid may also influence the anaerobic glucose decomposition; larger amounts of lactic acid are then formed. It is of interest that the

1. *Trypanosoma lewisi* is, according to our present knowledge, the only invertebrate forming ethyl alcohol. It is true that Piéri (1895) described the production of alcohol by anaerobically kept clams. There can be no doubt, however, that in that case, alcohol actually originated from the metabolism of bacteria developing in the water in which he kept his experimental animals.

aerobic as well as the anaerobic degradation of glucose is enhanced by carbon dioxide but only the aerobic degradation of glycerol. The exact nature of the carbon dioxide assimilation is not yet known; Searle and Reiner (1940) assume that it is related to oxidation-reduction processes and suggest (1941) that both the anaerobic carbon dioxide assimilation and the aerobic stimulation of the metabolism by carbon dioxide involve the formation of glucose carbonate and glycerol carbonate.

Under aerobic conditions the initial steps in that series of reactions are the same as under anaerobic conditions. But under aerobic conditions, the acetaldehyde is transformed into formic acid, carbon dioxide and water. The greater part of the formic acid is then completely oxidized to carbon dioxide and water. Another direct oxygenation may transform some of the acetaldehyde into acetic acid. The identified end products of aerobic sugar decomposition are therefore formic, acetic and succinic acid, ethyl alcohol and carbon dioxide. The R.Q. of these processes should theoretically be 0.8, but actually it was found to be 0.98. This indicates that there are still some gaps in our knowledge.

The fermentative processes of the protozoa inhabiting the intestine of *Zootermopsis nevadensis* and *Zootermopsis angusticollis* were studied primarily by Hungate (1939, 1943a). He showed that these protozoa decompose cellulose and he was able to identify about three-fourths of the end products of this decomposition. Among these were carbon dioxide, hydrogen and organic acids, the most important of the latter being acetic acid. In one case at least some lactic acid was also formed. It is to be noted that two types of fermentation occurred in which the various end products appeared in different proportions but, so far, these fermentations have not been found to be attributable to specific protozoan species of which large numbers would occur in this habitat. Hun-

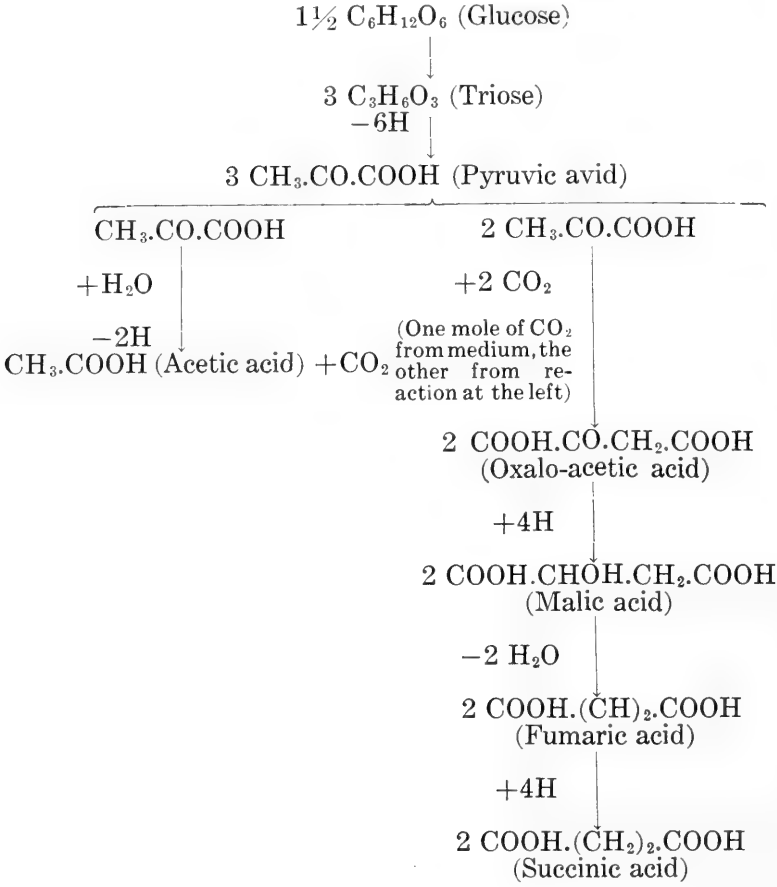
gate's experiments were carried out *in vitro,* but it is certain that the same type of metabolism prevails also when the parasites live within the intestine of their hosts, as the following observation of Cook (1932) indicates. He noticed that the termites harboring their normal intestinal fauna produced a gas that was not absorbed by strong alkali (and which must therefore be identified with the hydrogen found by Hungate), while defaunated ones did not, a clear indication that, *in vivo,* the hydrogen formation is also due to the intestinal protozoa. Additional data on this hydrogen formation will be found in the paper by Cook and Smith (1942).

Another case of mixed fermentations has been described for the ciliate *Tetrahymena gelei.* Thomas (1942) identified lactic, acetic and succinic acids as end products of the anaerobic carbohydrate metabolism of this organism. He considers the formation of the lactic acid as a process unrelated with those leading to the other acids, and, as already mentioned above, he is convinced that the lactic acid results from intermediate steps identical with those found in vertebrate muscle. His assumption of the independence of the two processes finds some support in his observation that the proportions in which the various substances are formed are variable, depending upon the medium in which the experiments are conducted. In phosphate buffer lactic acid predominates, while in bicarbonate media succinic acid is more abundantly formed.

The formulas (*cf.* Table 24) by which Thomas represents the formation of acetic and succinic acids are of considerable interest. Especially significant is the way in which the succinic acid is formed, since it is a process requiring an assimilation of carbon dioxide from the medium. That this actually happens has been proven beyond doubt. Thomas (*cf.* also van Niel, Thomas, Ruben and Kamen, 1942) used radio-active carbon as a tracer and

TABLE 24

PRODUCTION OF ACETIC AND SUCCINIC ACIDS BY *Tetrahymena gelei*. (ACCORDING TO THOMAS, 1942).



The above scheme results in the production of one mole of acetic acid, the uptake of one mole of carbon dioxide from the medium, and the formation of two moles of succinic acid.

recovered it exclusively from the carboxyl group of the succinic acid.

Finally, mixed fermentations were observed in malaria parasites, but only when these organisms were kept under aerobic conditions. As was mentioned in a previous section, their metabolism is of the pure glycolysis type when oxygen is completely absent. Wendel (1943) found that *Plasmodium knowlesi,* in the course of its aerobic sugar degradation, produced relatively large amounts of lactic acid, sufficient to account for about half the glucose decomposed. Furthermore, the quantitative relationships between oxygen consumption and sugar disappearance proved that the remaining half of the glucose could have been oxidized only partially. One of the end products of this process was probably pyruvic acid. Wendel and Kimball (1942) found that large amounts of this acid were formed during the aerobic incubation of blood containing the parasites. The aerobic sugar metabolism of *Plasmodium gallinaceum* is somewhat similar to that of *Plasmodium knowlesi* (Silverman, Ceithaml, Taliaferro and Evans, 1944). It leads to the production of carbon dioxide and lactic acid, but the carbon contained in these substances corresponded, in various series of experiments, to 58 to 93 per cent of that of the sugar consumed. Small amounts of pyruvic acid and traces of succinic acid were found, but they were not sufficient to account for all the missing carbon of the sugar. Whether a fixation of carbon dioxide is involved in these processes remains an open question; experiments with radio-active carbon in the medium yielded inconclusive results.

C o e l e n t e r a t e s . The nature of the fermentations occurring in this group is not well known. We do possess indications, however, that they are of a mixed type. Kramer (1937) working with isolated tentacles of *Anemonia sulcata* kept under anaerobic conditions, tested for the

presence of lactic acid. Employing the commonly used oxidation method, he found that acetaldehyde was indeed formed. But the time required for the completion of the reaction was considerably longer than if only lactic acid would have been present, and the ratio between the acid formed and the carbon dioxide liberated from bicarbonate did not correspond to the theoretical value. Other acids must, therefore, have been present which were responsible for these discrepancies.

W o r m s . Mixed fermentations have been found in the earthworm, in parasitic nematodes and in cestodes.

Lesser (1909a, 1910) studied the anaerobic metabolism of the earthworm and reported the production of carbon dioxide and of an organic acid which was tentatively identified as valeric acid. These findings were corroborated by Miyajima (1937)[1]. But a study of Lesser's data reveals clearly that not enough fatty acid was formed to account for the entire anaerobic glycogen loss. He did not search for lactic acid which, according to Davis and Slater (1928a), accumulates within the tissues of anaerobically kept earthworms. These investigators are even of the opinion that lactic acid is the only end product of the anaerobic carbohydrate metabolism of the earthworm, and that the appearance of fatty acids in Lesser's experiments was due to bacterial activity. They did not, however, make any quantitative glycogen determinations. It is therefore impossible to judge to what extent their assumption is well-founded.

The mixed fermentations occurring in *Ascaris* and *Parascaris* appear to be identical; the same end products have been found in both animals under both anaerobic and aerobic conditions. They have been studied by Weinland (1901, 1904), Flury (1912), Fischer (1924), Waechter (1934), von Brand (1934a), Krüger (1936), Toryu (1936) and Oesterlin (1937). All these workers

1. An abstract only of Miyajima's paper could be consulted.

agree that rather large amounts of carbon dioxide are produced; most of them also maintain that lower fatty acids are the most characteristic constituents of the excreta. Weinland was the first to recognize that valeric acid is the predominant one, but he found, in addition, some caproic acid. To these must be added small amounts of formic and butyric acids (Flury), perhaps propionic acid (Toryu), and acetic acid, as well as some unidentified higher acids (Oesterlin). Which of the isomers of valeric acid occurs is not certain; it is probably the normal acid (Waechter), although Flury suggested that it might be iso-valeric acid, and Krüger thought he had identified methyl-ethyl-acetic acid. No intermediate products have so far been isolated and the exact manner of the formation of the valeric acid is therefore not known. Weinland (1901) proposed the following equation:

$$4\ C_6H_{12}O_6 \longrightarrow 9\ CO_2 + 3\ C_5H_{10}O_2 + 9\ H_2$$

But this equation is hardly satisfactory since no hydrogen is liberated. Koenigs (*cf.* Weinland, 1901) proposed the equation:

$$13\ C_6H_{12}O_6 \longrightarrow 12\ C_5H_{10}O_2 + 18\ CO_2 + 18\ H_2O$$

which Weinland rejected because the amount of carbon dioxide actually produced was considerably higher than should be expected. One can, however, assume that the excess carbon dioxide originates from bicarbonate or from the breakdown of proteins. Jost (1928) suggested that the intermediate steps in Koenig's reaction are the formation of lactic, pyruvic, propionic and gamma-hydroxyvaleric acids. The connection thus suggested between the formation of lactic acid and that of fatty acids deserves consideration since small amounts of lactic acid were found amongst the excreta of *Ascaris* and *Parascaris* (von Brand, 1934a; Toryu, 1936). It has even been demonstrated that if minced material of *Parascaris* is employed instead of intact animals, the lactic acid form-

ed and the phosphoric acid liberated suffice to account for the total acidity obtained (Fischer, 1924).

From this observation Fischer postulates that carbohydrate decomposition proceeds along identical lines in the worm and in vertebrates; in other words, he does not believe that fatty acids are a normal end product of invertebrate anaerobic metabolism. But, as von Brand and Jahn (1942) point out, Fischer's observation may merely indicate that the chemical processes are not identical in minced material and in intact animals; they draw attention to the well-established fact that in yeast and other lower plants different metabolic end products may be found depending upon the conditions under which the experiments are conducted.

Another criticism against the assumption of fatty acids as end products of anaerobic carbohydrate metabolism was brought forward by Slater (1925). He found, in a saline solution in which ascarids had been kept, bacteria which, upon being cultivated in nutritive media, were capable of transforming sugar into volatile fatty acids. He assumed that such bacteria were responsible for the appearance of valeric and other fatty acids in the experiments of other authors. Slater, however, did not show that these bacteria develop in large numbers in the non-nutritive saline solutions commonly used in experiments with worms and did not investigate whether the postulated single end product, lactic acid, is a good substratum for the biological activity of the bacteria.

A few years later the same author, in his review on anaerobic life (Slater, 1928), developed more fully the idea that lactic acid is the only end product resulting from an anaerobic carbohydrate breakdown, both in vertebrates and in invertebrates. This conviction of his is quite clearly the reason why he rejects the experiments of his predecessors. But good reasons can be adduced to show that the fatty acids are produced by the animals

themselves and not merely by contaminants. Only a few of the most important of these reasons will be mentioned here; further evidence will be found in the papers of Weinland (1901) and of von Brand (1934).

In the first place the experiments summarized above concerning the trypanosomes and *Tetrahymena* were carried out under strictly sterile conditions. They therefore show conclusively that the production of acids other than lactic acid is quite possible in the case of *animal* protoplasm. Secondly, the presence of fat can be demonstrated within the excretory system of such helminths as *Fasciola* (Fig. 5) or *Moniezia*. In so far as the ascarids are concerned, the situation resembles, in principle, that encountered in the above flat worms, though the lower fatty acids cannot be demonstrated within the excretory system of ascarids by staining methods. It has, however, been shown (Weinland, 1902; Schimmelpfennig, 1903; Flury, 1912) that the same fatty acids which are recovered from the saline in which the worms were kept can also be isolated from the worm body proper. Weighing the available evidence, the writer considers as quite certain that the helminths are directly responsible for the appearance of fatty acids in their surroundings. However, no final, irrefutable proof is available, no experiment having been made in which these acids were isolated while the worms were kept under rigidly sterile conditions. With recent improvements in the technique of maintaining helminths aseptically such experiments should be possible in the not too distant future.

The experiments of Stannard, McCoy and Latchford (1938) with *Trichinella* larvae are of particular interest in that they reveal a type of metabolism which differs considerably from that of all other worms. These larvae produce no organic acid whatever, a fact which is of great importance because it shows that lactic acid does not necessarily appear, even in small amounts, amongst the ex-

creted end products of the anaerobic metabolism of parasitic worms. It is of interest, furthermore, to note that specific poisons like iodoacetate and iodoacetamide, as well as sodium fluoride and sodium arsenite were much slower in inhibiting the anaerobic processes in *Trichinella* than in suppressing the alcoholic fermentation of yeast or the glycolysis of muscle tissue. This is another clear indication that the *Trichinella* fermentations are quite different from those found in vertebrate muscle (Stannard, McCoy and Latchford, *l.c.*).

Having discussed the occurrence of the mixed fermentation in helminths we now turn to the question of how effective these processes are for energy production. Very little information is available on that point and the only data recorded concern *Ascaris*. Krummacher (1919) made some determinations of the heat production of this worm, but his experiments were conducted neither under clearly aerobic nor under definitely anaerobic conditions. Meier's (1931) data are of greater value. He found that anaerobically kept ascarids liberate 0.300 gram calorie per gram body weight per hour. The energy yield of these fermentations would, according to Meier, be 22 per cent of the maximal energy that could be derived from complete oxidation under aerobic conditions. Von Brand and Jahn (1942) consider this figure as unquestionably too high. They point out that Meier used two different sets of data for his calculation, his own on heat production and those of Weinland on carbohydrate consumption. Now, Meier's experimental periods lasted only from 4 to 12 hours, while Weinland's lasted several days. Since the rate of glycogen consumption decreases with increasing length of the experimental period (*cf.* prec. Ch.) the carbohydrate metabolism of Meier's worms must have been noticeably greater than he thought. He furthermore assumes that all the energy was derived from the carbohydrate fermentation. Schulte (1917), how-

ever, had calculated, by comparing the heat of combustion of entire worms with that of the glycogen content of fresh and of starving animals, that carbohydrate fermentations furnish only about 80 per cent of the total energy. Von Brand and Jahn (*l.c.*) remark that at the present state of our knowledge any calculation of the energy yield would be only approximate; their tentative estimate lies between 6 and 12 per cent.

Among cestodes, the mixed fermentations of only one species, *Moniezia expansa,* have been studied in some detail (von Brand, 1929, 1933a; Alt and Tischer, 1931). This worm produces daily, during its anaerobic metabolism, 0.44 g. carbon dioxide, 0.20 g. higher fatty acids, 0.16 g. lactic acid and 0.04 g. succinic acid. These acids account for 79 per cent of the total acidity of the saline medium determined by titration. The non-identified fraction may well consist of oxalic acid, which, according to Loeper and Tonnet (1931), accumulates within the body of an unspecified (human?) *Taenia* species, reaching 0.138 per cent of the weight of the animal during a 24-hour experimental period.

A r t h r o p o d s . Only one case of mixed carbohydrate fermentation has been described in arthropods. Von Kemnitz (1916) observed that, under anaerobic conditions, 100 g. of larval material of *Gasterophilus intestinalis* form 0.060 g. fat from glycogen, and liberate 0.276 g. carbon dioxide in 24 hours (*cf.* also Weinland, 1915). Since in the same period 0.723 g. glycogen are consumed, it is quite obvious that other substances must also have been formed. One of these is lactic acid, the formation of which has been proved definitely by Dinulescu (1932) and Blanchard and Dinulescu (1932); but the amounts found by these authors are hardly large enough to explain the discrepancy between the loss of glycogen and the sum of the end products identified. Von Brand (1934) points out that the only question studied so far is how

much lactic acid and fat accumulate within the body of the larvae; the possibility that some of the end products of anaerobic metabolism, perhaps lactic acid, may be excreted would also deserve attention.

2. FATS

We will consider here only fats proper since nothing whatever is known as to whether lipoids like cholesterol, phosphatids, *etc.*, are capable of furnishing energy under anoxic conditions. It may be noticed, in connection with this problem, that in a number of parasites which live in nearly anaerobic habitats, large amounts of unsaponifiable ether-soluble materials have been found (*cf.*, for *Goussia gadi*: Panzer, 1911 and 1913; for *Ascaris*: Flury, 1912; Fauré-Fremiet, 1913; Schulz and Becker, 1933; for *Fasciola*: von Brand, 1928; for *Moniezia*: von Brand, 1933a; for *Macracanthorhynchus*: von Brand, 1939).

A fat, as this term is used here, is a compound of fatty acids and glycerol. A fatty acid has only two oxygen atoms in its molecule and these two atoms are already combined with the end-carbon. Shifting processes similar to those mentioned above for carbohydrates are consequently not possible. A fatty acid molecule might, however, split into a hydrocarbon and carbon dioxide. A somewhat similar mechanism is assumed by Weinland (1906) to occur in minced material from pupae of *Calliphora vomitoria*. He found that the disappearance of fat under anaerobic conditions was accompanied by the production of carbon dioxide and hydrogen. These gases appeared in the proportion of 2 to 1. Weinland is of the opinion that they originated, at least in large part, from a decomposition of the carboxyl group of the fatty acid molecule, according to the formula:

$$C_{15}H_{31}COOH \longrightarrow C_{15}H_{31} + CO_2 + H$$

But such a reaction would leave one unsaturated linkage on the last carbon atom of the radical; one would have

to assume that other secondary processes occur that so far have not been elucidated. Weinland thought it possible that some oxygen dissolved in the minced material might give rise to a new fatty acid molecule with one carbon atom less than the one from which it originates.

While true higher fatty acids do not seem, on the whole, to be suitable substrates for anaerobic processes, higher hydroxyacids appear to be a better source of energy, at least from a theoretical standpoint. Though rare in invertebrates, they have been found in relatively large amounts in *Moniezia* (Oesterlin and von Brand, 1934). It is not yet known whether they are actually used by these tapeworms in case of oxygen deficiency.

The second chief component of a neutral fat, glycerol, has much more oxygen in its molecule than the fatty acids, and the oxygen atoms are more uniformly distributed amongst the carbon atoms. A shifting similar to that suggested in carbohydrates is therefore possible. Barnes and Grove (1916) assume that in larvae of *Attagenus undulatus,* kept with a very limited oxygen supply, the fat is first hydrolyzed into fatty acids and glycerol and that only this latter substance is used for the anaerobic production of energy. They argue that the amounts of carbon dioxide found were too large to be accounted for by a reduction of fatty acids, and that a derivation from glycerol was more plausible. These authors furthermore searched for hydrocarbons, which would result from fatty acids, but they found only traces of them. In judging Barnes and Grove's work one should recall the question raised in the preceding chapter, namely, whether the oxygen was excluded sufficiently in their experiments to prevent an aerobic oxidation of fat.

3. PROTEINS

Protein molecules are very large and complex and their anaerobic degradation is little understood, even in micro-

organisms in which it received more attention than in other invertebrates. But several degradation processes of amino acids are known which do not require molecular oxygen. They are listed here as given by Stephenson (1939). To investigate whether and to what extent these processes occur in invertebrates would be an interesting field for future study.

(a) Decarboxylation of the amino acids with formation of the corresponding amine:

$$R.CHNH_2.COOH \longrightarrow CO_2 + R.CH_2NH_2$$

(b) Hydrolytic deamination with formation of the hydroxyacid:

$$R.CHNH_2.COOH + H_2O \longrightarrow NH_3 + R.CHOH.COOH$$

(c) Hydrolytic deamination and decarboxylation with formation of the alcohol:

$$R.CHNH_2.COOH + 2H \longrightarrow CO_2 + NH_3 + R.CH_2OH$$

(d) Reductive deamination with formation of the saturated acid:

$$R.CHNH_2.COOH + 2H \longrightarrow NH_3 + R.CH_2COOH$$

(e) Reductive deamination and decarboxylation with the formation of the hydrocarbon:

$$R.CHNH_2.COOH + 2H \longrightarrow CO_2 + NH_3 + R.CH_3$$

(f) Deamination and desaturation at the alpha-beta linkage with formation of the unsaturated acid:

$$R.CH_2.CHNH_2.COOH \longrightarrow NH_3 + R.CH{:}CH.COOH$$

(g) Mutual oxidation and reduction by pairs of amino acids:

$$R.CH_2CHNH_2.COOH + 2R.CHNH_2.COOH + 2H_2O \longrightarrow$$
$$R.CH_2.COOH + CO_2 + NH_3 + 4H + 2R.CHNH_2.COOH$$
$$\longrightarrow R.CH_2.COOH + CO_2 + NH_3 + 2R.CH_2COOH + 2NH_3$$

(h) Anaerobic breakdown with production of hydrogen:

$$5C_5H_9O_4N + 6H_2O \longrightarrow 6CH_3.COOH + 2CH_3(CH_2)_2.COOH + 5CO_2 + H_2 + 5\ NH_3$$

In general, decarboxylation and deamination seem to liberate but little energy. These processes will, however, be of the greatest importance in transforming foreign protein into the specific protein of a given organism. On the other hand, as Stephenson (1939) remarks, the reaction (h) which releases hydrogen, "may perhaps be regarded as an anaerobic device for obtaining energy from amino acids without the use of oxygen or a hydrogen acceptor." But it is not yet known whether it occurs in invertebrates.

It has already been stated that our knowledge concerning the anaerobic protein metabolism of invertebrates is extremely scanty. The investigations performed so far have not gone beyond the identification of some end products, and even this was done only in a few isolated cases. It would therefore be premature to try to develop a general picture of anaerobic protein degradation. We can only present a brief review of the various papers that shed some light on the question.

Geimann (1936) observed that the ammonia content of the muscles of the medusa *Aurelia aurita* increases if the animal is kept for 2½ hours under anaerobic conditions.

Ammonia is also the most important identified water-soluble, nitrogenous end product of the anaerobic metabolism of *Ascaris*. About one-third of the excreted nitrogen is present in the form of ammonia; the other two-thirds can be precipitated with phosphotungstic acid and are obviously a mixture of various substances (Weinland, 1904a). Besides ammonia small amounts of amine bases, unidentified substances giving the biuret reaction, hydrogen sulfide and mercaptan are eliminated (Flury, 1912; Krüger, 1936). Urea, on the other hand, seems not to be formed by this worm (Chitwood, 1938).

The nitrogenous end products of the anaerobic metabolism of flukes are still less known. This is due to the

fact that most specimens, which have their intestinal crura filled with food material, regurgitate their contents when kept outside the host. One recovers therefore a mixture of true metabolic end products and of food in various stages of digestion. Flury and Leeb (1926) found ammonia, coagulated protein, albumoses, peptones and amino acids, besides traces of hydrogen sulfide, haemoglobin, oxyhaemoglobin and methaemoglobin, in a saline solution in which *Fasciola* and *Dicrocoelium* had been kept for several hours. It seems evident that some of these substances, especially the higher proteins and the haemoglobins, were not true excreta, *i.e.,* that they did not originate in the cellular metabolism of the worms.

In *Hirudo medicinalis* also Braconnier-Fayemendy (1933) found ammonia as one of the chief end products. The ammonia nitrogen represented 77.7 per cent of the total eliminated non-protein nitrogen in anaerobic conditions, as against 72 per cent in starving leeches kept under aerobic conditions. The corresponding figures for amino acid nitrogen were 1.14 and 1.27 per cent, and those for creatinine nitrogen 1.22 and 2.14 respectively.

The anaerobic protein metabolism of the earthworm must be different from that of leeches. Lesser (1909a) reported that only very little, if any, ammonia resulted from protein degradation in an atmosphere deficient in oxygen.

The curious observations of Harnisch (1939) on the larvae of *Chironomus thummi,* which seem to indicate that, during anaerobiosis, there occurs an increase in nitrogen which can be determined by Kjeldahl's method, have already been mentioned in the preceding chapter. A definitive evaluation of the significance of these data will only be possible when more experiments have been performed. In a recent paper Harnisch (1943) states that the larvae of *Chironomus bathophilus* excrete considerable amounts of ammonia when kept in nitrogen.

4. ANAEROBIC GAS METABOLISM

Carbon dioxide is the gas most commonly evolved when animals are kept under anaerobic conditions. As a matter of fact it was found in almost all the cases in which it was searched for. (Emerson, 1929, using a manometric method in experiments with *Amoeba proteus*, could not find any trace of carbon dioxide; but it is not clear from his brief account whether the organisms had so little resistance that they died when deprived of oxygen, or whether the method employed was not sensitive enough to register the production of small amounts of carbon dioxide.)

A. Liberation of carbon dioxide from inorganic substances. This process will always take place when the medium or the body of the experimental animal contains bicarbonates or carbonates and when, during the course of the anaerobic metabolism, acids are produced that are stronger than carbonic acid, such as, for example, lactic or lower fatty acids. As is well known—and this has been mentioned in numerous instances on previous occasions—the liberation of carbon dioxide from bicarbonates can be used as a convenient index of the extent of the anaerobic metabolism.

Carbon dioxide measurements can easily be made with manometric methods. One procedure consists in using two manometric vessels with the same amount of medium and the same number of animals, one for the anaerobiosis experiment, the other as control. To the contents of the control vessel is then added, at the beginning, through a side arm, a strong acid that liberates all the carbon dioxide bound up in the medium and in the bodies of the animals; the contents of the experimental vessel are acidified only at the end of the run. The increase in pressure found in the latter corresponds to the bicarbonates that were still present as such. The difference between this pressure increase and that computed from the data gain-

ed by the control allow one to calculate the carbon dioxide set free by organic acids during the experiment. In many cases, however, another fraction of carbon dioxide appears in the experimental vessel, namely, carbon dioxide originating directly from the degradation of organic material. In order to determine the magnitude of this fraction, one subtracts the total carbon dioxide pressure due to bicarbonates from the total carbon dioxide pressure (after acidification) actually observed in the experimental manometer, since this latter pressure is a resultant of the pressures of both fractions. This procedure, for the details of which the reader is referred to Dixon (1943), can conveniently be employed with small animals, like protozoa, or with tissue slices, but it obviously cannot be carried out with large experimental animals.

With the latter, one can determine the amount of lactic acid that has accumulated in the body during the anoxic period. Since one is fairly safe in assuming that the acids stored in the tissues will be almost entirely neutralized, one can calculate the amount of carbon dioxide set free during neutralization. In order to determine whether all the carbon dioxide excreted during the experimental anaerobic period is of this origin, or whether a certain amount was formed directly from organic material, it is only necessary to compare the calculated amount with that actually found during the anoxic period; any excess in the latter indicates that carbon dioxide originated also from the breakdown of some organic food substance. It must be emphasized that this procedure will yield reliable results only if the organic acids remain stored in the body as long as the oxygen is missing; if they are excreted, matters become more complicated, because the acids are not always neutralized before being eliminated. It can be demonstrated readily that much of the acid present in a saline in which parasitic worms were kept is in the free state (*cf.* von Brand, 1933a and 1938a, for the acids produced by *Moniezia* and *Eustrongylides*). No

effort has so far been made to study, in these cases, what fraction of the total carbon dioxide is of inorganic origin.

Another method, much used in recent years (*cf.* for example, Davis and Slater, 1928; Gilmour, 1941) is to study the gas metabolism during recovery from anaerobiosis. The accumulated metabolic end products are then removed through aerobic oxidation and the bicarbonates are resynthesized, a process which obviously results in the retention of carbon dioxide. If the normal and the post-anaerobic respiratory quotients are determined, the amount of retained carbon dioxide can be calculated. But this method has the same limitation as that outlined above: only if all the metabolic end products actually accumulate in the body and are not excreted, will it give reliable quantitative data concerning the origin of the anaerobically produced gas.

The reaction between inorganic substances and the anaerobically produced acids is of great biological importance especially if the latter are retained in the body; it works as a buffer mechanism that tends to prevent a dangerous lowering of the pH. This point has been investigated primarily by Collip (1920, 1921), Dotterweich and Elssner (1935), Dugal and Irving (1937, 1938), Dugal (1939, 1939a) and Dugal and Fortier (1941), who have shown that in clams kept under anaerobic conditions the carbon dioxide content of the mantle cavity fluid, as well as of the tissues themselves increases enormously. In Dugal's experiments the amount of carbon dioxide rose under anaerobic conditions from 6 to 150 cc. per 100 cc. of mantle cavity fluid. At the same time lactic acid and calcium accumulated but no decided shift in pH took place. The calcium was obviously derived from the calcareous shell which, in fact, became eroded. The process—which in this case does not lead to free carbon dioxide—can be formulated as follows:

$$2CaCO_3 + 2C_3H_6O_3 \rightarrow Ca(HCO_3)_2 + Ca(C_3H_5O_3)_2$$

If isolated tissues of *Anodonta hallenbeckii* are employed for experiments on anaerobiosis, a buffer action, similar in principle to the one just described, can be observed. It seems, however, that calcium carbonate plays here only a minor role. The chief buffer is another calcium compound, perhaps a calcium proteinate (Culbreth, 1941). Dotterweich and Elssner (1935) assumed the existence of a similar organic buffer in the case of *Anodonta cygnea*, where, however, it is of less importance than the inorganic buffer mechanism.

Deposits of calcium salts can probably be used in a comparable manner in other invertebrates. Thus, it has been suggested that the calcareous corpuscles of tapeworms may serve as an alkaline reserve (von Brand, 1933a). A similar function may be ascribed to the calciferous glands of earthworms. It has, in fact, been shown that these are used to buffer the carbon dioxide that accumulates under certain conditions in the body fluids of the worms (Dotterweich, 1933), but whether they serve also under anaerobic conditions to buffer organic acids is not yet known.

B. Liberation of carbon dioxide from organic sources. It is obvious that no carbon dioxide is produced directly from carbohydrates when the latter undergo lactic acid fermentation. In animals showing only this type of metabolic process, therefore, all the carbon dioxide found under anaerobic conditions must be derived from the neutralization of the acid. The quantitative investigations of Dugal (1939) on *Venus mercenaria* agree with this conclusion, although earlier experiments of Berkeley (1921) and of Collip (1920, 1921) on clams seemed to indicate that at least some carbon dioxide originated from metabolic processes proper. One should, of course, remember that, in anaerobically kept animals, proteins and in exceptional cases even fats may be consumed besides carbohydrates and that some carbon dioxide may be derived from these sources.

In the case of the eggs of *Arbacia, Chaetopterus, Mactra* and *Ostrea* (Ballentine, 1940), all of which probably ferment sugar to lactic acid, negligible amounts of carbon dioxide were derived directly from metabolic processes.

Besides lactic acid fermentation, there are also other fermentations which, theoretically, should not furnish any carbon dioxide directly, for example, the previously mentioned metabolic processes occurring in *Trypanosoma equiperdum*. Actually, however, small amounts of carbon dioxide do appear. Reiner, Smythe and Pedlow (1936) found, as mentioned above, a respiratory quotient of 0.062 instead of zero. This, we said, might well be due to secondary reactions on substances other than sugar or to the presence of leucocytes. Another well-established case of mixed fermentation, in which no carbon dioxide is derived from sugar degradation, is that of *Tetrahymena* (Thomas, 1942).

In many other types of anaerobic fermentation, however (*cf.* the preceding sections of this chapter), the production of carbon dioxide is definitely in direct dependence on the degradation of carbohydrate. The amount of gas produced (for example, by parasitic worms) is much too large to be explained by its liberation from inorganic substances or by its derivation from protein alone. A calculation from von Brand's (1934a) figures for the carbon dioxide and the organic acids produced by *Ascaris*, leads to the conclusion that at least 80 to 90 per cent of the carbon dioxide must have been derived directly from metabolic processes. Since the protein metabolism of these worms is not pronounced and since they consume no fat, there can be no doubt that most of the gas came from the breakdown of carbohydrate.

An especially clear-cut case is that of the *Trichinella* larvae. Stannard, McCoy and Latchford (1938) showed that the total bound carbon dioxide of the larvae and of

the solutions used in the experiments averaged only 5 c.mm., while the animals actually produced from 20 to 40 c.mm.

In general, not enough attention has been paid so far to the origin of the carbon dioxide produced during fatty acid or mixed fermentations. The frequent practice of studying chemically only the main anaerobic process, namely, the carbohydrate fermentation, and of ascribing to it all the carbon dioxide recovered, has, in several cases, resulted in data that may well be changed by subsequent investigations.

C. Production of combustible gases. Combustible gases, especially hydrogen and, to a much lesser extent, methane, are frequently formed during the fermentations of micro-organisms. A variety of mother-substances serve as their source. In invertebrates only a few cases of hydrogen formation have been described and methane has so far never been identified. Although some of the cases have already been mentioned in other sections, a brief survey of all available investigations will be presented here.

It is a well-established and repeatedly studied fact that one of the end products of the cellulose fermentation of the intestinal protozoa of termites is hydrogen (Cook, 1932 and 1943; Cook and Smith, 1942; Hungate, 1939 and 1943a—*cf.* Sect. 1, *C* of this chapter).

The symbiotic protozoa of the roach *Cryptocercus punctulatus* also produce hydrogen, and in addition, small amounts of a gas that gave rise to carbon dioxide upon combustion (Gilmour, 1940a).

A gas which is not absorbed by KOH and which is combustible is developed in bacteria-free cultures by *Trichomonas foetus* (Andrews and von Brand, 1938), but its nature has not yet been determined.

The only metazoon which produces large amounts of hydrogen during anaerobiosis is the pupa of *Calliphora*

vomitoria (Weinland, 1906). As was indicated previously, fat appears to be the mother-substance in this case. One may recall that Weinland used minced material; whether the same phenomenon takes place in intact specimens is not certain.

Traces of hydrogen have been described as liberated by *Ascaris* (Weinland, 1901), but von Brand (1934a) could not obtain confirmation of this finding. Very small amounts of hydrogen have also been found in larvae of *Attagenus undulatus* (Barnes and Grove, 1916) under conditions that were not absolutely anaerobic, but in which the oxygen supply was clearly deficient.

It is well established, on the contrary, that neither the earthworm (Lesser, 1910) nor the larva of *Tenebrio molitor* (Gilmour, 1941) produces even traces of combustible gases when kept under strictly anoxic conditions.

5. ENZYMES

That the anaerobic metabolism of invertebrates is governed by enzymes which exert their action even if the cellular structure is destroyed has been recognized long ago. Weinland (1902) was the first to show that the anaerobic metabolism of *Ascaris* remains unchanged if, instead of intact animals, minced material is used (as in the case of Buchner's *Pressaft*). In *Parascaris,* on the other hand, Fischer (1924) found that only lactic acid is formed and phosphoric acid liberated when the material is minced (experiments at the oxygen tension of atmospheric air), while it is well known that the chief end products of similarly kept intact worms are lower fatty acids.

For information concerning the corresponding phases of the anaerobic metabolism of insects, we refer the reader to the papers of Barnes and Grove (1916) and of Blacklock, Gordon and Fine (1930).

In recent years more intensive studies of the enzymes have been undertaken in cases of anaerobically kept in-

vertebrates. They are not yet sufficiently advanced, however, to give a uniform picture, as will appear from the following account.

Keilin (1925) showed, by examination of living *Galleria* by means of a micro-spectroscope, that the absorption bands of reduced cytochrome appear very rapidly when nitrogen or coal gas is passed over the animals.

Monier (1936) studied the behavior of glutathione during the asphyxiation of *Sipunculus nudus* and *Carcinus moenas*. In the blood of aerobically kept sipunculids the oxidized and the reduced glutathione were in the ratio of about 2:1, while in worms kept for 15 hours in a vessel with sea water, without contact with the atmosphere, only reduced glutathione was found. The latter had increased to about ten times its original value, but there is no information as to which organs furnished the additional amounts. In *Carcinus,* asphyxiated in a similar manner for the same length of time, the oxidized glutathione did not disappear completely from the gills or the hepato-pancreas; in this latter organ a distinct increase in reduced glutathione was observed, but none in the gills.

Gourévitch (1937) found relatively large quantities of flavine, a typical oxidation-reduction enzyme of anaerobic cells (4 to 12.5 gammas per g. of fresh tissue) in *Fasciola, Ascaris* and in the larvae of *Gasterophilus,* all animals that readily withstand oxygen deprivation. But as much, or even more, (up to 25 gammas per g. of tissue) was encountered in some typical aerobic organisms, like the earthworm, some coleoptera and lepidoptera. Therefore there seems to be no parallelism between flavine content and the ability to live anaerobically.

Ballentine (1940) observed that the eggs of *Arbacia, Chaetopterus, Mactra* and *Ostrea* are able to reduce ferricyanide under anaerobic conditions. This indicates

that within these cells systems of the type dehydrogenase-substrate-coenzyme must exist.

Relatively little work has been done so far with specific enzyme poisons. Chapheau (1932a) found that the anaerobic fermentations of oyster tissues were completely inhibited by M/20000 mono-bromine-acetic acid and Maloeuf (1937b) reported that the anaerobic carbon dioxide production of tissue slices of *Mytilus* was completely inhibited by M/50 mono-iodo-acetic acid. These findings substantiate the view expressed in a previous section of this chapter that the main anaerobic reaction in lamellibranchs is true glycolysis which, as is well known, is very easily influenced by mono-iodo-acetic acid. Maloeuf observed also that the anaerobic gas exchange of the same material was not affected by M/500 or M/1000 potassium cyanide nor by M/9 ethyl-urethane. This is interpreted as indicative of a lack of anaerobic oxidases or dehydrogenases.

Still fewer data are available on the actions of poisons on anaerobic fatty acid fermentations or on mixed fermentations. The only paper dealing with this topic, that of Stannard, McCoy and Latchford (1938), has already been mentioned. It may be recalled that in this case specific poisons were much slower in their action than is usual for lactic acid fermentation. Further studies along these lines with other organisms will, no doubt, reveal interesting points.

It may finally be mentioned that, as far as the author is aware, only one single attempt has been made to study, by means of poisons, the anaerobic nitrogen metabolism of invertebrates. Geimann (1936) observed that mono-iodo-acetic acid does not in any way suppress the formation of ammonia in *Aurelia*.

CHAPTER V

RECOVERY FROM ANAEROBIOSIS

All invertebrates, with the exception of the few which are adapted to permanent life in the complete absence of oxygen, become paralyzed after they have been deprived of oxygen for a certain time. Most terrestrial insects, for example, lose their motility in a few minutes, often in a few seconds. Other invertebrates that show better adaptations to anaerobic life, like many worms, may remain active for several days, but they too will finally become quiescent. In general, paralysis is due to the accumulation of non-oxidized substances within the body: these substances usually exert some toxic action. But if the anoxic period is not too long the animals recover upon readmission of oxygen. This recovery can be studied from various angles; we shall discuss here (1) the time required for recovery; (2) the post-anaerobic gas metabolism; and (3) the chemical nature of the recovery processes.

1. TIME FOR RECOVERY

The most generally used sign of recovery is the resumption of some form of movement: either—in the case of protozoa—amoeboid, flagellar or ciliary movement, or merely the functioning of contractile vacuoles or of other organelles, or—in the case of metazoa—actual locomotion or only the moving of some appendage. Reports of recovery are numerous in the literature: see in particular, Emery (1869), Plateau (1872), Bert (1878), Devaux (1891), Fielde (1904, 1904a), Collip (1920), Lee (1924), Wrede and Kramer (1926), Haldane (1927), Pantin (1930), Bruneteau (1931), Kitching (1939, 1939b), Kalmus (1935, 1942).

The time required for recovery, as measured by the resumption of locomotion, depends to a large extent upon

the length of the period of deprivation of oxygen. This is shown in Table 25 which consists of reports on insects, the group most thoroughly investigated. (For protozoa similar observations are recorded in Pantin's work (1930; *cf.* Fig. 6).

Kalmus (1942), has recently published an interesting study of the dependency of the recovery time on the length of the period of asphyxiation in *Drosophila.* He found that the average time was linearly related to the asphyxiation time.

Kalmus further observed that the time required by *Drosophila subobscura* for the resumption of movement

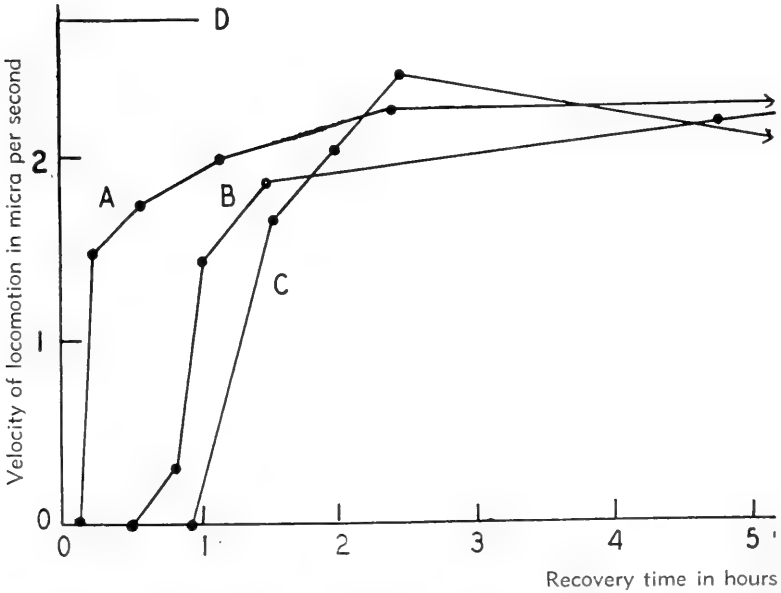


Fig. 6. Resumption of locomotion by marine amoebae of the *limax* type during recovery from anaerobiosis. *A*, after a 6¾-hour period of anaerobiosis (the amoebae were not entirely paralyzed at the end of this period). *B*, after a 4¾-hour period of anaerobiosis (the amoebae were entirely paralyzed). *C*, after a 25-hour period of anaerobiosis (the amoebae were entirely paralyzed). *D*, level of average pre-anaerobic velocity. (According to Pantin, 1930; with slight modifications.)

TABLE 25

DEPENDENCY OF THE RECOVERY TIME ON THE LENGTH OF THE PRECEDING PERIOD OF ANAEROBIOSIS IN INSECTS.

Species	Time of anaerobiosis	Time required for recovery	Source
Isoptera			
Zootermopsis nevadensis	1 to 3 hrs.	Immediate recovery	Cook, 1932
" "	6 hrs.	15 min.	" "
" "	9 hrs.	20 to 30 m.	" "
" "	24 hrs.	1 hr.	" "
" "	48 hrs.	12 hrs.	" "
Diptera			
Drosophila melanogaster	45 sec.	1 to 2 min.	Kalmus, 1935
" "	35 min.	15 min.	" "
" "	15 hrs.	6 to 7 hrs.	" "
Coleoptera			
Geotrupes stercorarius	48 hrs.	6 hrs.	Plateau, 1872
" "	74 hrs.	4 hrs.	" "
" "	96 hrs.	7¾ hrs.	" "
Hylobius abietis	23 hrs.	2½ hrs.	" "
" "	47 hrs.	3⅓ hrs.	" "
" "	72 hrs.	3½ hrs.	" "
" "	96 hrs.	5½ hrs.	" "
Leptinotarsa decemlineata	10 min.	4 min.	Bruneteau, 1931
" "	20 min.	9 min.	" "
" "	1 hr.	16 min.	" "
" "	2 hrs.	20 min.	" "
" "	19 hrs.	90 min.	" "
" "	24 hrs.	120 min.	" "
Hymenoptera			
Aphaenogaster fulva	30 min.	5 to 15 m.	Young, 1944
" "	2 hrs.	30 to 60 m.	" "
" "	4 hrs.	1 to 4 hrs.	" "
" "	8 hrs.	4 to 6 hrs.	" "
Camponotus herculeanus pennsylvanicus	1 hr.	15 to 30 m.	" "
" "	2 hrs.	20 to 45 m.	" "
" "	5 hrs.	35 to 50 m.	" "
" "	7 hrs.	50 to 60 m.	" "
" "	9 hrs.	1 to 2 hrs.	" "
" "	15 hrs.	2 to 3 hrs.	" "
" "	18 hrs.	2 - 3½ hrs.	" "
" "	24 hrs.	3 to 4 hrs.	" "
" "	30 hrs.	4 to 5 hrs.	" "
Formica cinerea	1 hr.	5 to 15 m.	" "
" "	2 hrs.	20 to 40 m.	" "
" "	4 hrs.	1 to 2 hrs.	" "
" "	6 hrs.	2 to 5 hrs.	" "
" "	9 hrs.	5 to 9 hrs.	" "
" "	14 hrs.	7 - 12 hrs.	" "
" "	18 hrs.	10 - 17 hrs.	" "
" "	24 hrs.	15 - 22 hrs.	" "
" "	28 hrs.	20 - 27 hrs.	" "

TABLE 25 (Continued)

DEPENDENCY OF THE RECOVERY TIME ON THE LENGTH OF THE PRECEDING PERIOD OF ANAEROBIOSIS IN INSECTS.

Species	Time of anaerobiosis	Time required for recovery	Source
Lasius umbrata	30 min.	10 to 20 m.	" "
" "	2 hrs.	1 to 2 hrs.	" "
" "	4 hrs.	1 to 3 hrs.	" "
" "	5 hrs.	2 to 4 hrs.	" "
" "	6 hrs.	2 to 5 hrs.	" "
" "	8 hrs.	3 - 5½ hrs.	" "
Tetramorium caespitum	30 min.	10 to 20 m.	" "
" "	1 hr.	25 to 40 m.	" "
" "	2 hrs.	1 to 2 hrs.	" "
" "	4 & 6 hrs.	1 to 3 hrs.	" "
" "	8 & 9 hrs.	1 to 4 hrs.	" "

was shortened if the flies were subjected to carbon dioxide at the end of the period of oxygen deprivation. He immobilized his animals in a stream of hydrogen for 2 minutes. One batch was then brought for 1 minute into 60 per cent carbon dioxide and these recovered after 2.7 ± 1.03 min. in air, while the control flies needed 4.7 ± 1.14 minutes. The difference of 2 minutes is considered significant.

According to the same author the time necessary for recovery varies in different species of *Drosophila* and even in mutants of the same species.

As far as the present writer is aware, all the experiments concerning the time required for the resumption of movement were performed with animals that are rather sensitive to the absence of oxygen. A comparative study with animals showing greater resistance would probably yield different results. Casual observations (von Brand, unpublished) on larval *Eustrongylides* showed that this worm resumes movement in about 30 minutes, after 18 hours without oxygen, in marked contrast with the long recovery periods found in many insects. (*cf.* Table 25).

It may be mentioned, finally, that animals exposed to respiratory poisons, like hydrocyanic acid, behave much like those directly deprived of oxygen. The revival of insects after exposure to such poisons has received considerable attention in connection with fumigation problems. It would lead us too far afield to review here the data accumulated in the literature of applied entomology. The reader is referred to the thorough papers of Bliss (1935), and of Broadbent and Bliss (1936), on *Drosophila,* among others.

2. POST-ANAEROBIC GAS EXCHANGE

Any animal in which the oxidations are normally aerobic will, when deprived of oxygen, experience a need for this gas, resulting in an oxygen debt. Whether and to what extent this debt is repaid during the subsequent exposure to air depends upon whether or not non-oxidized substances have accumulated in the body and also upon the chemical nature of the recovery processes. This is obvious since any excess oxygen is used to remove, by aerobic oxidations, the accumulated end products of the anaerobic metabolism. Another very frequent feature of the recovery respiration is the retention of carbon dioxide. We have mentioned previously that the liberation of carbon dioxide from inorganic substances is a common phenomenon in anaerobic metabolism. These substances —bicarbonates or carbonates—are rebuilt during the recovery period, and this resynthesis obviously requires carbon dioxide. In all cases of retention, pronounced changes in the respiratory quotient must occur. We shall discuss post-anaerobic respiration in two sections, the first one dealing with the oxygen consumption, the second with the carbon dioxide production and retention, and with the respiratory quotient.

A. Oxygen consumption. It is clear that, if *all* the end products of anaerobic metabolism were excreted, no repayment of an oxygen debt could be expected. It is,

however, questionable whether this condition actually occurs in any invertebrate capable of consuming oxygen. Adam (1932) and Harnisch (1933a) had observed, it is true, that the oxygen consumption of *Ascaris* was not markedly increased after a period of experimental anaerobiosis, but Harnisch found that isolated anterior parts of these worms contracted an oxygen debt, and Laser (1944) showed that, after 18 hours of anaerobiosis, the oxygen consumption of entire ascarids was definitely above normal for a period of about 2 hours. The repayment of the incurred oxygen debt is quite incomplete, due to the fact that *Ascaris* is capable of excreting a large percentage of the anaerobic end products.

Other cases of incomplete repayment are also known. A graph by Thomas (1942) representing the oxygen consumption of *Tetrahymena* under normal conditions and after 45 minutes in the absence of oxygen shows that this ciliate repays only about 25 per cent of its oxygen debt (the author does not discuss that point). Another case is that of the larva of *Eustrongylides ignotus,* studied by von Brand (1942). In that animal the excess oxygen consumption, after a 16 to 18-hour period of anaerobiosis, corresponded to about 30 per cent of the oxygen deficit. Both these organisms are known to excrete acids during the anaerobic period proper.

The case of *Zootermopsis nevadensis* is more difficult to understand. Cook (1932) stated that this organism does not show an increased oxygen consumption after anaerobiosis, but this was not corroborated by Gilmour (1940) who thinks that Cook's result must be explained by his failure to control the movements of his animals which were responsible for an abnormally high pre-anaerobic level of oxygen consumption. Gilmour found a repayment of 50 per cent, both in termites harboring their normal intestinal fauna and in defaunated ones. This low value is somewhat surprising since insects in

general seem incapable of excreting the end products of their anaerobic metabolism. A study of the chemical processes during recovery will be necessary before the significance of Gilmour's figure can be understood.

In a second group of invertebrates the oxygen debt is repaid in full. The first case to be mentioned is that of *Planorbis corneus,* investigated by Borden (1931). The calculation of the excess oxygen consumption of this organism is more complicated than in many other instances, because this snail contains relatively large amounts of haemoglobin. The actual period of anaerobiosis will therefore be shorter than the time during which the animals are kept in the absence of oxygen, since a certain amount of oxygen is stored in their blood. On the other hand, that oxygen which is required, during the post-anaerobic period, to transform the reduced haemoglobin into oxy-haemoglobin must be subtracted from the excess oxygen actually consumed, since obviously only the oxygen used in the cellular metabolism can be considered as true excess oxygen. Borden took these points into careful consideration; her findings are illustrated in Figure 7.

The situation is less complicated in animals which possess no respiratory pigment or which have only a small amount of blood. Davis and Slater found a total repayment of the oxygen debt in cock-roaches (1926a, *cf.* Fig. 8) and an almost total one in the earthworm (1928a). Curiously enough, Lesser (1910a) had previously described a diminished rate of oxygen consumption during the recovery period of this latter organism. Perhaps his animals had been damaged during the preceding anaerobic period. Bodine (1928) also reported an approximate total repayment of the oxygen debt in grasshoppers.

In a last group of invertebrates more oxygen is consumed than is necessary to make up for the deficit incurred during anaerobiosis. Thus in *Cryptocercus punc-*

tulatus, the oxygen consumed is three times the debt (Gilmour, 1940a), in the larva of *Tenebrio molitor* 1½ times (Gilmour, 1941), in the isolated hind femora of

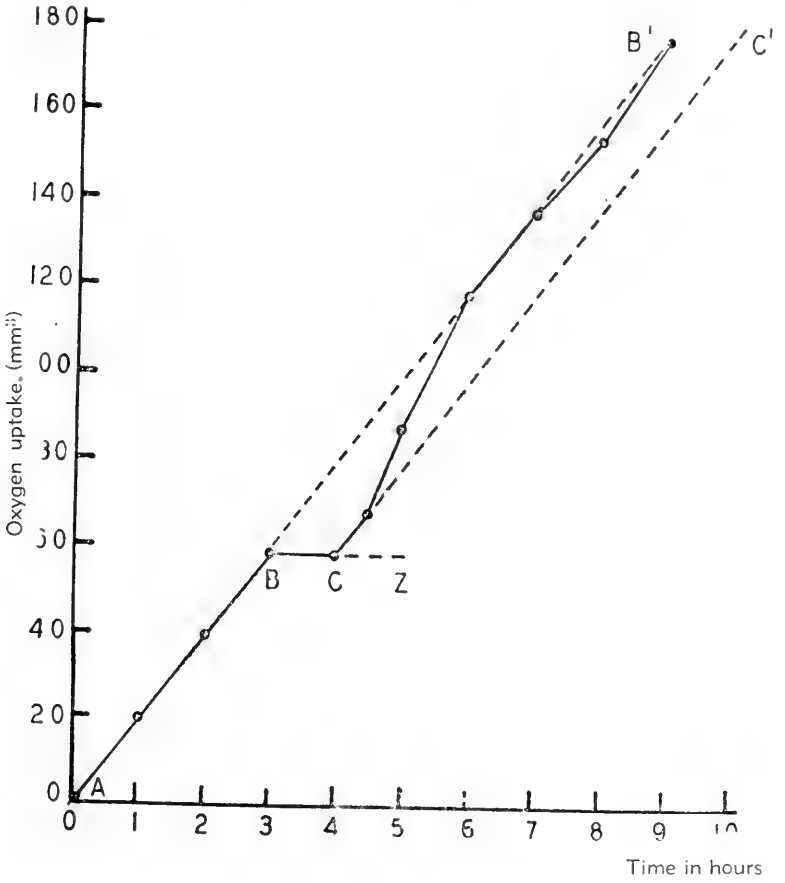


Fig. 7. Repayment of oxygen debt by *Planorbis corneus*. *AB*, normal oxygen uptake in aerobic medium. *BB'*, curve that would have resulted if the animal had been kept in aerobic medium. *BZ*, time of exposure to medium free of oxygen 2 hours. *BC*, true period of anaerobiosis (total time in oxygen-free medium minus time during which the oxygen bound to haemoglobin at the beginning of the exposure was still available), 61 minutes. *CB'*, oxygen uptake during recovery from anaerobiosis (the amount of oxygen used for the reoxygenation of the blood has been subtracted). *CC'*, theoretical curve that would have resulted if the animal had not repaid its oxygen debt. (According to Borden, 1931.)

the grasshopper *Melanoplus femur-rubrum* 1.9 times and in those of *Melanoplus differentialis* 1.25 times the debt (Gilmour, 1941a). It would be interesting to know whether the various tissues of these animals show a difference in the extent of repayment. This problem suggests itself because of the lower repayment (*cf.* above) observed by Bodine (1928) with intact animals. The

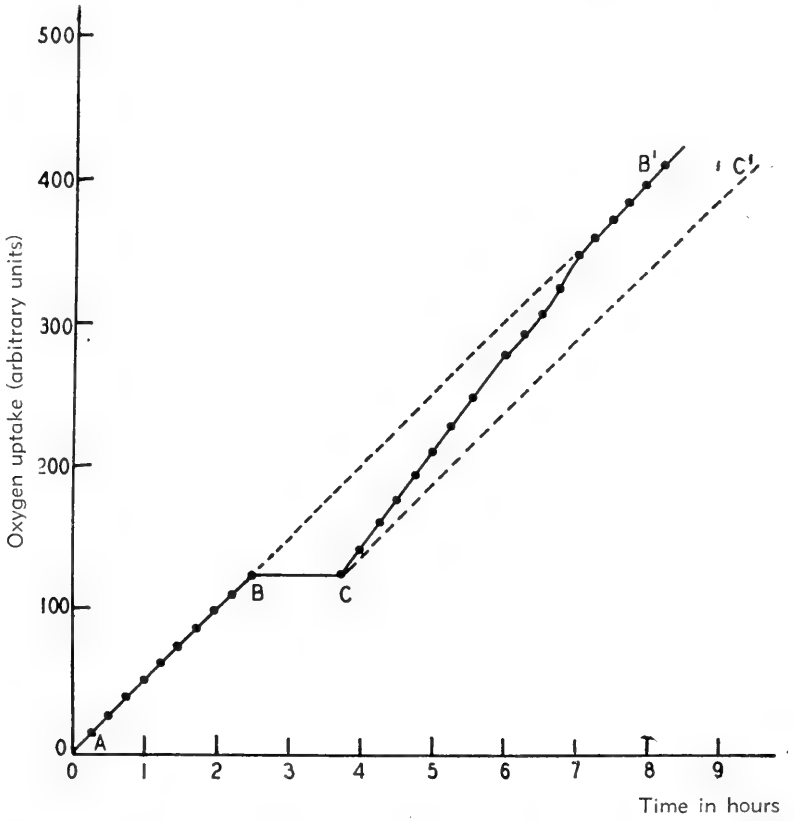


Fig. 8. Repayment of oxygen debt by *Periplaneta orientalis*. *AB*, normal oxygen uptake in aerobic surroundings. *BB'*, curve that would have resulted if the animal had been kept in aerobic surroundings. *BC*, time of exposure to anaerobic conditions (1¼ hour). *CB'*, oxygen uptake during recovery from anaerobiosis. *CC'*, theoretical curve that would have resulted if the animal had not repaid its oxygen debt. (According to Davis and Slater, 1926.)

probable reasons for the very high post-anaerobic oxygen consumption of the organisms mentioned in this paragraph will be discussed in a later section.

Besides the papers reviewed so far, which give quantitative data on the repayment of an oxygen debt, there are several others which only discuss the problem in a more general way, in particular those of Lund (1921), Dahr (1927), Jatzenko (1928), Kreps (1929), Hiestand (1931), van Dam (1935), Harnisch (1935a, 1935b, 1936, 1937b, 1939, 1941) and Gompel (1938). One more remark remains to be made here. The time required for the removal of the accumulated end products is occasionally much longer than in the examples used for illustrating the repayment of an oxygen debt. This happens, as has been shown especially by Gaarder (1918) for the *Tenebrio* pupa and by Jatzenko (1928) for lamellibranchs, when the anoxic period is long and the animals do not excrete large amounts of the end products.

We now turn to the question whether the post-anaerobic excess oxygen consumption shows characters differentiating it from normal respiration. According to Harnisch the post-anaerobic reaction is much more dependent on the oxygen tension, as observed in many different animals, especially in *Tubifex* (Harnisch, 1935a, 1935b, 1936), *Planaria* (1935b), *Chironomus* larvae (1936, 1937c) and *Ephemera* larvae (1939). Some of his curves are presented in Figure 9.

Harnisch (1935b) suggests two possible explanations of this phenomenon: (1) That the preceding anaerobiosis has brought about a fundamental change in the "internal milieu" and, consequently, in the normal course of aerobic energy production; (2) That the animals make use of two types of aerobic processes: the first, which would take place during normal respiration (primary aerobiosis), would be largely independent of the oxygen tension prevailing in the medium; the second would come into

play during recovery from anaerobiosis (secondary aerobiosis) and would be governed by the partial pressure

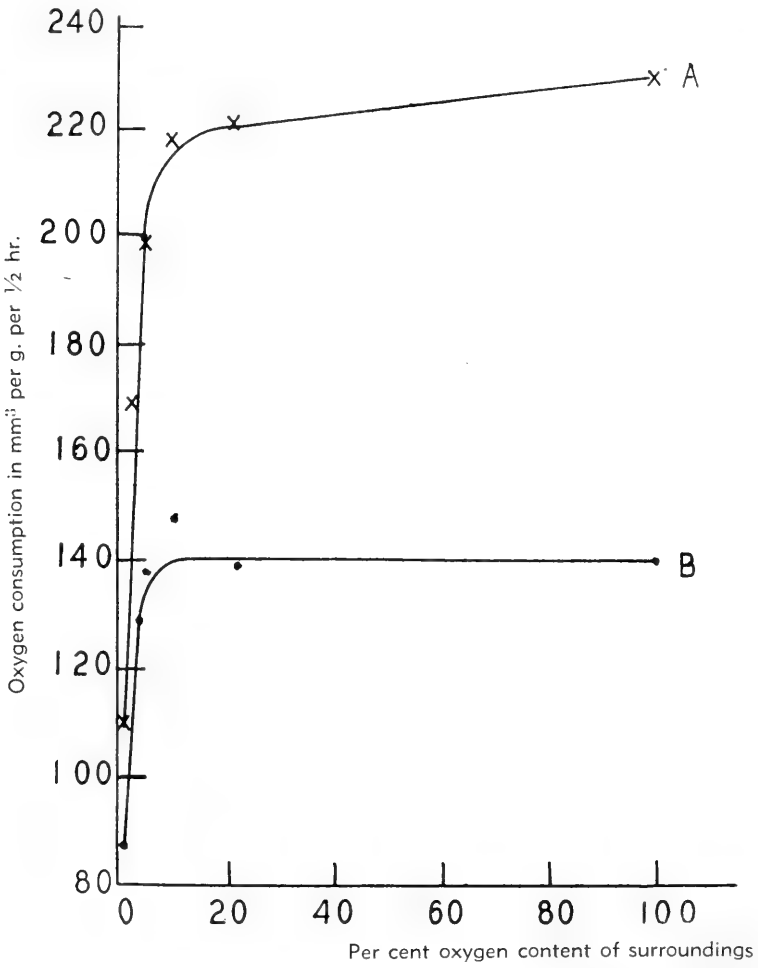


Fig. 9 Dependence of oxygen consumption on oxygen tension in larvae of *Chironomus thummi*. Batch *A*, which has been deprived of oxygen previous to the determinations, shows a more pronounced dependence of the oxygen consumption on the tension than batch *B*, which has been kept in well-oxygenated surroundings. (According to Harnisch 1936.)

of the oxygen in the medium. Harnisch is of the opinion that the experimental evidence does not favor the first hypothesis, and he brings forward the following arguments in favor of the second. (These arguments are derived largely from observations on the respiration of parasitic worms.) It is a well-established fact that the oxygen consumption of parasitic nematodes, trematodes and cestodes is dependent on the oxygen tension (*cf.* Harnisch, 1932a, 1933a, 1937a; Krüger, 1936; Laser, 1944). Contrary to what one finds in actinians this dependency is not materially changed if minced material is used instead of intact animals and consequently the distance through which oxygen has to diffuse cannot be the limiting factor of its availability. Harnisch, therefore, assumes that the greatest part, if not all, of the oxygen consumed by parasitic worms corresponds only to the post-anaerobic phase of the respiration (secondary aerobiosis) of free-living animals. Parasitic worms would thus show, at most, only traces of primary aerobic processes.

According to Harnisch (1937a, 1937b), an essential distinction between primary and secondary aerobiosis is that the enzymatic complex governing the primary processes is located within the cells, while that responsible for the secondary processes lies outside the cells, in the body fluid. Only the second of these complexes can therefore be removed by washing (when minced material is used). With this method it was found that in free-living organisms, like *Chironomus* larvae, only the post-anaerobic excess oxygen consumption disappears while in parasitic worms almost the entire oxygen consumption is eliminated.

Harnisch's evidence for a fundamental difference between primary and secondary aerobiosis seems good, on the whole. Von Buddenbrock (1939), however, raised against this view the objection that the dependency of

the post-anaerobic oxygen consumption on the tension is only a special instance of the rule that any increase in the respiratory rate will shift the critical oxygen tension at which the oxygen consumption begins to decline, towards a higher level. One could make a comparative study of the two opinions by investigating the relationships between the rate of oxygen consumption and the tension first in normal animals, kept, previous to the determinations, in well-aerated surroundings, then in "post-anaerobic" animals, and finally, in normal animals exposed to respiratory stimulants (*e.g.,* methylene blue, para-phenylene-diamine and others). If von Buddenbrock's view is correct the last group should show a curve similar to that of post-anaerobic animals; if Harnisch is right, it ought to resemble more that of the normal animals.

B. Production and retention of carbon dioxide, and the respiratory quotient. Regardless of whether or not the actual recovery processes result in the production of carbon dioxide, this gas is always formed during a post-anaerobic period. This is explained by the generally accepted view that the normal aerobic metabolism is resumed as soon as oxygen becomes available. It would, then, proceed side by side with the special reconstitution reactions. Rarely, however, if ever, is all the produced carbon dioxide actually excreted; a certain amount is usually retained and used to rebuild bicarbonates and carbonates destroyed during the anaerobic period.

The carbonate reconstruction requires, in most cases, a few hours, but the period of carbon dioxide retention does not always coincide exactly with that of excess oxygen consumption. As a rule, both seem to begin immediately upon the restoration of aerobic conditions, though there are exceptions. In snails (Dahr, 1927) the oxygen consumption is first at a low level, but it finally rises to high values. In *Zootermopsis* the carbon diox-

ide retention lasts somewhat longer than the excess oxygen consumption (Gilmour, 1940), in *Cryptocercus* both periods are of about equal length (Gilmour, 1940a), and in *Eustrongylides* the carbon dioxide retention ceases before the oxygen consumption has dropped to its normal level (von Brand, 1942). Some values for the amounts of carbon dioxide retained by worms and insects are given in Table 26.

The respiratory quotient of recovery is usually lower than that of normal metabolism. The decrease is especially noticeable immediately after the oxygen consumption begins; subsequently the values gradually return to normal again (*cf.* Table 26.). Lowered respiratory quotients have been observed, during recovery, (in addition to the instances mentioned in Table 26), in the *Tenebrio* larva and in grasshopper muscles (Gilmour, 1941, 1941a); carbon dioxide retention has also been shown to occur in *Tubifex* and in *Chironomus* larvae (Harnisch, 1936). (These later data are not included in Table 26 because in these cases no serial figures for the respiratory quotients have been presented).

It is obvious that any respiratory quotient determined during the recovery period is a composite of that of the normal respiration and that of the reconstitution processes. What are the values reached by the latter? In all cases in which only lactic acid is oxidized during recovery, the respiratory quotient would obviously be 1. But the nature of the end products of the anaerobic metabolism of invertebrates varies and, therefore, no general statement can be made about the values of the respiratory quotient.

Harnisch, on the basis of his observations on parasitic worms, has expressed the opinion that the respiratory quotients of the recovery processes are very low and might even reach the value of zero. He considers, as was said above, the oxygen consumption of these worms as

TABLE 26

RESPIRATORY QUOTIENTS (R.Q.) AND CARBON DIOXIDE RETENTION DURING A POST-ANAEROBIC PERIOD.

Species	Pre-anaerobic R.Q.	Duration of anaerobiosis in hrs.	Post-anaerobic R.Q. in 12 consecutive half-hour periods after restoration of aerobic conditions												Carbon dioxide retained in cmm. per g.
			1	2	3	4	5	6	7	8	9	10	11	12	
Worms															
Eustrongylides ignotus (larva)	1.06	16 to 18	0.76	0.53	0.58	0.58	0.68	1.02	1.09	1.11	1.15	0.96			322 1)
Tubifex tubifex	0.70	15 to 20	0.33	0.53	0.50	0.64									? 2)
Insects															
Chironomus thummi (larva)	0.7	14 to 15	0.5												? 3)
Cryptocercus punctulatus (defaunated)	0.69	1	0.26		0.54		0.64		0.54		0.66		0.77		81 4)
Cryptocercus punctulatus (with intestinal fauna)	1.09	1	0.51		0.76		0.80		0.85		1.11		1.13		81 4)
Periplaneta orientalis	0.89	½ to 1	0.71	0.72	0.85	0.86									59 5)
Zootermopsis nevadensis (defaunated)	0.76	4	0.12		0.28		0.42		0.45		0.47		0.57		28 6)
Zootermopsis nevadensis (with intestinal fauna)	1.05	4	0.71		0.75		0.79		0.86		0.91		0.93		21 6)

1) Von Brand, 1942. 2) Harnisch, 1935a. 3) Harnisch, 1936. 4) Gilmour, 1940a. 5) Slater, 1927. 6) Gilmour, 1940.

corresponding to the post-anaerobic metabolism of free-living organisms. It has, however, already been mentioned that other investigators could not obtain confirmation of Harnisch's findings; von Brand (1934a) and Krüger (1936) have shown that *Ascaris* forms more carbon dioxide in aerobic than in anaerobic conditions. The same authors (von Brand, *l.c.;* Krüger, 1937) have proven that the respiratory quotient of the aerobic processes of this worm lies between 0.9 and 1.0. They were able to separate the carbon dioxide correlated with the oxygen consumption from that coming from aerobic fermentation, by taking into consideration the acid production and correlating a certain amount of carbon dioxide with the latter. Von Brand (1942), finally, found definite indications that the respiratory quotient of the post-anaerobic processes of *Eustrongylides* is very similar to that of the normal aerobic metabolism (1.06). He observed that the quotient returned to normal as soon as the carbon dioxide retention ceased. Since, however, as mentioned above, the excess oxygen consumption of this worm lasts longer than the carbon dioxide retention, it follows that the respiratory quotient of the oxidations connected with the excess oxygen consumption must have been near unity. Otherwise an abnormal quotient should have been observed not only during the initial stages of the excess oxygen consumption but throughout its whole course.

3. THE CHEMICAL NATURE OF THE RECOVERY PROCESSES

It seems well established that, during the recovery of frog muscle from anaerobiosis, one fourth of the accumulated lactic acid is oxidized to carbon dioxide and water, and that the energy liberated thereby is used to resynthesize the remaining three fourths to glycogen (*cf.* Meyerhof, 1920). That a similar phenomenon occurs also in invertebrates appears probable, though there seem to be more possibilities than in vertebrates. In the

present state of our knowledge, however, we can only review the few recorded disconnected investigations.

So far only worms and insects have been studied. Dausend (1931) investigated the glycogen content of *Tubifex*, first in normal conditions, then after a stay of 22.7 hours in the absence of oxygen and finally in animals that were brought back to aerobic conditions for 4 and 6 hours after having endured the 22.7-hour deprivation of oxygen. He found a resynthesis of about 50 per cent of the anaerobically lost glycogen, *i.e.*, somewhat less than in vertebrates. But his conclusions are based on only a single series of experiments and, in view of the rather pronounced variations in glycogen content in invertebrates, a re-investigation on a broader basis is very desirable.

Von Brand (1937) made a large number of experiments with *Ascaris* and observed that 100 g. of worms may rebuild 70 to 150 mg. of glycogen during recovery. This corresponds to about 1/20 to 1/10 of the glycogen consumed during the preceding anaerobic period. But these figures might be somewhat low. During the recovery period at the same time that some glycogen is rebuilt, some of it may disappear as a result of the normal metabolic processes; the above figures show only the overall change. In any event, the resynthesis is much less pronounced than in vertebrates, a fact connected with the ability of these worms to excrete end products of the anaerobic metabolism. It is clear that the eliminated material is lost for resynthesis; only substances that actually have accumulated within the tissues can be used. Whether lactic or a lower fatty acid serves as substratum for these processes in *Ascaris* is not yet known.

In the case of *Tenebrio* larvae it has been proved definitely by Gilmour (1941) that lactic acid is resynthesized to carbohydrate. He found in his animals, at the beginning of the experiments, an average of 12.6 mg.

sugar per gram fresh weight; after a 4½-hour stay in nitrogen the value had decreased to 10.3 mg., but after a subsequent 5½ hours in air, it had again increased to 12.1 mg. As much as 1.8 mg. of sugar per gram of tissue had therefore been rebuilt. No comparable increase in glycogen was found, the glycogen content remaining practically unchanged during the period of recovery. Gilmour's findings are very interesting and show that the lower carbohydrates should not be neglected in studies of this kind, as is frequently done.

In the larvae of *Chironomus thummi* no glycogen resynthesis was found after the animals had been exposed for 14 to 22 hours to nitrogen and had then been allowed to recover for 18 to 48 hours in air (Harnisch, 1938). Very similar values were obtained at the two extremes of these exposure periods. Harnisch himself points out that a small resynthesis of glycogen is not necessarily excluded since some glycogen might have been used up during the normal metabolic processes of the rather protracted recovery period. At all events it cannot be questioned that a certain redistribution of glycogen within the various organs occurred. The use of specific stains revealed that, during recovery, the intensity of the glycogen reaction diminished within the fat body, but polysaccharide granules reappeared in greater number within the intestinal cells and the mesenchyme cells adjoining the muscle fibers. In both these types of cells most, and sometimes even all of the glycogen, had disappeared during anaerobiosis.

In the papers just reviewed a direct chemical determination of carbohydrates was made. We turn now to those investigations in which tentative conclusions as to the nature of the recovery processes were drawn from studies of the gas exchange.

Davis and Slater (1928a), found that in *Lumbricus terrestris* the excess oxygen consumption was about suf-

ficient to completely oxidize 1/5 of the lactic acid that was found to have accumulated in the body during anaerobiosis; thus, the proportion between excess oxygen and lactic acid was the same as in vertebrate muscle. It is therefore tempting to assume that the remaining 4/5 were resynthesized to glycogen, but direct glycogen determinations have so far not been made.

In the cockroach, on the contrary, the excess oxygen consumption was much too high to allow a similar conclusion (Davis and Slater, 1928). The ratio between lactic acid and excess oxygen would rather indicate that all the lactic acid was completely oxidized and that no resynthesis of carbohydrate occurred.

It was mentioned in a previous section that Gilmour observed in various insects an excess oxygen consumption far greater than expected. He (1941) emphasized the fact that in *Tenebrio* the above-mentioned synthetic processes leading to the formation of carbohydrate are sufficient to account for only a small part of this excess oxygen consumption, but he was unable to correlate the remainder with any specific process. On the whole, Gilmour's experiments (1940a, 1941a), on *Cryptocercus* and on grasshopper muscles led him to believe that the excessive repayment of the oxygen debt involves the oxidation of a greater part of the acids formed during anaerobiosis than occurs in vertebrates; in other words, his views and those of Davis and Slater have a common ground.

Still less is known as to whether any reactions involving fat or protein take place during recovery from anaerobiosis. Neither Harnisch (1938) nor Gilmour (1941) found any decided changes in the fat content during the recovery of the larvae of *Chironomus* and *Tenebrio,* with which they respectively worked.

Braconnier-Fayemendy (1933) showed that during a 48-hour period in the presence of air, following a 24-

hour period of deprivation of oxygen, leeches (*Hirudo medicinalis*) excrete a much larger quantity of nitrogenous substances than if they are exposed to the anaerobic conditions alone. This increase was observed in all determined fractions (total non-protein nitrogen, ammonia, amino-acids and creatinine). An increase in post-anaerobic ammonia excretion was also described by Harnisch (1943) for the larva of *Chironomus bathophilus*. In neither of these cases, however, is it clear whether the increased elimination was simply due to an impaired excretion during the anoxic period or to true recovery processes, *i.e.*, it is not yet definitely known whether these various substances themselves or their partially decomposed precursors had accumulated in the body.

SUMMARY

Partial transition from aerobic to anaerobic metabolism.

1. In nature, partial transition from aerobic to anaerobic metabolism takes place in many invertebrates when the oxygen tension in the surroundings drops below a certain level. The critical oxygen tension at which such a change occurs varies from species to species.

2. The evidence for an actual transition to a partly anaerobic metabolism under reduced oxygen tensions is based on: (a) the excretion or accumulation of end products of anaerobic metabolism, (b) an increase in the rate of carbohydrate consumption, (c) an increase in the respiratory quotient and (d) the accumulation of an oxygen debt during the stay in the oxygen-deficient medium.

3. The invertebrates are frequently divided into two groups according to their reaction to lowered oxygen tensions. In the first group the oxygen consumption remains constant over a wide range of tensions, while in the second it drops when the tension is still high. A strict distinction between these two groups is, however, not possible since the critical oxygen tension at which the oxygen consumption of a given species begins to decline is not fixed but depends largely on external and internal factors.

4. Experimentally, a partial transition to anaerobic metabolism can be induced, not only by lowering the oxygen tension of the surroundings, but also by eliminating a large part of the aerobic processes by means of various respiratory poisons and, occasionally, by changing the salinity of the medium.

Aerobic fermentations.

1. "Aerobic fermentations," that is, fermentations in the presence of an abundant supply of oxygen, have often

been reported in protozoa, in free-living and parasitic worms and in arthropods, but rarely in molluscs, and never yet in echinoderms; they might occur in coelenterates.

2. The best evidence for aerobic fermentations is the direct demonstration that non-oxidized or partly oxidized substances are formed when oxygen is plentiful. The respiratory quotient and the rate of carbohydrate consumption are not infallible criteria.

3. The assumption that oxidations remain incomplete in the presence of a surplus of oxygen (aerobic fermentations) because the organisms were previously adapted to an anaerobic life and are now incapable of utilizing oxygen does not hold in the case of blood parasites in which the ability to utilize oxygen from the erythrocytes has been demonstrated.

4. Some aerobic fermenters can perhaps obtain energy from both the *synthesis* and the *breaking down* of fat, the former in the absence of air, the latter in aerated media. (This is in contrast with what generally happens in most animals: usually in aerobic organisms the synthesis of fat from carbohydrates serves to store energy for future need, and liberation of energy during this synthesis is only incidental; in anaerobic organisms, on the contrary, the fatty acids formed are waste end-products, and the energy production during fat synthesis is the essential feature.)

5. The relative importance of aerobic fermentations and of other metabolic processes in supplying energy has been studied primarily in parasitic worms. It has been claimed that aerobic fermentations alone furnish all the energy required by the worms and that the energy gained by aerobic oxidations is wasted. Recent investigations, however, indicate that the two processes are interdependent and that the energy derived from both sources may be used by the parasites in aerated surroundings.

The sources of energy in anaerobiosis.

1. Lower carbohydrates serve as mother-substances for anaerobic processes both in the presence and in the absence of oxygen. This has been shown especially in the case of protozoa; the data concerning metazoa are rather scanty.

2. *Glycogen* (stored in the body) is the most common substrate for anaerobic energy-yielding processes. Its storage and its anaerobic utilization have been demonstrated for representatives of all the major phyla of invertebrates with the exception of the coelenterates in which the question of anaerobic glycogen consumption has not yet been studied. The rate of anaerobic degradation is, in the great majority of the cases investigated, much higher than that of the corresponding aerobic reaction, a fact which is to be expected if the same total amount of energy is to be produced since anaerobic processes yield much less energy than aerobic ones.

3. *Starch* is commonly utilized under anoxic conditions by many parasitic protozoa, and its degradation products are known to promote also the development of intestinal worms.

4. The anaerobic utilization of *cellulose* is relatively rare but has been demonstrated in termite- and in rumen-protozoa.

5. The available data concerning anaerobic *fat* consumption are in most cases open to question; the best evidence was obtained with minced material from *Calliphora* pupae.

6. The anaerobic *protein* metabolism is poorly developed in most invertebrates. It is probably related with the synthesis of new protoplasm in many organisms that habitually live in surroundings very poor in oxygen. But in some cases, as in *Hirudo,* the anaerobic protein degradation seems also to have a function in the production of energy.

Anaerobic processes.

1. The anaerobic processes liberating energy from carbohydrates are more varied in invertebrates than in vertebrates. Lactic acid fermentation occurs in both groups, but well authenticated cases of fatty acid fermentations and of mixed fermentations (with lactic acid, fatty acids, dibasic acids or even occasionally alcohol as end products) are recorded only for invertebrates. In some cases the fatty acids (higher and lower) are the only organic end products, though it is possible that lactic acid is formed as an intermediate substance.

2. No reaction in which fatty acids are attacked under anaerobic conditions is known to occur in invertebrates; but glycerol may serve as a substrate for anaerobic metabolism.

3. Little is known on anaerobic protein degradation. Ammonia is the most commonly formed end product.

4. Carbon dioxide is found in practically all experiments with anaerobically kept invertebrates. It may originate directly from the breakdown of organic food material, or it may be set free from inorganic substances under the action of acids.

5. Combustible gases, especially hydrogen, are formed occasionally during anaerobiosis; the best-known cases have been reported among parasitic protozoa.

6. The few available data on anaerobic enzymes in invertebrates are too few to allow one to make any general statement as to their action.

Recovery from anaerobiosis.

1. Many invertebrates which become paralyzed when deprived of oxygen revive when brought back to aerated surroundings. The time required for the resumption of movement depends on the species and also on the length of the preceding anaerobic period.

2. The oxygen debt incurred under anaerobic conditions is usually repaid upon readmission of air. The extent of the repayment is different in various species, depending on the chemical nature of the recovery processes and also on whether or not non-oxidized substances were excreted during the anoxic period.

3. Carbon dioxide is very frequently retained during recovery and is used in the resynthesis of bicarbonates that had been decomposed in the preceding anaerobic processes. This usually leads to abnormally low respiratory quotients at least during the initial stages of recovery.

4. The chemical nature of the recovery reactions appears to be more diversified in invertebrates than in vertebrates. Carbohydrate resynthesis has been observed in worms and insects, but this phenomenon is, in most cases, less pronounced than in vertebrate muscle. In other invertebrates, especially in insects, a much greater proportion of the accumulated end products, sometimes even all of them, are oxidized and no resynthesis of glycogen takes place.

5. The data on the nitrogen metabolism of invertebrates during recovery from anaerobiosis are insufficient to permit one to decide whether any special resynthesis processes take place or whether delayed oxidations are completed.

PART III

GENERAL ADAPTATION TO THE LACK OF OXYGEN AND ORIGIN OF ANAEROBIOSIS

CHAPTER I

THE PLACE OF ANAEROBIC PROCESSES IN THE GENERAL METABOLISM OF INVERTEBRATES

Our survey of the reactions of invertebrates to the lack of oxygen showed that there are various degrees of resistance to asphyxiation. Some organisms, like the sapropelic ciliates and a number of sewage protozoa, seem to be *obligate anaerobes, i.e.,* they thrive only if oxygen is practically absent. Others are *facultative anaerobes, i.e.,* they normally live in surroundings rich in oxygen, but can withstand the absence of that gas for a certain length of time. The majority of invertebrates reviewed (but not necessarily the majority of *all* invertebrates) fall into this category. A last group can be termed *obligate aerobes.* These show only a negligible resistance to the lack of oxygen; such are the cephalopods.

While this classification is quite convenient for the discussion of ecological relationships, it is clearly insufficient to characterize the various modes of energy production and to bring out the relative importance of anaerobic processes in the life and metabolism of various animals. Von Brand and Harnisch (1933) proposed the following classification which is based on the mode of energy production.

(A) Monobionts: animals gaining their energy exclusively through anaerobic reactions. (1) *Anaerobic organisms:* animals injured even by low oxygen tensions. (2) *Anoxybiotic organisms*: animals capable of living in

either the presence or the absence of oxygen though gaining their energy exclusively from anaerobic reactions. The aerobic reactions which occur in the presence of oxygen would not be related to the production of energy.

(B) Amphibionts: animals capable of gaining energy from both aerobic and anaerobic processes. (1) *Euroxybiotic organisms:* animals which normally gain their energy through aerobic metabolism, but whose anaerobic functions are well enough developed to allow a long survival under anaerobic conditions. (2) *Stenoxybiotic organisms:* animals which normally gain their energy through aerobic metabolism and which do not survive long under anaerobic conditions, either because anaerobic functions are not well developed, or because the animals are injured by the accumulating end products of the anaerobic metabolism.

Since this classification was published, new facts have become known and a revision is necessary. The group "anoxybiotic organisms," as defined above, is no longer justified. The newer data on the metabolism of parasitic nematodes indicate clearly that these animals can derive at least part of their energy from aerobic reactions. Future investigations will probably reveal similar relationships in cestodes and trematodes. Another change is necessitated by our increased knowledge concerning the prevalence of energy-producing fermentations in the presence of air in many invertebrates. We, therefore, propose the following revised classification.

(A) Animals that gain their energy solely through anaerobic reactions; they are injured by relatively low oxygen tensions. Example: sapropelic ciliates.

(B) Animals capable of gaining energy from both aerobic and anaerobic reactions.

(1) *Aero-fermenters:* animals with a metabolism characterized by the prevalence of incomplete

oxidations in aerobic conditions (so-called "aerobic fermentations").

(a) Euryanoxybiotic aero-fermenters: animals with a well-developed anaerobic metabolism, allowing them to survive for a long time under anaerobic conditions. Example: many parasitic worms.

(b) Stenanoxybiotic aero-fermenters: animals in which the anaerobic metabolism is not well enough developed to allow long survival under anaerobic conditions. Example: trypanosomes.

(2) *Holo-oxidizers:* animals in which the metabolism in the presence of air consists exclusively or predominantly of complete oxidations.

(a) Euryanoxybiotic holo-oxidizers: animals with well-developed anaerobic functions under anaerobic conditions. Example: some parasitic worms (*Trichinella* larvae), probably *Owenia*.

(b) Stenanoxybiotic holo-oxidizers: animals with a poorly developed anaerobic metabolism, not sufficient to allow survival for long periods under severe lack of oxygen. Example: many insects, cephalopods.

The group *A* in this classification calls for some further discussion. While the obligatory anaerobic invertebrates are injured rapidly by oxygen at a tension of about one atmosphere and probably at somewhat lower tensions (Wetzel, 1928; Liebmann, 1936; Lackey, 1932; Trager, 1934; Westphal, 1934; Hungate, 1939, 1941, 1942; Hastings, 1944), it has never been ascertained whether extremely low tensions are also toxic. Indeed, the fact that termite flagellates can be cultured successfully in

tubes which are overlayed with vaseline may well indicate that low oxygen concentrations are not toxic, since a vaseline seal is not perfectly air-tight. Similarly, it is not certain that the culture methods employed for the rumen protozoa excluded the last traces of oxygen. The sapropelic ciliates have never been cultured successfully in the laboratory; they survived only for short periods under the experimental conditions to which they were subjected. As to the sewage protozoa, apparently no attempt has been made so far to study the influence of low oxygen concentrations on them.

Assuming that there are non-toxic concentrations of oxygen for the organisms just mentioned, a very important problem which has not yet been tackled is whether these animals are able to consume that oxygen. This problem will be difficult to solve, since it presupposes the establishment of bacteria-free cultures. The view recently expressed by Krogh (1941) is of great interest in this connection. He writes: " . . . from the observations on many different animals, from worms and insects to diving mammals, the impression remains that the continued provision of quite small and in themselves absolutely insufficient amounts of oxygen is essential for the successful resistance against asphyxiation. . . . It may be a more fundamental phenomenon, perhaps analogous to the necessity of metabolizing a little carbohydrate along with fats." The above protozoa should be suitable material on which to test whether Krogh's view applies also to unicellular animals. If such experiments should yield positive results the organisms in question would, strictly speaking, not be true anaerobes. (The setting aside of these organisms in a special group will, nevertheless remain justifiable, since their metabolism would, under any condition, be characterized mainly by anaerobic processes, and since it appears necessary to separate them from typical euryanoxybiotic aero-fermenters which are much less sensitive to the toxic effects of oxygen).

CHAPTER II

BASIS FOR THE DIFFERENCES IN ANAEROBIC FUNCTIONS AMONG INVERTEBRATES

We shall discuss here the three following problems: (1) Why is oxygen injurious to anaerobic invertebrates? (2) Why can some organisms be deprived of oxygen, while others cannot? and (3) How is anaerobiosis related to oxidation-reduction potentials?

1. WHY DOES OXYGEN INJURE ANAEROBIC INVERTEBRATES?

Regardless of whether future investigation will show that the protozoa of the group A in our classification are strictly anaerobic, or utilize oxygen when they have access to it, or even require traces of that gas, the fact remains that they are injured at least by higher concentrations of oxygen. The question as to the immediate cause of this injurious action has been studied primarily by bacteriologists. Jahn (1941) has recently summarized their findings and discussed to what extent these can be applied to protozoa.

He mentioned the following theories: 1. Oxygen is itself toxic; 2. Anaerobes do not contain catalase and are therefore not capable of destroying the toxic hydrogen peroxide which is formed under the action of oxygen; 3. The growth of anaerobes is dependent upon the presence of a low oxidation-reduction potential in the medium, the attainment of which is prevented by oxygen; 4. Oxygen forms a loose chemical complex with the respiratory system of obligatory anaerobes, and thereby inhibits its activity.

According to Jahn there is little evidence for the first two theories; though, of the two, he prefers the second for explaining the principle of Cleveland's (1925, 1925b) method of eliminating parasitic protozoa from a variety of hosts by oxygenation. The second theory has also

received strong support recently through Laser's (1944) work on *Ascaris*. This worm is not a strict anaerobe, but a euryanoxybiotic aero-fermenter which succumbs to high oxygen tensions. Laser demonstrated, by a direct chemical method, that in ascarids killed by oxygen an accumulation of hydrogen peroxide occurs. The catalase content of these worms is very low (Lesser, 1906) and not sufficient to counteract the rapid hydrogen peroxide production occurring at high oxygen tensions, but it does suffice to destroy the amounts formed at low tensions, or the peroxide may be eliminated in some secondary oxidation reactions catalyzed by methaemoglobin.

As to the third theory Jahn writes: "The chief difficulty in interpreting experiments pertaining to the effect of E_H on growth is that it is necessary to change O_2 tension in order to change E_H. This makes an experiment containing only one variable seemingly impossible to execute, and the theory, therefore, has not been amenable to experimental approach."

On the fourth theory no experimental evidence has yet been presented.

Thus the available evidence is much too incomplete to allow any conclusion on the mechanism of injury by oxygen. A comparative investigation of the catalase content and the susceptibility of invertebrates to oxygen might be the most promising approach.

2. WHY CAN SOME INVERTEBRATES TOLERATE A LACK OF OXYGEN WHILE OTHERS CANNOT?

The ability to withstand the deprivation of oxygen rests, presumably, on two conditions: (*A*) the organisms should be able to gain sufficient energy from the anaerobic metabolism and (*B*) they should be immune to the injury usually occasioned by the accumulation of metabolic end products. These two conditions will now be discussed.

A. Ability to gain sufficient energy from anaerobic metabolism. The only direct measurements of the energy

gained by anaerobically kept invertebrates are those made on *Ascaris* by Meier (1931). He found a heat production of 0.300 g. cal. per gram per hour, but a comparison with worms kept under aerobic conditions is not possible since no data for the latter are available; furthermore *Ascaris* is an organism which under natural conditions leads a predominantly anaerobic life. There is a good deal of indirect evidence, however, that many normally aerobic invertebrates can gain all the energy needed for the maintenance of their normal life processes even during protracted periods of oxygen deficiency. This can reasonably be assumed in all those previously reviewed cases in which the organisms survive for a long time without visible damage. In many free-living or parasitic protozoa and worms the anaerobic energy production must be relatively high since the animals are able to move freely in the absence of oxygen. In other instances, as in *Trichinella* larvae and in most clams, the energy requirements are doubtless lowered since the muscular movements cease; but the anaerobic processes are still potent enough to maintain the animals alive. A last group which includes the majority of the hitherto studied insects, consists of animals that are incapable of gaining much energy under anaerobic conditions, as evidenced by their short survival time. It is not possible at present to decide whether their biochemical organization, perhaps their cellular enzyme complex, does not allow fermentations, or whether some organ or tissue is rapidly injured by the accumulation of end products of the anaerobic metabolism.

One may approach this problem experimentally by studying more thoroughly the resistance of the various tissues of a given animal against asphyxiation and, at the same time, determining the rate of anaerobic metabolism. Such a study might reveal that the inability of the nervous system to withstand the deprivation of oxy-

gen is responsible for the early asphyxiation of many invertebrates. It has been observed (Buck and Boche, 1938; Richards, 1941) that in insects kept under anoxic conditions chromatin clumping occurs in the nuclei of various tissues. Richards considers this change not as the actual cause of death, but rather as a criterion of asphyxiation. It is of significance that this clumping was observed especially in the cells of the central nervous system of *Culex* larvae. As to the mechanism of the clumping, Richards (personal communication) thinks that a lowering of the cellular pH may explain it, as is suggested by an observation of Nassonov (1932a) on a similar phenomenon. It is indeed possible that sufficient lactic acid accumulates during asphyxiation to change the reaction of the cell content. If this view is correct, the inability of the nerve cells to eliminate or detoxicate the end products of the anaerobic metabolism would be largely responsible for the early death of insects kept in anoxic surroundings.

B. Immunization against the injury caused by the accumulation of metabolic end products. It is a well-known fact that a vertebrate muscle loses its ability to contract in the absence of oxygen or when the oxygen supply is insufficient, as in fatigue, because of the accumulation of non-oxidized substances, primarily lactic acid. A similar toxicity of the end products of the anaerobic metabolism can be observed in many invertebrates. The actual accumulation of lactic acid has been demonstrated repeatedly in animals that are not well adapted to life in anoxic environments, for example, the earthworm (Davis and Slater, 1928a), echinoderms (Boyland, 1928) cockroaches (Davis and Slater, 1926), and other insects (Gilmour, 1941). But no quantitative determination has yet been made of the concentrations which bring about reversible paralysis or which injure the animals irreversibly.

Invertebrates which can live anaerobically either have a mechanism for the excretion of toxic end products or they elaborate non-toxic end products.

The importance of the excretion mechanism, as an adaptation to anaerobic life, seems to have been first emphasized by Lesser (1912). The facts proving that the ability to excrete organic acids and other end products of the anaerobic metabolism into the medium is widely distributed amongst invertebrates have already been mentioned previously. The accumulation of acids within certain body fluids should perhaps also be regarded as true excretion, as when, for example, considerable amounts of lactic acid and perhaps other acids accumulate in the fluid of the mantle cavity of clams.

The interesting question why some invertebrates are able to eliminate these substances from their tissues while others are not can unfortunately not be answered yet. No correlation with the morphological structures which serve excretory purposes seems possible.

In this connection a word should be said also of the elimination of non-toxic end products of anaerobic metabolism. Among them we may consider primarily higher fatty acids and true fat. As explained previously such substances represent the main end products in some free-living protozoa, some parasitic worms and arthropods. If the periods of anaerobiosis are not excessively long, the fatty substances may simply be deposited within the body where they cause no damage. This happens, for example, in protozoa like *Stentor,* or in parasites like the *Gasterophilus* larva. However, even a completely non-toxic substance does not accumulate indefinitely. One finds, therefore, that in animals which live permanently in oxygen-free or oxygen-poor habitats and which elaborate fat, the latter is excreted just like a toxic substance would be. This is illustrated in the case of *Fasciola hepatica.* A modified form of this behavior is encountered in *Moniezia*

expansa, where some fat is excreted but nevertheless large amounts accumulate in the proglottids. Such an accumulation is of no consequence since the proglottids of tapeworms are only ephemeral structures.

A different detoxication mechanism was suggested by Harnisch (1939). According to him certain anaerobic processes in insects may liberate oxygen which, in turn, could be used to detoxicate poisonous substances. Further experiments seem, however, necessary before the possible significance of this assumption can be judged.

3. RELATION OF ANAEROBIOSIS TO OXIDATION-REDUCTION POTENTIALS

The problem of the relations of anaerobiosis to oxidation-reduction potentials has been studied rather extensively by the bacteriologists, and, for a theoretical discussion, the reader is referred to a review by Clark (1934).

Certain metabolic processes are directly dependent on the attainment of a definite potential. For example, the liberation of hydrogen by a bacterial culture is possible only when the reduction intensity of the culture medium reaches that of hydrogen at one atmosphere pressure; some anaerobic bacteria indeed are known to exceed that intensity, that is, to produce a "hydrogen overvoltage" and thus to liberate hydrogen (Clark, 1924).

It is also a well-established fact that bacteria are able to condition the oxidation-reduction potential of their media (*cf.,* for summaries, Rahn, 1932 and Stephenson, 1939), partly through their oxygen consumption, partly through the elaboration of metabolic end products of low reduction potential. (The latter mechanism is assumed when the potential reached by the cultures is lower than that of deoxygenated sterile controls.)

With invertebrates, similar studies can be carried out only in bacteriologically sterile cultures since it would otherwise not be possible to distinguish between the action

of the test animals and that of the bacteria. Jahn (1935), using sterile cultures of *Chilomonas paramaecium,* a normally aerobic organism, observed that the E_H of the medium decreased from the second day after inoculation for a period of several days and then rose again to almost the original level. Thus the curves resembled those found with cultures of aerobic bacteria. In this case the oxygen consumption of the protozoa present in various numbers at different times and the diffusion of oxygen into the medium are probably sufficient to explain the observed changes, there being no necessity of assuming the production of reducing substances.

Organisms better adapted to anaerobic life than *Chilomonas* would probably produce lower potentials. It would, then, be of particular interest to study the time-potential curves in pure cultures of *Trichomonas foetus.* This organism elaborates an inflammable gas which may be hydrogen (Andrews and von Brand, 1938). In conformity with the findings on bacterial cultures, pronounced changes in the oxidation-reduction potential should be expected. As Clark (1924) remarks: "It is extremely difficult to conceive of molecular oxygen playing any part in the activity of a cell that is producing a hydrogen overvoltage and tearing to pieces by reductive action materials which resist strong chemical reducing agents." Applied to the present case this would mean that once hydrogen production begins, the molecular oxygen that may still be in the medium would play no further role. (When oxygen is present at the tension of the atmospheric air, however, it is being utilized by *Trichomonas,* as Riedmueller (1936) has demonstrated.)

In nature, inorganic constituents, and especially the bacterial flora present in almost any habitat will be much more potent than the metabolism of the invertebrates in influencing the oxidation-reduction potential of the surroundings.

The question then arises of the effect that the oxidation-reduction potential of the *medium* has on invertebrates. Efimoff, Nekrassow and Efimoff (1928) reported that the E_H of hay infusions changes from −322 mv. shortly after the preparation of the infusions to +422 mv. ten months later. This change was probably due primarily to bacterial activity. They further observed that the appearance and disappearance of various protozoan species was most rapid during the periods of rapid change in potential.

Jahn (1933a) studied the growth rate of *Chilomonas* in bacteria-free cultures in which definite potentials had been established by the addition to the basic medium, of H_2O_2 and of compounds containing the SH-radical. The results suggested that the growth rate of the ciliates was influenced by the potential, but the evidence was not quite conclusive. Jahn (1933a, 1934) himself pointed out that an alternative explanation could be found in a possible influence of the various amounts of reduced sulfur present in the cultures.

Another interesting study is that of Jacobs (1941) who investigated the oxidation-reduction potential of pure cultures of bacteria in relation to the cultivation of *Endamoeba histolytica*. He observed that excystment and growth of the amoebae occurred at potentials of −114 to −150 mv., but that growth was more pronounced in the range of −300 to −500 mv; in other words, this organism seemed to require, in culture, a rather strongly reducing medium. Jacobs' results are in fair agreement with the fact that *Endamoeba histolytica* usually lives in surroundings with quite low oxygen tensions.

As to the influence of the oxidation-reduction potential of the surroundings on *metazoa*, we have the following few data. Kollath and Erhardt (1936) studied the survival *in vitro* of the fluke *Opisthorchis felineus* in

media with a range of potentials varying from −330 to +227 mv. and found the maximum survival at −81 mv. Hutchinson, Deevey and Wollack (1939) investigated the potential of lake waters, about 0.5 m. above the mud, in cases where the latter harbored different chironomids. If the water had a potential above +400 mv. *Tanytarsus* was present; if the potential was below +300 the fauna was of a true *Chironomus* type; while *Endochironomus,* or mesotrophic *Chironomus* characterized the intermediate range. The authors point out, however, that their study has no direct bearing on the question of the influence of the potentials on the respiratory physiology of mud-dwellers, but "that the relationship here established is primarily of typological interest, indicating the reducing power of the mud as it affects the open water." In the mud itself, *i.e.,* in the immediate surroundings of the animals, lower potentials must be expected, but these seem to have been measured in only one case, in which a value of +150 mv. was found.

The last question to be discussed is whether the intracellular oxidation-reduction potential varies with the oxygen content of the medium and whether there are differences in this respect between animals that are purely or predominantly aerobic and those that are adapted to a predominantly anaerobic life. The intensity of the intra-cellular potential is usually expressed by the rH value, that is, by the negative logarithm of the hypothetical hydrogen pressure in equilibrium with the oxidation-reduction system in question; this mode of expressing the potential will be used in the following discussion.

Needham and Needham (1926) observed that the normally aerobic *Amoeba proteus* maintains an unchanged rH of about 18 in water saturated with air, oxygen or hydrogen. With *Nyctotherus cordiformis,* on the contrary, which occurs in the presumably oxygen-poor intestine of frogs, the rH was between 19 and 20 under

aerobic conditions and between 9.5 and 10.5 under anaerobic conditions. The internal rH of *Opalina ranarum,* another frog parasite, seems to be even lower, as the above authors (1927) deduce from observations by Becker (1926) that the protoplasm of *Opalina* reduces Janus Green, a dye having an electrode potential below that of indigotine monosulphonate. They then conclude: "It seems that in the facultative anaerobe the oxidation-reduction potential can be adjusted to the environment, while in the strict aerobe changes in the environment which would lead to such an alteration in the rH bring about death." The first part of this statement is confirmed by the observations of Nassonov (1932) that the internal rH of the *Ophryoscolecidae,* typical anaerobic ciliates of the rumen of herbivores, changes readily with that of the surroundings. But the second part has not been corroborated.

Ssinitza's (1936) findings are in disagreement with those of most other workers. According to her the rH of the blood of *Chironomus plumosus* was lowered during anaerobiosis, while that of *Psectrocladius brevicalcar* was slightly raised. This latter larva, however, resists asphyxiation much better than the former.

The majority of investigators, on the contrary, found that in normally aerobic invertebrates (amoebae, reproductive cells of echinoderms, tissues and blood of snails and insects) the rH is lowered when oxygen is excluded from the surroundings. The rH in aerobic conditions is listed as lying between about 12 and 20, and in anaerobic conditions between about 5 and 9. For details the following papers should be consulted: Cohen, Chambers and Reznikoff, 1928; Chambers, Pollack and Cohen, 1929; Aubel and Lévy, 1929, 1929a, 1930, 1930a; Chambers, Cohen and Pollack, 1932; Alexandrov, 1932; Machlis and Green, 1933; Cohen and Chen, 1933; Cham-

bers, Beck and Green, 1933; Chambers, 1933; Clark, 1934.

As was pointed out by Chambers, Cohen and Pollack (1932) some of the discrepancies in the literature can probably be explained by the fact that not enough attention was paid to the "capacity" factor, *i.e.,* to the total reducing power of the cells. Occasionally the amounts of dye injected were so large as to overtax this reducing power.

It should furthermore be kept in mind that all the measurements recorded deal with the "overall" potential of the cells. Jahn (1941) recently remarked that "the colloidal nature of protoplasm makes possible the existence of different E_H values in different phases of the substance, and the differential adsorption of oxidized and reduced material at interfaces may produce a potential different from that in any of the phases." This conception may, in the future, if precise data can be obtained, lead to the discovery of important mechanisms for the adaptation or non-adaptation to anaerobic life in various organisms.

A final observation, that possibly is related to the internal oxidation-reduction potential, may be mentioned here. Nassonov (1932) found that the macronucleus of typically aerobic protozoa (*Paramaecium, Stentor* and others) normally does not stain with vital dyes, unless the organisms are damaged. The macronuclei of protozoa living in oxygen-poor or oxygen-free surroundings (rumen ciliates, *Balantidium* and others) stain rapidly in perfectly normal specimens.

CHAPTER III

THE ORIGIN OF ANAEROBIOSIS IN INVERTEBRATES

The idea that, in phylogenetic development, the anaerobic type of life preceded the aerobic has been expressed repeatedly (Pütter, 1905; Snyder, 1911; Clark, 1924; Kollath, 1935; Koschtojanz, 1935a; Szent-György, 1939), but it seems not to have received much attention from biologists and physiologists. Those who discussed it were, in general, not inclined to accept it. Krogh (1916), for example, who is a recognized authority on respiration, designated the view that the anaerobic metabolism is more primitive than the aerobic as a "singular proposition" although he conceded that it "probably contains an element of truth, in so far as the initial stage in the breakdown of the foodstuffs is probably not oxidative." Jordan (1934) rejects the idea because the elimination of the end products of the anaerobic metabolism seems to require special mechanisms; and from this standpoint the anaerobic metabolism cannot be more primitive than the aerobic.

Snyder (*l.c.*), the most ardent proponent of the phylogenetic precedence of anaerobiosis, formulates the chief evidence as follows:

"1. What appear to be the simplest forms of life are anaerobic still and the majority of lower organisms, both plant and animal, can live under anaerobic conditions more or less continuously.

"2. The fundamental chemical processes of the cell in all organisms, even the highest, are anaerobic, phenomena of oxidation being of secondary or ulterior importance.

"3. The relations of anaerobic and aerobic life are genetic and we have clear evidence of the gradual evolution of the latter from the former, an increasing need

of oxygen accompanying an increasing complexity of chemical and morphological organization.

"4. This increase in need of oxygen, as complexity increases, is paralleled in the growth of the individual organism, the ability to endure complete abstraction of oxygen varying inversely with age and size.

"5. The oxygen of the atmosphere appears to have been formed exclusively by plant action. In the beginning there was probably no oxygen free: if there had been any, it would have been very quickly absorbed by the unoxidized substances of the earth's crust or the quantity would have been so small as to be practically negligible."

Since Snyder wrote his article some new information has become available on several of the points he discusses, and, though we look upon his paper as a stimulating piece of work, we find ourselves in disagreement with most of his statements.

To point 1: Whether the simplest forms of life are still anaerobic is a debatable matter. According to Snyder they are represented by the anaerobic bacteria, but, to the writer's knowledge, no generally accepted phylogenetic relationship of the various groups of bacteria has been established as yet. As to the statement that the majority of lower organisms can live more or less continuously under anaerobic conditions, our review[1] constitutes sufficient proof to the contrary in so far as the animals are concerned.

To point 3 (in part): The assumption that an increasing complexity in morphological organization increases the need for oxygen is contradicted by the fact that amongst protozoa the strictest anaerobes are found amongst the most specialized and complex forms, for example, the sapropelic and rumen ciliates. In no phy-

1. Part I, Chapter III.

lum can a connection between the ability to live anaerobically and the morphological organization be established. Even looking upon the animal kingdom as a whole one fails to perceive the postulated phylogenetic relationship. The anaerobic functions found in different groups are most frequently related to ecological factors. One would, furthermore, not be justified in speculating whether a certain animal became established in an anaerobic habitat because it had inherited from its ancestors well-developed anaerobic functions, or whether the latter became more pronounced during adaptation to environments with progressively depleted oxygen supply (for exceptions to this statement, *cf.* below, Chapter 4).

To point 4: Snyder believes that the theory of recapitulation offers support to his idea of anaerobiosis as the primitive condition. He mentions some organisms in which the developmental stages are more resistant than the adults when deprived of oxygen. Koschtojanz (1935a) has recently again emphasized this point. Such cases undoubtedly do occur; the reproductive cells of echinoderms, for example, are more resistant than the adults. But this behavior is by no means universal. Thus the juvenile stages of many trematodes seem to require more oxygen than the fully developed worms. (It may be added that the recapitulation theory itself has lost some of the prestige that it enjoyed thirty years ago.)

Much more weight must be given to the arguments contained in Snyder's point 2, point 3 (in part) and, perhaps, also point 5.

To point 2 and point 3 (in part): Snyder emphasizes that the function of oxygen is largely that of a waste remover, or, in the expressive words of Clark (1924) that of "a scavenger cleaning up the waste products of metabolism by combustion." It is indeed generally acknowledged that the initial steps of practically all the energy-producing processes within the animal body re-

quire no oxygen; this is well illustrated in the glycogen cycle in muscular contraction. Clark summarized the relationships of the two processes in these words: "What we find in general is the reducing tendency of the isolated cell; what we find in particular are special mechanisms for the use of molecular oxygen." It is then obviously very tempting to assume that the anaerobic processes are the primitive ones and that those connected with the uptake of molecular oxygen are secondarily acquired. If one does not do so, one would have to assume two fundamentally different kinds of life, an anaerobic and an aerobic one. Snyder already pointed out "that the adaptation of anaerobic organisms to an oxygen habit is conceivable and may be understood, whilst in the light of our present knowledge the reverse is not."[1]

To point 5: That the atmosphere of the primitive earth was devoid of oxygen is a hypothesis for which Snyder cites the authority of such eminent scientists as Lord Kelvin (1899) and Arrhenius (1907). The chief argument seems to be that in the earth's crust vast amounts of oxidizable but actually unoxidized materials occur which at one time or another must have been exposed to the atmosphere; if oxygen had been present, these substances should have been oxidized. It is then assumed that all the oxygen of the present-day atmosphere has been liberated by the photosynthetic activity of plants; Snyder himself is inclined to ascribe the main role in this process to marine algae rather than to a terrestrial flora.

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1. Snyder has in mind a hypothetical aerobic organism which does not possess intermediate anaerobic processes. His statement should not be understood as intimating that an animal endowed with the intermediate reactions cannot become adapted to anaerobic life. Most "anaerobic" invertebrates living today are probably derived from oxygen-consuming ancestors. This is illustrated in the case of intestinal worms, the derivation of which will be discussed in greater detail in Chapter 4.

Since Snyder's work was published new geophysical theories have been evolved and various other possible sources have been suggested for the origin of the atmospheric oxygen. According to Nutting (1926) the atmosphere (when the earth had a temperature of 5000° or above) contained up to 90 per cent oxygen. Enormous amounts of this gas were removed by oxidations as the temperature fell. But the protective action of the superficial layer of oxides thus formed prevented a complete depletion of the oxygen. According to other theories (Chamberlin and Salisbury, 1906; Nichols, 1941), water vapor may become dissociated in the upper atmosphere by various mechanisms, *e.g.,* by the impact of white-hot planetesimals, or by the radiation emitted from the sun. The hydrogen so formed would escape the gravitational control of the earth while the oxygen would remain in the atmosphere. Nichols (1941) finally points out that even some volcanic gases may liberate molecular oxygen from water, while other gases would tend to remove it from the atmosphere.

Thus the geologists are evidently not in agreement on this question. The present writer does not feel competent to judge the relative merit of these various theories and he doubts whether the point can be used at all as an argument *pro* or *contra* in this discussion.

Weighing all the evidence one finds, then, that the only strong argument in favor of the idea that the most primitive form of life was anaerobic is based on the ubiquity of reducing processes and on the fact that these anaerobic reactions are the first in the chain of events; the reactions involving molecular oxygen occur only later. From this viewpoint a fairly strong case for the theory of anaerobic precedence *as such* can be made. Whether invertebrate life was originally purely anaerobic, however, is quite a different question.

Whatever the origin of the bulk of the atmospheric oxygen may have been, true animal life must have been preceded by plant life since plants represent the ultimate source of animal food. One can safely assume, therefore, that the primitive animals had access to, at least, some oxygen. Whether they were originally able to utilize it, we obviously cannot say.

From the point of view of economy of food—supposing that nature considers economy—it may be pointed out that anaerobic processes involve more waste, the more so since the reactions possible to animal protoplasm depend on organic materials as mother-substances. Oxidative processes using molecular oxygen are much more economical; *i.e.,* with the same amount of food-substance available, a much richer aerobic than anaerobic fauna can be supported. Could it not be, then, that the type of life that we call animal life became possible only after the development of aerobic processes?

The fact that most animals which possess pronounced anaerobic functions are nevertheless capable of utilizing oxygen when they have access to it, suggests that they are only invaders of anaerobic habitats, that they have become adapted to them. The sapropelic ciliates may represent an exception; though, as explained previously, we do not yet know whether or not they are capable of utilizing molecular oxygen of low tension. In whatever way future investigation may decide this question, it should not be overlooked that a function once possessed by an organism may be lost again. If it should be demonstrated that these ciliates are incapable of utilizing any oxygen, one would not be justified in assuming that this is necessarily a primitive condition. One might as well argue that the necessary enzyme mechanism was "discarded" once they became established in their peculiar habitat. One could compare such a hypothetical

event with the secondary loss of eyes in many cave animals.

On the whole, the present author is inclined to believe with Koschtojanz (1935, 1935a) that both the anaerobic and the aerobic metabolism should be regarded as original traits of true animal life. The various degrees of development of anaerobic functions in the members of the contemporary fauna offer no objection to such an assumption. Both types of metabolic processes are coupled more or less closely with each other, and with a variety of secondary processes (*e.g.,* various methods of waste removal). The balance struck between the various combinations of reactions seems to decide to what degree a certain animal species is dependent or independent of molecular oxygen.

CHAPTER IV

ANAEROBIOSIS AND THE ORIGIN OF ENDOPARASITISM

It was shown previously that many parasites live in surroundings that are extremely poor in oxygen, or, sometimes, even entirely devoid of it. It is not surprising, therefore, that the question of the origin of the anaerobic functions which these organisms exhibit has been correlated with that of their parasitic nature.

The first author to have suggested such a correlation was Bunge (1889). According to him the worms and insect larvae occurring within the intestinal tract of vertebrates belong to classes of which the free-living representatives show, at least in some cases, a remarkable resistance in anaerobic media. He mentions in particular the parasitic cestodes and nematodes and, as corresponding free-living organisms, the mud-dwelling turbellaria and nematodes. Thus the intestinal parasites would be derived from free-living animals that were anaerobes before acquiring parasitic habits. Anaerobic muds can, according to him, be regarded as predisposing habitats ("Vorschule") for intestinal parasitism. He considers it, however, as certain that the mud-dwellers, and therefore ultimately also the parasitic worms, were derived from aerobic ancestors.

Bunge's views have been rather widely quoted, but Alsterberg (1922) has raised some objections to them. He points out, first, that large groups of animals can usually be classified so as to bring out apparent phylogenetic relationships at the will of an author, but such classification is of relatively little value. Secondly, he emphasizes that there is a rather fundamental difference in behavior between intestinal parasites and free-living invertebrates enduring the adverse conditions of anaerobic habitats. The latter, with perhaps a few exceptions,

cease to feed when the oxygen is removed, while the intestinal parasites are then still able to assimilate food. A third objection is that it is questionable whether mud, considered from a nutritional standpoint, would serve as a good "Vorschule" for a future establishment in the intestinal tract. Mud is usually very poor in nutritive material utilizable by metazoa, while the intestine has an abundant supply of it.

The present writer considers Alsterberg's first and third points as rather weighty, but cannot regard the second argument as conclusive. The facts reviewed previously indicate that both in free-living animals and in parasites marked differences in the degree of tolerance towards the lack of oxygen are encountered; but these differences seem to be only quantitative, not qualitative.

There are still other objections to Bunge's views. The lack of oxygen is not the only adverse condition that intestinal parasites have to contend with. They must also resist the action of the digestive enzymes of their hosts, be it through the development of anti-enzymes, or otherwise. They must be able to live in an environment of relatively high osmotic pressure, many must be quite independent of the hydrogen ion concentration, *etc.* It is not easy to understand how all these prerequisites could have been acquired in the mud.

There are other possibilities. Pintner (1922) visualizes a gradual phylogenetic development of the parasitism of worms by the following steps: ecto-parasitism ——→ parasitism in mesenchyme ——→ in lymph ——→ in blood ——→ within the respiratory tract ——→ within the intestinal tract.

While this scheme obviously does not require a mud-ancestry, it is not too satisfactory from the viewpoint of gradual adaptation to anaerobic conditions. The passage from the respiratory tract to the intestine must involve quite abrupt changes in oxygen supply; a gradual acqui-

sition of anaerobic habits can hardly have come about in that manner.

Much more satisfactory in this respect is Stunkard's (1937) view. Taking into consideration the bionomics, development and morphology of the flat-worms, he developed the idea that Turbellaria, Mesozoa, Cestoda and Digenea have "descended concomitantly from a common ancestral group of planula-like ancestors." According to him, the predecessors of the present-day parasites belonging to these groups became parasites of invertebrates in early geological times, long before they invaded vertebrates. This view obviously opens new angles to the question of adaptation to the extreme conditions that parasites encounter in vertebrate hosts. It has been indicated previously that the tissues and perhaps also the intestines of many invertebrates are not so poor in oxygen as those of vertebrates; the other adverse conditions mentioned above may likewise not be so pronounced in the former as in the latter. A gradual adaptation to extreme conditions would thus have occurred through a successive invasion of various hosts.

The idea that the ancestors of *some* parasitic worms might have been mud-dwellers is not, however, to be dismissed altogether. The nematodes, which are extremely resistant to all kinds of injurious agents, might have passed from a non-parasitic life in the mud to that of parasites in the intestine of vertebrates. It is of interest in this connection to recall that Moore (1931) found mud-dwelling nematodes with such pronounced anaerobic functions that they appeared to be normally anaerobic.

As to Bunge's view that ultimately the parasitic worms and arthropods are derived from aerobic ancestors, it seems well-founded. It is supported by the fact that all species investigated so far are capable of consuming oxygen when it is available. In most instances they even *require* oxygen at some stage during their life cycles.

Another problem is that of the role played by the anaerobic functions of some intestinal worms in the development of their wandering habits when they invade the body of a host as larvae. In the case of *Ascaris,* for example, as is well known, the larvae, developed from the eggs and set free within the small intestine, do not settle down there at once, despite the fact that the small intestine represents the normal habitat of the grown worms. On the contrary, they bore into the intestinal wall, penetrate into the blood vessels and are carried by the blood stream to the lungs, *via* liver and heart. There they break out from the blood vessels, penetrate into the air passages and wander up the trachea to the throat; they are then swallowed and, passing through the esophagus and stomach, they finally again reach the small intestine whence they started. This circuit takes about ten days and during this time the larvae grow considerably. Pintner (1922) developed the idea that the biological reason underlying this curious phenomenon must be sought in the fact that many intestinal worms survive in their normal environment only because of their ability to live anaerobically. But to live anaerobically they require glycogen. The wanderings of the larvae would be undertaken to permit the formation of a glycogen reserve; during their stay in the blood stream they have access to so much oxygen that an aerobic metabolism would be possible, and to so much sugar that a glycogen synthesis can take place.

This theory is ingenious, but does not seem to be backed by the experimental evidence. Stepanow-Grigoriew and Hoeppli (1926) pointed out that even old *Ascaris* larvae (embryonated eggs stored for six months), contain glycogen, and the same holds for young filariform larvae of *Strongyloides stercoralis.* It is true that the glycogen content of these nematode larvae increases during their wanderings. (This was noticed both by Step-

anow-Grigoriew and Hoeppli (*l.c.*) and by Giovannola (1936), the latter working without knowledge of either Pintner's or Stepanow-Grigoriew and Hoeppli's papers.) But this glycogen increase is not dependent on the migratory process. It has been shown that a comparable polysaccharide accumulation takes place in dog hookworms when they develop directly within the small intestine. In order to separate the worms from the larvae that had gone through the circuit, a tracheotomized dog was used in which no larva could go over from the trachea to the esophagus (Stepanow-Grigoriew and Hoeppli). It might be remarked too that the larvae of parasitic nematodes are all very small and have, therefore, a high surface volume ratio. Since the intestine is usually not completely devoid of oxygen, they should be able to get, before they have grown to larger sizes, rather significant amounts of oxygen. One can hardly conceive that they would die of asphyxiation before the abundant food available in their surroundings would allow the building up of a sufficient amount of reserve material for the transition to a fermentative type of metabolism, even if their original glycogen stores would not be sufficient for this purpose. We are thus led, on the basis of these arguments, to reject Pintner's theory.

As to the *parasitic protozoa* only one attempt seems to have been made to consider their anaerobic functions as a clue to their ancestry. Lauterborn (1916) pointed out a curious general similarity between the sapropelic protozoan fauna and the rumen- or termite-protozoa, and also between their respective habitats. In the three habitats a pronounced cellulose fermentation takes place, the concentration of solutes is relatively high, there is little or no free oxygen present, but a large quantity of carbon dioxide and combustible gases. These common features in the environment may have been responsible for a series of rather pronounced similarities in the or-

ganization of the animals: the development of the pellicle into a kind of skeleton, the prevalence of a deeply sculptured pellicle, the torsion of the body which occurs in many forms, the development of very long cilia and of tufts of flagella and finally the accumulation of large reserves of polysaccharides. Lauterborn is of the opinion that these similarities may not be without significance for the question of the origin of these parasites; from no other habitat is the transition in external conditions less abrupt than from the sapropelic to the intestinal. Alsterberg's main objections (*cf.* above) to a derivation of intestinal worms from mud-dwelling ancestors are not valid in this instance. The sapropelic ciliates are obviously well fitted to assimilate food in the absence of oxygen; indeed, as has been shown before, they are even harmed by oxygen. Since some of them seem capable of utilizing cellulose, their environment is also quite rich in food material. The same holds true even for forms that cannot do so; pure bacteria feeders or scavengers will find a rich source of food in the bacteria developing on the decaying organic matter that is characteristic of a typical sapropelic mud. Of course, one cannot think of a direct connection between the present-day sapropelic protozoa and the rumen- or termite-protozoa. They are all highly specialized forms and, to the writer's knowledge, not even common genera occur. They, most likely, have a long history behind them. As to the general similarities in organization, they may just be an expression of convergent development, but the derivation of the three faunas from the same ancestral mud-dwellers still remains a possibility.

SUMMARY

The place of anaerobic processes in the general metabolism of invertebrates.

1. A new classification of the chief energy-yielding metabolic reactions occurring in invertebrates is proposed which shows the relative importance of aerobic oxidations and of aerobic and anaerobic fermentations.

2. It is emphasized that truly anaerobic invertebrates are found only amongst the protozoa, and that even these may consume or perhaps require traces of oxygen for their continuous well-being.

Basis for the differences in anaerobic functions among invertebrates.

1. Of the various theories proposed to explain why anaerobic protozoa and other animals that live habitually in environments with low oxygen content are injured by oxygen, only one is supported by experimental tests, the one which holds that the injury is due to the toxic action of the hydrogen peroxide formed from the oxygen present, the catalase content being insufficient to destroy the peroxide.

2. Some invertebrates withstand well the deprivation of oxygen, others are rapidly paralyzed or killed. The former are evidently able to gain sufficient energy for their life processes through fermentations. (In many cases, however, the energy requirements are lowered when the oxygen is withdrawn, due to cessation of muscular movements.) As to the organisms which are injured or succumb, they are either incapable of gaining sufficient energy through anaerobic processes, or some specialized tissue in them, like the nervous tissue, is irreversibly damaged, or some toxic metabolic end products accumulate in their body.

3. Some organisms have adapted themselves to anaerobic life by excreting toxic end products, others by elaborating, instead, substances which are relatively non-toxic.

4. Invertebrates are capable of conditioning, in culture,

the oxidation-reduction potential of the medium in much the same way as do bacteria. In nature, inorganic constituents and the bacterial flora will be more important than the metabolism of the animals themselves in determining the oxidation-reduction potentials of a given habitat. *Endamoeba and Opisthorchis,* two organisms living in nature in surroundings poor in oxygen, survive best, *in vitro,* in media having low potentials.

5. The data on the adjustability of the internal oxidation-reduction potential to the oxygen content of the surroundings in purely aerobic animals and in invertebrates with well-developed anaerobic functions fail as yet to demonstrate a distinctive difference between the two groups.

The origin of anaerobiosis in invertebrates.

1. Several arguments (phylogenetic considerations, theory of recapitulation, geophysical theories concerning the origin of the atmospheric oxygen), which have been put forward in support of the idea of the phylogenetic precedence of anaerobiosis, are not well grounded. The only argument which has some weight is the ubiquity of reducing processes and the fact that anaerobic reactions occur first in the chain of energy-producing metabolic processes.

Anaerobiosis and the origin of endoparasitism.

1. The idea that intestinal worms and insect larvae are derived from anaerobic mud-dwelling ancestors seems, on the whole, not well-founded, although the theory may perhaps still be held for intestinal nematodes.

2. The theory that the wanderings of many parasitic nematode larvae within the body of vertebrate hosts, before the parasites settle down in the intestine, are correlated with the necessity of leading, in the latter habitat, an anaerobic life, is not supported by experimental evidence.

3. A derivation of the present-day sapropelic-, rumen- and termite-protozoa from the same mud-dwelling ancestors appears possible from a physiological standpoint.

APPENDIX

In this appendix some tables are presented which list the fauna occurring in the following anaerobic habitats: sapropelic habitat, sewage tanks and anaerobic zone of lakes. Only the faunas described in some representative papers have been included in the tables. The faunas occurring in other anaerobic or near-anaerobic habitats have been omitted, partly because it would be premature to establish a list (as for the oxygen-minimum layer in the sea), partly because not enough is known to decide whether many animals found in near-anaerobic habitats (*e. g.*, some parasitic habitats) really lead a predominantly anaerobic life.

TABLE 1

LIST OF ANIMALS FOUND IN THE SAPROPELIC HABITAT (ACCORDING TO LAUTERBORN, 1916)

ONLY THE PROTOZOA, ROTATORIA AND GASTROTRICHS ARE TYPICAL MEMBERS OF THIS FAUNA, THE OTHER ANIMALS ARE FOUND ONLY OCCASIONALLY IN THIS HABITAT.

1. Protozoa:
   a. Rhizopods: *Actinosphaerium eichhorni, Amoeba chlorochlamys, Arcella vulgaris, Cochliopodium vestitum, Difflugia acuminata, Heterophrys myriopoda, Pamphagus armatus, Pelomyxa palustris.*

   b. Flagellates: *Chloromonas pulcherrima, Cryptomonas ovata, Cyathomonas truncata, Euglena acus. Euglena viridis, Eutreptia viridis, Heteronema acus, Heteronema spirale, Hexamitus inflatus, Mastigamoeba trichophora, Menoidium pellucidum, Phacus oscillans, Phacus pleuronectes, Physomonas socialis, Pteridomonas pulex, Rhynchomonas nasuta, Spondylomorum quaternarium, Trepomonas agilis.*

   c. Ciliates: At the surface: *Aspidisca turrita, Bursaria truncatella, Campanella umbellaria, Chilodon cucullus, Coleps amphacanthus, Coleps hirtus, Euplotes patella, Frontonia leucas, Halteria grandinella, Loxodes rostrum, Loxophyllum meleagris, Paramaecium aurelia, Paramaecium caudatum, Pleuronema inflatum, Spirostomum ambiguum, Spirostomum teres, Stentor coeruleus, Urocentrum turbo, Uroleptus rattulus, Vorticella longifilum.*

In the mud proper: *Amphileptus claparedii, Blepharisma lateritium, Blepharisma musculus, Caenomorpha medusula, Caenomorpha uniserialis, Chaenia limicola, Cristigera pleuronemoides, Dactylochlamys pisciformis, Dinophrya lieberkuehnii, Discomorpha pectinata, Lacrymaria aquaedulcis, Lagynus elegans, Legendrea loyesae, Loxocephalus granulosus, Loxocephalus luridus, Metopus contortus, Metopus pyriformis, Metopus sigmoides, Mikrothorax sulcatus, Opisthodon niemeccense, Pelamphora buetschlii, Pelodinium reniforme, Perispira ovum, Plagiopyla nasuta, Saprodinium dentatum, Sphaerophrya soliformis, Spathidium lieberkuehni, Tropidoatractus acuminatus.*

2. Rotatoria:

Atrochus tentaculatus, Diglena biraphis, Diplacidium compressum, Diplacidium trigona, Floscularia atrochoides, Lepadella salpina, Lepadella triptera, Rotifer vulgaris.

3. Gastrotrichs:

Aspidiophorus paradoxus, Chaetonotus arquatus, Chaetonotus nodicaudus, Chaetonotus serraticaudus, Dasydytes bisetosus, Dasydytes longisetosus, Dasydytes saltitans, Gossea antennigera, Lepidoderma rhomboides, Stylochaeta fusiformis.

4. Turbellaria:

Macrostomum appendiculatum.

5. Nematodes:

Diplogaster sp., *Dorylaimus* sp., *Trilobus* sp.

6. Oligochaeta:

Slaviana appendiculata.

7. Crustaceans:

Canthocamptus sp., *Cyclops* sp., *Lathonura rectirostris.*

8. Tardigrades:

Cysts of *Macrobiotus.*

9. Insects:

Larvae of *Eristalsis tenax.*

TABLE 2

PROTOZOAN FAUNA OF SEWAGE TANKS (ACCORDING TO LACKEY, 1932)

1. **Rhizopods:**

   Present in small numbers or infrequently: *Chlamydophrys stercorea, Dimastigamoeba gruberi, Hartmanella hyalina, Vahlkampfia albida, Vahlkampfia guttula, Vahlkampfia limax.*

   Always present in the absence of oxygen: *Chlamydophrys minor, Vahlkampfia fragilis.*

2. **Flagellates:**

   Present in small numbers or infrequently: *Anthophysa vegetans, Bodo caudatus, Bodo lens, Bodo mutabilis, Cercobodo crassicauda, Cercobodo longicauda, Cercobodo ovatus, Chilomonas paramaecium, Clautriavia parva, Cyathomonas truncata, Dinomonas vorax, Distigma proteus, Entosiphon sulcatum, Euglena gracilis, Helkesimastix faecicola, Heteronemas acus, Hexamitus inflatus, Mastigella simplex, Menoidium incurvum, Monas amoebina, Monas minima, Monas vulgaris, Notosolenus orbicularis, Oicomonas termo, Peranema trichophorum, Petalomonas carinata, Petalomonas mediocanellata, Pleuromonas jaculans, Tetramitus descissus, Tetramitus pyriformis.*

   Always present in the absence of oxygen: *Bodo glissans, Mastigamoeba longifilum, Mastigamoeba radiosa, Mastigamoeba reptans, Mastigamoeba viridis, Trepomonas agilis.*

3. Ciliates:

   Present in small numbers or infrequently: *Colpoda cucullus, Colpoda inflata, Cyclidium glaucoma, Glaucoma scintillans, Hexatrichia caudatum, Paramaecium putrinum, Plagiopyla nasuta.*

   Always present in the absence of oxygen: *Holophrya* sp., *Metopus sigmoides, Saprodinium putrinum, Trimyema compressa.*

TABLE 3

ANIMALS OCCURRING IN THE ANOXIC REGIONS OF LAKES (IN PART ACCORDING TO WELCH, 1935)

THE ORGANISMS ENUMERATED BY WARD (1940) WERE FOUND IN A POND.

Organisms	Source
1. Protozoa	
a. Rhizopods:	
Actinosphaerium eichhorni, *Amoeba proteus*	Imel, 1915
Dactylosphaerium radiosum, *Difflugia globostoma*	" "
Difflugia sp., *Pelomyxa* sp.	Juday, 1908
Pelomyxa palustris, *Pelomyxa villosa*	Moore, 1939
b. Flagellates:	
Cryptomonads	Lindeman, 1942
Monas sp., *Peranema* sp.	Juday, 1908
Peranema sp.	Imel, 1915
c. Ciliates:	
Caenomorpha sp., *Coleps* sp., *Colpidium* sp.	Juday, 1908
Colpidium sp.	Imel, 1915
Lacrymaria sp., *Paramaecium* sp.	Juday, 1908
Paramaecium bursaria	Imel, 1915
Prorodon sp.	Juday, 1908
Stentor coeruleus	Imel, 1915
Uronema sp.	Juday, 1908
Vorticella sp.	Imel, 1915
2. Nemathelminthes	
Anguillula sp.	Juday, 1908
Hydromermis sp.	Eggleton, 1931
Mermis sp.	Thienemann, 1918
Trilobus bastian	Moore, 1939
3. Gastrotricha	
Chaetonotus sp.	Juday, 1908
Chaetonotus sp.	Moore, 1939
"*Gastrotrich*"	Imel, 1915
4. Rotatoria	
Philodina sp.	Birge & Juday, 1911
Rotaria rotatoria, *Rotaria tridicus*	Moore, 1939

TABLE 3 — (Continued)

Organisms	Source
5. Oligochaeta	
Limnodrilus claparedianus, Limnodrilus hoffmeisteri	Eggleton, 1931
Limnodrilus sp.	Juday, 1908
"*Oligochaetes*"	Thienemann, 1918
Tubifex sp.	Juday, 1908
Tubifex sp.	Lindeman, 1942
6. Mollusca	
Musculium rosaceum, Musculium truncatum	Eggleton, 1931
Pisidium compressum, Pisidium sp.	" "
Pisidium idahoense	Juday, 1908
Pisidium sp.	Thienemann, 1918
7. Crustacea	
Candona sp.	Juday, 1908
Candona exilis, Candona reflexa	Moore, 1939
Canthocamptus staphylinoides (cysts & active state)	" "
Cyclops albidus	Ward, 1940
Cyclops bicuspidatus	Imel, 1915
Cyclops bicuspidatus (cysts)	Birge & Juday, 1911
Cyclops bicuspidatus (cysts)	Moore, 1939
Cyclops sp.	Thienemann, 1918
Cyclops sp. (nauplii), *Cypria elegantula*	Ward, 1940
Cypria exsculpta, Cypria lacustris	Moore, 1939
Daphnia pulex	Ward, 1940
Pallasea quadrispinosa	Thienemann, 1918
Simocephalus exspinosus	Ward, 1940
8. Insecta (larvae)	
Chaoborus sp.	Lindeman, 1942
Chaoborus sp.	Thienemann, 1918
Chaoborus sp., *Chironomus* sp.	Juday, 1908
Chironomus fasciventris, Chironomus plumosus	Eggleton, 1931
Chironomus (plumosus group)	Thienemann, 1918
Chironomus punctipennis	Birge & Juday, 1911
Chironomus tentans	Muttkowski, 1918
Chironomus utahensis	Eggleton, 1931
Chironomus sp., *Palpomyia* sp., *Protenthes* sp.	Lindeman, 1942
Protenthes choreus	Muttkowski, 1918
Protenthes culiciformis	Eggleton 1931
Tanypus sp.	Thienemann, 1918

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