RESPIRATION AND UTILIZATION OF ENDOGENOUS CARBOHYDRATE
IN HETERAKIS GALLINAE, A CAECAL NEMATODE
OF THE DOMESTIC FOWL

by

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REVIEW OF LITERATURE

A. INTRODUCTION

Helminthiasis is widespread and has involved a medical and veterinary problem since ancient times. Stoll (1947) has estimated that at least eight hundred million human beings are infested with more than two billion helminths. There are also thousands of species of parasites which do not infest man, but which have as their natural hosts other animals, or plants.

Despite the extensive distribution of parasitic worms and their enormous economic and medical importance, little is known concerning their life processes. What is known consists, for the most part, of poorly related details which have accumulated over the past fifty years, and from which few generalizations can be drawn. Very few biochemists or physiologists have devoted themselves to more than a desultory examination of the life processes of parasitic helminths. To quote Willard H. Wright (1950):

"The science of parasitology, like other sciences has over the years gone through many different cycles and many changing trends. Unlike bacteriology, in which marked advances have been made in recent years in the biochemistry and physiology of bacteria, parasitology is far behind in this vital phase. Somewhat blindly, we have been endeavoring to learn something of host-parasite relationships, chemotherapy, immunology, and of the manner in which
parasites damage their hosts without an adequate understanding of their physiological requirements and the method by which they carry on their metabolism in the varying types of environment in which they exist within the bodies of their intermediate and definitive hosts. We need more parasitologists interested in physiology and biochemistry, and more physiologists and biochemists interested in parasitology."

In the present thesis the results obtained from a study of the respiratory metabolism of *Heterakis gallinae* will be presented. These results are the first from a long-term investigation of the physiology and biochemistry of this parasite which has been planned at the Institute of Parasitology. *H. gallinae* was chosen as the object of investigation because of its availability and relatively large size (up to 10 mm. in length). No previous study has appeared in the literature.

B. REVIEW OF THE LITERATURE ON *HETERAKIS GALLINAE*

*H. gallinae* is a nematode which inhabits the caeca of poultry. The chief economic importance of the species arises from its role as a vector for enterohepatitis or "blackhead" in turkeys (Graybill and Smith, 1920; Swales, 1948).

The history of the nomenclature has been described by Clapham (1933). Before 1923, the worm was known as *H. papillosa*.

The life cycle of *H. gallinae* is direct, with no
intermediate host and no migration through the body (Riley and James, 1921; Ackert, 1927; Clapham, 1933). Un-embryonated (uninfective) eggs are discharged with the caecal droppings of the host and, under favourable conditions, complete embryonation on the ground in 9-14 days. When ingested by the fowl, fully embryonated (infective) eggs hatch in the small intestine within 12 hours, and within 48 hours pass into the caecum. For the next four days, the larvae bury their heads (and possibly feed) in the mucosal glands of the caecal wall; after this time, they are found free in the lumen of the caecum (Uribe, 1927). Complete maturity is attained in two months.

The only experimental observations on *H. gallinae* were made by Li (1933) who fed charcoal to chickens and recovered, from the caeca, *H. gallinae* with charcoal particles in the intestine, thus indicating that the worm fed upon the ingesta of the host.

The remainder of this review of literature, therefore, must necessarily be a review of some of the observations which have been made upon the physiology of other helminths. Reviews on this subject have been published by von Brand and Jahn (n.d.), McCoy (1944), Hobson (1948), and Bueding (1949).
C. SURVIVAL OF PARASITIC HELMINTHS IN VITRO

Helminths parasitizing the intestine of warm-blooded hosts are particularly difficult to keep alive in vitro. Tissue parasites, and intestinal parasites of cold-blooded hosts appear to be much hardier in vitro. Wardle (1937) kept the cestode *Moniezia expansa* (from the intestine of the sheep) alive for only twelve hours in a balanced Ringer solution, whereas von Brand (1938) has kept larval *Eustrongylides ignotus* (a tissue nematode of a fish) alive in nutrient medium for as long as four years.

Intestinal parasites are normally contaminated with microorganisms which multiply rapidly in vitro, especially in nutrient medium, and produce metabolic end-products which are toxic to the worms. The presence of the end-products of bacterial metabolism also interferes seriously with the estimation of the end-products of parasite metabolism. It would, therefore, be of great advantage to maintain helminths in vitro under sterile conditions. A few cestodes and trematodes, and fewer nematodes, have been obtained free of contaminating microorganisms. Among the latter were larval *E. ignotus*, which was dissected aseptically from its cyst in the muscle of the killifish, *Fundulus heteroclitus* (von Brand, 1938), and *Ascaris lumbricoides* (from the intestine of the pig) which has been made free of microorganisms by chemical means (Fairbairn and Reesal, 1950).
Intestinal helminths will withstand exposure to a relatively broad range of hydrogen ion concentration, which generally corresponds to that of their normal environment. Davey (1938b) found that nematode species from the duodenum of the sheep could withstand a pH as low as 3.6 in vitro, whereas nematode species occurring farther down in the intestine were killed when the pH fell below 4.6.

In order to survive in vitro, most intestinal parasites require a temperature which is similar to that of their normal environment. Some of the tissue and blood parasites appear to be able to withstand a broader range of temperature. No generalization can be made, however, until more observations are available (Hobson, 1948).

Some investigators, of whom Weinland (1901) was one, maintained that oxygen is not essential for the survival of parasitic helminths (in this case, *A. lumbricoides*). Other workers, including Slater (1925), have maintained that oxygen is essential. Toryu (1935) found that the survival of *Parascaris equorum* (*Ascaris megaloccephala*) was ten hours longer in boiled saline than in saline saturated with air or oxygen. Fairbairn and Reesal (1950) observed that *A. lumbricoides* lived longer and more actively in nitrogen than in air. On the other hand, Slater (1925) and Davey (1938b) found that intestinal helminths (*A. lumbricoides* in the former instance, sheep nematodes in the latter) did
not survive as long under anaerobic conditions as they did in air. The worms became immotile after a short period of anaerobiosis, but the motility reappeared on the admission of air. It is interesting to note that Slater and Davey used hydrogen to produce anaerobic conditions, and it is possible that hydrogen may have been toxic to the helminths, either directly or through its effect on bacterial metabolism.

The fact that parasites survive actively in an atmosphere of pure nitrogen is not proof that oxygen is never required for their normal metabolism. It must be remembered that all observations have been made upon worms surviving in vitro, under conditions which do not necessarily correspond to the normal behaviour of the parasites in vivo.

*Litomosoides carinii* (Bueding, 1949a) and larval *Trichinella spiralis* (Stannard, McCoy and Latchford, 1938) showed a loss of motility in nitrogen. Von Brand and Simpson (1945) found that *E. ignotus* larvae did not tolerate complete anaerobiosis, but could survive favourably at low partial pressures of oxygen. These three worms are tissue parasites (in the phase of their life cycles which was studied) living in an environment which may often contain a higher oxygen tension than the intestine. This
does not imply, however, that a relatively high oxygen tension in the environment guarantees aerobiosis, because some parasitic helminths show a primarily anaerobic existence even in the presence of a rich supply of oxygen (Bueding, 1949b). The effect of oxygen on parasitic worms is therefore variable, and seems to depend upon some intrinsic aspect of metabolism, rather than the actual presence of oxygen in the environment.

Although the effect of oxygen on the survival of parasites has been widely studied, the effect of carbon dioxide has been generally overlooked. Weinland (1901) and Fairbairn and Reesal (1950), observing *A. lumbricoides*, and Toryu (1935), *P. equorum*, noted that saturation of the medium with carbon dioxide greatly increased the survival time of the worms. Toryu found that the anaerobic survival time of *P. equorum* was increased by almost 40 hours in the presence of carbon dioxide. This phenomenon was explained by the fact that the intestinal contents contain a large amount of carbon dioxide. Toryu (1934) analysed the contents of the intestine of the horse and found that the jejunum contained 70.5 volumes per cent carbon dioxide of which 8.2 volumes per cent existed as free gas. Von Brand and Jahn (n.d.) recognized the fact that the environment of intestinal parasites is saturated with carbon dioxide, and
suggested that it might act as an external buffering agent for the worm. The effect of carbon dioxide, as will appear from the results presented in this thesis, is undoubtedly of greater importance than has been believed hitherto.

D. RESPIRATION OF PARASITIC HELMINTHS IN VITRO

Although it is evident that some parasitic helminths will survive anaerobically, it is also established that they will consume oxygen if it is available. For this reason, no helminth which has been studied can be considered an obligate anaerobe (Bueding, 1949b).

The oxygen uptake in air of various parasitic helminths which have been studied is presented in Table I. The symbols used in this table and in the remainder of the paper are defined as follows:

- \( Q_{O_2} \) = cu. mm. of oxygen consumed per hour per mg. dry weight
- \( Q_{O_2}^{(WW)} \) = cu. mm. of oxygen consumed per hour per mg. wet weight
- \( Q_{O_2}^{(N)} \) = cu. mm. of oxygen consumed per hour per mg. nitrogen
- \( Q_{CO_2}^{(WW)} \) = cu. mm. of carbon dioxide produced per hour per mg. wet weight
- R.Q. = respiratory quotient = \( Q_{CO_2}^{(WW)}/Q_{O_2}^{(WW)} \)

It has been generally observed, and can be seen from Table I, that the rate of respiration increases as the size of the species decreases. Krüger (1940) obtained positive...
correlation of the oxygen consumption of individual *Ascaris lumbricoides* with the surface area as determined by Meeh's formula (surface area = $KW^{2/3}$). Rogers (1948) and von Brand (1942), however, could not compare different species by this means. It is possible that, like other small animals, the increased respiration is due to increased activity and intrinsic metabolic needs rather than surface area alone.

The rate of oxygen consumption of many parasitic worms increases with increasing oxygen tension in the atmosphere (Harnisch, 1933; Rogers, 1947b). This phenomenon does not depend upon a possible anaerobic nature of the worms as was suggested by Harnisch (1933; 1937). The larva of *Trichinella spiralis*, which is thought to be aerobic was found to be quite independent of oxygen tension (Stannard, McCoy and Latchford, 1938). The same observations were obtained for *Moniezia benedini* and *Fasciola hepatica* (Bueding, 1949b) which are thought to be primarily anaerobic.

It is now believed that the oxygen tension in the intestines of animals is much greater than has been suspected or determined previously. Rogers (1949a) found that the oxygen tension in the small intestine of the rat varied from 8.9 - 30.2 mm. of mercury, and that in the small intestine of the sheep, from 4.0 - 12.7 mm. of mercury. The oxygen tensions were measured electrometrically *in situ*, and the
### TABLE I

THE OXYGEN CONSUMPTION OF SOME PARASITIC HELMINTHS

<table>
<thead>
<tr>
<th>Parasite (in order of decreasing size)</th>
<th>$Q_{O_2}$</th>
<th>$Q_{O_2}(ww)$</th>
<th>R.Q.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ascaris lumbricoides</em> (pig)</td>
<td>1.3</td>
<td>0.080</td>
<td>1.1-1.2</td>
<td>Laser (1944)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(muscle pulp)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.03-0.04 (females)</td>
<td></td>
<td>Harwood and Brown (1934)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.05-0.07 (males)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ascaridia galli</em> (chicken)</td>
<td>2.5</td>
<td>0.85-1.01 (0.96)</td>
<td></td>
<td>Rogers (1948)</td>
</tr>
<tr>
<td><em>Bunstrongylides ignotus</em> (larvae) (killifish)</td>
<td>5.6</td>
<td>0.140</td>
<td>1+</td>
<td>von Brand (1942)</td>
</tr>
<tr>
<td><em>Litomosoides carinii</em> (rat)</td>
<td>3.9</td>
<td>0.780</td>
<td>0.44</td>
<td>Bueding (1949)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.850</td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.04M glucose)</td>
<td>(0.04M glucose)</td>
<td></td>
</tr>
<tr>
<td><em>Ancylostoma caninum</em> (dog)</td>
<td></td>
<td>0.400</td>
<td></td>
<td>Harwood and Brown (1934)</td>
</tr>
<tr>
<td><em>Heterakis gallinae</em> (chicken)</td>
<td>3.6</td>
<td>0.98</td>
<td>0.61-0.88 (0.74)</td>
<td>Glocklin, present thesis</td>
</tr>
<tr>
<td><em>Nematodirus spp.</em> (sheep)</td>
<td>5.1</td>
<td>0.55-0.69 (0.66)</td>
<td></td>
<td>Rogers (1948)</td>
</tr>
<tr>
<td><em>Nippostrongylus muris</em> (adult) (larva) (rat)</td>
<td>6.8</td>
<td>0.64-0.76 (0.69)</td>
<td>0.72</td>
<td>ibid.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Neoaplectana glaseri</em> (Japanese beetle)</td>
<td>12.6</td>
<td>0.55-0.67 (0.59)</td>
<td></td>
<td>ibid.</td>
</tr>
<tr>
<td><em>Trichinella spiralis</em> (larva) (pig, rat, man)</td>
<td>1.7</td>
<td>1.13</td>
<td></td>
<td>Stannard, McCoy and Latchford (1938)</td>
</tr>
</tbody>
</table>
highest tensions were found closest to the pylorus. Although this technique has not yet been widely used in this respect, it is probable that the estimation of the oxygen content of the intestine, so obtained, is much more accurate than the older chemical methods.

Some parasitic helminths have been found to possess a high efficiency of utilization of oxygen even at low tensions. Rogers (1949b) showed that at a partial pressure of oxygen equivalent to 5 mm. of mercury, Nippostrongylus muris consumed oxygen at a rate which was almost 40 per cent that in air. Furthermore, Rogers (1949c) showed the presence of haemoglobin in N. muris, Nematodirus spp., and Haemonchus contortus. Davenport (1949a) demonstrated the presence of haemoglobin in A. lumbricoides, N. muris, and Strongylus spp. The parasite haemoglobins were found to have slightly different spectroscopic properties and a much greater affinity for oxygen than the haemoglobins of the corresponding hosts. The affinity of the parasite haemoglobins for carbon monoxide was less than that for oxygen. It was suggested by Rogers, and Davenport, that the haemoglobins of parasitic helminths possibly acted as oxygen carriers even at low partial pressures of oxygen. If this is so, then intestinal parasites (especially the small ones) may lead a more aerobic existence in vivo than was formerly thought to be possible.
Von Brand (1942) and Fenwick (1938) demonstrated the presence of an increased oxygen consumption after anaerobiosis, in larval Eustrongylides ignotus and larval A. lumbricoides (respectively). The suggestion was made that an oxygen debt was incurred during anaerobiosis, and the existence of an essential aerobic metabolism was implied. Bueding (1949a) found that Litomosoides carinii showed no such phenomenon.

Bueding (1949a; 1949b) found that L. carinii and Schistosoma mansoni had a high rate of exogenous glucose utilization with a corresponding increase in oxygen consumption (see Table I). Rogers and Lazarus (1949) found no change in oxygen uptake by Neoaplectana glaseri on addition of glucose, but they did observe that the respiratory quotient increased from 0.59 to 1.1, indicating, possibly, a high rate of aerobic fermentation of this substrate. Added glucose had no effect on the oxygen consumption or carbon dioxide production by Trichinella spiralis larvae (Stannard, McCoy and Latchford, 1938), or E. ignotus larvae (von Brand, 1945). The addition of fructose or galactose had an effect similar to glucose, but if a positive effect, it was not so great quantitatively. Few investigations have been carried out with other carbohydrates or carbohydrate intermediates.
The fact that glucose (or other substances) does not appear to affect the respiration of some worms does not indicate that it cannot be utilized, but rather that it is not being utilized. In other words, the worms may not be feeding under the conditions of the experiment.

Although an effort has been made to identify lower fatty acids excreted by intestinal helminths (Bueding, 1949b; von Brand, 1950), no one appears to have considered the possibility of using lower fatty acids as substrates in the study of helminth metabolism. The fact that butyrate, propionate, and acetate are known to be the major end-products of cellulose degradation in the alimentary tracts of animals emphasizes the desirability of investigating the utilization of these substances by the parasites.

The effect of inhibitors has been found to be variable. Von Brand (1945) showed that the oxygen uptake of larval _E. ignotus_ was inhibited by cyanide and azide, and to a lesser extent by urethane. Larval _T. spiralis_ (Stannard, McCoy and Latchford, 1958) and _L. carinii_ (Bueding, 1949a) were also sensitive to cyanide. On the other hand, Rogers (1948) found that cyanide exerted only a partial inhibition upon the oxygen consumption of _Ascaridia galli_ and _N. muris_. Similar results were obtained
for *A. galli* by Mitchell, Nabrit and Smith (1949). Laser (1944) showed that the oxygen consumption of *A. lumbricoides* was not affected by cyanide or azide.

Herrick and Thede (1945) reported that cytochrome oxidase was lacking from *A. lumbricoides*. A similar claim, however, was made by Bueding (1949a) for the cyanide-sensitive *L. carinii*. As suggested by Moulder (1950), the cyanide-sensitive enzymes are probably heavy-metal proteins, but need not include cytochrome oxidase.

The catalase content of *A. lumbricoides* was claimed to be very low (Laser, 1944), although no data were reported. Similar findings were made by Pennoit-de-Cooman and van Grembergen (1942) for the cestodes *Moniezia benidini*, *Taenia pisiformis*, and *Cysticercus pisiformis*. Magath (1918), using a rather uncertain method, produced evidence for the presence of a small amount of catalase in the body wall of *A. lumbricoides*. Magath's hypothesis was that the catalase functioned as an anti-enzyme to protect the worms against digestion by the host. On the other hand, specific anti-enzymes were demonstrated by Harned and Nash (1932) and Collier (1941).

**E. CARBOHYDRATE METABOLISM OF PARASITIC HELMINTHS**

This phase of parasite metabolism has been reviewed
recently in an excellent article by von Brand (1950).

With the exception of *Ancylostoma caninum* (von Brand and Otto, 1938), adult *Trichinella spiralis* (Oliver-Gonzales and Bueding, 1948), and, possibly, *Litomosoides carinii* in vitro in a glucose-free medium (Bueding, 1949a), parasitic helminths generally contain large endogenous stores of glycogen. Von Brand (1950) has suggested that such organisms, living in an anaerobic or semi-aerobic habitat, probably consume carbohydrate to a greater extent than other substances because the fermentation of carbohydrate yields more energy than does fat or protein. This, of course, is only speculation, although it had been shown many times that the glycogen stores of parasitic helminths are reduced during starvation (von Brand, 1934; 1937; 1938; 1945b; Toryu, 1935). Von Brand (1941) found that the fat stores of *A. lumbricoides* did not decrease during starvation. The glycogen content of several parasitic helminths is shown in Table II.

Females of *Ascaris lumbricoides*, *Parascaris equorum*, and *Ascaridia galli* were reported to contain more glycogen than males (von Brand, 1937a; Toryu, 1935; Reid, 1944, respectively). The difference was shown to be due to a large store of glycogen in the reproductive tissues of the female, the glycogen content of the other tissues being similar in both sexes.
TABLE II
THE GLYCOGEN CONTENT OF SOME PARASITIC HELMINTHS (FRESH)

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Glycogen % of wet wt.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moniezia expansa (sheep)</td>
<td>0.35 - 5.25</td>
<td>Wardle (1937)</td>
</tr>
<tr>
<td>Ascaris lumbricoides (pig)</td>
<td>3.5 - 6.5</td>
<td>von Brand (1937b)</td>
</tr>
<tr>
<td>Parascaris equorum (horse)</td>
<td>2.9 - 3.8</td>
<td>Toryu (1933)</td>
</tr>
<tr>
<td>Ascaridia galli (chicken)</td>
<td>3.6 - 4.7</td>
<td>Reid (1945a; 1945b)</td>
</tr>
<tr>
<td>Eustrongylides ignotus larva (killifish)</td>
<td>4.8 - 9.0</td>
<td>von Brand (1938)</td>
</tr>
<tr>
<td>Strongylus vulgaris (horse)</td>
<td>3.5</td>
<td>Toryu (1933)</td>
</tr>
<tr>
<td>Litomosoides carinii (rat)</td>
<td>1.23</td>
<td>Bueding (1949a)</td>
</tr>
<tr>
<td>Ancylostoma caninum (dog)</td>
<td>1.5</td>
<td>von Brand and Otto (1938)</td>
</tr>
<tr>
<td>Heterakis gallinae (chicken)</td>
<td>1.46 - 4.01</td>
<td>Glocklin (present thesis)</td>
</tr>
</tbody>
</table>

Bueding (1949a) demonstrated a rapid synthesis of glycogen by *L. carinii* from glucose added to the medium. He showed, also, the presence of two carbohydrate fractions in the worms. One of these was glycogen, and amounted to about 1.2 per cent of the body weight. The other was completely
fermentable, and comprised 4.6 per cent of the body weight. The increase in carbohydrate produced by exogenous glucose was confined to the glycogen fraction. No suggestion was made as to the identity of the non-glycogen carbohydrate. The glycogen fraction was shown to be similar to mammalian glycogen, as was glycogen isolated from *A. lumbricoides* (Baldwin and King, 1942), *Moniezia expansa* and *Fasciola hepatica* (Oesterlin and von Brand, 1934).

There has been considerable quantitative work done on the utilization of glycogen by various helminths under aerobic and anaerobic conditions *in vitro*. Table III is a partial reproduction of Table 2 from von Brand's review (1950).

Von Brand (1938) pointed out that the glycogen consumption of larval *E. ignotus* showed a greater similarity to that of free-living forms than it did to other parasitic helminths. The ratio of aerobic to anaerobic glycogen consumption in *E. ignotus* was 1:3, whereas for *A. lumbricoides* it was 1.0:1.3 and for the earthworm, *Lumbricus terrestris*, 1:5.

Table III shows clearly that, in most parasitic helminths, there is little difference between the aerobic and anaerobic consumptions of glycogen. This is very different from that found for free-living invertebrates (as illustrated...
by *L. terrestris* which exhibit a marked Pasteur effect, the carbohydrate being oxidized completely in the presence of oxygen to derive the maximum energy from a minimum of fuel. In parasitic worms, the presence of oxygen does not appear to diminish markedly the intensity of fermentation, and this in turn may indicate a limited requirement for oxygen. The amount of carbohydrate consumed aerobically is so similar to that consumed anaerobically, that a Pasteur effect is scarcely evident (von Brand, 1950). Since fermentation is, at best, an inefficient process, the large glycogen stores found in parasitic helminths, may be considered as a provision against unfavourable conditions.

It is wise to call the utilization of glycogen in this case "fermentation" rather than "glycolysis". Glycolysis is a term denoting the breakdown of glycogen, which has come to be applied specifically to the breakdown of glycogen to lactic acid. The formation of lactic acid occurs in the filaria *Litomosoides carinii* and in *Schistosoma mansoni* (Bueding, 1949b). In the case of most parasitic worms, however, the end-products of carbohydrate utilization occur as a variety of lower fatty acids. Until more is known of the nature and mechanism of the formation of these end-products, the process cannot correctly be called "glycolysis". For references concerning the end-products
of the carbohydrate metabolism of helminths, see the article of Bueding (1949b) and von Brand (1950).

**TABLE III**

**THE GLYCOGEN CONSUMPTION OF PARASITES**

(Gm. per 100 Gm. in 24 Hours at 37 to 41°C.)

<table>
<thead>
<tr>
<th>Species</th>
<th>Glycogen Consumption</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anaerobic</td>
</tr>
<tr>
<td><strong>TREMATODES:</strong></td>
<td></td>
</tr>
<tr>
<td><em>Fasciola hepatica</em></td>
<td>2.6</td>
</tr>
<tr>
<td><em>Schistosoma mansoni</em></td>
<td>79 to 96</td>
</tr>
<tr>
<td><strong>CESTODES:</strong></td>
<td></td>
</tr>
<tr>
<td><em>Moniezia expansa</em></td>
<td>1.0</td>
</tr>
<tr>
<td><em>Railletina cesticellus</em></td>
<td>4.8</td>
</tr>
<tr>
<td><strong>NEMATODES:</strong></td>
<td></td>
</tr>
<tr>
<td><em>Ascaris lumbricoides</em></td>
<td>1.4</td>
</tr>
<tr>
<td><em>Parascaris equorum</em></td>
<td>1.4</td>
</tr>
<tr>
<td><em>Ascaridia galli</em></td>
<td>3.6</td>
</tr>
<tr>
<td><em>Eustrongylides ignotus</em></td>
<td>0.7</td>
</tr>
<tr>
<td>(larvae)</td>
<td></td>
</tr>
<tr>
<td><em>Litomosoides carinii</em></td>
<td>34 to 41</td>
</tr>
<tr>
<td><em>Heterakis gallinae</em></td>
<td>3.29 (21 hr.)</td>
</tr>
</tbody>
</table>

* indicates total carbohydrate consumption in glucose-containing medium.
In Figure 1 are presented graphs, reproduced from the report of Toryu (1934), illustrating the anaerobic and aerobic utilization of glycogen by *Parascaris equorum*. Under anaerobic conditions, particularly, the rate of utilization of glycogen decreases as the worms continue to starve *in vitro*. For comparative purposes, a similar graph has been constructed from the data of von Brand (1937a) on the anaerobic utilization of glycogen by *A. lumbricoides*. This is shown in Figure 2.

Von Brand (1937b) claimed that 1/20 to 1/10 of the glycogen utilized anaerobically was resynthesized when *A. lumbricoides* was restored to air. The normal variation in glycogen from worm to worm was considerable, and an entire worm had to be sacrificed for each determination. The apparent increase may, therefore, have been within the range of experimental error and natural variation. There is no other information available on this phenomenon.

Reid (1944; 1945a; 1945b) has studied the depletion of glycogen stores of *Ascaridia galli* *in vivo*, and has compared it with worms maintained *in vitro*. It was found that starvation of the host (chicken) decreased the glycogen stores of the worms to the same extent as when they were kept *in vitro* in a nutrient-free medium. Prolonged starvation of the host resulted in the elimination of the parasite. Similar observations were made by Weinland (1901)
for *A. lumbricoides*. Von Brand (1939) found that starvation of the host of larval *E. ignotus* (killifish) caused no change in the glycogen content of the worm, which is a tissue parasite at this stage. The starvation of the host of *Ancylostoma caninum* (dog) also caused no change in the metabolism of the hookworm. This blood-sucker is thought to derive its carbohydrate nutrient from the blood sugar of the host (von Brand and Otto, 1938), which remains constant even during starvation.

It will be apparent from this review that one cannot generalize, to any great extent, upon the behaviour of parasites under experimental conditions, nor upon the interpretation of the behaviour observed. Parasitic helminths appear to vary greatly in the details of metabolism, and this may be, in part, a reflection of their high degree of adaptation to specific hosts. The chemical basis for host-parasite relations is completely unknown.

Details from a study of the metabolism of *Heterakis gallinae* will be presented in the subsequent pages. It is hoped that these results, added to those already obtained for other helminths, may be another step toward the ultimate understanding of helminth metabolism.
Figure 1
Decrease in Glycogen Content of *Parascaris equorum* (*Ascaris megaloccephala*) aerobically and anaerobically.
(from Toryu, 1935)

Figure 2
Decrease in Glycogen Content of *Ascaris lumbricoides* anaerobically
(from von Brand, 1937a)
MATERIALS AND METHODS

A. THE CULTURE OF H. GALLINAE EGGS

Mature, dead female *H. gallinae* were obtained from the caeca of fowl slaughtered at a commercial market. The eggs were released by gently rubbing the worms over a fine mesh sieve dipped into a Petrie dish containing distilled water. The suspension of eggs so obtained was set up for culture by the method of Swales (1948). After 15 to 20 days, about 50 per cent of the eggs were fully embryonated and infective.

B. THE INFECTION AND MAINTENANCE OF THE HOST

Young chickens (5 to 12 weeks old) were each given 1000 to 3000 embryonated eggs. The eggs were thoroughly mixed with poultry-mash and fed to the birds so that each consumed 200 to 600 eggs per day for five days. This method proved to be more effective than giving the birds the total number of eggs in one dose by capsule. Chickens infected by the former method contained, on an average, from 100 to 200 worms each. The actual variation, however, was from 10 to 500 worms per bird. The variation in the number of worms in birds infected by capsule was as great, but the actual number of worms was considerably less. In some experiments, naturally infected birds were used. These, also, showed a
marked variation in the number of worms harboured.

The chickens were fed commercial chicken feed with a supplement of fish liver oil during the winter months when they were kept indoors. Most of the birds were treated for coccidiosis with sulphamethazine before they were infected with *H. gallinae*.

C. PREPARATION OF *H. GALLINAE* FOR EXPERIMENTATION

The caecal contents of freshly killed chickens were stripped into a beaker of 1.0 per cent sodium chloride solution. Except for short intervals, all subsequent operations were carried out in a water bath maintained at 41°C. The worms were obtained free from macroscopic débris by repeated transfer to fresh saline. They were then counted, and on the basis of the number found, divided into a given number of samples. This sampling was conducted by transferring one worm at a time into successive beakers of saline, until the supply was exhausted. In this manner, errors due to variations in size, sex and weight of the worms were minimized.

A sample, composed of 50 to 125 worms, had a wet weight that varied from 15 to 50 mg.

In most experiments, 1.0 ml. of Krebs Ringer phosphate, pH 7.3, was used as the medium (Umbreit, Burris
and Stauffer, 1949, p. 119). For the absorption of carbon
dioxide, 0.10 or 0.15 ml. of 20 per cent potassium hydroxide
solution and a fluted 2 cm. square of No. 2 Whatman filter
paper were placed in the centre well of the flask.

For experiments in which gases other than air were
employed, gassing was accomplished by evacuating the flasks
five times to a residual pressure of 70 mm. mercury, and
refilling the flasks with the desired gas or gas mixture.
Fisher "Cello Seal" grease gave a satisfactory vacuum-
tight seal for this purpose. It was used routinely because,
unlike anhydrous lanoline, it retained its consistency
at 41°C.

Gas mixtures were prepared over 25 per cent
sodium chloride solution in order to decrease losses by
solution. Nitrogen and carbon dioxide were freed of oxygen
by passing them through a column of heated copper filings
(Elliott, 1949). The complete elimination of oxygen from
tank nitrogen gas was demonstrated by observing the absence
of oxygen consumption in a flask containing a piece of
yellow phosphorus in the centre well.

The sample of worms was blotted quickly on filter
paper. In this semi-dry condition, the worms adhered together
so that they could be transferred in one mass, by means of
a slender, hooked needle, into the main chamber of the flask which had been previously warmed in a shallow pan of warm water. The flasks were set upon their corresponding manometer joints, placed in the water bath, gassed (if necessary) and equilibrated for 15 minutes before the first reading was taken.

For the determination of oxygen consumption and carbon dioxide production in air and at various partial pressures of oxygen, Warburg's direct method was employed (Umbreit, Burris and Stauffer, 1949, p. 17-19). Readings were taken at 15 minute intervals for the first 30 minutes, and at 30 minute intervals thereafter. The experiments were concluded at the end of four hours, since at the end of this time the rate of oxygen consumption had sometimes begun to decline and, in addition, the bacteria in the medium had multiplied many-fold as evidenced by Gram-stained smears.

At the end of the experiment, the worms were examined for viability, and those not used for analysis were discarded.

E. CHEMICAL ANALYSIS OF E. GALLINAE

1. Preparation for Analysis

The use of separate samples for determination of glycogen and total reducing substance was inconvenient and
wasteful, especially since the supply of material was limited. It was therefore necessary to find a reliable method of using one sample of worms for both analyses.

The following procedure was developed and found to be satisfactory. The sample of worms was blotted on filter paper and was homogenized, in an all-glass Potter homogenizer, in 3.0 ml. of ice-cold 0.01N sulphuric acid. Fermentation was stopped immediately in this concentration of acid. To 0.5 ml. portions of the homogenate were added, immediately, 0.5 ml. of 1N sulphuric acid or 0.5 ml. of 60 per cent potassium hydroxide solution for subsequent determination of total reducing substance or glycogen, respectively.

2. Analysis for Carbohydrate

The worms were prepared as quickly as possible for carbohydrate utilization experiments. The zero-time sample was taken usually from 2 to 2½ hours after removing the worms from the caeca of the hosts. The other samples of worms were set up in test tubes, Thunberg tubes, or Warburg flasks in a water bath at 41°C. Entire samples were removed at required intervals of time and were homogenized immediately in 0.01N sulphuric acid and prepared for analysis. When Warburg flasks were used, manometric readings were taken for four hours, after which time, the remaining flasks were left stationary.
in the water bath until required for sampling.

Glycogen was determined by the method of Good, Kramer and Somogyi (1933). Total reducing substance was determined after hydrolysis of the homogenate in 5N sulphuric acid (Bueding, 1949a). Following neutralization, and dilution to a convenient volume, protein was precipitated by adding to 1.0 ml. of the diluted hydrolysate 3.0 ml. of glass-distilled water, and 0.5 ml. each of 1.8 per cent zinc sulphate solution and 0.1N sodium hydroxide solution (Somogyi, 1930). Non-fermentable residue was determined by substituting for the water a 10 per cent suspension of bakers' yeast which had been washed repeatedly until no inherent reducing power remained. The yeast was kept in the refrigerator and was warmed to room temperature before using. Its fermenting power was tested in each experiment by permitting it to act for 15 minutes upon a standard glucose solution. Reducing power was determined by the method of Nelson (1944). The variation in glycogen or total reducing substance between samples from the same lot of worms was less than four per cent.

3. Analysis for Substances other than Carbohydrate

Nitrogen was determined by the method of Johnson as described by Umbreit, Burris and Stauffer (1949, p.161).
The variation between samples was sometimes as great as ten per cent.

Catalase was determined, in worm homogenates made in ice-cold distilled water, by the method of Jolles, as described by Summer and Somers (1943, p. 171).
EXPERIMENTAL AND DISCUSSION

A. GENERAL OBSERVATIONS ON THE SURVIVAL OF H. GALLINAe IN VITRO

Dead worms, only, were recovered from the caeca of chickens which had been killed several hours previously. No experiments were conducted to determine the limit of survival time of the worms in the caeca after the death of the host. It was convenient to remove the caeca immediately after killing the fowl, and the worms, so obtained, were always alive and very active if kept at 41°C, which approximated the body temperature of the chicken.

H. gallinae survived well in 1.0 per cent sodium chloride solution, but lived somewhat longer in a balanced buffered solution such as Krebs-Ringer phosphate, pH 6.4 to 7.4. The pH of the caecal contents was found to lie between 6.8 and 7.3. The maximum survival time of the worms in Krebs-Ringer phosphate was three days. Addition of glucose to the medium (or the use of Tyrode's solution) caused a rapid multiplication of bacteria. Unless the medium was well buffered, the acid end-products of the bacteria quickly decreased the pH below 5, and the worms died within 30 hours. Davey (1938b) found that the presence of glucose did not increase the survival time of the sheep nematode, Ostertagia circumcincta. Glucose was found to be advantageous for the
survival of larval *Eustrongylides ignotus* (von Brand and Simpson, 1944) and *Fasciola hepatica* (Stephenson, 1947).

*H. gallinæ*, set up in various media in Thunberg tubes, were gassed with oxygen-free nitrogen. The survival of the worms did not differ from that in air, although increased motility was observed. This is similar to the findings of Fairbairn and Reesal (1950) for *Ascaris lumbricoides*, and quite unlike the results of Slater (1925) and Davey (1938b) who used hydrogen instead of nitrogen for producing anerobic conditions.

**B. VARIABILITY WITHIN AND BETWEEN EXPERIMENTS**

The sampling procedure, previously described, produced good replicate results within any one experiment. The analytical data from two experiments are presented in Table IV. The experimental variation between samples was found to be less than five per cent (except in the case of the nitrogen determinations).

On the other hand, the variation in results between experiments was found to be rather large (Table V). There was a 20 to 30 per cent variation for most of the determinations although the variations in wet weight per worm and glycogen content were considerably larger. The greatest part of this variation was probably caused by differences in age, size, and nutrition of the various lots of worms.
### TABLE IV

**IISAN ANALYTICAL DATA WITHIN EXPERIMENTS**

<table>
<thead>
<tr>
<th>Determination</th>
<th>Mean Value</th>
<th>Extremes</th>
<th>No. of Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet Weight (gm.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>0.0438</td>
<td>0.0426 - 0.0445</td>
<td>5</td>
</tr>
<tr>
<td>b.</td>
<td>0.0345</td>
<td>0.0342 - 0.0350</td>
<td>4</td>
</tr>
<tr>
<td>Nitrogen (% of wet wt.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>3.01</td>
<td>2.88 - 3.16</td>
<td>5</td>
</tr>
<tr>
<td>b.</td>
<td>3.57</td>
<td>3.52 - 3.60</td>
<td>4</td>
</tr>
<tr>
<td>Glycogen (% of wet wt.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>2.35</td>
<td>2.30 - 2.38</td>
<td>4</td>
</tr>
<tr>
<td>b.</td>
<td>1.88</td>
<td>1.85 - 1.91</td>
<td>4</td>
</tr>
</tbody>
</table>

### TABLE V

**IISAN EXPERIMENTAL DATA COMPILLED THROUGHOUT THE PROJECT**

<table>
<thead>
<tr>
<th>Determination</th>
<th>Mean Value ± S.D.*</th>
<th>Extremes</th>
<th>No. of Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet Weight per Worm (mg.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry Weight</td>
<td>0.47 ± 0.17</td>
<td>0.17 - 0.83</td>
<td>86</td>
</tr>
<tr>
<td>Nitrogen (% of wet wt.)</td>
<td>25.3 ± 3.9</td>
<td>19.4 - 32.2</td>
<td>20</td>
</tr>
<tr>
<td>Glycogen (% of wet wt.)</td>
<td>3.06 ± 0.59</td>
<td>2.32 - 4.52</td>
<td>34</td>
</tr>
<tr>
<td>Total Reducing Subst. (% of Wet wt.)</td>
<td>2.75 ± 0.74</td>
<td>1.46 - 4.01</td>
<td>20</td>
</tr>
<tr>
<td>$Q_0^2$</td>
<td>4.68 ± 0.93</td>
<td>3.47 - 6.67</td>
<td>17</td>
</tr>
<tr>
<td>$Q_0^2$ (ww)</td>
<td>3.61 ± 0.53</td>
<td>3.00 - 4.68</td>
<td>25</td>
</tr>
<tr>
<td>$Q_0^2$ (N)</td>
<td>0.979 ± 0.156</td>
<td>0.555 - 1.179</td>
<td>21</td>
</tr>
<tr>
<td>$Q_0^2$ (ww)</td>
<td>34.8 ± 6.6</td>
<td>31.2 - 40.8</td>
<td>10</td>
</tr>
<tr>
<td>$Q_0^2$ (ww)</td>
<td>0.700 ± 0.122</td>
<td>0.292 - 1.008</td>
<td>20</td>
</tr>
<tr>
<td>R.Q.</td>
<td>0.74 ± 0.08</td>
<td>0.61 - 0.88</td>
<td>20</td>
</tr>
</tbody>
</table>

\[ S.D.* = \sqrt{\frac{\sum x^2 - \bar{x}^2}{N}} \]

For definition of other terms see page 8.
C. KANOETRIC EXPERIMENTS ON H. GALLINAE

1. Gas Exchange in Air

a. Variation due to Rate of Shaking

For the first few experiments, the rate of shaking was 105 per minute through a travel of 4 cm. At this rate, the oxygen consumption began to decline after about three hours, and a considerable number of dead worms were generally found in the flasks at the end of the experiment. In later experiments, a shaking rate of 98 per minute was found to be as efficient and was less damaging to the worms; the linearity of the rate of oxygen consumption continued for four hours or more. At a rate of 80 oscillations per minute, there was a marked decrease in oxygen consumption. Data from three experiments are illustrated in Figure 3.

Because dead or moribund worms were found in flasks which had been shaken at the more rapid rate, it may be assumed that the shaking caused some physical damage to the worms. Healthy worms were very active during the experiment, and this activity possibly augmented the equilibrating effect of the shaking even at the moderate rate of 98 per minute. There was no evidence of clumping or entangling of the worms in the flask as was noticed by Rogers (1949b) for Nematodirus spp., etc.
The oxygen consumption was found to vary from experiment to experiment as indicated in Table V. The variation of the oxygen consumption as compared with the variation in wet weight and surface area of the eggs is shown in Table VI.

FIGURE 3

The Variation in Oxygen Consumption Due to the Rate of Shaking.
b. Variation of the Oxygen Consumption with the Size of the Worm.

The oxygen consumption was found to vary from experiment to experiment as indicated in Table V. The variation of the oxygen consumption as compared with the variation in wet weight and surface area of the worm is shown in Table VI.

<table>
<thead>
<tr>
<th>Number of Worms per Sample</th>
<th>Mean wet weight per worm (mg.)</th>
<th>Relative Surface area per gm.</th>
<th>$Q_{O_2}(ww)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>68</td>
<td>0.63</td>
<td>1.00</td>
<td>0.97</td>
</tr>
<tr>
<td>70</td>
<td>0.51</td>
<td>1.07</td>
<td>0.98</td>
</tr>
<tr>
<td>87</td>
<td>0.51</td>
<td>1.08</td>
<td>0.83</td>
</tr>
<tr>
<td>100</td>
<td>0.51</td>
<td>1.08</td>
<td>0.61</td>
</tr>
<tr>
<td>53</td>
<td>0.49</td>
<td>1.08</td>
<td>1.11</td>
</tr>
<tr>
<td>88</td>
<td>0.43</td>
<td>1.14</td>
<td>0.79</td>
</tr>
<tr>
<td>125</td>
<td>0.35</td>
<td>1.22</td>
<td>0.89</td>
</tr>
<tr>
<td>54</td>
<td>0.34</td>
<td>1.24</td>
<td>0.85</td>
</tr>
<tr>
<td>100</td>
<td>0.33</td>
<td>1.29</td>
<td>0.78</td>
</tr>
<tr>
<td>100</td>
<td>0.31</td>
<td>1.29</td>
<td>0.75</td>
</tr>
<tr>
<td>110</td>
<td>0.25</td>
<td>1.36</td>
<td>1.05</td>
</tr>
<tr>
<td>90</td>
<td>0.24</td>
<td>1.41</td>
<td>0.98</td>
</tr>
</tbody>
</table>
Relative surface areas were calculated from Meeh's formula (surface area = $K W^{2/3}$; where $K$ is a constant, and $W$ is the wet weight) (Krüger, 1940). Since a relative, rather than an absolute, relationship was sought, the value for $K$ was assumed to be unity. These relative values were obtained by giving the lowest number, calculated by Meeh's formula, the arbitrary value of 1.0. The other values were then calculated accordingly. Krüger (1940) found that the oxygen consumption of individual *Ascaris lumbricoides* was proportional to the surface area. Rogers (1948) could not compare different species on this basis. Table VI does not indicate any correlation between the rate of oxygen consumption and the size of *H. gallinae*. The results presented in this table were taken at random from experiments throughout the project.

The rate of oxygen consumption of *H. gallinae* calculated on a basis of percentage dry weight, or percentage nitrogen, also proved to be very variable (see Table V).

c. **Effect of Glucose**

The addition of glucose had no effect upon the oxygen consumption or carbon dioxide production by *H. gallinae*. The results from one experiment are presented graphically in Figure 4. In this experiment, glucose
Figure 4

The Effect of the Addition of Glucose on the Gas Exchange
dissolved in Krebs-Ringer phosphate (final concentration in the flask was 0.015M glucose) was tipped into the flask from a side-arm after 75 minutes respiration in air. The $Q_{O_2}(ww)$ remained constant at 0.865 and the R.Q. at 0.72.

The respiration of another fowl nematode, *Ascaridia galli* was also found to be unaffected by the addition of glucose to the medium (Rogers and Lazarus, 1949). As discussed previously, the effect of glucose on the respiration of parasitic helminths has been found to be variable. Intestinal helminths do not seem to be much affected by the addition of glucose to the medium; however, this may only indicate that these worms do not feed in vitro. On the other hand, there is probably very little glucose present in the host intestine, especially at the level of the caecum in the chicken. Glucose is easily and quickly absorbed, in the upper part of the intestinal tract, even when present at hypotonic levels (Best and Taylor, 1945).

d. Effect of Sodium Azide and Determination of Catalase

After 45 minutes respiration in 1.0 ml. of Krebs-Ringer phosphate, pH 6.4, 0.1 ml. of a solution of sodium azide, dissolved in Krebs-Ringer phosphate,
was tipped into the flask from a side arm. Oxygen consumption was recorded for 60 minutes, after which time the flasks were removed from the manometers and the azide-medium was quickly replaced with warm, fresh Krebs-Ringer phosphate. The flasks were replaced on the manometers, and the respiration was observed for an additional 60 minutes.

\( \text{M/400 azide inhibited the oxygen consumption by 25.6 per cent, and M/1200 azide, by 19.3 per cent. After removal of the azide, 93.0 and 88.3 per cent (respectively) of the control respiration was attained. Experimental data are presented in Table VII. Only one experiment of this nature was attempted, and the results are, therefore, inconclusive.} \)

**TABLE VII**

**THE EFFECT OF SODIUM AZIDE ON OXYGEN CONSUMPTION**

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Conditions</th>
<th>( Q_{O_2} \text{(ww)} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 60</td>
<td>no azide</td>
<td>0.976 0.997</td>
</tr>
<tr>
<td>60 - 120</td>
<td>azide added</td>
<td>(M/400) (M/1200)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.726 0.809</td>
</tr>
<tr>
<td>125 - 195</td>
<td>azide removed</td>
<td>0.909 0.879</td>
</tr>
</tbody>
</table>
Mitchell, Nabrit and Smith (1949) found that 1/400 azide produced a 25 per cent inhibition of the oxygen consumption of *Ascaridia galli*, which is also an intestinal parasite of chickens.

Azide is known to react with catalase to form a spectroscopically distinct, inactive substance (Sumner and Somers, 1943). The amount of catalase present in *H. gallinae* was therefore determined. K values obtained were linear and could be extrapolated to a K₀ value which was found to be 39.3 per gm. worm. The corresponding K₀ value obtained for whole human blood (haematocrit, 50 per cent) was 301 per gm. The catalase content of the worms was therefore 1/7.7 that of whole blood. A portion of the homogenate which had been heated in a boiling water bath for 20 minutes showed no catalase activity.

Burris and Little (1949) have stated that catalase is absent only from obligate anaerobic bacteria and certain micro-aerophilic bacteria. Laser (1944) claimed that *Ascaris lumbricoides* contained very little catalase. He gave no comparative values (i.e. against liver or erythrocytes), however, and
what was meant by "low catalase content" is not clear. Laser attributed the death of *A. lumbricoides*, under high oxygen tensions, to poisoning by hydrogen peroxide which could not be removed adequately by the small amount of catalase present. *H. gallinae* was also found to die rather quickly under high oxygen tensions. A tension equivalent to 380 mm. of mercury (50% oxygen) was sufficient to cause the death of a considerable number of worms. It is possible that hydrogen peroxide was formed in toxic amounts under high tensions of oxygen since the R.Q. was found to decrease with increasing oxygen tensions, indicating the possibility that some oxidative end-product besides carbon dioxide was formed. There is no evidence that peroxide was formed, however, and there are undoubtedly many unknown factors involved in the decrease of R.Q. with increasing oxygen tensions. Until more is known of the oxidative mechanisms of the worm, further speculation would be premature.

e. Effect of Carbon Dioxide

Because of the necessity of using Warburg's
direct method for this study, it was impossible to determine the effect of carbon dioxide on the gas exchange of *H. gallinae*. It is possible that the complete absence of carbon dioxide might alter the true picture of oxygen consumption. Laser (1942) found that carbon dioxide tended to stabilize the oxygen consumption of mammalian tissue slices, and that R.Q. values were valid only if calculated on short-term experiments in the presence of a constant tension of carbon dioxide.

To obtain a more valid estimation of the respiration of *H. gallinae*, a method which does not require absorption of carbon dioxide would be necessary. Such methods are available but they presented disadvantages which could not be overcome. One method possible was that of Dixon and Keilin (Umbreit, Burris and Stauffer, 1949; Dixon, 1943). In this method, the carbon dioxide formed could be absorbed at the end of the experiment by the addition of alkali to the flasks. Although Dixon-Keilin flasks were available, they had a volume of about 15 ml. which would require more
material than could be prepared at one time. Another alternative was Warburg's indirect method, which would employ either flasks of markedly different volumes, or flasks of similar volume containing markedly different volumes of fluid. The former instance would again require too much material for an experiment, and the latter modification was impossible because the limiting volume of the small flasks available prevented much alteration in the volume of fluid. It was therefore necessary to rely upon Warburg's direct method for comparable results, even though the results obtained must be treated with reservation.

2. Gas Exchange in Various Tensions of Oxygen

The oxygen consumption and carbon dioxide production of *H. gallinae* were determined in oxygen at partial pressures equivalent to 7.6, 38, 76, 266, and 380 mm. of mercury (1, 5, 10, 35, and 50 per cent oxygen). Gas mixtures were made up in oxygen-free nitrogen. An air control was run with each experiment. The data obtained are presented in Figure 5 and Table VIII.

At an oxygen tension equivalent to 7.6 mm. of mercury (1.6 oxygen) *H. gallinae* was found to have an initial *Q_{O2}^{ww}* of almost 44 per cent that in air. The worms at all
Figure 5

Gas Exchange at Various Oxygen Tensions
tensions of oxygen were very active, much more so than it
was possible for them to be \textit{in vivo} in the semi-solid
caecal contents. Therefore, as suggested by Rogers (1949b),
the maximum oxygen consumption found \textit{in vitro} may be
considerably higher than that occurring \textit{in vivo} where less
energy is needed for motion. It is possible that the worms
may utilize efficiently the small amount of oxygen which
may be present in the caecum. The presence or absence of
oxygen in the fowl caecum has not been determined.

\textbf{TABLE VIII}

\textbf{RESPIRATION IN VARIOUS TENSIONS OF OXYGEN}

<table>
<thead>
<tr>
<th>Oxygen tension (mm. Hg.)</th>
<th>( Q_{O_2} ) (ww) 1st 30 min.</th>
<th>( Q_{O_2} ) (ww) 1st 60 min.</th>
<th>R.Q. 1st 50 min.</th>
<th>Corresponding value for air ( Q_{O_2} ) (ww) R.Q.</th>
<th>( Q_{O_2} ) (ww) as % of air value</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.6 (1% )</td>
<td>0.326</td>
<td>0.274</td>
<td>2.04</td>
<td>0.746</td>
<td>0.81</td>
</tr>
<tr>
<td>38 (5% )</td>
<td>0.791</td>
<td>0.765</td>
<td>0.88</td>
<td>0.977</td>
<td>0.78</td>
</tr>
<tr>
<td>76 (10% )</td>
<td>0.770</td>
<td>0.747</td>
<td>0.82</td>
<td>0.887</td>
<td>0.83</td>
</tr>
<tr>
<td>266 (35% )</td>
<td>1.320</td>
<td>1.320</td>
<td>0.85</td>
<td>0.744</td>
<td>0.81</td>
</tr>
<tr>
<td>380 (50% )</td>
<td>1.502</td>
<td>1.392</td>
<td>0.67</td>
<td>0.842</td>
<td>0.88</td>
</tr>
</tbody>
</table>

Although no study was carried out to determine
whether or not haemoglobin is present in \textit{H. gallinae}, the
pink colour of the worms indicated that haemoglobin might
be present as in other nematodes (Davey, 1949a; 1949b;
Rogers, 1949c). If such is the case, it is possible that
haemoglobin would show the low loading-tension which characterizes other parasite haemoglobins. This again suggests that the worms would be able to make use of exceedingly low oxygen tensions.

At an oxygen tension equivalent to 7.6 mm. of mercury, the rate of respiration of *H. gallinae* fell off markedly after the first 30 minutes. This was due in part to the limiting amount of oxygen present, although there may have also been a diffusion error. Because of the ill effects caused by rapid shaking, the shaking rate was not increased from 98 per minute even at low oxygen tensions. The worms remained active at low oxygen tensions, however, which may have reduced a possible diffusion error.

In oxygen at a partial pressure equivalent to 380 mm. of mercury (50% oxygen), the rate of respiration fell off after 60 minutes. At the end of the experiment there were many dead worms in the flasks, and those which remained alive were moribund. Possibly some toxic end-product was formed, or some necessary catalyst or pigment was permanently oxidized at high tensions of oxygen.

At an oxygen tension equivalent to 7.6 mm. of mercury, the respiratory quotient of *H. gallinae* was more than twice that in air. The R.Q. decreased with increasing
oxygen tension until, at a partial pressure of oxygen equivalent to 380 mm. of mercury, it was 0.65 as compared with 0.89 in air. Laser (1944) found that the R.Q. of *Ascaris lumbricoides* decreased from 1.1 in air to 0.6 in pure oxygen. In the case of mammalian tissue (retina, chorion, tumour, etc.), however, Laser (1937) found that the R.Q. decreased with decreasing oxygen tension. A discussion concerning the significance of R.Q. values will appear subsequently.

Since the rate of oxygen consumption in air varied from one experiment to another, the $Q_{O_2}$ values obtained at the various oxygen tensions could not be compared directly. For this reason these values were compared with the mean $Q_{O_2}$ in air for the series. For convenience in comparing the results with those obtained by other workers, $Q_{O_2}$ (dry weight) values were calculated. The data are presented in Table IX. The relationship between $Q_{O_2}$ and oxygen tension is shown graphically in Figure 6, and is compared with results obtained by Laser (1944) for *A. lumbricoides*, and by Rogers (1949b) for *Nippostrongylus muris*. The three curves are similar in shape at oxygen tensions less than that of air. At higher tensions of oxygen, the curve for *H. gallinae* takes on a sigmoid shape which is not shown by *A. lumbricoides* (Rogers' results did not extend above an oxygen tension equivalent to
### Table IX

**The Rate of Oxygen Consumption in Various Tensions of Oxygen**

<table>
<thead>
<tr>
<th>Oxygen tension (mm. mercury)</th>
<th>QO₂ (1st 30 min.)</th>
<th>Corresponding QO₂ in air to mean QO₂ in air</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.6</td>
<td>1.58</td>
<td>3.54</td>
</tr>
<tr>
<td>38</td>
<td>3.74</td>
<td>4.61</td>
</tr>
<tr>
<td>76</td>
<td>3.14</td>
<td>3.62</td>
</tr>
<tr>
<td>152</td>
<td>4.94</td>
<td>2.86</td>
</tr>
<tr>
<td>266</td>
<td>5.68</td>
<td>3.80</td>
</tr>
<tr>
<td>380</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

**Figure 6**

Variation of Rate of Oxygen Uptake with the Oxygen Tension
150 mm. of mercury). The relationship in the case of *H. gallinacea*, possibly, may not be truly sigmoid. Values obtained at the higher tensions of oxygen may be displaced to the right due to experimental variation or some other factor.

Tang (1933) demonstrated that the *Q*$_{O2}$ of many organisms showed a hyperbolic relationship to the oxygen tension (especially at low tensions) which could be described by the equation:

$$A = \frac{P}{K_1 + K_2P}$$

where $A$ is the *Q*$_{O2}$, $P$ is the oxygen tension in mm. of mercury, and $K_1$ and $K_2$ are constants. When $P/A$ was plotted against $P$, Tang obtained a straight line which had as its ordinate-intercept, $K_1$, and as its slope, $K_2$.

The data obtained for *H. gallinacea* were treated according to the above equation, and the graph obtained by plotting $P/A$ against $P$ is shown in Figure 7. At oxygen tensions less than 152 mm. of mercury, the relationship is linear; at higher tensions of oxygen, the results are irregular. $K_1$ (ordinate-intercept) is 3.0 and $K_2$ (slope) is 0.27. Rogers (1949b) found that for *Nippostrongylus muris*, $K_1$ was 1.2 and $K_2$ was 0.14, for *Nematodirus spp.*, 2.6 and 0.15, respectively, and for *Haemonchus contortus*, 10 and 0.14 respectively. Laser's (1944) results, plotted
Figure 7
Relationship Between P/A and P
in the same manner, did not intercept the ordinate, and gave a slope \((K_x)\) of 1.84. It is interesting to note that for the results of Rogers and those on *H. gallinae*, \(K_1\) increased with increasing size of the species.

Equations similar to that developed by Tang (1933) have been derived for the dissociation curves of oxyhaemoglobin (*Barcroft, 1928*) and oxyhaemocyanin (*Stedman and Stedman, 1928*). Other similar equations have been developed for the absorption of gases on a solid surface (*Langmuir, 1918*), and for cell respiration, assuming the transportation of oxygen by an intermediary carrier or enzyme (*Warburg and Kubowitz, 1929; *Gerard, 1931*). Tang (1935) stressed the fact, however, that the equation expressing the relationship between the \(Q_O_2\) and the oxygen tension is purely empirical, and that any similarity to other equations describing absorption, dissociation, or oxidation-reduction does not establish an actual identity with them. One cannot attach too much significance to the constants obtained until more data are available.

### 3. Anaerobic Production of Carbon Dioxide

Carbon dioxide was produced by *H. gallinae* under anaerobic conditions. Carbon dioxide retention (in Krebs-

References cited from Tang (1933).
Ringer phosphate) was negligible in most experiments. The rate of carbon dioxide production was not linear, but decreased with time until at the end of four hours, the rate was less than half that at 30 minutes. No alkali-insoluble gas, for example, ammonia, was given off. The results of two experiments are given in Table X.

**TABLE X**

THE ANAEROBIC PRODUCTION OF CARBON DIOXIDE

(KREBS-RINGER PHOSPHATE)

<table>
<thead>
<tr>
<th></th>
<th>$\frac{Q \text{N}_2}{\text{CO}_2}$ (ww)</th>
<th>$\text{CO}_2$ produced per gm. worm in 4 hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>a.</td>
<td>30 min. 456 60 min. 433 240 min. 226</td>
<td>1206</td>
</tr>
<tr>
<td>b.</td>
<td>509 418 206</td>
<td>1278</td>
</tr>
</tbody>
</table>

In one experiment, the carbon dioxide production was determined for 30 minutes in air, then the flasks were gassed with pure nitrogen and, after 105 minutes, carbon dioxide production in air was again determined. The results are shown in Table XI. The amount of carbon dioxide produced anaerobically was less than that produced aerobically. This has been shown to be the case in other helminths: *Parascaris equorum* (Toryu, 1934), *Ascaris lumbricoides* (von Brand, 1938), and *Trichinella spiralis*
larvae (Stannard, McCoy and Latchford, 1938). It appears probable that in these helminths, aerobic carbon dioxide production is the sum of that due to fermentation and that due to complete oxidation of substrates.

**TABLE XI**

**COMPARISON OF CARBON DIOXIDE PRODUCTION IN NITROGEN AND IN AIR.**

<table>
<thead>
<tr>
<th></th>
<th>$Q_{CO_2}(ww)$</th>
<th>R.Q.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>599</td>
<td>0.77</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>114</td>
<td></td>
</tr>
<tr>
<td>Air</td>
<td>548</td>
<td>0.73</td>
</tr>
</tbody>
</table>

**D. UTILIZATION OF ENDOGENOUS CARBOHYDRATE BY H. GALLINAE**

1. **Amounts of Glycogen and Total Reducing Substance Present.**

Glycogen amounted to $2.75 \pm 0.74$ per cent, and total reducing substance, to $4.68 \pm 0.93$ per cent of the wet weight of *H. gallinæ* (Table V). The determinations, in all cases, were made after the worms had survived *in vitro* for approximately three hours. The initial rate of carbohydrate utilization was very great, as will be shown presently, and so, the actual content of glycogen and total
reducing substance in vivo might have been almost twice the amount found. The nature of the reducing substances other than glycogen was not determined, but their reaction with the highly specific aldehyde reducing agents (Nelson, 1944) and their complete fermentation by yeast, make it appear probable that they are carbohydrate of some kind.

Bueding (1949a) discovered a fermentable reducing substance in Litomosoides carinii which he called "total carbohydrate". The glycogen amounted to only 20 per cent of this substance when the worms were kept in a glucose-free medium.

2. Aerobic Carbohydrate Utilization

Aerobically, both the glycogen and total reducing substance in H. gallinae decreased in vitro, rather rapidly at first, and then at a gradually decreasing rate. The data from a typical experiment are presented in Table XII and Figure 8. The data in the table and the points on the graph marked ♦ indicate the presence of metabolic carbon dioxide and will be discussed later. All other data are for worms surviving in a complete absence of carbon dioxide.

This particular experiment employed Warburg flasks, and the gas exchange was determined for the first four hours.
The $Q_{02}$ was 1.109, the $Q_{CO2}$ (ww) 0.909, and the R.Q. 0.82.

In four hours, 22.8 micromoles of glycogen and 24.2 micromoles of total reducing substance (both expressed as glucose) were utilized per gram worm. Only 49.6 micromoles of oxygen were consumed, although 136.8 and 145.2 micromoles of oxygen would have been necessary to oxidize this amount of carbohydrate (respectively). These results were confirmed in several experiments. Evidently, most of the carbohydrate disappearing was not being oxidized but was being fermented even in the presence of oxygen.

**TABLE XII**  
THE UTILIZATION OF ENDOGENOUS CARBOHYDRATE IN AIR

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Per cent of initial glycogen remaining</th>
<th>Per cent of total reducing substance remaining</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>56.1</td>
<td>67.3</td>
</tr>
<tr>
<td>10</td>
<td>26.0</td>
<td>43.9</td>
</tr>
<tr>
<td>24</td>
<td>15.0</td>
<td>22.1</td>
</tr>
<tr>
<td>$x_{24}$</td>
<td>$x_{20.1}$</td>
<td>$x_{36.8}$</td>
</tr>
</tbody>
</table>

3. Anaerobic Carbohydrate Utilization

Carbohydrate was utilized only slightly more quickly anaerobically than aerobically. The anaerobic
Figure 8

The Utilization of Endogenous Carbohydrate in Air
decrease in total reducing substance was somewhat greater than was glycogen. This may indicate the anaerobic fermentation of a reserve carbohydrate which augments the energy from the fermentation of glycogen, much of which is fermented aerobically as well.

Data from one experiment are presented in Table XIII and Figure 9. In this experiment, the worms set up in test tubes or Thunberg tubes gassed with nitrogen, and parallel air and nitrogen samples were taken. No respiratory study was made.

In 21 hours, 28.8 and 32.9 mg. glycogen were consumed aerobically and anaerobically (respectively) per gm. worm. The ratio aerobic: anaerobic was therefore 1:1.14. The corresponding ratio for total reducing substance was 1:1.23. This compares well with the ratio found for most other parasitic helminths, with the exception of larval *Eustrongylides ignotus* (von Brand, 1938), and is quite unlike that found for free-living invertebrates. For further details see Table III and page 17.
TABLE XIII
THE ANAEROBIC UTILIZATION OF ENDOGENOUS CARBOHYDRATE

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>% of initial glycogen remaining</th>
<th>% of initial total red. subst. remaining</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>air nitrogen</td>
<td>air nitrogen</td>
</tr>
<tr>
<td>4</td>
<td>68.8</td>
<td>66.0</td>
</tr>
<tr>
<td>8</td>
<td>47.0</td>
<td>44.2</td>
</tr>
<tr>
<td>21</td>
<td>28.6</td>
<td>18.4</td>
</tr>
</tbody>
</table>

Figure 9
The Utilization of Endogenous Carbohydrate
Aerobically and Anaerobically
4. Effect of Carbon Dioxide on Carbohydrate Utilization

It was discovered, early in the project, that the presence of accumulated metabolic carbon dioxide had a carbohydrate-sparing effect. This has been indicated in Table XII and Figure 8 by the data marked *. The effect of carbon dioxide on carbohydrate utilization was followed subsequently during experiments in air and nitrogen, in the presence of metabolic and added carbon dioxide. Both aerobically and anaerobically, an accumulation of metabolic carbon dioxide showed a carbohydrate-sparing action which was even more pronounced in the presence of five per cent added carbon dioxide. Data from the experiments are presented in Table XIV.

The nature of the carbohydrate-sparing action is not as yet known. The carbon dioxide may have been built into a substrate of low molecular weight, and thus used more or less directly, or it may have been incorporated into glycogen, or some other carbohydrate, since the sparing effect on the total reducing substance seemed to be somewhat greater than the glycogen. Whether carbohydrate was not used so rapidly, or whether it was replaced as quickly as it was used, cannot be known until the intermediary mechanisms have been studied.
Roger and Lazarus (1949) have found that bicarbonate was removed from the medium by cell-free extracts of *Nematodirussp.* and *Ascaridia galli*, but no details or further results have been published. The only other observations on the effect of carbon dioxide have been on survival time of *Parascaris equorum* and *Ascaris lumbricoides* (Toryu, 1935; Fairbairn and Reesal, 1950, respectively). It is not known if carbon dioxide exerted a carbohydrate-sparing effect in these worms. If such an effect was present, however, the increase in survival time in the presence of carbon dioxide might have merely been an indication that the worms were starving to death more slowly than under conditions where carbon dioxide was unavailable.

Various organisms have been shown to fix carbon dioxide into over 17 different compounds (Wood, 1946); included among these are glycogen and intermediates of the tri-carboxylic acid cycle. There has been some evidence to show that the tri-carboxylic acid cycle, or a modification thereof, functions in some parasitic helminths (Massey, 1949). However, even if such intermediates were formed from the fixation of carbon dioxide, and subsequently oxidized, it would not explain a carbohydrate-sparing effect under anaerobic conditions. The problem of the fixation of carbon dioxide may indeed be vital to the understanding of helminth metabolism.
### TABLE XIV

**The Utilization of Endogenous Carbohydrate in the Presence and Absence of Carbon Dioxide**

*Glycogen and T.R.S. expressed as glucose.*

<table>
<thead>
<tr>
<th>Time (hr.)</th>
<th>Atmosphere</th>
<th>Glycogen</th>
<th>Total Reducing Subst.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mg. per</td>
<td>% of Initial Glycogen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gm. Worm</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>Nitrogen 5% CO₂</td>
<td>40.5</td>
<td></td>
</tr>
<tr>
<td>4.25</td>
<td>Nitrogen (no CO₂)</td>
<td>37.1</td>
<td>91.7</td>
</tr>
<tr>
<td>4.25</td>
<td>Nitrogen-Metabolic CO₂ (about 0.5%)</td>
<td>30.2</td>
<td>74.7</td>
</tr>
<tr>
<td>0</td>
<td>Nitrogen-Metabolic CO₂ (about 0.5%)</td>
<td>23.0</td>
<td></td>
</tr>
<tr>
<td>4.25</td>
<td>Nitrogen (no CO₂)</td>
<td>17.5</td>
<td>76.2</td>
</tr>
<tr>
<td>4.25</td>
<td>Nitrogen (no CO₂)</td>
<td>15.8</td>
<td>68.8</td>
</tr>
<tr>
<td>0</td>
<td>Air 5% CO₂</td>
<td>43.5</td>
<td></td>
</tr>
<tr>
<td>4.0</td>
<td>Air-Metabolic CO₂ (about 1.5%)</td>
<td>36.3</td>
<td>83.4</td>
</tr>
<tr>
<td>4.0</td>
<td>Air-Metabolic CO₂ (about 1.5%)</td>
<td>33.2</td>
<td>76.3</td>
</tr>
<tr>
<td>4.0</td>
<td>Air (no CO₂)</td>
<td>31.6</td>
<td>72.8</td>
</tr>
</tbody>
</table>
DISCUSSION

The results which have been presented show clearly that *H. gallinae* utilizes oxygen when this is available, and that the utilization is efficient at low partial pressures. Thus, at a partial pressure equivalent to 7.6 mm. of mercury the rate of oxygen consumption was equal to 44 per cent of the rate in air. Since the great *in vitro* activity of the worms, even at low oxygen tensions, is probably much higher than the natural *in vivo* activity, it is possible that the oxygen requirement of this species can be satisfied by the very small amounts of oxygen which may be present in the caecum of the host (Rogers, 1949b). This would be an interesting and important adaptation of the parasite to the environment provided by the host.

On the other hand, *H. gallinae* survives as long under strictly anaerobic conditions as it does in air, and during this period consumes only slightly more endogenous carbohydrate than it does in air. Under aerobic conditions, furthermore, the oxygen consumed accounts for only 1/3 of the carbohydrate disappearing. These considerations provide strong, though indirect, evidence for a large aerobic fermentation. *H. gallinae* cannot be classified as a strict aerobe, nor as a strict anaerobe.
Possibly it is best considered as being microaerophilic in nature, i.e. with its metabolism adapted to the utilization of oxygen at low partial pressures, but with most of its energy requirements being provided by fermentations. It may, however, be a facultative aerobe, and although having no essential requirement for oxygen, nevertheless possess the ability to utilize small amounts of this gas as they become available.

The carbohydrate-sparing effect exerted by carbon dioxide, especially upon the non-glycogen carbohydrate in both air and nitrogen, may be significant (Table XIV). The possible importance of carbon dioxide has been overlooked by most workers in the field of parasite metabolism. Observations by Toryu (1935) and Fairbairn and Reesal (1950) upon its beneficial effect on survival in vitro, and by Rogers and Lazarus (1949) upon the utilization of bicarbonate from a medium by worm tissue extracts, have not yet been supported by quantitative data. Von Brand and Jahn (n.d.) suggested that, since the normal environment of intestinal parasites is usually saturated with carbon dioxide, the parasites may depend upon carbon dioxide as an extra-cellular buffer. However, the effect of carbon dioxide appears to be much more extensive than this, especially since the data for *H. gallinae* indicate that
fixation may take place. Although the importance of carbon
dioxide utilization in physiological experiments, such as
those which have been reported for *H. gallinae*, can be
demonstrated clearly, it is equally clear that other
methods, such as the use of radio-isotopes, will be
required before the mechanism of utilization can become
known.

The carbohydrate-sparing effect of carbon dioxide
emphasizes further the unreliability of the absolute
values obtained for oxygen consumption and carbon dioxide
production by the use of Warburg's direct method. It is
possible that the respiratory mechanisms, as well as the
fermentative mechanisms, may be affected by carbon dioxide.

The values obtained for the respiratory quotient
are comparable but not absolute. R.Q. is used conventionally
as an indication of the type of substance being oxidized,
it has a value of 1.0 for carbohydrate, 0.71 for fat, and
0.80 for protein. In the case of most parasitic helminths,
however, if *H. gallinae* may be taken as an example, the
R.Q. is not a measure of the substrate being utilized.
The apparent carbon dioxide production is the result of
a balance struck between the production of carbon dioxide
from oxidation and aerobic fermentation, and its consumption
by fixation. The relative importance of each factor contributing to the net production observed, cannot be estimated at the present time. It is apparent, however, that the R.Q. depends upon the conditions of the experiment as well as upon the substrate which is being utilized.

Although the comparison of one helminth with another is tempting, and sometimes useful, in our present state of knowledge such comparisons must be made with caution. Helminths which react in a similar manner under one set of conditions, react differently under other conditions. Furthermore, parasitic worms, unlike free-living forms, are completely dependent upon their environment. Many parasites show a strict host-specificity, and a location-specificity within a host. Environmental differences, such as may occur at different locations in the intestine of a given host, may be sufficient to establish significant metabolic dissimilarities between species of parasitic helminths.

Finally, it must be remembered that this study of *H. gallinae*, as is the case in most of the studies made on other helminths by other workers, has been made in vitro. A parasite in a substitute environment does not necessarily compare in behaviour with its counterpart in vivo. The fact that intestinal parasites are much more active in vitro
than in vivo suggests that important, but less obvious, differences may also be present. Most in vitro work has involved parasites dying from starvation or other causes which, of course, is not a physiologically normal state. With acquired skill in rearing parasitic helminths in more adequate media, a closer approach to a truly normal physiological state may be attained. Meanwhile, examination of the nutrition and intermediary metabolism must first be made in vitro if the actual metabolic relationship of the parasite to the host is to be ascertained.
Summary

1. Literature concerning the metabolism of parasitic helminths was reviewed.

2. Heterakis gallinae were divided into samples by a sampling technique which gave replicates showing a variation of less than five per cent (except for nitrogen determinations). The experimental variation between experiments was much greater.

3. The $Q_02$ of H. gallinae in Krebs-Ringer phosphate at pH 7.4 and 41°C. was 3.61 ± 0.53. The $Q_02$ (wet weight) was 0.979 ± 0.156. The rate of oxygen consumption was constant for at least four hours.

4. There was no relationship between the $Q_02$ and the mean size of the worms in the sample.

5. Addition of glucose had no effect upon the oxygen consumption or carbon dioxide production.

6. M/400 sodium azide inhibited the rate of oxygen consumption by 25.6 per cent, and M/1200 azide, by 19.4 per cent. Approximately 90 per cent of the initial rate of respiration was recovered after removal of the azide.

7. The catalase content of H. gallinae was 1/7.7 that of same weight of human blood.

8. A hyperbolic relationship was obtained between $Q_02$ and oxygen tensions up to 152 mm. of mercury. Above this tension the results were irregular. The initial rate of
oxygen consumption at an oxygen tension equivalent to 7.6 mm. of mercury was almost 44 per cent that in air. The R.Q. at this tension of oxygen was 2.04, and at a tension equivalent to 380 mm. of mercury was 0.67 as compared with a mean of 0.82 in air.

9. *H. gallinae* produced less carbon dioxide anaerobically than in air.

10. Glycogen amounted to 60 per cent of total reducing substances which were completely fermented by washed bakers' yeast.

11. Both glycogen and total reducing substances decreased aerobically and only slightly more quickly anaerobically. The rate of utilization decreased with time. The consumption of total reducing substances was slightly greater anaerobically than was the glycogen consumption.

12. Oxygen consumed during respiration in air accounted for about 1/3 of the carbohydrate which disappeared.

13. Carbon dioxide had a marked carbohydrate-sparing effect which was somewhat more pronounced in the total reducing substances than in the glycogen.
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