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# Physiological Observations upon Larval *Eustrongylides*. IX. Influence of Oxygen Lack upon Survival and Glycogen Consumption.

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The larvæ of *Eustrongylides ignotus* are parasitic nematodes which lead in their natural habitat, encysted in *Fundulus heteroclitus*, a more or less completely aerobic life.[1,2] In view of the fact, however, that many parasitic round worms have extremely well developed anaerobic functions, it appeared of interest to investigate some reactions of this helminth to the lack of oxygen. The influence of the latter on the survival of the worms and their glycogen consumption will be discussed in the following sections. All experiments were conducted under strictly sterile conditions; the technic used in isolating worms under aseptic conditions has been described previously.[3]

*Survival Under Lack of Oxygen.* It has been shown[4] that larval *Eustrongylides* survive more than 30 months *in vitro* if kept at 20°C in rubber stoppered tubes containing sterile nutrient media. The two series upon which this statement was based have now come to an end and the results, not heretofore published, are summarized in Table I. That

TABLE I.
Survival of Larval *Eustrongylides ignotus* at 20°C in Sterile Media. Rubber-stoppered test tubes (25 x 150 mm) containing each 30 cc medium and one worm. Series A: Bacto-Broth 1.6%, NaCl 0.85%, glucose 0.5%. Series B: Bacto-Proteose-Peptone 1.0%, NaCl 0.85%, glucose 0.5%.

| | No. of worms at beginning | No. of worms living after specified month | | | |
|---|---|---|---|---|---|
| | | 36 | 42 | 48 | 50 |
| Series A | 14 | 1 | 0 | | |
| " B | 12 | 5 | 3 | 2 | 0 |

some worms survived as much as 4 years outside of their host is quite remarkable in view of the fact that most parasitic worms die rather rapidly when removed from their natural surroundings. Since the tubes containing these worms were tightly closed with rubber stoppers, it appeared likely that the gaseous atmosphere had undergone profound changes during the time of incubation. In the later stages of these series it became possible to analyze, in a van Slyke manometric apparatus, the gas present above the culture fluid after the worms had died. The average composition, in cases of larvæ having died after periods of 36 to 49 months after isolation, was 0.95% $O_2$ (extremes 0.37 and 1.73%) and 2.63% $CO_2$ (extremes 1.40 and 5.90%). These observations indicate that the worms tolerate a rather severe lack of oxygen.

The corresponding changes in atmosphere were more closely followed in another series in which the tubes were kept at 37°C. The same large size tubes were used as in the preceding series; the fluid medium consisted of 25 cc 1% peptone + 0.5% NaCl + 0.5% glucose and each tube contained only one worm. At the intervals specified in Fig. 1 4 worm tubes were used for analyses of the enclosed air. Despite the fact that the oxygen content diminished rather rapidly, falling below 1% after about 3 months, and that a pronounced accumulation of carbon dioxide occurred, all the worms were alive when the tubes were opened. It should be noted that the present data can only be used to show at what oxygen and carbon dioxide tensions the worms survive. They are insufficient to allow reliable calculations concerning the intensity of the gaseous exchange of the worms since control experiments showed that some changes in the atmosphere occur also in tubes containing no larva. These changes are probably due in part to the spontaneous oxidation of some constituent of the medium, in part to the oxidation of substances contained in the stopper.

While the worms are obviously not damaged

[1] von Brand, T., *J. Parasitology*, 1938, **24**, 445.

[2] von Brand, T., *Biol. Bull.*, 1942, **82**, 1.

[3] von Brand, T., and Simpson, W. F., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 245.

[4] von Brand, T., and Simpson, W. F., *J. Parasitology*, 1944, **30**, 121.

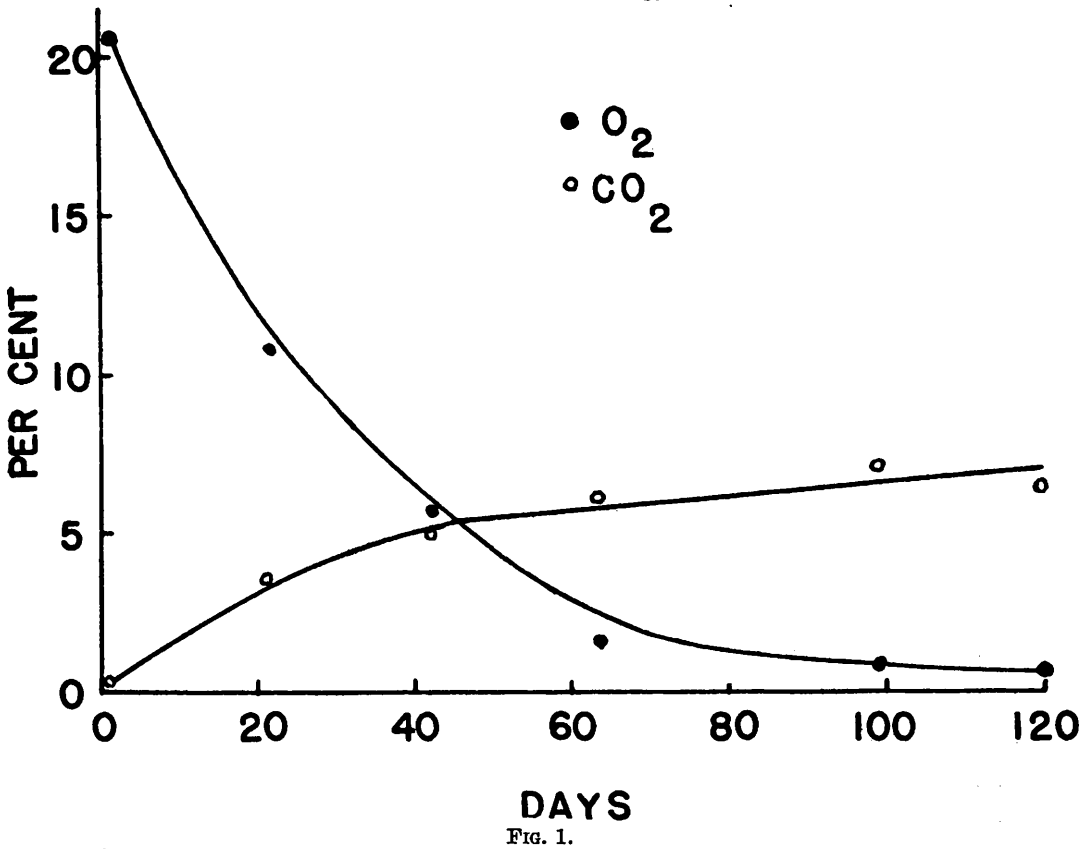


FIG. 1.
Oxygen and carbon dioxide in the atmosphere enclosed above the culture fluid in tubes containing larval *Eustrongylides* during incubation at 37°C.

by relatively low oxygen tensions, they do asphyxiate under complete lack of oxygen. A series of 30 was set up in standard size test tubes almost completely filled with Brewer's thioglycollate medium (Infusion from 37.5 g meat per 100 cc, 1% thio-peptone, 0.5% NaCl, 0.5% glucose, 0.2% dipotassium-phosphate, 0.1% Na-thioglycollate, 0.05% agar, 0.0002% methylene blue, initial pH 7.5) which were tightly closed with rubber stoppers. That anaerobic conditions actually prevailed is indicated by the fact that the methylene blue remained completely reduced throughout the course of the experiment. The average survival of these worms was 18 days (extremes 4 and 25 days). It now became necessary to ascertain whether the brief survival was actually due to the lack of oxygen, or to a toxic action of some constituent of the medium. To this purpose a new series was established in test tubes filled only to about one-third with the above medium and these tubes were closed with cotton stoppers only. In all 21 tubes employed the movements of the individual worm was sufficient to mix the contents to such an extent that enough oxygen diffused from the surface to oxidize the methylene blue throughout the entire column within 2 days. The average survival of these larvæ was 98 days (extremes 10 and 161 days). The difference between the 2 series proves that the ingredients of the medium are non-toxic and not responsible for the early death of the anaerobically kept worms.

*Influence of Anaerobiosis on Glycogen Consumption.* In order to study the influence of lack of oxygen on the glycogen consumption of the larvæ, glycogen determinations according to von Brand's[5] modification of Pflueger's method were carried out on freshly isolated worms and on worms kept for 6 days at 37°C

[5] von Brand, T., *Skandin. Arch. Physiol.*, 1936, **75**, 195.

TABLE II.
Glycogen Consumption of Larval *Eustrongylides ignotus* Under Aerobic and Anaerobic Conditions, 37°C.

| Medium | Beginning | | | | | After 6 days aerobiosis | | | | | | After 6 days anaerobiosis | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. of det. | No. of worms | Avg wt per worm, mg | Glycogen % | pH of medium | No. of det. | No. of worms | Avg wt per worm, mg | Glycogen % | Glycogen change mg/g worm/day | pH of medium | pH change | No. of det. | No. of worms | Avg wt per worm, mg | Glycogen % | Glycogen change mg/g worm/day | pH of medium | pH change |
| NaCl 0.85% | 4 | 20 | 65 | 8.28 | 6.7 | 4 | 21 | 69 | 6.8 | —2.4 | 5.6 | —1.1 | 4 | 24 | 56 | 3.9 | —7.3 | 4.3 | —2.4 |
| NaCl 0.85% + 0.2% glucose | 4 | 23 | 90 | 7.81 | 6.5 | 4 | 24 | 57 | 7.1 | —1.1 | 5.31 | —1.2 | 4 | 24 | 57 | 4.0 | —6.4 | 4.41 | —2.1 |
| Peptone 0.5% + 0.5% NaCl | 4 | 24 | 56 | 6.61 | 7.0 | 4 | 20 | 69 | 6.6 | +0.01 | 6.02 | —1.0 | 4 | 24 | 62 | 2.5 | —6.9 | 5.01 | —2.0 |
| Peptone 0.5% + 0.5% NaCl + 0.2% glucose | 4 | 24 | 58 | 7.11 | 7.0 | 4 | 26 | 66 | 6.8 | —0.5 | 5.96 | —1.4 | 4 | 26 | 60 | 2.7 | —7.3 | 5.1 | —1.9 |

in various solutions under aerobic and anaerobic conditions respectively (Table II). The test tubes were not closed by a stopper or cotton plug, but their sterility was maintained by placing over their open end an inverted loosely fitting glass vial, thus allowing an easy interchange with the surrounding atmosphere. Anaerobic conditions were established by placing the rack supporting the tubes into a desiccator in which the oxygen was absorbed by an alkaline solution of pyrogallol (Buchner's procedure). The completeness of the absorption was ascertained by withdrawing a gas sample and analyzing it in a van Slyke manometric apparatus.

The results summarized in Table II show that, under aerobic conditions, the glycogen consumption was greatest in pure saline, indicating that the nutritive material contained in the other series exerted a glycogen sparing action. This confirms earlier observations extending over longer periods.[4] Under anaerobic conditions, on the other hand, no such difference could be observed. The worms are apparently unable to utilize either peptone or sugar contained in the medium when oxygen is completely lacking. The evidence for this assumption is perhaps not quite convincing; direct determinations in the medium were not carried out, because the evaporation taking place under the condition of the experiments excludes the possibility of gathering reliable data. The glycogen consumption itself under anaerobic conditions was about three times that found under aerobic conditions, if for such comparison only the glycogen disappearance in a non-nutritive medium is used. This, of course, is due to the fact that an anaerobic carbohydrate breakdown yields but little energy.

The pH of all the solutions employed decreased during the experiments and this decrease was much more pronounced under anaerobic than aerobic conditions. This is in agreement with the previously reported excretion of acids by anaerobically kept Eustrongylides larvæ.[1]

A number of comparable series were carried out at 20°C. Despite the fact that they extended over 3 weeks each no definite conclusions could be reached. At this temperature

the daily rate of aerobic glycogen consumption is very low[4] and consequently the variations in glycogen content between various batches of worms may falsify any result. It was unfortunately not possible to conduct the experiments over a longer period, because too many worms die after 3 weeks anaerobiosis.

*Summary.* Some specimens of larval *Eustrongylides ignotus* could be maintained for 4 years *in vitro*. The worms survived for long periods in media showing a pronounced partial lack of oxygen, but they did not tolerate complete absence of oxygen for very long periods. Anaerobically kept worms seemed not to feed but they consumed from their body reserve about three times as much glycogen as worms starving under aerobic conditions.

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## Effect of Acute A-V Fistula on Circulation Time and Auricular Pressure in Dogs.

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The physiological effects of A-V fistula have long been the subject of extensive experimental investigation. Added impetus has been given to this work by successive wars. The existence of such effects as the Nicoladoni-Branham phenomenon, increased pulse pressure, decreased diastolic pressure, and the relationship of these changes to the size and location of the fistula, have been definitely established. On the other hand, another group of physiological effects or the explanation thereof, such as increased cardiac output,[1,2,3] increased blood volume, and increased pressure in the large veins proximal to the fistula,[4,5] have been matters of considerable controversy. Finally there are some effects which have been studied incompletely or not at all. The present investigation was undertaken to determine the effect of A-V fistula, first, on femoral and jugular circulation times and the relationship between the two, and second, on auricular pressures.

*Method.* Thirty observations were made on 5 dogs. The animals were anesthetized with nembutal intravenously, 1 grain per 5 lb of body weight. The right femoral artery and vein, and right jugular vein were exposed under rigid aseptic conditions. Right femoral arterio-venous fistulæ were made in the manner described by Holman.[4] Femoral and jugular circulation times, using the cyanide method described by Robb and Weiss,[6] were determined before and after the fistula was made. All injections into the femoral vein were made at a point just proximal to the site of the fistulous opening into the vein. The injections into the jugular vein were made at a point midway between the lower edge of thyroid cartilage and sternum. Next, auricular pressures were determined with the fistula open and closed. A No. 10 French catheter, with tip cut off, was introduced into the right auricle by way of the right jugular vein according to the method of Richards *et al.*[7] Heparin was added to the saline in the catheter to prevent clotting. In one dog (No. 5) auricular pressures were not studied until 5 months after the fistula was made. All readings were made in duplicate and mean readings were determined.

1 Lewis, T., and Drury, A. N., *Heart*, 1923, **10**, 301.

2 Ellis and Weiss, S., *Am. Heart J.*, 1929-1930, **5**.

3 Grollman, A., *Cardiac Output*, Hopkins, 1932.

4 Holman, E., *Arterio-venous Aneurysm*, Macmillan Company, 1937.

5 Reid, M. R., and McGuire, J., *Annals Surg.*, 1938, **108**, 643.

6 Robb and Weiss, S., *Am. Heart J.*, 1932-1933, **8**.

7 Richards, Cournand, Darling, Gillespie, and Baldwin, *Am. J. Physiol.*, 1942, **136**, 115.