Studies on Lipid Metabolism of Ascaris During Starvation[1]

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INTRODUCTION

Evidence of lipid metabolism in *Ascaris* is meager. Previous investigators (Weinland, 1901; von Brand, 1934 and 1941) found no change in the ether-extractable material of whole worms during starvation under aerobic or anaerobic conditions. An exception to these observations is reported by Schulte (1917) who observed a slight increase in the total lipid of whole *Ascaris* after 24 hr starvation under anaerobic conditions. In contrast with the observations on whole worms, there is cytological evidence of lipid catabolism in lateral-line tissue (Mueller, 1929) and intestinal wall (Hirsch and Bretschneider, 1937) of the parasite during starvation.

Determinations of the distribution of lipids in *Ascaris* (Fairbairn, 1957 and Beames, 1965) show that some 60-70% of the total fat in the worm is located in the reproductive system and eggs. It seems possible that some lipid catabolism could occur in the muscle and/or gut tissue during starvation but be masked in analyses on whole worms by the lipid synthesis associated with egg formation. Recently, Harpur (1962) has shown that CO_2 in *Ascaris*-holding solution has a beneficial effect on the worm's metabolism. His work suggests that environmental gases might markedly influence lipid metabolism in *Ascaris*.

This paper reports experiments that were carried out to measure and compare the total lipids of *Ascaris* held in nonnutrient salt solutions under air, air plus CO_2, nitrogen, or nitrogen plus CO_2 for various periods of time. Also reported are determinations made on the total lipid of body wall and reproductive system plus eggs of *Ascaris* starved in a salt solution under aerobic and anaerobic environment.

MATERIALS AND METHODS

Adult females of *Ascaris lumbricoides suum* were collected at the packing house and transported to the laboratory in warm basal salts solution made up according to Harpur (1962) or, in one instance, Ellison, et al. (1960).

The wet weight was determined and each worm was placed in a 25 × 200 mm culture tube with 40 ml of basal salts solution.

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Groups of 5 culture tubes, arranged in series, were connected to a manifold so that 100 to 150 tubes could be accommodated at one time. Either air, 95% air/5% CO_2, N_2, or 95% N_2/5% CO_2 was bubbled through each tube via the manifold. All experiments were carried out at a constant temperature of 37 C. The salt solution was changed every 24 hr, and the eggs from each tube were collected and saved for analysis with each worm. Worms that failed to survive were removed daily.

Total lipid for each worm was estimated by gravimetric analysis. The worm with its accumulated eggs was extracted with 20 volumes of chloroform:methanol (2:1, v/v). In the determinations on individual tissues, a longitudinal incision was made and the gut and reproductive system were removed. The gut was discarded and the body wall and reproductive system plus accumulated eggs were extracted separately with chloroform:methanol (2:1, v/v). In all determinations the lipid extract was transferred to a tared beaker, washed according to Folch, et al. (1951) and the solvent was evaporated under a stream of air. The beaker and lipid residue were then dried to a constant weight.

Results and Discussion

Results of the total lipid determinations of whole worms are presented in Figure 1. Each bar represents the mean for one experiment. A total of 132 worms was used and they were distributed as follows: 42 were maintained in air, 30 were maintained under 95% air/5% CO_2, 28

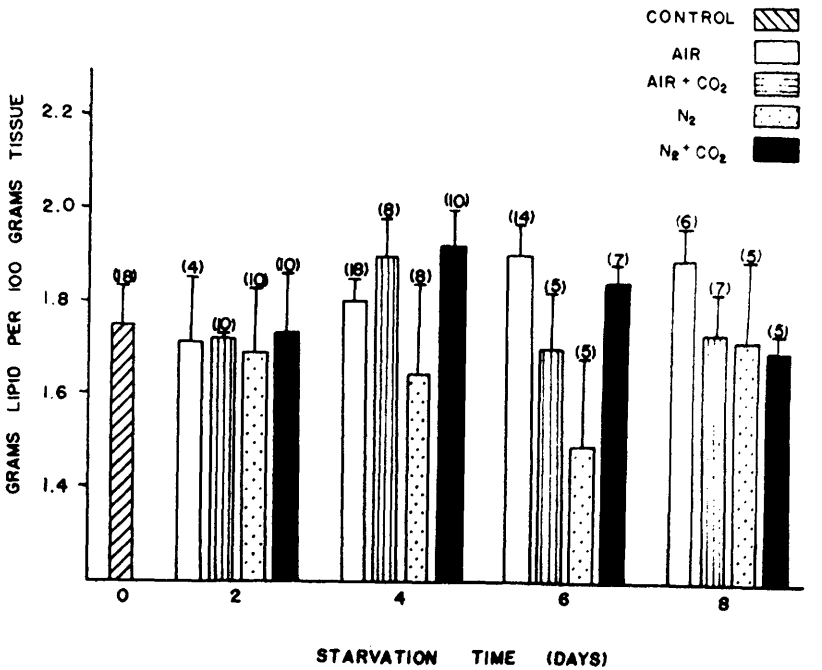

Figure 1. Analysis of total lipid of female *Ascaris lumbricoides suum* during starvation. Each bar represents a mean value. The line at the top of each bar represents the standard deviation of the mean. The number in brackets indicates the number of worms analyzed.

were maintained under nitrogen, 32 were maintained under 95% nitrogen/5% CO_2 and 18 were unincubated controls. All values are based on wet weight of the worm at the beginning of the experiment.

Total lipid values of worms starved for two days under aerobic or anaerobic conditions and in the presence or absence of CO_2 are very close to the control value. The results on total lipid of worms starved 4, 6 and 8 days respectively vary considerably from the control. To determine the significance of the observed variations the difference between each mean and the control mean was evaluated at the 0.1 and 0.05 level by the *lsd* (least significant difference) method. Since the number of worms (n) represented by each of the mean values was not the same, the standard deviation of the mean value (s_d) for each comparison was calculated as follows: $s_d = \sqrt{s^2(n_1 - n_2)/n_1n_2}$. The *lsd* for each comparison was calculated by the formula: $ts_d = lsd$. Appropriate t values were taken from a statistical table. The degrees of freedom were 132.

The difference between the control and experimental means was not significant at the 0.05 level. Only 3 means, the one for 6 days under nitrogen and the means for 4 and 6 days under 95% nitrogen/5% CO_2 were significant at the 0.1 level. There is no consistent trend with any of the values. It is reasonable to conclude that no real change occurs in the total lipid from the whole worms starved under aerobic or anaerobic conditions. Carbon dioxide has no influence on the total lipid of *Ascaris* under the experimental conditions employed. These results compare closely with those of von Brand (1941).

In a second series of experiments the total lipid of the body wall and reproductive system plus eggs was determined for worms starved 2, 4 and 6 days under aerobic and anaerobic conditions. The experiments were terminated after 6 days because the animals were in poor condition. After 8 days starvation, dissection of the worm was usually impossible.

Means for each experiment are presented graphically in Figure 2, A. and B. A total of 131 worms was used and they were distributed as follows: 30 were maintained under air, 30 were maintained under 95% air/5% CO_2, 22 were maintained under nitrogen, 29 were maintained under 95% nitrogen/5% CO_2 and 20 were unincubated controls.

Although the results are variable, the means presented in Figure 2 are generally much higher than control values. The difference between each mean and the control was analyzed for significance by the *lsd* method. Total lipid of the body wall after 2, 4 and 6 days starvation under nitrogen is significantly greater (0.05 probability level) than the total lipid of the body wall control. Mean values of body wall lipid for worms starved under air, 95% air/5% CO_2, and 95% nitrogen/5% CO_2 are not significant at the 0.05 level. When worms are starved under anaerobic conditions and in the absence of CO_2, apparently there is a net gain in the total lipid of the body wall.

All mean values for total lipid in the reproductive system under the various experimental conditions are higher than the control. With three exceptions (day 6 under nitrogen, day 3 under 95% nitrogen/5% CO_2 and 4 under 95% air/5% CO_2) the observed differences are significant at the 0.1 level. The increase in lipid occurs within the first 2 days of starvation and then remains rather constant. The mean value for 2 days starvation was compared with the mean values for 4 and 6 days starvation in the same environment. The differences were not significant at the 0.05 level. The presence or absence of CO_2 does not influence the total lipid of the reproductive system plus eggs under aerobic or anaerobic conditions. The results suggest that lipid synthesis continues at a rapid rate in *Ascaris* even during starvation. This supports the observations

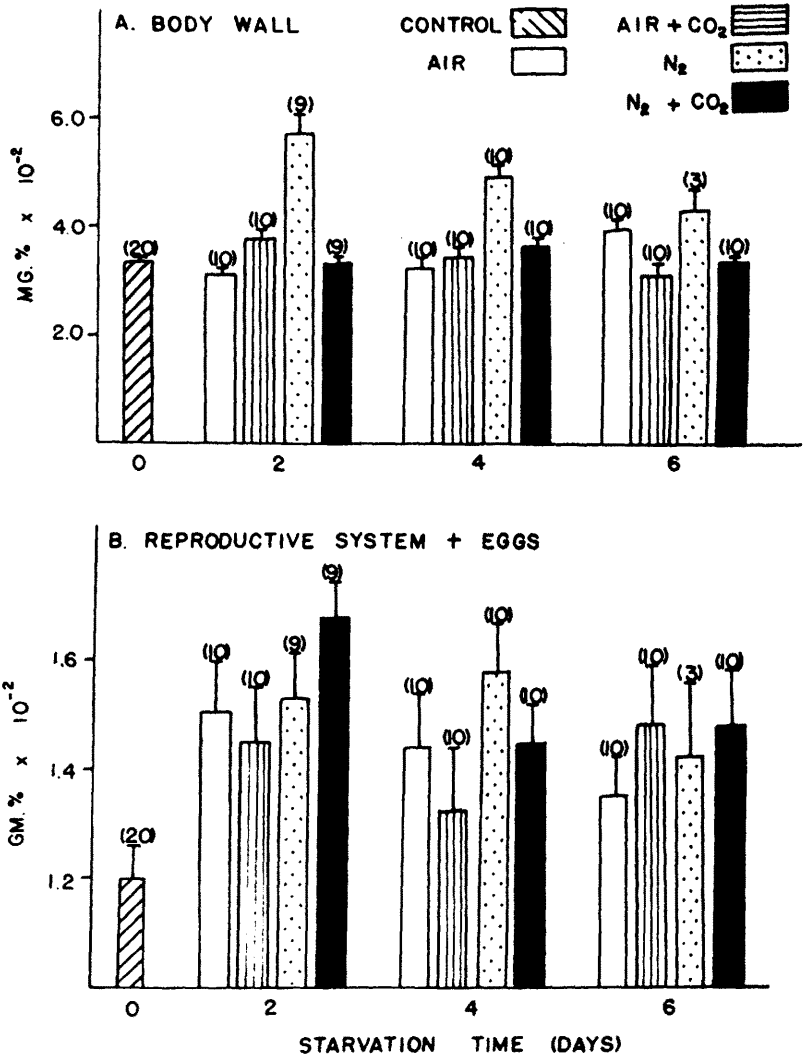

Figure 2. Analysis of the total lipids of A., body wall, and B., reproductive system plus eggs of female *Ascaris lumbricoides suum*. Each bar represents a mean value. The line at the top of each bar represents the standard deviation of the mean. The numbers in brackets indicate the number of worms analyzed.

reported by Schulte (1917). He suggested that carbohydrate catabolism provided the precursors for lipid synthesis.

In summary, there is little observed change in the total lipid of intact worms starved for periods of time under aerobic or anaerobic conditions. Separate analysis of the total lipid of body wall and reproductive system plus eggs suggests, however, that some increase does occur in these tissues during starvation.

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LITERATURE CITED

Beames, C. G., Jr. 1965. Neutral lipids of *Ascaris lumbricoides* with special reference to the esterified fatty acids. Exp. Parasitol. 16:291-299.

von Brand, T. 1934. Der Stoffwechsel von *Ascaris lumbricoides* bei Oxybiose und Anoxybiose. Z. vergl. Physiol. 21:220-235.

von Brand, T. 1941. Aerobic fat metabolism of *Ascaris lumbricoides*. Proc. Soc. Exp. Biol. Med. 46:417-418.

Ellison, T., W. Thomson, and F. Strong. 1960. Volatile fatty acids from axenic *Ascaris lumbricoides*. Arch. Biochem. Biophys. 91:247-254.

Fairbairn, D. 1957. The biochemistry of *Ascaris*. Exp. Parasitol. 6:491-554.

Folch, J., I. Ascoli, M. Lees, J. Meath and F. LeBaron. 1951. Preparation of lipid extracts from brain tissue. J. Biol. Chem. 191:833-841.

Harpur, T. 1962. Maintenance of *Ascaris lumbricoides* in vitro. A biochemical and statistical approach. Can. J. Zool. 49:991-1011.

Hirsch, G. and L. Bretschneider. 1937. Die Arbeitsräums in den Darmzellen von *Ascaris:* die Einwirkung des Hungerns; die Sekretbildung. Cytol. Fujii Jubilee (Jubelfeier) p. 424-436.

Muller, J. 1929. Studies on the microscopic anatomy and physiology of *Ascaris lumbricoides* and *Ascaris megalocephala*. Z. Zellforsch. 8:361-403.

Schulte, H. 1917. Versuch über Stoffwechselvorgänge bei *Ascaris lumbricoides*. Pflug. Arch. gesch. Physiol. 166:1-44.

Weinland, Ernst. 1901. Über Kohlenhydratzersetzung ohne Sauerstoffaufnahme bei *Ascaris*, einen tierischen Gärungsprozess. Zeit. Biol. 42:55-90.