

and 583 males. The total average number of eggs passed daily was found to be 2,335,000. Dividing this figure by the total number of females recovered, it is found that each female *Trichocephalus vulpis* lays a daily average of 2035 eggs. Dividing the total daily egg output by the total number of worms recovered, it is found that each 1350 eggs in the feces represents one worm in the host. The average daily egg output in the different animals varied considerably (*i. e.*, from 833 to 3379). The lighter infections showed a larger daily egg output per worm.

The ratio of male to female whipworms harbored by the dogs was found to be 1:2. However, a compilation of the number of male and female *Trichocephalus trichiurus* found at autopsy by various workers gives a ratio of 1:1.2.

*Conclusions.* While the results of the present study on the egg output of *Trichocephalus vulpis* cannot be directly applied to that of *T. trichiurus*, they may provide some idea of the true output of the latter. If comparison is permitted with the results obtained by previous workers on *T. trichiurus*, it may be concluded that Leuckart's and Moosbrugger's calculations are much closer to the true ones than are those of Manalang and Correa and Mellone.

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### Studies on Embryonation and Hatching of the Eggs of the Dog Whipworm, *Trichocephalus vulpis*.\*

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The embryonation of *Trichocephalus vulpis* eggs was studied after mechanical agitation by centrifugalization, immersion in brine for a short period, and in sodium chloride solutions of different strengths. The extra-corporeal hatching of fully-embryonated *T. vulpis* eggs was also studied.

The eggs were collected from the feces of infected dogs by sieving, alternate sedimentation and decantation, and finally, in all experiments except the first, by brine centrifugal flotation. All embryonation experiments were carried out at temperatures of 33° to 36°C.

In the experiment to determine the effects of centrifugalization and

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immersion in brine on the embryonation of *Trichocephalus vulpis* eggs, 4 types of cultures were studied. The eggs in the first type were collected by sedimentation alone and served as control; in the second, by sedimentation followed by centrifugalization at 2600 r.p.m. for 2 minutes; in the third, by sedimentation followed by immersion in brine for 5 minutes; in the fourth, by sedimentation and brine centrifugal flotation. In the experiment to determine the effects of sodium chloride on the embryonation of *T. vulpis* eggs, saline solutions from 1/1000 N to 3 N were used. Eggs in distilled water served as control.

The results showed that almost 100% of the eggs subjected to centrifugalization and immersion in brine were completely developed after 13 and 14 days respectively, and eggs subjected to both, after 9 days of incubation. Only 15% of the control eggs were fully embryonated after 19 days of incubation. Eggs cultured in sodium chloride solutions of 1/1000 to 1/10 N completed their development in 12 days, while control eggs in distilled water required 23 days. Eggs cultured in a 5/10 N solution required 16 days to complete their development, and a small percentage showed a partial collapse of the inner layer of the egg shell, although the larvae within appeared quite viable. About 40% of the eggs cultured in normal salt solution contained vermiciform larvae after 12 days of incubation. However, there was no further development, and after 19 days all eggs appeared to be degenerate. There was no development of eggs in a 3 N solution, and all eggs were dead within 5 days.

The extra-corporeal hatching of *Trichocephalus vulpis* eggs was studied at a temperature of 37°C in artificial gastric juice (0.8% pepsin in an 0.5% solution of HCl) and artificial pancreatic juice (1.5% solution of pancreatin in distilled water brought to a pH of 7.3 with Na<sub>2</sub>CO<sub>3</sub>).

The eggs did not hatch in gastric juice, nor did hatching occur in distilled water. Up to 2% hatched in pancreatic juice alone, but never a larger percentage. Eggs placed in pancreatic juice following a previous immersion in gastric juice showed a much higher percentage of hatching. Thus, 10% of eggs previously placed in gastric juice for 30 minutes, were hatched after 19 hours in pancreatic juice, and from 25% to 50% of eggs, previously placed in gastric juice for 20 hours, were hatched after 2 hours in pancreatic juice. *In vivo* studies on hatching confirmed the *in vitro* findings. Hatching was never found to occur in the stomach of dogs, but always in the small bowel. However, hatching occurred more rapidly in the experimental animal's digestive tract. Eggs introduced by stomach tube were found hatching within 30 minutes.

*Summary and Conclusions.* (1) Since *Trichocephalus vulpis* eggs are stimulated in their development by previous subjection to mechanical agitation by centrifugalization, and to immersion in brine, the results of studies on these, and probably other nematode eggs subjected to these influences are not applicable to eggs developing in nature. (2) Sodium chloride in low dilutions (1/1000 N to 1/10 N solutions) stimulates the embryonation of *T. vulpis* eggs. Higher concentrations (1 to 3 N solutions) inhibit development, collapse the inner layer of the egg shell, and finally kill the embryo. It is not unlikely that other salts present in the soil in small concentrations may also influence the embryonation of these eggs. Thus, the salt content of soils may conceivably play an important rôle in the epidemiology of whipworm disease. (3) From 25% to 50% of *T. vulpis* eggs can be hatched by subjecting them in turn to artificial gastric juice for 20 hours and artificial pancreatic juice for 2 hours. The fact that eggs must remain in the gastric juice for a period of time before they will hatch in pancreatic juice suggests that the rapidity with which eggs pass through the stomach of the host may influence the rate of hatching, and thus the resulting intensity of infection in the host.

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**Sulfanilamide and Sulfapyridine in Treatment of Experimental *B. Friedländer* (*Klebsiella pneumoniae*) Infections of Mice.**

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Buttle, Parish, McLeod and Stephenson<sup>1</sup> found sulfanilamide in dose of 0.025 g by oral administration twice daily practically ineffective in the treatment of mice inoculated intraabdominally with varying amounts of 18-hour broth culture of *B. friedländer*; of 70 treated mice only 12 survived the minimal amount of culture for 12 days, whereas 4 out of 40 untreated controls remained alive. All treated and control mice inoculated with larger amounts of culture perished so that the compound was found to give only temporary protection. Bliss, Feinstone, Garrett and Long,<sup>2</sup> using type B of

<sup>1</sup> Buttle, G. A. H., Parish, H. J., McLeod, M., and Stephenson, D., *Lancet*, 1937, 1, 681.

<sup>2</sup> Bliss, E. A., Feinstone, W. H., Garrett, A. W., and Long, P. H., *Proc. Soc. EXP. BIOL. AND MED.*, 1939, 40, 619.