The calculated organic phosphorus figures are interesting in view of the controversy in the literature concerning the effect of insulin on this fraction. The changes that we found, an increase in the organic partition of about 3% in over 80% of the cases following insulin injection, are not of sufficient magnitude to warrant definite conclusions, but appear to indicate that there is a tendency, under the conditions of this experiment, for the blood organic phosphorus to increase during insulin activity. Such a change is in contrast with the results of Kerr[5] but in agreement with those of Briggs, Koechig, Doisy, and Weber[2] and those of Kay and Robison.[4]

Conclusions. It may be concluded, then, that after insulin injection, (a) the blood inorganic phosphorus of rats decreases only on the high fat diet, (b) the other determined phosphorus fractions vary only slightly, (c) there does not appear to be a loss of phosphorus from the blood, and (d) there is a tendency for the organic phosphorus of the blood to increase.

8210 C

Intermediate Hosts of Aelurostrongylus Abstrusus of the Cat.

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The conception of the life history of the lungworm *Aelurostrongylus abstrusus* of the cat is based upon the work of Cameron as set forth in a series of papers by this author. No information, however, in regard to the development of intermediate forms of the first stage larvae in the supposed intermediate host, the mouse, is available from his studies.

Necropsies of 500 housecats of the city of San Francisco revealed the presence of *Aelurostrongylus abstrusus* in 8 instances. Twenty-two autopsies performed on cats from 20 other localities in California added 2 more cases. This material has been used in the present study.

The first stage larvae of *Ael. abstr.* behave in a manner usual among larvae of Synthetocaulinae known at the present time, according to our observations. Fry and Stewart[1] experimenting with

[1] Fry, W., and Stewart, T. Th., *Parasitology*, 1932, **18**, 34.

these larvae state: "The larvae in this instance lived 18 days, which is opposed to Cameron's opinion that the larvae can not live longer than a fortnight in the free state." These authors believe that they may exist even longer under favorable conditions. In our experiments larvae have been found alive after a stay of 5 weeks in water. At this time we had to use them for other purposes. Water was chosen as a medium by reason of convenience. Under natural conditions the larvae may thrive on moist ground. According to Cameron: "The larvae of this species are carried to the exterior in the droppings, ingested by mice, continue their development and become encysted in these animals, and ultimately, when the rodent is eaten by a cat develop into the adult lungworms." (p. 58.)[2]

"The faeces are eaten by mice and the larva migrates to various situations among the muscles and in the subcutaneous tissue, where, within 3 weeks it assumes an infective encysted form." (p. 66.)[3]

Cameron bases these conclusions upon his experimental work. One of his experiments—apparently his chief experiment—may be cited (pp. 102-103)[4] (pp. 59-60).[3] Forty-six mice were infected artificially. Eighteen mice died spontaneously owing to outbreaks of sarcosporidiosis and of rat bite fever. Eleven mice later showed cysts containing extra-intestinal larval nematodes. Six mice were negative as shown by investigation. Eleven mice were preserved for further investigation. However, Cameron did not find stages of development linking the larvae fed to the experimental animals to those observed in the 11 mice or in any of the 46 mice.

In 4 series of experiments undertaken by the writers in the course of the last 2 years to produce evidence of the development of the larvae in the mouse the result was negative. The experiment in one group may be briefly recorded. Twenty-four mice were fed with the first stage larvae of *Ael. abstr.* They subsequently were killed in groups of 2, after 1, 2, 3, 7, 14, 21, and 28 days. At no time, however, were larvae detected outside the intestine. The remaining 10 mice were destroyed 40 days after the beginning of the experiment. No larvae or cysts containing larvae were recovered from these mice. Thereafter they were fed to 2 kittens. Ten weeks later the kittens were autopsied. In neither of the animals could lesions of the lungs, embryos, eggs or adults of the lungworm be detected.

Recently Baudet[5] repeated Cameron's experiments of infecting

2 Cameron, Th. W. M., *J. Helminth.*, 1926, **4**, 53.

3 Cameron, Th. W. M., *J. Helminth.*, 1927, **5**, 55.

4 Cameron, Th. W. M., *Vet. J.*, 1928, **85**, 97.

5 Baudet, E. A. R. F., *Tijdschr. v. Diergeneeskunde*, 1933, **60**, Afl. 18, 1.

mice with the larvae of *Ael. abstr.* with the same negative results; he knew of no reasons for this negative outcome.

Consequently an inquiry was undertaken by the writers to determine the nature of the apparently still unknown intermediate host of the lungworm.

We used in our work larvae either taken directly from the lungs or those isolated from the faeces of affected cats. We, however, were not able to raise the first stage larva itself from the unsegmented egg of the parasite as Cameron was able to do. He reiterates with emphasis this fact—to have raised the larvae outside the vertebrate host in the body of female worms of *Ael. abstr.* and corroborates this statement with camera lucida drawings (p. 57).[3] Cameron makes no mention, however, of the technique applied to secure these results, which, successfully attained, would be extraordinary in any species of Synthetocaulinae.

Several fruitless attempts were made by the writers to infect small vertebrates and certain insects, which occasionally might be taken by cats as food. Positive results were obtained finally by the introduction of different mollusks. Snails of the genus *Epiphragmophora Doering,* 1873, proved to be the most suitable intermediate hosts. *Helminthoglypta* (E.) *californiensis,* Lea; *Helminthoglypta* (E.) *nickliniana,* Lea; and *Helminthoglypta* (E.) *arrosa,* Gld.* were infected successfully. Furthermore, third stage larvae could be raised in *Helix aspersa,* Mueller, in *Agriolimax agrestis,* Linné, and in *Ariolimax columbianus,* Gld. These observations show again that lungworm larvae of Synthetocaulinae are linked rather with a wide group of correlated intermediate hosts than with a single species. In a few of them, the larvae encounter optimal conditions and develop in considerable numbers; meanwhile, even after heavy infestations, in others only a restricted number of larvae result due to less favorable conditions. In the case of *Ael. abstr.,* Epiphragmorphora in its different species is the most favorable intermediate host in California. In the other snails and especially in the slugs named above the final number of third stage larvae remained restricted, the time for their development varied, and their life span appeared shortened.

The invasion of the mollusks takes place in the manner previously described in other Synthetocaulinae.[6] Shortly after invasion, movements of the larvae decrease and they finally coil up. Their length

* We are indebted to Dr. G. D. Hanna, of the Academy of Sciences, San Francisco, who assisted us in the classification of the snails.

[6] Hobmaier, M., *Z. f. Parasitologie,* 1934, **6**, 642.

increases to a less extent than their width. Fat and protein granules develop and conceal their inner structure. The first molt occurs about 10 days after infection. The larva now appears stout and its body becomes more delicate. The cuticle loosens from the body first in the head and tail regions. Finally it may surround the second stage larva as a wide sheath (Fig. 1). The cuticular parts of

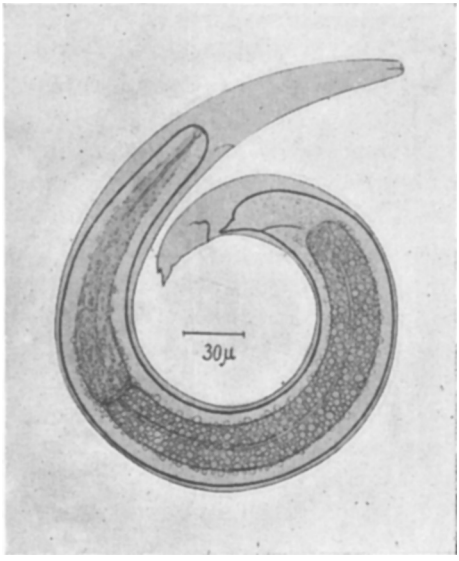

FIG. 1.
Aelurostrongylus abstrusus. Second stage larva from *Helminthoglypta* (E.) *arrosa* Gld.

the entrance of the esophagus, of the excretory pore, and of the cuticular rectum of the first stage larva remain attached to the sheath as ordinarily. No ecdysis follows. The enclosed second stage larva measures 380 to 400μ in length by 36 to 38μ in width just below the esophageal junction. It attenuates toward both ends, more, however, toward the head region than toward the tail. The newly formed tail is short and it ends straight and sharp pointed. The undulating sigma form and the dorsal spine of the first stage larva are absent on it. The tail is only 20μ long. The esophagus is more slender and it measures about 160μ in length. It shows only one slight bulb at its junction with the intestine. With the approaching second molt the accumulated fat and protein granula begin to dissolve. This process proceeds from the esophageal region slowly toward the posterior parts of the larvae.

Four to 5 weeks or even later after the beginning of the experi-

ment the second and final molt of the larva in the mollusk may be observed. The reasons for this variation in time are not definitely known. Chief factors may be the species of intermediate hosts involved, the varying localization of the larvae in the mollusks and above all certain seasonal factors. The newly formed larva, representing the third or infestive stage, is slender in appearance compared with the second stage larva (Fig. 2). Nothing is known at

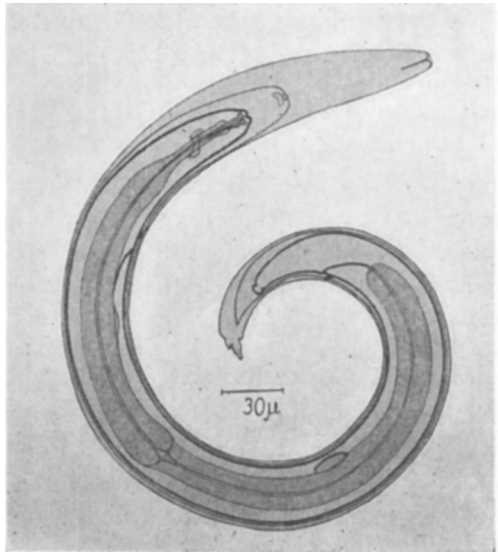

FIG. 2.
Aelurostrongylus abstrusus. Third stage larva from *Helminthoglypta* (E.) *arrosa* Gld.

the present time about the metabolism of the larva during its stay in the mollusk. The accumulation of steadily increasing quantities of fat and protein granules beginning with the time of the immigration of the larva and ending with its first molt seems to indicate that fluids of the host tissues are used as foodstuff during this period. Meanwhile the formation of the final third stage larva may be accomplished by the exclusive use of these reserves. The second phase of the intramolluscan life of the young nematode is characterized by the transformation of the reserves into well defined cellular structures. After the second molt has occurred the dissolution of the fat and protein granules progresses until the body of the larva appears clear in its lines. The stay of the third stage larva may extend over months, but otherwise no particular changes are to be observed during this time.

The larva enclosed in its 2 sheaths measures, according to its state of contraction, 450 to 520μ in length by 26 to 28μ in width, after a stay of about 6 weeks in its intermediate host. The length may increase a little during the following months. Both ends of its body are slightly attenuated. The mouth opening is simple. The esophagus, measuring 200 to 225μ in length has now a more muscular appearance. It is still composed of 3 parts but has only one slight bulb at its junction with the intestine. The excretory pore opens 90μ behind the head of the larva. The intestine is colorless. It, however, later may show a slightly yellowish brown color. It forms a small tube with a diameter of about 18μ. It ends in a small cuticular rectum 26 to 30μ in length. The primordial cells are situated near the middle of the intestine. The anus ends 30μ in front of the tip of the tail. Immediately behind the anus the larva attenuates to a short stout tail with a little flat knob on its end. It carries no papillae. After artificial liberation of the larva from its sheaths the knob stretches to the extent that the little appendix may present itself in the shape of a short finger. The larva is now more resistant toward injury possibly caused by microscopic investigation than previously. Slight pressure releases the larva from its sheaths. It moves forth and back for some time in the surrounding tissues; finally, however, it coils up, but death may be delayed for a few days. Larvae have been kept in mollusks under observation for a period of 6 months. At the end of this time little change had taken place in larvae located in Epiphragmophora. In *Helix aspersa* they appeared enclosed in stronger tubercle formations surrounded by yellowish caseous material. The larvae themselves were feeble but most of them still were alive. In the other mollusks mentioned above the greater number of larvae had disappeared already or were found to be lifeless.

Third stage larvae raised in the different species of Epiphragmophora and of *Helix aspersa* as intermediate hosts were fed separately to uninfected cats and kittens. The different species of slugs, however, were fed simultaneously to the animals. Adult lungworms of *Ael. abstr.* were recovered from the experimental animals in all cases by autopsies. An account of the development of the adult stages and of the possible rôle of mice in the life cycle of the parasite will be presented in subsequent papers.

Conclusion. The occurrence of the lungworm *Aelurostrongylus abstrusus* of the cat in California was established. About 2% of the cats investigated were found infected with the parasite. Different species and varieties of Epiphragmophora were found serving as

principal intermediate hosts. Other snails and slugs mentioned above seem to be of minor importance. The development of the third stage larvae is described and subsequent infections of cats and kittens are recorded. Mice fed with first stage larvae of the lungworm escaped infections. The intermediate hosts of the lungworm *Aelurostrongylus abstrusus* are not, as Cameron states, mice, but certain mollusks.

8211 P

Route of Transmission of St. Louis Encephalitis Virus in Mice.*

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Histopathological studies by Webster and Fite[1] suggest that the virus isolated from the St. Louis outbreak of encephalitis, travels by way of the central nervous system in mice. The present report confirms and extends these findings.

In order to determine the portal by which mice could be infected, they were given virus by the nasal and gastro-intestinal routes. Three series of mice each consisting of 4 to 6 animals, were given a concentrated suspension of the virus in their food over 2 to 3 days. Not one of these mice became infected. Three animals, upon whom a laparotomy was performed, were injected intrastomachally with 0.2 cc. of a thick virus suspension. None of these showed evidence of the disease.

Mice could be infected regularly with the intracerebral virus, within an incubation period of 6-8 days, by placing 0.03 cc. of a 1% suspension into one nostril, while 0.1% failed to give regular infection. The virus was transmitted serially by the intranasal route for 28 passages, using a 10% suspension, but its infectivity, by this route, did not increase.

After 28 passages the virus was infective by the intranasal route, at approximately a 1 :250 dilution.

In order to trace the route of infection from the nasopharynx,

*This research was aided by grants from New York and Rockefeller Foundations.

[1]Webster, L. T., and Fite, S. L., *J. Immun.*, 1934, **26**, 344; *Am. J. Path.*, 1934, **10**, 666.