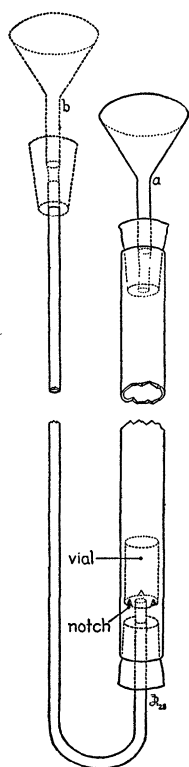


a simpler apparatus that I have used in the zoological department of the University of Texas for a number of years. This embodies the principle of Courtney's device for dehydration, namely, that alcohol, being lighter than water, is admitted from below.



The apparatus is illustrated in the accompanying cut. It is made up of strong-walled glass tubing of about 18 mm bore, fitted in the bottom with a fresh one-hole rubber stopper. Through this is run a thin glass tubing bent up as shown in the illustration. The narrow glass tube should somewhat overtop the larger one. The objects to be treated are placed in "baskets" consisting of short shell vials about 15 mm in outside diameter, each provided with two or three holes ground in the bottom to insure diffusion of fluids. The holes can be ground very readily *under water* by means of a sharp-edged carborundum stone. In the grinding operation the stone is guided by the tips of the thumb and index finger, which should be protected against abrasion by strips of adhesive tape. If the objects are smaller than the holes, these may be closed with small tufts of cotton. A label is added to each "basket"; with a little care the mixing of labels is entirely avoided. After dehydration is completed, the baskets with their contents are readily dropped into a dish of alcohol.

For economy of reagents it is well to have tubes of a half-dozen different lengths, say six, eight, ten, twelve, fifteen, eighteen inches, and a couple of dozen "baskets" to accommodate a variable number of specimens assembled at any one time.

For washing, a funnel with stem pushed half way into a suitable one-hole rubber stopper, is placed in position *a* of the illustration. Tap water may be run through without unduly shaking delicate specimens, an advantage over the Kornhauser apparatus (SCIENCE, March 27, 1924, p. 464). For dehydration the funnel is changed to position *b* (dotted lines), since alcohol must enter from the bottom.

Dehydration may be as gradual as desired. To control the rate of addition of alcohol I have utilized Long's capillary siphon method.¹ Siphons are prepared by drawing out glass tubing to capillary fineness; these are then calibrated. Thus with the appropriate siphon one can regulate the flow of alcohol so that dehydration will be completed in a couple of hours or a couple of weeks.

With no more handling of the objects, the apparatus may be used for fixing and mordanting as well as washing and dehydrating—one fluid is allowed to run out and the next is run in, changes that require only a few seconds.

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SPECIAL ARTICLES

THE LIFE CYCLE OF SPIROCERCA SANGUINOLENTA—A NATURAL NEMATODE PARASITE OF THE DOG

IN an earlier study¹ the writer found that mature third-stage larvae of *Spirocerca sanguinolenta* were commonly encysted in the mesentery and omentum and on the parietal wall of the stomach and adjacent intestine of the Asiatic hedgehog, *Erinaceus dealbatus*, and that these larvae, when fed to experimental puppies and kittens, excysted in the stomach of these animals, penetrated the stomach wall, passed through the gastro-epiploic veins into the portal circulation, thence *via* the capillaries of the liver and lungs into the left heart, and upon reaching the aorta became attached to the intima and burrowed into the aortic wall, where they produced extensive lesions. In view, however, of the fact that this infection occurs natu-

¹ J. A. Long, *Anat. Rec.*, 29: 319, 1925.

² E. C. Faust, "Migration Route of *Spirocerca sanguinolenta* in its Definitive Host," *Proc. Soc. Exp. Biol. Med.*, 25: 192-195, 1927.

rally in dogs in areas in which the hedgehog is not present, it seemed necessary to assume that the dog may become naturally infected from some other source.

The family relationship of this nematode, as well as the morphological studies of Seurat,² suggest that certain insects which harbor the encysted larvae are the most likely source of this infection for dogs. In fact, Grassi³ believed that he was dealing with this species when he fed dogs encysted nematode larvae obtained from the cockroach, *Blatta orientalis*, and on autopsy five to fifteen days later found spiruroid larvae embedded in the wall of the stomach and small intestine. The writer's experience has, however, shown that the true *Spirocerca* larvae pass directly through the stomach wall almost immediately after excystment, and between the fifth and thirtieth days are all to be found attached to or embedded in the wall of the aorta and its immediate offshoots. Furthermore, examination of hundreds of cockroaches of several species in heavily infected foci in Peking and Amoy, China, has failed to reveal any larvae of *Spirocerca sanguinolenta* in these insects, although several specimens of other spiruroid larvae have been obtained (Amoy). Likewise, controlled feeding experiments in Peking, in which mature eggs of *S. sanguinolenta* from esophageal tumors in dogs were fed to *Blatta orientalis*, *Periplaneta americana* and *P. australasiae*, have always given negative results. More recently (April, 1928) thirteen specimens of the dung beetle, *Canthon* sp., were obtained from an area near Peking from which heavily infected hedgehogs had been commonly obtained. On dissection five of these beetles were found to harbor in the thoracic and adjacent leg muscles a relatively heavy infestation of larvae morphologically indistinguishable from those of *S. sanguinolenta*. These were fed to a dog born and reared in the laboratory. Twelve days later the dog was autopsied. Examination showed all organs to be negative except the aorta, which contained serpiginous tunnels in the intima and aneurisms in the wall characteristic of early infections with *S. sanguinolenta*. Larvae were also recovered from the tunnel as well as from the deeper layers of the aortic wall, and corresponded in every detail to the larvae fed.

The series of examinations as well as experimental evidence strongly favor the view that the first inter-

mediate host of *Spirocerca sanguinolenta*, at least in North China, is a beetle, *Canthon* sp., while neither dissections nor experimental evidence provide any grounds for believing that cockroaches in China serve in this capacity. Furthermore, it seems altogether probable that the larvae which Grassi (*l. c.*) obtained from the cockroach, *Blatta orientalis*, and fed to dogs, were not those of *Spirocerca sanguinolenta*. In the first place, the nodules obtained on the stomach wall and other parts of the intestinal tract from five to fifteen days after feeding correspond neither in type nor position to the lesions produced by larvae of *S. sanguinolenta* up to thirty days after feeding. In the later stages of naturally infected dogs, nodular growths in which the adult worms are found do occur along the esophageal tract, but they are for the most part outgrowths into the lumen of the esophagus, and develop some time after thirty days' incubation and migration of the worms. Grassi was probably dealing with a spiruroid species of larva of which the dog was an abnormal host, but for which there was sufficient adaptation to cause digestion of the cyst wall and permit the larva to burrow into the wall, there to re-encyst. Cram⁴ has already called attention to this phenomenon in spiruroid species. It is altogether likely, therefore, that the hedgehog is an abnormal host for *S. sanguinolenta*, which, however, is able to utilize this insectivorous mammal as a satisfactory medium in which to re-encyst and live almost indefinitely.

The larval hosts of *Spirocerca sanguinolenta* in Algeria, listed by Seurat (*l. c.*) purely on morphological grounds, include six species of coprophagous beetles, and a large number of amphibians, reptiles, birds and mammals. These should be divided into two categories, the one comprising dung beetles and possibly other coprophagous insects, constituting the primary (true) intermediate hosts; and the other, including all insectivorous and omnivorous vertebrates, in which the larvae, failing to pass through into the blood stream, become "side-tracked" and encyst in or on the various abdominal viscera. These latter are secondary (reservoir) intermediate hosts. Their importance as a source of infection for dogs is probably considerable, since the larvae appear to remain viable in these secondary intermediate hosts for long periods of time and even increase in size there, and since these hosts are more commonly eaten by dogs than are coprophagous beetles.

Finally, reference should be made to the cat, which is not known to be a natural host of this nematode but which serves as an excellent experimental animal for tracing the migration route during the early stages

² L.-G. Seurat, "Formes Larvaires des Nématodes Parasites Hétéroxènes," *Bull. Soc. Sci. France et Belge*, ser. 7, 49: 297-377, 1916.

³ B. Grassi, "Beiträge zur Kenntniss des Entwicklungscyclus von fünf Parasiten des Hundes," *Centralbl. Bakt. Parasitenk.*, 4: 614-615, 1888.

⁴ E. B. Cram, *Journ. Parasitol.*, 11: 117, 1924.

of the infection. The primary lesions in the aortic wall of the cat are, however, much less intense than they are in the dog, and it seems not unlikely that in the cat the infection becomes abortive before the worms are able to mature.

The complete life cycle of *Spirocerca sanguinolenta*, therefore, needs include only two hosts, the insect (primary larval) and the dog (definitive), although a wide variety of vertebrates which are insectivorous or which ingest insects accidentally may become intercalated as reservoir intermediate hosts in the cycle. With the exception of the wolf and the fox, the dog is the only known definitive host of this worm. It is not unlikely, however, that other Canidae may also be found to be natural definitive hosts.

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VISCOSITY CHANGES DURING EARLY CLEAVAGE STAGES OF FUNDULUS EGGS

In the course of a series of experiments carried on during the summer of 1927, it was noted that the removal of the protoplasmic material of one of the first two blastomeres of the egg of *Fundulus heteroclitus* is accomplished with little or no difficulty at certain times, while at other times it is practically impossible. The observations made on this point contribute to our knowledge of the relative viscosity of protoplasm¹ during the cleavage stages and should, I think, be reported in this place.

As is well known, the egg of *Fundulus* is relatively large and the first two blastomeres may very easily be seen under even the low power of the binocular dissecting microscope. Being large, the blastomeres of the early cleavage stages contain a considerable amount of protoplasm which bulges within the chorion. It was the purpose of the experiments to remove all the protoplasm of one of the first two blastomeres. This was found to be quite possible during the period commencing shortly after the completion of the first cleavage furrow and ending shortly prior to the formation of the second cleavage plane. Before and after these stages, it was impossible to remove all the protoplasm of the cell without injury to the other blastomere and without the loss of a considerable amount of yolk. A brief examination of the physical state of the cellular material showed the reason for this.

The experiments were performed with a fine glass needle by means of which one of the blastomeres was punctured, allowing the cytoplasm to flow out. Thus

they resemble the microdissection studies carried on in a much more refined way by Chambers and his coworkers. The toughness of the cortical membrane of the fish egg precludes the use of the finer method in these experiments. Nor is it necessary. By means of the microscopically fine glass needle it is possible to puncture one blastomere of the egg in any part and to express the contained cytoplasm. The behavior of the extruded material and the rapidity of the outflow may be directly observed. These give definite information as to the relative viscosity of the protoplasm. Very briefly stated, the results of such tests follow.

A short time before the formation of the first cleavage plane, a puncture with the needle results in the outflow of all the contained protoplasmic material. When examined, it is quite evidently very fluid. This stage is soon replaced by another at which the cytoplasm in the cortical region begins to thicken. This gelation of the cellular material involves not only the actual cortex of the cytoplasmic mound, but also crosses the cell in the region of the future cleavage plane. In this way, the future line of division is foreshadowed even before the margins of the cell have indented. It is probable that the gelation is initiated in this mid-region. The cytoplasm at this time is so stiff in the outer region that the puncture of the cell results only in the extrusion of the medially located cytoplasm. This leaves a hollow shell of relatively rigid cytoplasm in the center of which may be a yolk or water-filled space. In other cases the entire cortex may crumple in. From the cortical area, this stiffening spreads centripetally, accompanying cleavage until both daughter cells show a high relative viscosity throughout. At this stage the removal of all the cytoplasm in one blastomere can not be accomplished without at the same time removing a large amount of yolk and in all probability injuring the sister blastomere.

The gelation effect is almost immediately reversed, so that the protoplasm of the cells again becomes very low in its relative viscosity. It is during this stage that it is expressed most easily, with very little loss of the yolk material, and no deleterious effects to the neighboring cell. The blastomeres remain in this state until just before the second cleavage, when the cortex again stiffens slightly. This is accompanied by a change in the cell itself similar to that found just prior to the first cleavage. The cytoplasm in the region to be occupied by the new line of division also stiffens. This region of higher viscosity is continuous with that of the cortex of the cells. There is every reason to believe that the cycle of changes just described for the so-called resting phases between the first and the second cleavages is repeated between

¹ For literature on this subject see L. V. Heilbrunn, *Quart. Rev. Biol.*, II, p. 230, 1927.