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Development of *Clonorchis sinensis* Eggs to Cercaria Stage in Laboratory Bred Snails, *Bithynia fuchsiana.*

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Various investigators (Muto,[1, 2] Faust and Khaw,[3] Yamaguti[4]) have in the past described the cercaria of *Clonorchis sinensis* but none of these investigators have given complete experimental proof for their statements. Such proof would seem all the more necessary because these different descriptions did not, on the whole, agree well with each other. Correct knowledge of the cercaria of *C. sinensis* is of special value because with such knowledge it is possible to examine and discover which snail acts as first intermediate host of this parasite.

In order to obtain the cercaria which, beyond question, is that of *C. sinensis,* it is obviously necessary to infect snails with *Clonorchis* eggs and to achieve a full development to the cercaria stage in these snails. Although several investigators (Nagano,[5] Faust and Khaw) realized the importance of this fact, their attempts were unsuccessful so far as complete experimental proof was concerned.

Instead of using old snails collected from rivers and ponds as has been done by former investigators, the present writers used laboratory-bred young snails which may be regarded as not only free from a previous infection with larval stages of trematodes but also as being much more easily infected than older snails. The following is the report of a successful experiment concerning the development of *C. sinensis* eggs to the cercaria stage in laboratory-bred young snails, *Bithynia fuchsiana.*

In the first part of April, 1938, a large number of *B. fuchsiana* were collected in the vicinity of Peiping and kept in an aerated aquarium. Within a few days, eggs of this species of snails were

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1 Muto, M., *Chuo Igakkai Zasshi* (J. Centr. Med. Assn.), 1918, 138, Japanese text.

2 Muto, M., *Ibid.*, 1919, Japanese text.

3 Faust, E. C., and Khaw, O. K., *Am. J. Hyg.*, Monographic Series, 1927, **8**, 1.

4 Yamaguti, S., *Z. f. Parasit.*, 1935, **8**, 183.

5 Nagano, K., *Trans. 6th Congress, F.E.A.T.M., Tokyo,* 1926, **1**, 379.

laid on the glass wall of the aquarium and were immediately removed to another aquarium. Young snails hatched out within a few weeks' time and had already grown to a size of 2-3 mm by the end of May. On June 2nd, a cat, which had been experimentally infected with a large number of *Clonorchis* cysts, was killed and the eggs in the gall bladder were carefully collected and washed. The young snails, together with *Clonorchis* eggs, were put into a small dish which was then covered with fine gauze to prevent the snails from leaving the dish. This dish was carefully put back into the aquarium still covered with the gauze and after one day the gauze was removed and the snails allowed to wander freely in the aquarium. Empty egg shells were found in the feces of the snails which not only showed that the snails had eaten the *Clonorchis* eggs in the dish during the time of their confinement, but probably also that the miracidium in the egg had hatched out in the digestive tract of the snail. The temperature of the water in the aquarium varied from 22°-26°C during the time of the experiment. *Clonorchis* eggs from the gall bladders of other killed cats were put into the aquarium about 15 times during the months of June, July and August in order to afford additional opportunity for the snails to become infected.

On September 11th, 3 months and 8 days after the infection, 2 snails were crushed for examination. One of them was found to be infected. The positive one contained many mature, free swimming cercariae together with many rediae. On September 15th, 3 more snails were crushed. Two contained rediae only and the third one both rediae and cercariae. This showed that the attempt to obtain a complete development of *Clonorchis* eggs to the cercaria stage in the experimentally infected laboratory bred snails, *B. fuchsiana,* had been successful. Consequently the cercariae thus obtained must be regarded as genuine ones of *C. sinensis.*

The cercaria experimentally produced in our snails is an oculate, lophocercous one. The oral sucker is protrusible. On the dorsal margin of the mouth opening there are 4 small, penetrating teeth arranged closely in a horizontal row and, in addition, there are 3 other horizontal rows of minute teeth in that region. The cephalic glands are 14 in number and their ducts are arranged in 4 groups in the formula, $3 + 4 + 4 + 3$, and open into the dorsal margin of the mouth opening. The outer margin of the ventral sucker is not well differentiated. The tail possesses a dorso-ventral fin along its posterior half.

From the above description, it is evident that while the important characteristics of the *Clonorchis* cercaria experimentally produced

in our snails do not agree with those described by Muto and Faust and Khaw, they do agree well with those given by Yamaguti, and therefore we may conclude that Yamaguti's description is certainly based on a study of the genuine cercaria of *C. sinensis.*

Concerning the first intermediate host of *C. sinensis, Parafossarulus striatulus japonicus* was believed to have been incriminated by Muto in Japan, *Melania hongkongensis* by Faust and Barlow,[6] and *Parafossarulus striatulus, P. sinensis, Bithynia fuchsiana* and *B. longicornis* by Faust and Khaw, in China. As proved by the present experiments, *B. fuchsiana* should unquestionably be regarded as one of the first intermediate hosts of *C. sinensis.* Yamaguti's description of the cercaria of *C. sinensis* from *P. striatulus* var. *japonicus* leads us to the conclusion that this also should be regarded as one of its first intermediate hosts. Hsü and Chow[7] have reported *Clonorchis* cercariae in *P. striatulus,* in Canton, identical with those described by Yamaguti, and therefore *P. striatulus* should also be regarded as a first intermediate host of this worm. The result of our experiments indicates that the cercaria described by Faust and his coworkers as being that of *C. sinensis* is, in reality, the cercaria of another fluke; therefore *M. hongkongensis, P. sinensis* and *B. longicornis* cannot be accepted as first intermediate host for *C. sinensis* in China unless definite proof be given that this is true.

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Type-Specific Polysaccharides of *C. diphtheriae.*

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Polysaccharides derived from gravis, intermediate, and mitis types of *C. diphtheriæ* were found to be group-specific.[1] Recent findings,[2] however, indicate that any of these different cultural types may be present in a single serological type. Because of this an attempt was made to extend our previous study to include polysaccharides of different serological types of *C. diphtheriæ* with the object of determining the existence of type-specific polysaccharides.

6 Faust, E. C., and Barlow, H., *Am. J. Hyg.,* 1924, **4**, 69.

7 Hsü, H. F., and Chow, C. Y., *Chinese Med. J.,* 1937, **51**, 341.

1 Wong, Sam C., and T'ung, T., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 422.

2 Sia, R. H. P., and Huang, C. H., *Ibid.,* in press.