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Simple Technique for Studying Schistosome Worms in Vitro.

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The study of adult parasitic helminths is often handicapped by difficulties in keeping them alive for prolonged observation outside the body of their host. According to Coutelen[1] the first attempts at artificially rearing helminth parasites were made by Lönnberg in 1892 and Tower in 1900, using cestodes. Reichenow and Wülker[2] referred to the use of artificial media by Meggit in preserving the life of cestodes for several days and by Meier, who through frequent changes of media kept alive certain trematodes and cestodes from fish for as long as 21 days.

In metabolic studies of helminth parasites such as undertaken by Alt and Tischer[3] and Brand[4] observations must be made on living parasites *in vitro*. Such studies might have been more exhaustive if the life of the parasites could be maintained for several days instead of a few hours. In testing the anthelmintic value of a drug preliminary *in vitro* experiments seem an essential step and for such purposes intestinal helminths are usually used. Ryoji,[5] however, studied the action of certain organic antimony compounds on *Clonorchis sinensis in vitro* and Oesterlin and Krainick[6] tested the action of about 80 organic substances on many kinds of parasites including adult schistosomes. The latter authors remarked that the adult schistosomes recovered from experimentally infected mice do not remain alive in serum for long, probably as a result of bacterial growth in the medium.

In the course of chemotherapeutic studies in experimental schistosomiasis japonica the present authors devised a simple technique by which it was possible to keep adult schistosomes alive in artificial medium for weeks and if necessary for months without difficulty. This technique may prove useful in future studies in which it is

[1] Coutelen, F., *Ann. de Parasit.*, 1927, **5**, 1.

[2] Reichenow, Ed., und Wülker, G., Leipzig, 1929, p. 125.

[3] Alt, H. L., and Tischer, O. A., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 222.

[4] Brand, Th., von., *Z. f. Vergleichende Physiol.*, 1933, **18**, 562.

[5] Ryoji, S., *Okayama Igakkai Zasshi*, 1927, **39**, 1809 (Abstract) *Trop. Dis. Bull.*, 1930, **27**, 959.

[6] Oesterlin, M., und Krainick, H., *Zentralb. f. Bakt., Parasit. u. Infekt.*, 1934, **132**, 222.

desired to keep adult schistosomes or allied parasites alive for prolonged observation *in vitro*.

The difficulties in maintaining in artificial medium the life of such adult helminths as the schistosomes obtainable in an aseptic condition are mainly 2, accidental bacterial contamination and inspissation through evaporation of the medium. These difficulties can be surmounted by the use of a flask of the type employed in tissue culture work but smaller in size, which any competent glass blower can make to order (Fig. 1, A). The body of the flask is about 4 cm. in diameter and not more than 0.7 cm. in depth to permit observation under an ordinary low power objective (*e. g.*, Leitz No. 3) of the microscope without disturbing the contents. The mouth of the flask should be relatively wide, about 1.2 cm. in diameter, so as to allow easy introduction of the parasites and should be provided with a tight fitting rubber cap, which can be extemporized from the severed bulbous end of an ordinary rubber teat (Fig. 1, B, C). The flasks plugged with cotton wool and wrapped up in paper should be sterilized in the hot air sterilizer while the rubber caps placed in a petri dish are sterilized by steam under atmospheric pressure.

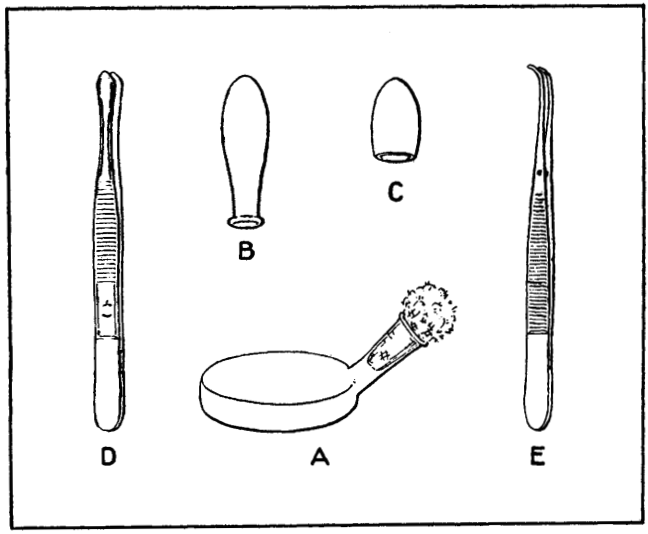

FIG. 1. For explanation of lettering see text.

The schistosomes are obtained from the large portal veins of an animal, preferably a rabbit on account of its large size, which has been infected 8 to 10 weeks previously with cercariae from snails. Rigid aseptic technique must be practiced in laying open the main

portal veins entering the hilum of the liver. In extracting the worms from the lumen of the veins a pair of forceps with round and smooth tips (Fig. 1, D) should be used to avoid injury to the parasites. They are then first transferred to a small covered petri dish containing warm sterile Locke's or saline solution and later distributed in a number of culture flasks into each of which exactly 2 cc. of the medium to be used have been previously introduced by means of a 10 cc. pipette under bacteriological technique. In carrying the worms over the neck of the flask a pair of fine, curved forceps (Fig. 1, E) are of advantage. The culture flasks with their mouths covered by rubber caps are then placed in an incubator at 37°C. to simulate the natural environment of the schistosomes.

Daily or, when indicated, hourly observations should be made of the culture to ascertain the condition of the worms. Any culture, in which there is evidence of bacterial contamination by a cloudy appearance of the medium should be discarded. The condition of the worms may be judged by their movements. Those showing no movements can be induced to do so by gently heating the flask over an electric hot plate unless they are actually dead. No worm should be regarded as dead without passing this test. Worms becoming sluggish after a few days will show renewed activity upon a change of fresh medium.

Almost any animal blood sera will serve as satisfactory media. We have tried horse, sheep and rabbit serum and ascitic fluid from cases of cirrhosis of liver with success. The addition of Locke's solution or dextrose does not improve the efficiency of the media. The duration of life of the worms in different media varies somewhat depending probably more upon the inherent vitality of the parasites rather than upon the nature of the medium.

Table I shows the results of a typical experiment. In this experiment it would seem that ascitic fluid and sheep's serum are more efficient than horse or rabbit serum but in another experiment some worms lived for 21 days in rabbit serum. With changes of fresh

TABLE I.

Duration of Life in Days of Schistosomes in Different Media without Change.

	Horse serum	Sheep serum	Rabbit serum	Ascitic fluid
Copulating pairs—Males	(2) 13-14	(2) 18	—	(2) 13-14
Females	(2) 9-11	(2) 14-15	—	(2) 19-20
Mature males	(6) 9-22	(7) 13-19	(9) 7-10	(4) 14-16
Mature or almost mature females	(2) 8-17	(3) 8-9	—	—
Immature females	(5) 8-16	(5) 10-14	(8) 7-9	(5) 17-20
Aver.	13.4	14.4	8.2	16.3

Figures in parentheses indicate number of worms studied.

medium at intervals it is possible to prolong the life of the schistosomes for 2-3 months. In one experiment in which the medium (in this case rabbit serum) was renewed at intervals of 1-2 weeks 2 schistosomes lived for 82 days.

It was also found that not more than 4 or 5 worms should be placed in each flask. Overcrowding shortens the duration of life of the parasites as shown by the experiment recorded in Table II.

TABLE II.
The Effects of Overcrowding. The Worms Were All Mature Males Uniform in Size. Plain Ascitic Fluid Was the Medium Used. No Change of Medium.

No. of flasks	No. of worms in each flask	Aver. duration of life in days
3	3	16
3	6	13
3	9	11
3	12	10
1	30	8

Summary. A simple technique is described for maintaining the life of adult schistosomes *in vitro* over a period of several weeks which, with frequent changes of medium, may be extended to 2½ months. This is made possible by the use of small tissue culture flasks, which prevent bacterial contamination and desiccation of the media. The latter may be either horse, sheep or rabbit sera or human ascitic fluid.

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Action of Various Organic Antimony Compounds on Schistosoma Japonicum in Vitro.

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By using the technique described by Lee and Chu[1] studies were made of the lethal action of the following 4 antimony compounds on adult *Schistosoma japonicum in vitro.*

Trivalent Antimony Compounds

Sodium antimonyl tartrate; chemically pure, supplied by E. Merck, Germany.

Fouadin. This is a 6.3% solution of a colorless powder, antimony III-pyrocatechin-disulphonate of sodium, supplied by Bayer and Company, Leverkusen, Germany.

[1] Lee, C. U., and Chu, H. J., in press.