Growth and Regeneration of *Schistosoma mansoni in vitro.**† (22649)

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The development of technics for the cultivation of the parasitic trematodes of man has progressed slowly. Studies on the most important human pathogens in this group, the schistosomes, have dealt primarily with the longevity of adult worms under artificial conditions in media containing serum and with attempts to define the constituents of serum essential for their survival. *Schistosoma japonicum* has survived *in vitro* for as long as 82 days in a rabbit serum medium(1), and for almost 5 months in horse serum-Ringer's solution containing washed red cells(2). Mature *S. mansoni* have been maintained for 18 days in ox serum(3), and for 2 months in horse serum(4). Egg production has been reported in a human serum system(5). With a chemically defined medium, a survival time for *S. mansoni* of 5 days has been noted(6).

Smyth(7) has emphasized that in studies on helminths *in vitro* criteria for demonstration of "growth" as contrasted to mere "survival" need to be developed. In this paper we will present evidence that growth of immature schistosomes maintained *in vitro* occurred, as manifested by an increase in size and by regeneration of amputated portions of certain of the worms.

Materials and methods. Specimens of immature *S. mansoni* were obtained employing aseptic technics from the portal system of white mice infected percutaneously in our laboratory. In addition, schistosomulae were recovered from peritoneal washings and from washings of liver fragments of mice inoculated with cercariae by the intraperitoneal route; these infected mice were supplied through the courtesy of Dr. Henry van der Schalie of the University of Michigan.

Infected mice were lightly etherized and then killed by cervical fracture. The peritoneal cavity was flushed with culture fluid (CF) in the case of mice infected by inoculation. The portal vessels and liver were then removed to separate Petri dishes containing CF and worms recovered by the teasing apart of the tissues under a dissecting microscope. Mice that had been infected by the percutaneous route were given 0.4 ml of heparin sodium (Liquaemin, Organon) intraperitoneally 5 minutes before being sacrificed.

The worms were transferred with Pasteur pipettes through several changes of fluid and then placed singly or in pairs in rubber-stoppered or screw-capped test tubes (15 x 150 mm) containing 1.4 ml of CF. The culture fluid was that used by us for maintenance of human cells *in vitro*, with the single omission of soybean trypsin inhibitor. It consisted of 45% bovine amniotic fluid(8), 45% Hanks' balanced salt solution(9), 5% inactivated horse serum, and 5% beef embryo extract, with penicillin, streptomycin and phenol red as described(8). Whenever necessary, the pH was adjusted to about 7.4 prior to use by gassing with 5% CO_2 in air. Cultures were maintained in an upright position at 37°C. At 2- to 4-day intervals, the CF was withdrawn and fresh medium substituted. The basic medium was supplemented in certain experiments by the addition to each tube at weekly intervals of fresh washed mouse red blood cells. After each addition, the erythrocytes were left in the cultures for 2 to 4 days and then removed. Cells for this purpose were obtained from young female white mice; such mice were heparinized, sacrificed as described above, and bled from the exposed heart. The cells were washed and centrifuged 3 times in CF. Approximately 0.3 ml of packed cells was resuspended in 2.0 ml of medium and 2 drops of the resulting suspension added to each culture. Final concentration

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of red cells in the culture medium was determined to be equivalent to a hematocrit of 3 to 5.

All subsequent manipulations of the cultures, with exception of photographic procedures, were carried out in a warm room at 32°C. The schistosomes were observed daily under a compound or dissecting microscope. This procedure was facilitated by gently rotating the culture in an almost horizontal position until the worm adhered to the tube wall out of the fluid. Serial camera lucida drawings were made in order to document changes in size. Inasmuch as constant contraction and extension of the worms was occurring, exact determination of length was difficult. Therefore, at each observation period 3 to 6 outline drawings were made per worm. A "map measurer" calibrated by mensuration of the projected image of a slide micrometer, was run along the axis of each outline. Length was arbitrarily derived by averaging sets of measurements. Serial drawings of worms less than 0.5 mm could not be obtained, since worms of this size could not regularly be induced to adhere to the wall of the culture tube. In a number of instances, interval photographs were taken. Tracings of these photographs were transferred to graph paper for calculation of total area as a measurement of body growth. Results generally corroborated data on length as obtained by multiple camera lucida drawings.

The CF, removed from each tube at the first change of medium, was cultured in thioglycollate broth and on Sabouraud's agar to reveal bacterial or fungal contaminants. Similar control cultures were repeated at intervals. No bacterial contamination was encountered, but growth of a mold resulted in loss of 3 worms.

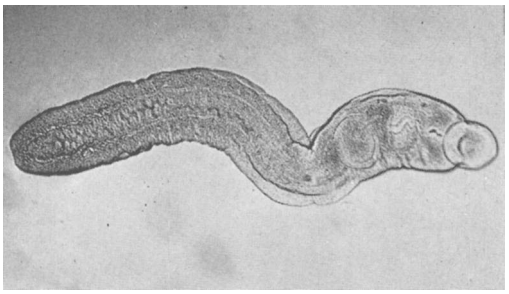

FIG. 1. Male schistosome (#905) recovered from mouse on 16th day of infection and photographed on 11th day *in vitro*. Length approx. 2 mm.

Results. A total of 78 schistosomes was studied *in vitro* for various periods of time. Although all worms showed some increase in length, irrespective of initial size, the survival period *in vitro* of worms measuring less than 0.5 mm was limited. The results of experiments with schistosomulae recovered from the peritoneal cavity of mice 6 to 10 days after injection of cercariae are summarized in Table I. In this series, erythrocytes were not added to the medium. A similar experiment was initiated using 11 worms obtained from mice on the 16th day of infection. For 2 weeks these were maintained in individual cultures on CF alone; at that point 5 of the worms were given red cells which appeared to increase activity and prolong slightly their survival. Two worms were alive after 35 days in culture, at which time the experiment was terminated; one, a female (#911) had increased in length from 0.98 mm to 4.58 mm, while the other, a male (#905) had increased from 1.52 to 4.08 mm. These schistosomes are pictured in Fig. 1 and 2. The results of serial measurements on the 16-day-old schistosomulae are summarized in Table II.

Immature schistosomes, derived from mice 30 or more days after percutaneous infection,

TABLE I. Survival of Schistosomulae *In Vitro*.

No. of worms in group	Time in days between infection of mice and recovery of worms	Original length (mm)*	Final length (mm)*	Avg days survival *in vitro*
6	6	.12–.25	.25– .50	3.3
6	9	.15	.50– .75	10.5
4	10	.25	.75–1.2	10.7

* Length estimated; serial measurements not done.

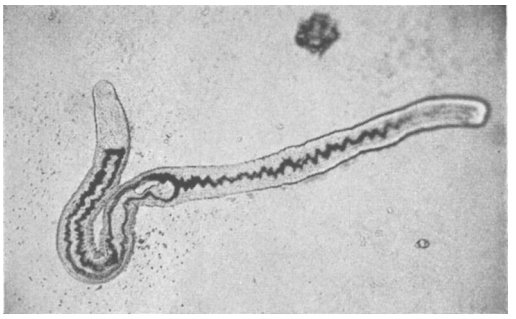

FIG. 2. Female schistosome (#911) recovered from mouse on 16th day of infection; photographed on 24th day *in vitro* and 24 hr after red cells had been added to the medium. Length 2.4 mm.

and having an initial length exceeding 1.5 mm, generally survived for many weeks on CF supplemented with red cells. Thus, 40 trematodes in this category remained alive and displayed vigorous feeding activity for periods as long as 87 days. Serial measurements on 3 male worms and 1 female in this group are presented graphically in Fig. 3. Accelerated somatic movement and peristaltic activity usually followed ingestion of the added red cells. After an exposure of 8 to 24 hours to the erythrocytes, ceca previously almost devoid of pigment would become distended with black material; usually such pigment disappeared within 48 hours following the withdrawal of red cells from the system.

Three pairs of larger worms (3-5 mm), each pair consisting of a male and a female, were placed in separate tubes. All began to copulate *in vitro*. In one case this attitude was maintained for 2 months, while the other pairs parted and rejoined at irregular intervals. No eggs were produced.

No systematic study was undertaken of the ability of schistosomes to regenerate amputated portions, but observations were made on a limited number of worms accidentally traumatized during recovery procedures. Four worms that lacked the posterior ⅓ to ½ of the body were noted to regenerate the missing part *in vitro* within 10 to 20 days. Ceca, cut just proximal to the point of union, were seen to unite and form the usual single posterior trunk during the regenerative period. In one specimen, a large cyst-like bladder developed in the regenerating posterior third. Although the excretory system remained imperfect, this individual survived during the 75-day observation period. Two schistosomes lacking the anterior third of the body were observed for 23 days, during which time there was no noticeable diminution of activity.

TABLE II. Survival and Growth *In Vitro* of 16-Day Schistosomulae.

Worm No.	Original length (mm)*	Final length (mm)*	% size increase	No. days survival
901	1.0	1.23	23	21
902	.94	1.01	7	26
904	.46	.61	33	25
905	1.52	4.08	168	(35)†
906	.62	.92	48	23
908	.67	1.09	63	25
909	.63	.87	38	17
910	.54	.67	24	17
911	.98	4.58	367	(35)†
912	.41	.61	49	17
913	.58	.61	5	17

* Averages derived from camera lucida drawings.

† ( ) = alive at end of experiment.

Discussion. The results of these preliminary studies indicate that a nutrient medium suitable for the cultivation of mammalian cells will also induce growth of *S. mansoni in vitro* as evidenced by an increase in length and by the regeneration of somatic amputations. That this nutrient system is not opti-

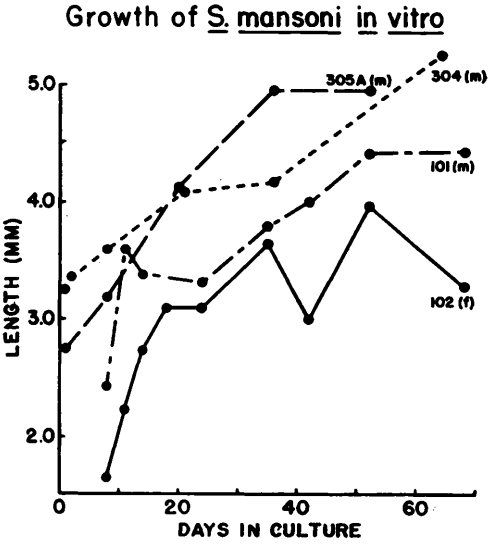

FIG. 3. Serial measurements derived from camera lucida drawings of 4 schistosomes maintained *in vitro* on CF supplemented with red cells.

mal is clearly indicated by the limited survival periods of the small specimens. The apparent stimulatory effect of ingested red cells on the larger worms requires further investigation. It is to be noted that Moore and Meleney(10) have suggested that the defective maturation of *S. mansoni* in the peritoneal cavity of the mouse may be a reflection of the absence of blood upon which to feed. Although not yet ideal, the *in vitro* system here described offers a convenient means for the direct and prolonged observation of feeding schistosomes. The technic should, therefore, prove useful for a variety of experimental purposes, as well as offering a baseline for the development of improved media in the future.

Summary. Preliminary investigations on the growth of *Schistosoma mansoni in vitro* are reported. In a nutrient medium composed of bovine amniotic fluid, beef embryo extract, and horse serum, schistosomulae generally showed evidence of growth. The duration of survival was directly related to initial size. Worms that initially approximated 1.0 mm or greater in length were found to survive many weeks *in vitro;* the survival period of smaller schistosomulae was of much shorter duration. The addition of red cell suspensions to the basic medium was followed by active feeding and accelerated somatic movements. Growth of the schistosomes *in vitro* was manifested by increases in length of male worms to a maximum of 168%, and of female worms to 367%, and also by the observed regeneration of amputated portions of certain of the worms.

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Action of Thyroid Hormones on Brain Metabolism of Newborn Rats. (22650)

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Investigations have shown that the metabolism of the brain is better protected against interference, especially of the thyroid hormone, than other tissues(1a). It was reported(1) that thyrotrophic hormone can increase the brain oxidation of hypophysectomised rats, although it can seldom influence that of normal adult rats. It seemed that after hypophysectomy a regulating mechanism, which is responsible for the constancy of the brain metabolism, is disturbed. During the last few years it seemed promising to continue these experiments on animals whose anterior pituitary function was not yet fully developed. The brain of newborn rats seemed also to be particularly interesting owing to the extraordinary resistance of these animals to anoxaemia which enables them to survive in a carbon monoxide atmosphere(2).

Contrary to previous experiences with adult animals these investigations showed some significant changes after treatment with thyroid hormones.

Methods. Litters of Wistar rats were 5 to 11 days old at investigation, and weighed 8 to 20 g each. Each litter was divided equally,