Cultivation of the Sparganum of *Spirometra mansonoides in Vitro* with Prolonged Production of Sparganum Growth Factor[1] (37342)

T. W. CHANG, M. S. RABEN,[2] J. F. MUELLER, AND L. WEINSTEIN

New England Medical Center Hospital and the Department of Medicine, Tufts University School of Medicine, Boston, Massachusetts 02111; and State University of New York, Upstate Medical Center, Syracuse, New York 13210

Rats infected with the sparganum of *Spirometra mansonoides* by subcutaneous implantation contain in their serum a growth hormone-like substance called sparganum growth factor (SGF) (1–3). Infestation of hypophysectomized rats causes growth of the animals, but growth ceases about 4–5 wk after worm implantation, probably due to the development of neutralizing antibody to the growth factor. During *in vitro* cultivation of the sparganum, it was found that SGF appeared in the medium if a complex medium containing embryo extract and serum was used but not with unfortified medium 199. Addition of purified plasma proteins was stated to substitute partially for serum and embryo extract (3). We have cultivated the sparganum first in cell cultures and subsequently in cell-free medium 199 containing only 3–5% calf serum as supplement. The worms continued to produce SGF in the medium for 6 mo, the longest period observed thus far.

Methods. Spargana were supplied by Dr. Justus F. Mueller and were either used on arrival or were implanted subcutaneously in Charles River normal or hypophysectomized rats and later removed for *in vitro* studies. Monolayer cell cultures included primary cultures of human amnion, rhesus monkey kidney, rat embryo, hamster embryo, and cell lines of W1-38 and L-cells. Primary cultures were grown in Eagle's medium or medium 199 containing 10% calf serum and maintained in Eagle's–199 mixture (3:1) containing 5% serum. Cultures of tissue fragments embedded in chicken plasma clot were prepared using heart, lungs, kidney and liver from rat fetuses. The tissues were grown and maintained in the same media used for cells.

The spargana were pipetted into containers containing either monolayer cell culture of human amnion, monkey kidney, L-cells or W1-38, or medium containing varying amounts of calf serum without cells. The volume of the medium was adjusted to 1 ml/worm. At this volume, the cultures contained approximately 5×10^5 cells/ml of medium. The cultures were incubated at 37°.

The growth-promoting effect of SGF was assessed in female hypophysectomized rats weighing about 90 g. The assay was similar to that used for pituitary growth hormone except that a single injection was given rather than daily injections because of the prolonged effect of SGF. The activity of the media was determined by the amount needed to produce a gain in weight of at least 1 g/day for 7–10 days, which is the gain produced by the daily injection of about 10 μg of pituitary growth hormone.

Results and Discussion. Tables I and II show the results of cultivation. The spargana survived in all cell cultures despite varying amounts of serum in the medium. On the other hand, in the absence of cells, most of the worms did not survive with or without serum in the medium. During the first 2 wk of incubation, the accumulation of acidity in the medium was so rapid that the medium

[1] This investigation was supported by Grant AM-01567 from the National Institute of Arthritis and Metabolic Diseases and Training Grant 276 from the Institute of Allergy and Infectious Diseases of the National Institutes of Health, U.S. Public Health Service.

[2] Recipient of a Research Career Award, National Institutes of Health, U.S. Public Health Service.

TABLE I. *In Vitro* Cultivation of Sparganum of *S. mansonoides*.

Condition of incubation				
Amnion cells	Serum in medium 199 (%)	Day of incubation 5	10	20
No	No	6/15[a]	14/15	
No	5	1/5	4/5	
Yes	No	1/15	3/15	6/15
Yes	1	0/6	0/6	0/6
Yes	2	0/4	0/4	0/4
Yes	5	0/10	0/10	0/10[b]

[a] No. deaths/no. inoculated.

[b] Subsequently transferred into cell-free medium containing 5% calf serum. No death was observed thereafter.

had to be changed every 2 days. As the incubation continued, less acid was formed. After 1 mo incubation, there was only a slow change in pH in the medium. From then on, the medium needed to be changed only once every 7–10 days. Cell cultures containing spargana began to show cytopathic changes 2–3 days after the addition of the worms. Gradually, the cells became round and detached from the glass surface. By the end of 1 wk, only a few cells remained. The worms were then transferred into new cell cultures for further cultivation.

The worms which failed to survive showed changes before death, including sluggishness in peristaltic movements, swollen segments starting from the tail end, branching of the body and fragmentation of the entire worm. The worms which survived continued to grow in length and showed none of these changes. At the end of 4 mo, the worms reached a length varying from 50 to 110 mm from an initial length of about 10 mm (Fig. 1). Active peristaltic-like movements could easily be seen on tapping the container.

TABLE II. Cultivation of Sparganum in Various Types of Cell Cultures.

Types of cell culture[a]	Day of incubation 5	5	5
L-cells	0/6[b]	0/6	0/6
Monkey-kidney	0/6	0/6	0/6
W1-38	0/6	0/6	0/6
Human amnion	0/10	0/10	0/10

[a] Cell cultures were maintained in medium 199 containing 5% calf serum.

[b] No. of deaths/no. inoculated.

Because amnion cell culture can be maintained in serum-free medium, our attention was focused on the use of amnion cells. Worms that had been in amnion cultures for 3 wk were transferred into cell-free medium containing 5% calf serum initially and 3% serum in later transfers. The worms continued to grow in size and to excrete SGF into the medium (Table III). The growth-promoting activity of the medium was comparable to that of serum from worm-infested rats, though perhaps somewhat weaker. A single injection of 0.3 to 1.0 ml of the medium injected intraperitoneally stimulated hypophysectomized rats to grow at a rate of at least 1 g/day for a period of about 7–10 days. Some worms that had been grown in cell cultures until they produced only minimal pH changes were transferred into amnion cell cultures containing no serum. In spite of viability of the sparganum, production of SGF was not detected.

TABLE III. Demonstration of SGF in Medium in Which Spargana Were Cultivated by Assay in Hypophysectomized Rats.[a]

	Duration of cultivation of spargana (mo) 2	4	6
Wt gain of at least 7 g in 7 days	10/10[b]	3/3	4/4

[a] A single injection of 0.3 to 1 ml/rat was given intraperitoneally.

[b] No. gaining wt/no. injected.

Medium containing SGF and serum from worm-infested rats were tested for their *in vitro* effect on cell growth. The addition of varying amounts of these preparations to cell suspensions did not affect the time required to form a monolayer, starting from varying concentrations of trypsinized cells. There was no detectable effect on the growth of human

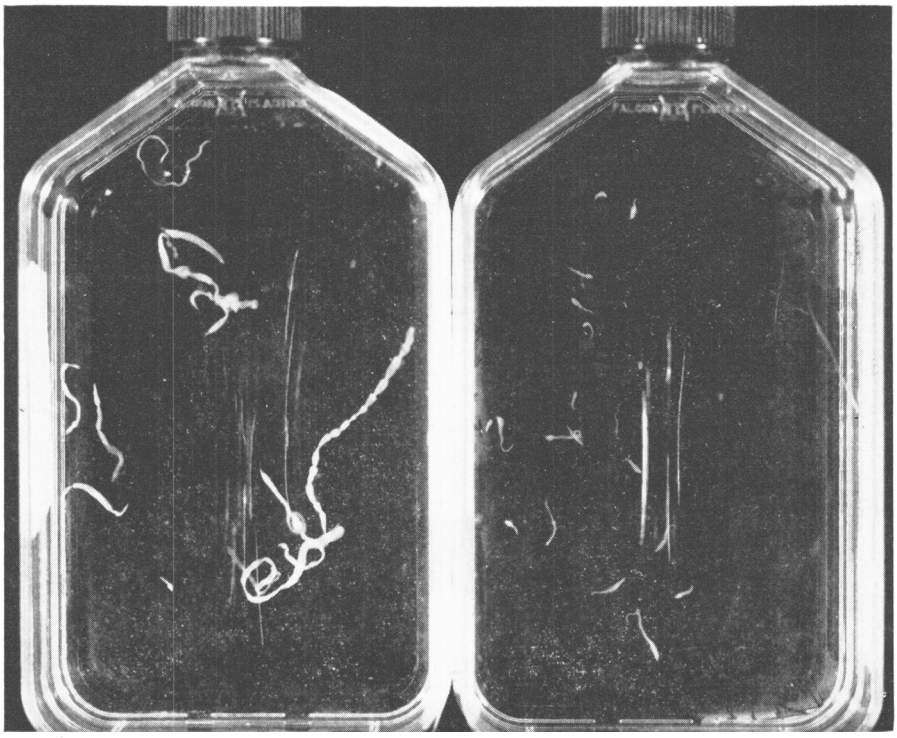

FIG. 1. Growth of spargana in culture. The bottle on the right contains spargana at the beginning of cultivation. The bottle on the left contains spargana after 6 mo of cultivation. A single peristaltic wave is seen in small spargana while multiple waves are present in larger ones. The straight lines are scratch marks on the bottles.

amnion, L-cell, W1-38 or hamster embryo monolayer cell cultures. The addition of varying amounts of these preparations to monolayer cultures of these cell types did not change the appearance of the cultures. The growth of tissue explants of rat embryonic kidney, liver, lung and heart was not accelerated by the addition of rat serum containing SGF. The serum did not differ from noninfested rat serum in its effect on the uptake of ^{3}H-thymidine in L-cells.

Thus, we have observed that the sparganum of *Spirometra mansonoides* could be maintained *in vitro* in medium 199 enriched only with 3–5% serum with continued production of SGF for at least 6 mo, and that, despite the *in vivo* effect of SGF, no *in vitro* growth-stimulating effect could be demonstrated. The prolonged output of SGF by cultured spargana in a medium with a relatively low protein content may provide a better source of SGF for chemical and physiological studies than serum from worm-infested rats.

Summary. If cultured initially in a cell culture, spargana of *Spirometra mansonoides* could subsequently be maintained in medium 199 enriched only with 3–5% serum with growth of the worms and continued production of sparganum growth factor (SGF) for at least 6 mo. Although growth-promoting *in vivo,* SGF failed to stimulate the growth of cultured cells or tissue explants.

1. Mueller, J. F., and Reed, P., J. Parasitol. **54,** 51 (1968).

2. Mueller, J. F., J. Parasitol. **54**, 795 (1968).

3. Steelman, S. L., Glitzer, M. S., Ostlind, D. A., and Mueller, J. F., *in* "Recent Progress in Hormone Research" (E. B. Astwood, ed.), Vol. 27, p. 97. Academic Press, New York (1971).

Received Nov. 21, 1972. P.S.E.B.M., 1973, Vol. 143.