cuprous ion is toxic to bacteria under anoxia from a concentration of 1×10^{-6} M upwards.

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BIOLOGY

Teratogenic Effect of Trypan Blue on Rat Embryos cultivated *in vitro*

GILLMAN *et al.* first observed the teratogenic activity of trypan blue in 1948 (ref. 1), and the dye has since become widely used as a teratogenic agent for laboratory work. The recent development of a system for the cultivation of post-implantation rat embryos *in vitro*[2] has made it possible to investigate the direct action of trypan blue on the isolated embryo.

Five- to ten-somite rat embryos (10–11 days) surrounded by the amnion and yolk sac placenta were explanted on to plasma clots and incubated for 18–45 h. Before cultivation the embryos were treated in one of the following ways: (1) injection of a 0·05 per cent solution of trypan blue in glucose–Tyrode's solution into the yolk sac followed by immersion of the embryo in a 0·05 per cent solution of trypan blue in embryo-extract for 30 min at room temperature; (2) injection of glucose–Tyrode's solution into the yolk sac followed by immersion of the embryo in embryo-extract for 30 min at room temperature; (3) immersion of the embryo in a 0·05 per cent solution of trypan blue in embryo extract for 30 min at room temperature; (4) immersion of the embryo in embryo-extract for 30 min at room temperature. The purpose of soaking the embryo for 30 min at room temperature was to allow diffusion of the dye before development was resumed in incubation. After cultivation the embryos were examined as whole mounts, and selected specimens were examined histologically.

The results of the experiments are shown in Table 1.

Table 1. RESULTS OF TRYPAN BLUE INDUCED MALFORMATIONS

	No. of embryos treated	No. of normal embryos	No. of abnormal embryos
(1) T.B. + G.T. injected and soak	32	17	15 (47%)
(2) G.T. injected and soak	19	19	0
(3) T.B. + E.E. soak	14	13	1 (7%)
(4) E.E. soak	12	12	0

T.B., trypan blue. G.T., glucose–Tyrode. E.E., embryo extract.

It can be seen that only embryos treated with trypan blue developed abnormalities. Of the 32 embryos injected with trypan blue, 15 (47 per cent) exhibited one or more abnormalities which were similar to those described by Waddington and Carter[3]: oedema and swelling of the head and neural folds, failure of neural folds to close properly, dilatation of the pericardial sac, enlargement of the heart, sub-ectodermal blister, haematoma of the trunk and tail regions, and general retardation of development. Of the 14 embryos immersed in trypan blue without injection, only one exhibited abnormalities.

These results show that trypan blue has a direct teratogenic effect on the rat embryo. Gillman *et al.* observed that the critical period of the rat embryo *in utero* to the teratogenic action of trypan blue injected into the maternal circulation is day 7–9. The work recorded here shows that the embryo is still sensitive to trypan blue after day 9; as first suggested by Wilson *et al.*[4], the sensitivity ceases *in utero* after day 9 because the yolk sac which completely envelopes the embryo at this time prevents the dye from reaching the embryo. The single abnormality among the embryos soaked in trypan blue may be the result of the dye leaking into the yolk sac through a small puncture made during explantation.

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A Culture Medium for Snail Cells and Tissues

As a first step in providing a suitable medium for *in vitro* culture of snail-borne stages of trematode parasites, we have developed a suitable medium for *in vitro* culture of snail cells and tissues. The culture medium devised and tested in our laboratory is capable of sustaining cells and promoting growth for as long as 60 days. Presumably it will maintain snail cells and tissues for a much longer time, but lack of media has prevented us from running longer experiments.

The snail tissues cultured in our experiments were taken from the foot muscle, mantle, oesophagus, oviduct and gonad of *Helix pomatia* Linn. (Euthyneura : Stylommatophora : Helicidae), *Planorbina* (= *Australorbis*) *glabratus* (Say) (Euthyneura : Basommatophora : Planorbidae) and *Pomatiopsis lapidaria* (Say) (Streptoneura : Mesogastropoda : Hydrobiidae). Foot muscle and gonad were the two tissues mostly used. Prior to removing the tissues, each snail's shell was cracked by gently applying pressure with pliers. The snail (unanaesthetized and unnarcotized) was then freed from its shell by cutting the columellar muscle with a scalpel. The tissues were removed with sterile micro-knives, minced into small pieces, washed several times in a sterile saline solution and placed in sterile plastic Petri dishes containing 5 ml. of snail media.

Especially important in culturing snail tissues are the following factors: sterility, proper antibiotic mixture, addition of snail extract, temperature and *p*H. Several of the tissues were maintained better at different respective *p*Hs, but all the cultures seemed to do better at 15° C (we tested temperatures of 0°–40° C at 5° intervals).

Gonadal tissues of all 3 snail species were maintained for as long as 60 days in a total of 5 experiments at *p*H 7·0. Several observations are particularly pertinent: (1) Actively motile mature sperm were evident continually through the 60 days; (2) the various prophase I stages of meiosis, especially diakinesis, were seen in great abundance in the 5 experiments involving all 3 species; numerous metaphase I cells were also seen; (3) after 14 days many mitotic metaphase cells were seen in one primary explant culture of *Helix pomatia*, indicating actual cell division and quantitative growth of the cultures; (4) in addition to the experiments using primary explants, as many as three successful sub-cultures were made on gonadal and oviduct tissues.

Foot muscle tissues, maintained at *p*H 8·5, appeared in good condition at the end of 60 days for two of the snail