The Effects of Trypan Blue on Chick Embryos Cultured in vitro

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INTRODUCTION

Various malformations in rats and mice have been observed to be caused by administration of the vital dye, Trypan blue (Gillman, Gilbert, Gillman, & Spence, 1948; Fox & Goss, 1956, 1957; Hamburgh, 1952, 1954; Waddington & Carter, 1952, 1953). Recently Waddington & Perry (1956) reported a teratogenic effect of Trypan blue on Amphibian embryos. The present paper deals with the effects of this dye on cultured chick embryos.

MATERIAL AND METHODS

Hen's eggs were incubated at 38° C. to the desired stage of development. Cultures were set up according to the ring technique described by New (1955). Precautions to maintain sterile conditions were observed throughout. An aqueous 1 per cent. solution of Gurr's vital Trypan blue was diluted with Pannett Compton saline to 0.05 per cent., which had been found to be a suitable concentration. A few drops of the dye solution were placed on the upper surface of the treated blastoderms. The embryos were kept for about half an hour at room temperature before incubating to allow proper diffusion of the dye before development was resumed. The dye was left in contact with the treated embryos throughout the whole culture period.

A total of 28 embryos at the primitive-streak or early head-process stage, and 13 embryos at the medullary-plate stage, were treated and studied. Twenty-six control embryos at the primitive-streak or early head-process stage received a few drops of saline instead of the dye and were maintained under identical conditions. Both the controls and the experimental cultures were incubated for 14–16 hours, after which they were fixed in acetic alcohol and serially sectioned at 10 μ. In some experiments the entire embryos were stained in borax carmine, differentiated in acid alcohol, and sketched before sectioning.

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EXPERIMENTAL RESULTS

Of the 28 embryos treated at the primitive-streak or early head-process stage, 18 showed abnormalities. Fifteen of the abnormal embryos showed malformation of the somites. In some regions somitic mesoderm was not formed at all (Text-fig. 2). In others it was formed but the somites were irregular and asymmetrical (Text-fig. 6). Extensive failure of notochord development occurred in 4 specimens.

All the drawings were made from photographs.

The embryo illustrated in Text-figs. 1, 2, and 3 was in an early head-process stage when treated. On sectioning it was found that the notochord was absent except in a few anterior sections. The mesoderm showed a tendency towards somite formation and in part there was a median somite strand (Text-fig. 3). The presence of such median somitic mesoderm in the absence of notochord has also been described by Waddington (1932) and Waddington & Perry (1956).

In two cases the brain and the optic vesicles were found to be very small. Shortening of the axis of the embryos was also observed in 9 treated embryos.

In nine cases the heart did not show its characteristic flexure and was represented by a somewhat dilated straight tube for a considerable time, although the
heart of the control at the same stage showed the characteristic flexure to the right.

**TEXT-FIG. 4.** Embryo treated at primitive-streak stage showing blister (b) over the blastoderm. × 66.

**TEXT-FIG. 5.** Section of embryo shown in Text-fig. 4, through blister. × 110.

**TEXT-FIG. 6.** Section showing irregular somite (s) formation. The notochord (ntch) appears to be big for the neural plate. It may be a somite on the right side moving towards the centre in the absence of the notochord. × 80.

**TEXT-FIG. 7.** Embryo treated at the medullary-plate stage showing shortening of the axis and inhibition of somite formation. × 47.

Large transparent blisters were seen in 4 embryos, especially posteriorly between the ectoderm and mesoderm (Text-fig. 4). Sections of the embryo passing
through the blister showed suppression of the neural plate beside the blister, failure of notochord formation, and abnormalities in the somites (Text-fig. 5).

Of 13 embryos treated at the medullary-plate stage 7 showed more or less similar abnormalities. The anterior portion of the embryo appeared to have developed normally in these embryos; but shortening of the axis, malformation of somites, and transparent blisters were quite common (Text-fig. 7). Six of 13 cultures treated at this stage were incubated for 42–44 hours to study the effect of dye on the development of the eye, but the eyes appeared to develop normally. However, in two cases the eye and the nearby neural tissue of the brain showed an unusual amount of cell degeneration.

None of the malformations described were observed in the control embryos.

**DISCUSSION**

Some of the abnormalities such as transparent blisters overlying the embryo, and abnormal hearts reported for mice by Hamburgh (1952, 1954) and by Waddington & Carter (1952, 1953), and for rats by Gillman et al. (1948) and Fox & Goss (1956, 1957), have also been observed in the present study with chick embryos. In both mice and rats ocular defects such as eyelessness (anophthalmia) or absence of lens, &c., seemed to be quite common; but in the chick embryos which were incubated for 42–44 hours for the study of the development of eye no defects were seen, though in two cases the eye showed unusual cell degeneration.

Cases of pseudencephaly have been described in mice at birth by Waddington, but in the present work the embryos were not kept alive long enough for this effect to be observed. In mice and rats the nervous and circulatory systems seemed to be severely affected by the Trypan blue treatment, and minor effects on notochord and somites were also observed; in contrast, the somites and notochord of Amphibia seem to be much affected. Instances of mesodermalization consisting of suppression of the notochord with the conversion of notochord material into somitic material have been described by Waddington & Perry (1956). In the present study the majority of malformations were found in somites. Where notochord failed to appear a median somite strand was found, as observed in Amphibia by Waddington & Perry; but with the data available it cannot be said with certainty that mesodermalization of notochord material had occurred.

**SUMMARY**

1. Chick embryos at the primitive-streak or early head-process and at the medullary-plate stages were treated with Trypan blue during culture *in vitro*.

2. The dye produced many abnormalities such as malformation of somites, shortening of the axis, the formation of large blisters on the embryo, and delayed heart flexure. In some specimens notochord failed to form and a median somite strand was found in these cases.
ACKNOWLEDGEMENTS

I wish to thank the Government of India for the award of a research grant under the scheme of grant-in-aid for fundamental research, which enabled me to work on the problem. My grateful thanks are due to Professor Waddington, F.R.S., for reading through the manuscript and making useful suggestions.

REFERENCES


(Manuscript received 28: iv: 59)