A STUDY OF THE MECHANISM OF ACTION OF COLCHICINE ON EMBRYOS OF MICROHYLA ORNATA

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Introduction

The importance of —SH groups in the embryonic development, particularly during morphogenetic movements has been emphasized by several workers. In a recent investigation (Diwan, 1964) it has been found that colchicine, an —SH inhibitor, produces abnormalities in the brain region and also causes a fall in the inducing capacity of Hensen’s node. It has also been shown that the effects of colchicine on the morphogenesis of chick embryos could be completely reversed by subsequent treatment with cysteine hydrochloride. These studies have emphasized the importance of —SH containing proteins stressed by several workers. (For a recent review, see Brachet, 1964.)

Though several workers (Keppel and Dawson, 1939; see Beatty, 1951) have studied the effects of colchicine on amphibian development, the mechanism of its action has not been discussed so far. It was felt desirable to undertake such a study. In the present work, therefore, an attempt has been made to elucidate the possible mechanism of action of colchicine on the early development of Microhyla ornata.

Materials and Methods

The eggs of Microhyla ornata were collected from the local ponds. These eggs were separated by dissection but the jelly was not removed. They were then put into the solutions of colchicine in 10 HF solution. After some preliminary experiments a concentration of 0.0025 g/l (62 × 10^{-7} M) was found to be suitable. Eggs of various stages (Shumway 7—8, 10—11, 14—15) were left in this solution for 2 or 4 hours after which they were transferred to 10 HF solution for further cultivation. For each experiment controls were reared in 10 HF solution. The experiments were conducted at 22°C.

The embryos were examined on the second day and the typical morphological features were recorded. They were then fixed in Bouin’s solution. A total number of 800 embryos were studied, about 100 being sectioned for microscopical examination.

Experimental Results

I. Effects of Colchicine on Early Development

It will be seen from the experimental results that in most batches of eggs subjected to the action of colchicine, there was considerable variation in the degree of abnormality produced. However, on the whole, the
effects were stronger; the earlier the stage at which the eggs were placed in the chemical, and longer it remained there.

Out of 400 experimental embryos 150 were treated at cleavage stage (stage 7—8), 150 at gastrula stage (stage 10—11) and the remaining 100 were treated at early neurula stage (stage 14—15). The embryos treated at early neurula stage do not develop abnormalities.

In the experimental embryos treated at earlier stages, a number of different kinds of abnormalities were produced. The main types are:

1. Suppression of gastrulation. In some batches of eggs treated in early stages (stage 7—8) the process of gastrulation was completely inhibited. The cells divide usually but do not assume any regular form. They are in most cases, loosely grouped above the undivided yolk mass thus resulting in the formation of a ball of rather featureless cells.

2. Oedema. The treated embryos are extremely swollen and full of fluid in the tissue spaces.

3. Microcephaly. Embryos treated at gastrula stage often exhibited various degrees of reduction of head. On sectioning, such embryos showed a variety of abnormalities. Perhaps the most interesting of these consisted in the reduction of both the size and the differentiation of the brain (Fig. 3). The notochord is normal in majority of the cases and the neural tube overlying it is much reduced. In some cases the notochord is completely absent and the somite mesoderm from each side unites in the midline giving an irregularly segmented mass. The absence of parts of brain occurred in a few cases and in one exceptional case only the hindbrain was present.

4. Eye-defects. With few exceptions, a considerable number of embryos treated at gastrula showed size and structural abnormalities of eyes. In majority of these cases, there was no true eye cup, but simply a solid mass of closely packed cells which had not differentiated into separate retinal and pigment layers (Fig. 1). In some specimens the pigment cells were clumped in one or more groups at the medial side of the eye cup whereas, normally, they form a complete layer around it.

5. Loss of pigmentation. As compared to the controls, the experimental larvae showed reduction in pigment. Defects in pigmentation consist of a decrease in both the number of melanophores and the amount of melanin. In extreme cases a strong reduction in the number of melanophores all over the body was observed.

II. Cytological Effects of Colchicine

Embryos treated at the gastrula stage (stage 7—8) and the neurula stage (stage 14—15) were fixed together with controls after 20—21 hours of development. They were then sectioned at 4 μ, stained in iron haematoxylin and observed under the oil immersion lens.