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INFLUENCE OF CHLORAMPHENICOL ON THE EMBRYOS AND LARVAE OF TOAD, *BUFO ANDERSONII* (BOULENGER)

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Embryos and larvae of toad, *Bufo andersonii* were reared in 0.05%, 0.1%, 0.2%, 0.3% & 0.4% solutions of chloramphenicol from early blastula stage onwards at room temperature for 10 days. Chloramphenicol did not affect hatching but caused severe mortality, dehydration, skin depigmentation and growth inhibition of the larvae. This effect was correlated with the amount of chloramphenicol present in the rearing medium. Frequency of mortality was low in larvae treated with 0.05% and 0.1% chloramphenicol but was much greater in those treated with 0.2%, 0.3% and 0.4% chloramphenicol.

INTRODUCTION

Chloramphenicol is one of the major antibiotic derived from *Streptomyces venezuelae*. It is a bacteriostatic drug, like other microbial toxins it interferes with the enzymes controlling the synthesis of proteins and inhibit protein synthesis (Kenneth & Williams, 1958; Shaw, 1984; Liversage & Smith, 1988). Chloramphenicol is known to have profound effects on growth, development and differentiation processes in animals (Globus & Liversage, 1975). Its influence on these processes during ontogeny in anuran embryos has not been studied at all. Therefore, the background information motivated the present study of the effects of chloramphenicol on development and growth in embryos and larvae of the toad, *Bufo andersonii*.

MATERIAL AND METHODS

Early blastulae of toad, *Bufo andersonii* were used in these experiments. Eggs were divided into 6-groups of 100 eggs each. One group (Control-G1) was reared in tap water and the other groups were reared in 0.05% (G2), 0.1% (G3), 0.2% (G4), 0.3% (G5) and 0.4% (G6) solutions of chloramphenicol. A known quantity of this antibiotic was dissolved in in 1 ml of water and then diluted to give a solution of required concentration. The experiment was carried out at room temperature (30-32^{0}C) and lasted for 10 days. After hatching the larvae were fed boiled spinach and their medium was changed every two days. Some of the larvae from each group were fixed in Bouin's solution. The larvae of all the six groups were carefully observed after anaesthetising in 1 : 4000 aqueous solution of MS222 (Sandoz). Number of tadpoles surviving in each concentration of chloramphenicol and their stage of development was noted and shown in Table I. The data thus obtained was analysed statistically by using significant differences from control larvae. The significant level was set at $P < 0.05$.

RESULTS AND DISCUSSION

Chloramphenicol treatment had no apparent effect on embryos. At the time of hatching the larvae of all the 6-groups were similar in size but those of chloramphenicol

treated groups were distinctly less developed morphologically than the controls (G1). The differences were further accentuated during the subsequent days. The tadpoles of groups 2-6, which survived remained small in size and were malformed. Hatching was normal in both controls and chloramphenicol treated groups. It was soon followed by heavy mortality among the latter; none died among the controls. By the end of 2nd day most of the larvae treated with 0.3% concentration of chloramphenicol died. In 0.4% (G6) 70 larvae had died during the first day and by the end of 3rd day none of the larvae in this concentration had survived. There was a heavy mortality in the larvae exposed to 0.2% (G4); 0.3% (G5) and 0.4% (G6) solutions of chloramphenicol. The mortality rate was nigh in larvae exposed to higher concentrations of chloramphenicol. Treatment of larvae for longer duration resulted in complete inhibition of body growth. These results are in confirmity with the observations of the previous workers (Kenneth & Williams, 1958).

Table I. Influence of chloramphenicol on embryos and larvae of toad, *Bufo andersonii* (Boulenger).

Chloramphenicol (Conc.)	% Survivor	Length in mm	Stage of development
(G1) Control	100	20 ± 2.03	34
(G2) 0.05%	10	10 ± 0.308**	25
(G3) 0.1%	8	7 ± 0 .441**	25
(G4) 0.2%	2	6 ± 0 .396**	23
(G5) 0.3%	0 (all died on 4th day)	5 ± 0 .442**	23
(G6) 0.4%	0 (all died on 3rd day)	4.5 ± 0 .165**	19

** Significantly different from controls. $P < 0.05$.

Stages are equivalent to those described in a normal Table of *Bufo melanostictus* (Khan, 1965).

19 stage - Gill bud; 23 stage - Opercular fold; 25 stage - Hind limb bud; 34 stage - 3 segments of the leg are clearly demarcated; the thigh, shank and foot.

Rearing in media containing chloramphenicol did not affect the time of hatching of the embryos because they hatched at the same time simultaneously with their respective controls. It is possible that the egg jelly surrounding the embryos before hatching protected them against the toxic influence of the chloramphenicol; but once they were out of the jelly and directly exposed to high concentrations of chloramphenicol they succumed to it. However, the jelly cannot be presumed to have completely prevented the influence of chloramphenicol reaching the embryonic cells and tissues. This is because the chloramphenicol treated larvae at hatching showed retarded and

abnormal features which must be attributed to chloramphenicol effects. The embryos given low concentration of chloramphenicol appeared similar in size and morphology but even among them there were several differences at the histological and cellular levels. It can be assumed therefore, that the influence of chloramphenicol in the rearing medium did go through the egg jelly to affect the embryo.

The data in Table I, thus show that the early developmental stages of the toad embryos and larvae are adversely affected by chloramphenicol. The specific mechanism of this action is not known. It has been suggested that protein synthesis inhibitors produce embryo lethality but few or no overt terata (Ritter, 1977). Chloramphenicol is a well known inhibitor of protein synthesis. It is a bacteriostatic drug, readily penetrates into the body cells and exerts its toxic and growth inhibiting effect (Kenneth & Williams, 1958; Burnet & Liversage, 1964). Cytotoxic agents can interfere with the feed back control of cellular cholesterol synthesis (Flynn *et al.*, 1987). Since, cholesterol is an important component of cell membrane associated with the normal embryonic differentiation and development (Chepnik & Waldman, 1983). It is possible, that the growth inhibiting effects of the drug may be due to its effects on the metabolism of cholesterol or of proteins.

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