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FINAL REPORT

PROJECT B-299

THE USE OF TADPOLES FOR TOXICITY

By

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SUMMARY

The preliminary study of the toxicity of sodium chromate with tadpoles indicated that a solution of 10 mg per liter sodium chromate retarded metamorphosis of the tadpoles. When this study was repeated with much larger numbers of organisms, a statistically significant support of our hypothesis was not obtained. Many difficulties were encountered: tadpoles will not metamorphose after a 24 hour period of starvation, the time required for metamorphosis is partially dependent upon crowding in the aquarium; they should be in a shallow water; a high salt concentration as recommended by Carolina Biological Supply Company, retards growth. Once an individual tadpole has increased in size more than others in the acquarium then it becomes cannibalistic and the significantly smaller individuals disappear. This was partially true even in the control.

It is concluded that for long term toxicity studies of soluble salts, the use of tadpoles will not be recommended for inclusion in Standard Methods.

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I. INTRODUCTION

In the attempt to evaluate the carcinogenic properties of sodium chromate, this chemical was added to the medium in which tadpoles were observed. While microscopic observations of skin cells failed to show chromosomal aberations in the squamous epithelial cells, it was observed that the tadpoles in the control medium metamorphosed much earlier than those in the chromate solution.

On this basis, the grant was requested for the opportunity to study large enough numbers of tadpoles to eliminate chance as the cause for the retardation of metamorphosis by chromate.

II. METHODS

The tadpoles for the preliminary observations obtained from Carolina Biological Supply Company in January, 1967, because of the company's proximity to Atlanta, Georgia. The present project was inaugurated in June, 1966, when no tadpoles were available in North Carolina. Attempts to use tadpoles from Rochester, New York were unsuccessful; too much time was consumed in travel and they died very quickly. Work was abandoned until tadpoles were again available locally.

When tadpoles were available, they were distributed into a large number of containers with several different concentrations of chromates with and without potassium iodide. It soon became apparent that too much time was required to handle the large numbers of different media that were involved with four series of four concentrations. The early (November) tadpoles from Carolina Biological Supply Company did not respond well in our laboratory. The tadpoles obtained nearer the normal date for ovulation responded better or lived longer under our laboratory conditions, but the controls did not metamorphose. A mass of frog eggs were obtained from a local pond and extra pond water was returned to the laboratory. No salts were added, but the pond water was kept in light so that algae developed. No other food was used. Only four pans of tadpoles were prepared: plain pond water, pond water plus 0.1 mg per liter potassium iodide, and each of these controls plus 10 mg per liter sodium chromate. Each of these four pans contained 17 or more tadpoles to begin. The water was changed once a week.

Other attempts were made to grow tadpoles in Holtfreters solution which contains:

3.5 g NaCl	0.10 g $CaCl_2$
.05 g K Cl	.2 g $NaHCO_3$

in one liter. A large volume of this was made up in a jar for daily renewal of the frogs environment. The iodide and chromate was added in 1.0 ml aliquots as needed. These tadpoles were generally obtained from the Carolina Biological Supply House, but died off before significant results could be obtained.

Because of the different times between ovulation and metamorphosis of individual species of frogs the local species, Rana pipiens, was chosen because its normal larval period lasts 90 days.

III. RESULTS

The results of the four sets of tadpoles grown in pond water are given in Table 1. The tadpoles were very difficult to maintain in their original numbers. Analysis of the data shows no hastening of metamorphosis by the potassium iodide. This would indicate that this element is not deficient in the local pond water. (Goiter in this area has been uncommon).

The presence of the chromate in the presence or absence of iodide showed no retardation in metamorphosis.

All color and structural changes proceeded in the chromate solutions as normally as in the controls. The cannibalism of the smaller tadpoles by the faster growing individuals in the controls was frustrating, for the controls disappeared rapidly enough that the numbers remaining ruled out a statistical evaluation. The long period of time before any deaths occurred indicates reasonable care of the organisms.

Variation in the growth rate of individuals is typical of any biological experiment, but the magnitude of the variation observed was much greater than expected. The two individuals in Figure I are the same age from the same solution.

It may be noted that the tadpoles which were not ready to metamorphose within the normal period of the larval stage died soon after the passage of the normal period. The experiments were carried out in a laboratory without windows and with a thermostatic temperature control. The question of a lack of sunlight and temperature cycling may explain the poor number of metamorphosis in the tadpoles.

TABLE 1

TIMES TO DEATH OR METAMORPHOSIS OF INDIVIDUAL TADPOLES
OF THE RANA PIPIENS SPECIES

Control	Control plus potassium iodide	Sodium Chromate	Sodium Chromate plus potassium iodide
	To death and removed		
Days	Days	Days	Days
89	89	54	54
89	90	85	85
97	97	85	89
108	99	85	89
129	108	131	90
129	108	131	101
131	136	10 eaten*	104
8 eaten*	140		104
	165		107
	165		129
	165		135
	7 eaten*		135
			136
			140
			168
	Times to Metamorphosis		
117	117	105	130
137		130	134
158		160	137
			160

*Days to disappearance not recorded.

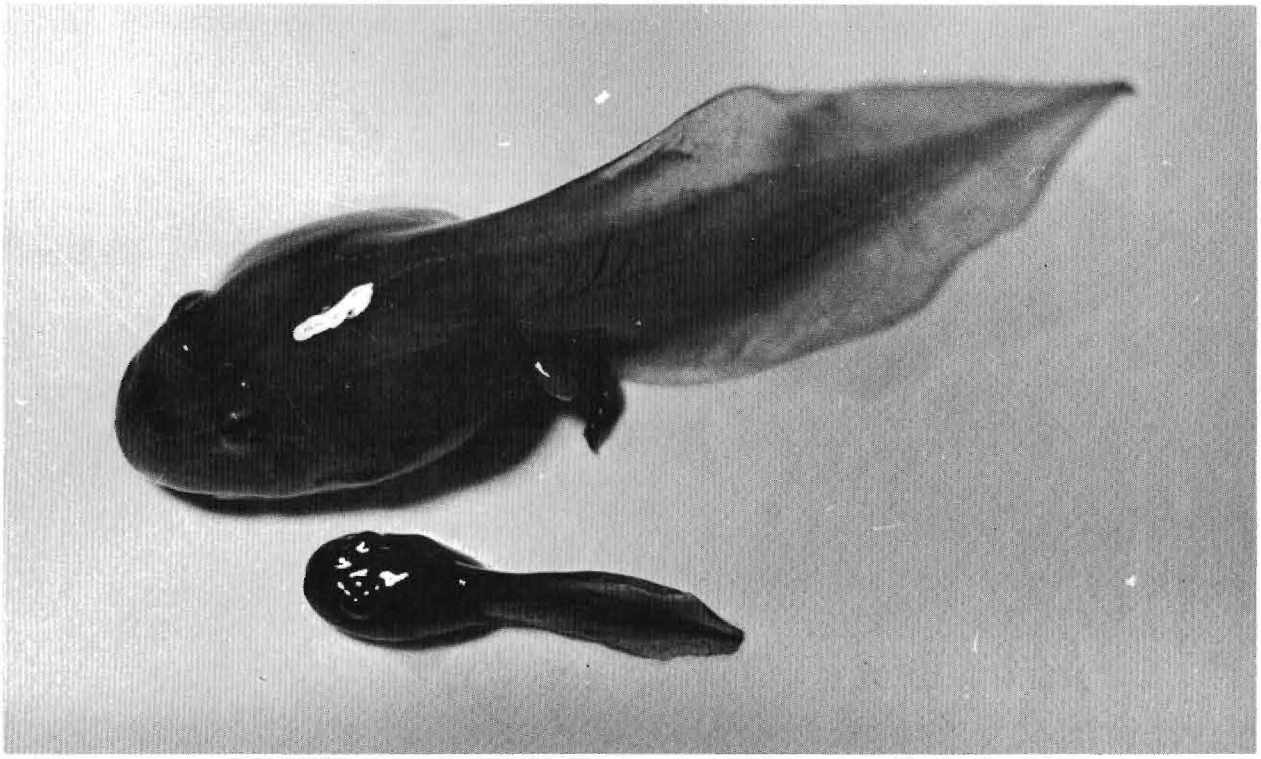

Figure I. Variations in size of two tadpoles of the same age which were grown in the same solution.

IV. DISCUSSION

The fact that tadpoles from a common egg mass in a pond show large variations when brought into the laboratory probably explains why earlier experiments indicated a toxicity from sodium chromate toward metamorphosis. That is, slow growing individuals were placed in the sodium chromate solution by chance. When a large enough number of individuals was used, the variations developed in each medium. Thus, no conclusions about a special cause of the variation could be drawn.

The cannibalism in the controls and its absence in the chromate solutions may be significant, but it is not germaine to this study.