

paper electrophoresis, with Veronal buffer (pH 8.6) (6). Hemoglobin A was the only type detected in these samples (7).

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13 October 1958

New Method for the Rapid Determination of Lathrogenic Agents

Abstract. Salamander and toad embryos will develop grossly observable tumors of the notochord if they are placed for 3 or more days in a solution of the lathyrism-producing chemicals beta-aminopropionitrile or aminoacetonitrile. A technique for using tumorigenesis in amphibian embryos as a biological indicator for other lathrogenic agents is presented.

Rats fed diets containing large amounts of meal prepared from the sweet pea *Lathyrus odoratus* or containing a crystalline factor isolated from

Lathyrus seeds develop a syndrome known as experimental lathyrism. The skeletal and other mesenchymal tissue changes of the syndrome can also be produced by feeding small amounts of the nitriles beta-aminopropionitrile (BAPN) or aminoacetonitrile (AAN). In amphibians, the changes can be produced by rearing embryonic forms in water containing either the crystalline factor of the *Lathyrus* seed or one of the nitriles. They are characterized by distortions of the limbs and jaws and by tumors of the notochord.

Interest in experimental lathyrism is increasing, probably as a part of the recent general interest in diseases of the connective tissues, and probably because of the similarity between induced lesions in the experimental disease and such human afflictions as slipped epiphyses, degenerative arthritis, and dissecting aortic aneurism (1).

Only a few compounds, notably the nitriles, have been shown to produce lathyrism, and the mode of action by which these chemicals produce the lesions has not been explored. Recently Dasler (2) has shown that semicarbazide will produce lesions of osteolathyrism in the rat. Since semicarbazide is a known aldehyde blocking agent, it occurred to my coworkers and me that other water-soluble aldehyde blocking agents might also produce lathyrism.

Salamander and toad embryos reared for 3 days in water containing a lathrogenic nitrile exhibit gross tumors of the notochord. It seemed likely that these animals could be used as a biological screening tool for new lathrogenic agents.

Ten to 20 embryos of the salamander *Amblystoma punctatum* or of the toad *Bufo americanus* in early tail bud stages were placed in finger bowls containing 100 ml of various concentrations (0.01 to 100 mg/100 ml) of an aldehyde-blocking agent in spring water (Table 1). These agents were selected from chemicals frequently used in blocking the periodic acid-Schiff histochemical reaction. The embryos were examined daily under the dissecting microscope for evidence of tumor formation, which is the first gross sign of lathyrism. The known tumorigenic agents, BAPN and AAN, were used as controls. The experiments were usually terminated after 1 week. All chemicals tested except sodium bisulfite, hydrazine hydrate, and hydroxylamine hydrochloride produced tumors (Table 1) (Fig. 1).

Dasler's (2) finding that semicarbazide produced osteolathyrism in rats opened a relatively new field for the investigation of connective tissue metabolism. Dasler pointed out that finding new osteolathrogenic agents might aid in locating the metabolic defect in the disease. Our technique provides a rapid

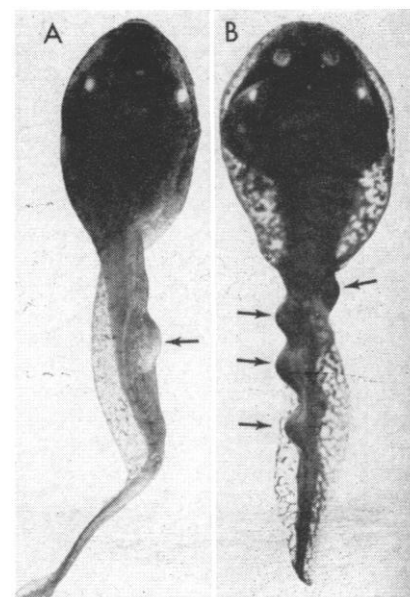


Fig. 1. Single (A) and multiple (B) tumors in tadpoles after immersion for 1 week in beta-aminopropionitrile.

and accurate screening method for seeking such agents. Our findings suggest that the defect might be concerned with carbohydrate metabolism of the connective tissue ground substance (3).

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3. The technical assistance of Johnnie Goodrich is gratefully acknowledged. The 1-benzyl-1-phenylhydrazine HCl and the 4-phenyl-3-thiosemicarbazide used in this study were provided through the courtesy of M. S. Burstone of the National Institute of Dental Research. I am indebted to Ulrich Weiss of the National Institutes of Health for the thiosemicarbazide and to Waldeman Dasler of Chicago, Ill., for the beta-aminopropionitrile and the aminoacetonitrile. This investigation was supported in part by grant D-822 from the National Institutes of Health, U.S. Public Health Service.

14 October 1958

Effect of Ultraviolet Light on Pectolytic Enzyme Production and Pathogenicity of *Pseudomonas*

Abstract. Ultraviolet radiation-induced mutants of the soft rot bacterium *Pseudomonas marginalis* were selected for loss of pathogenicity for lettuce and witloof chicory. The avirulent mutants differed from the parent pathogen in their inability to synthesize pectolytic enzymes in culture or to ferment sodium pectate or sodium polygalacturonate as the sole carbon source in media.

In his pioneer work in 1909, Jones (1) postulated that parasitism in the soft rot bacteria seemed to be associated directly with the ability to produce pec-

Table 1. Effects of various aldehyde blocking agents on tumor formation in salamander and toad embryos.

Blocking agent	Result	Lowest effective concn. (mg/100 ml)
Amino antipyrine HCl	Tumor	50
Hydrazine hydrate	No tumor	
Phenylhydrazine*	Tumor	1
1-Methyl-1-phenylhydrazine	Tumor	0.5
1-Benzyl-1-phenylhydrazine HCl	Tumor	1
Semicarbazide†	Tumor	1
Thiosemicarbazide	Tumor	10
4-Phenyl-3-thiosemicarbazide	Tumor	3
Hydroxylamine HCl*	No tumor	
Sodium bisulfite†	No tumor	
Urea†	Tumor	1
BAPN	Tumor	1
AAN	Tumor	1

* Baker. † Fisher.