paper electrophoresis, with Veronal buffer (*p*H 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 Lathyrogenic 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 lathyrogenic 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.

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.

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 lathyrogenic nitrile exhibit gross tumors of the notochord. It seemed likely that these animals could be used as a biological screening tool for new lathyrogenic 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-Shiff 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 osteolathyrogenic agents might aid in locating the metabolic defect in the disease. Our technique provides a rapid 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*).

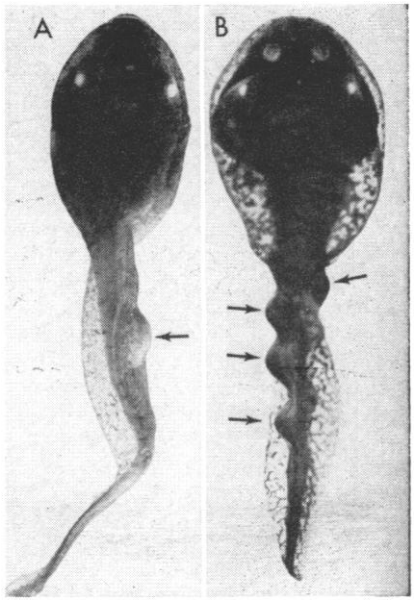

Fig. 1. Single (*A*) and multiple (*B*) tumors in tadpoles after immersion for 1 week in beta-aminopropionitrile.

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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-

tolytic enzymes. This viewpoint has been restated more recently (*2*). As far as can be ascertained from the literature, there appears to be no direct experimental evidence that loss of ability by a soft rot bacterium to produce pectolytic enzymes has resulted in loss of pathogenicity. The purpose of the present communication is to report evidence bearing on this aspect of the host-pathogen relationship.

Pseudomonas marginalis (Brown) Stevens, strain P1 pathogenic for lettuce, was selected for study. The bacterium was isolated originally from witloof chicory (*Cichorium intybus* L.) in 1949 (*3*). Cell-free culture filtrates of the pathogen are now known to cause soft rot, russet spotting, and vascular browning of lettuce and to contain protopectinase (or macerating enzyme complex), pectin depolymerase, and pectinmethylesterase (*4*).

Bacterial suspensions of *P. marginalis* in water were irradiated with ultraviolet light (15-watt General Electric germicidal lamp) at a distance of 22.5 cm for varying periods of time up to 10 minutes. Cells surviving exposure were plated out on yeast extract-nutrient agar, and 17 single-colony isolates were tested for pathogenicity. Ten of these showed complete inability, in repeated tests, to infect leaves of witloof chicory and head lettuce (*Lactuca sativa* L.) following needle inoculations with cells grown on yeast extract-nutrient agar.

Five isolates chosen at random from the ten avirulent isolates failed in repeated tests to produce pectolytic enzymes in cultures of lettuce broth and Uschinsky broth, even though they formed heavy, fluorescent growth in the broths. Under similar conditions the pathogenic P1 strain exhibited protopectinase, depolymerase, and pectinmethylesterase activity. Furthermore, the addition of 0.15-percent Seitz-sterilized pectin to an Uschinsky broth culture of an avirulent isolate (strain M4) failed to induce the formation of pectolytic enzymes. Although the mutants have been in culture over 6 months and have been transferred at approximately biweekly intervals, they have not reverted to a virulent state, and they have not produced pectolytic enzymes in culture.

To ascertain whether the avirulent M4 and the virulent P1 strains differed in other respects, the two were compared morphologically, culturally, and physiologically by means of standard methods (*5*). The strains were found to be similar with respect to characteristics previously reported for P1 (*3*), except for their reactions in synthetic broths containing 0.15- to 0.5-percent sodium pectate or sodium polygalacturonate or 1-percent sucrose as the sole carbon source. The avirulent strain M4 failed to ferment these substances, whereas the virulent P1 strain produced alkali from the pectic substrates and acid from sucrose.

Recently it was reported that ultraviolet irradiation of the soft rot bacterium *Erwinia aroideae* produced some specific nutritional mutants which showed a loss of pathogenicity resulting from the inability of the mutants to find on the host tissue the required nutrilites for growth (*6*). Five avirulent strains and strain P1 of *P. marginalis* were grown in minimal broth and minimal agar to determine whether a nutritional mutation was involved in these cultures also. For this purpose eight different, simple synthetic broths [including Davis and Mingioli broth and Entner and Stanier broth (*7*)] and four agar media were used. Inorganic salts or asparagine were used as nitrogen sources, while sodium acetate, glycerol, dextrose, or asparagine were used as carbon sources. Noble (Difco) or washed agar were used for the solid media. Cells washed with 0.8-percent sodium chloride were used to inoculate the media. In all cases, the growth of the five avirulent strains on the minimal media was comparable to that of the virulent parent strain, indicating that a nutritional mutation was not involved.

It is known that the presence of inhibitory substances in plant tissue may affect the host-pathogen relationship (*8*), but in the present study unheated, blended lettuce extracts did not inhibit the growth of the avirulent M4 strain in culture. In addition, it is recognized that there are saprophytic species of *Pseudomonas* and other microorganisms which are able to form pectolytic enzymes in culture but are unable to invade plant tissue (*9*). In the case of these saprophytes, failure to cause disease must be due to some mechanism other than the inability to produce pectolytic enzymes. Although pectolytic enzymes play a decisive role in soft rots of plants, it is quite possible that other enzymes play some role in pathogenesis by *P. marginalis*. In the present study, however, assays for other enzymes were not made.

In summary, the results in the present study of the soft rot pathogen *P. marginalis* indicate that loss of pathogenicity by the radiation-induced mutants is genetic in nature and is linked with their inability to form pectolytic enzymes and with their consequent inability to attack the pectic substrates present in inoculated host tissues.

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6 October 1958

Intracellular Impulse Conduction in Muscle Cells

Abstract. A hypothesis, suggested previously by morphological studies, for impulse conduction from the sarcolemma to the contractile material via the sarcoplasmic reticulum is discussed. The relation of reticulum morphology and cell size to speed of contraction in smooth and striated muscle agrees with the hypothesis and thus supports it. Additional support comes from evidence concerning an unusual morphological relationship between the sarcolemma and contractile fibrils in striated muscle of amphioxus.

Studies of the fine structure of cells by recently developed physical means, such as electron microscopy, have stimulated the formulation and study of hypotheses of the mechanisms of various cellular functions. The worth and durability of these hypotheses will depend ultimately, of course, on sound physiological demonstration that the mechanisms are in reality those described by the hypotheses. It is valuable in the meantime, however, to formulate hypotheses, provided that they suggest new lines of experimental investigation. Such investigation, when carried out, will lead to validation or rejection of the hypothesis.

It is the purpose of this report to discuss a hypothesis of intracellular impulse conduction in muscle cells that has been suggested by cytological observations recently made with the electron microscope. As is shown, the hypothesis is justified in that it brings the previously paradoxical time-distance relationships in muscle into proper order and suggests investigation directed toward the demonstration of the presence of intracellular membrane potentials and depolarization phenomena.

The paradox referred to is as follows: Striated muscle cells, which average 50 to 100 μ in diameter, contract very quickly and across the entire diameter of the cell very soon after the action potential has passed over the cell membrane. Smooth muscle cells, on the other hand, which average 6 to 10 μ in diameter, contract slowly, and parts of the cell come into play only at relatively long