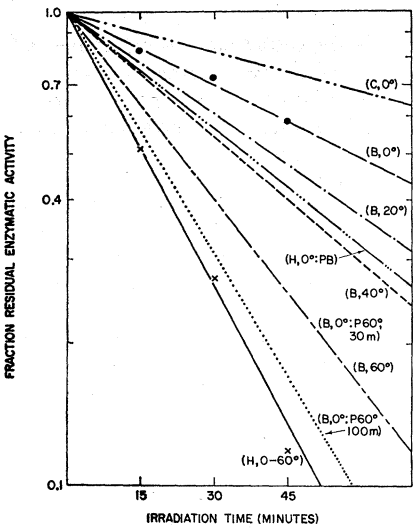

Fig. 1. Effect of temperature, urea, and substrate on the measured level of ultraviolet inactivation of trypsin. The labeling on the curves is explained in the text. Data are shown for only two curves for clarity of presentation; other data showed comparable variances.

by BAEE assay has a ΔH^* of 2500 ± 500 cal/mole from zero (B, 0°) to 60° (B, 60°), approaching the "Hb-measured rate" at 60°C.

4) Without exposure to urea or thermal treatment, an amount of BAEE, which would normally be completely hydrolyzed in 5 minutes, is added in buffer (*p*H 8) 10 to 30 minutes prior to assay; the inactivation rates measured by a subsequent Hb assay are more characteristic of "BAEE-measured rates" than of Hb rates (H, 0°:PB).

The results suggest that radiation causes previously inaccessible H-bonds—necessary for activity—to become available for urea attack or else unstable to the molecular swelling it produces. Thus it appears that the effects of urea or heat, or both, are additive with those of irradiation. This result was predicted previously on the basis of my hypothesis (*7, 8*) that inactivation by physical means proceeds by the sequential rupture of the disulfide and hydrogen bonds making up a "weak-link" structure which is instrumental in "latching" the enzyme together.

The calculated efficiency of 2537-A light in promoting protein inactivation through the rupture of disulfide bonds (*9*) indicates that the data presented here are probably in accord with this hypothesis. Also consistent are the observations of McDonald (*10*) and Monier (*11*) that trypsin molecules having different sensitivities to thermal aging and urea denaturation are produced by x-irradiation of dilute solutions. However, Liener (*12*) recently reported that irreversible trypsin inactivation results from the rupture of only one disulfide bond, rather than my predicted two. This expected correlation between inactivation and increase in —SH titer is now being investigated.

The "reactivation" proposed in the fourth postulate had also been previously anticipated (*8*). However, although my evidence indicates that damaged molecules are reactivated through interaction with BAEE, it is difficult to exclude the possibility that a significant portion of the reversal is produced by the change in *p*H from 4.5 to 8. Probably the reactivation contributions of substrate and *p*H can be best differentiated with a nonproteolytic enzyme, since the possibility of tryptic autodigestion interferes with the interpretation of some of the critical control experiments.

The reversal by BAEE of inactivation which would have been measured by Hb assay indicates that portions of the two activities are probably inactivated by a common mechanism (*13*) (weak link ?). In addition, protease assays employing casein (C, 0°) without urea suggest that the *reactivation capability* differs among substrates. For example, in one experiment the rate of inactivation measured by casein assay (no urea) had a $\Delta H^* = 4750 \pm 500$ cal/mole from 0° to 60°C, as compared with 2500 ± 500 for the BAEE assay.

These and the results cited in paragraphs 2) and 3) above, plus the evidence reviewed in the first paragraph, lead to a tentative conclusion of overlapping sites—where the hydrolytic apparatus would be common but the elements which could form specific attachment would vary with the substrate. Unfortunately, these preliminary results are as yet insufficient for *unequivocal* specification of the architecture of the sites. However, they provide strong evidence concerning some of the steps whereby inactivation proceeds and, therefore, warrant reporting at this time. It is hoped that extension of these studies will permit a specification of the secondary and tertiary structure critical for enzymatic activity as well as define the structural correlation between the two activities.

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13. Chymotryptic contamination is probably not the explanation of the effects reported here.

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2 January 1959

Lack of Abnormal Hemoglobins in Alaskan Eskimos, Indians, and Aleuts

Abstract. In an examination of the blood of 708 Eskimos, 200 Aleuts, and 44 Indians in Alaska for abnormal types of hemoglobin, only normal hemoglobin A was detected. It may be concluded that abnormal hemoglobins in these races are rare if they occur at all.

Although hemoglobins other than normal adult hemoglobin A are found with varying frequency in various racial groups (*1*), these genetic variants of hemoglobin have been found primarily among African or Asian populations. Since the Eskimos, Aleuts, and Indians of Alaska may be of Asian origin, we wished to determine whether any abnormal hemoglobins were characteristic of these Alaskan racial groups. The possibility was also considered that the moderate anemia which is prevalent in Eskimos in western Alaska (*2*) might be related to the presence of an abnormal variant of hemoglobin.

Hemoglobin samples from 593 Eskimos from all parts of Alaska and 25 Indians from central Alaska were tested by paper electrophoresis in Veronal buffer (*p*H 8.6), by alkali denaturation (*3*), and for solubility (*4*). Blood cells from 42 of these persons with moderately low hemoglobins were further examined for osmotic fragility, and the absorption spectra of the hemoglobins were measured. No abnormalities were found in any of the tests.

An additional 334 blood samples were sent to the University of Texas (*5*). Of these, 200 were from Aleuts, 115 were from Eskimos, and 19 were from Indians. The hemoglobins were analyzed by

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*).

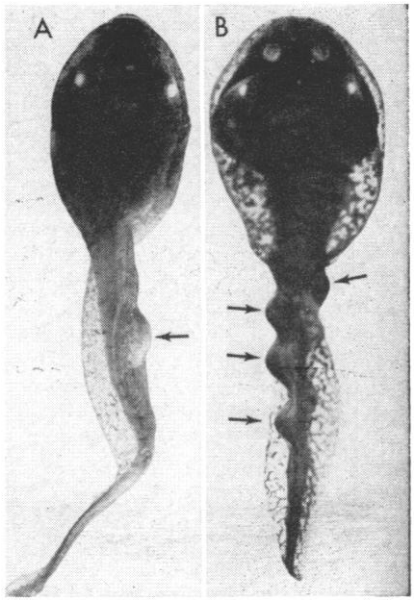

Fig. 1. Single (*A*) and multiple (*B*) tumors in tadpoles after immersion for 1 week in beta-aminopropionitrile.

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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-