THE DEMONSTRATION OF A POSSIBLE COMMON MECHANISM OF LATHYROGENIC ACTIVITY*

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It may prove useful to define lathyrogenic agents as a class of compounds which, when given to normal, growing animals, prevent the formation of either intra- or intermolecular covalent crosslinkages in collagen. Operationally, this may be demonstrated by an increased extractibility of collagen by neutral salt solutions as shown by Levene and Gross (1959). The collagen in these extracts contains a paucity of interchain crosslinkages (Martin et al., 1961).

Although it is conceivable that there is more than one mechanism of lathyrogenic activity, it is preferable to assume a single mechanism for the purpose of formulating a working hypothesis. Levene (1961) showed that there are four classes of compounds which produced lathyrism in chick embryos: aminonitriles, ureides, hydrazides and hydrazines. He also showed that the lathyrogenic activity of these compounds could be modified by the simultaneous injection of an aldehyde such as pyridoxal, but not pyridoxine or pyridoxamine. These data suggest that the lathyrogens could prevent the normal crosslinking reaction by reacting with active aldehyde groups in the collagen molecule. However, Orloff and Gross (1963) showed that radio-active β-aminoproprioni-

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trile (β-APN), a powerful lathyrogen, was not bound to the soluble collagen. This demonstrated that β-APN does not act by combining with intrinsic aldehyde groups, although it does not rule out the possible role of exogenous aldehydes in the crosslinking reaction. Levene (1961) also demonstrated that in the chick embryo iproniazid and nialamide, strong inhibitors of monoamine oxidase, had little lathyrogenic activity whereas isonicotinic acid hydrazide, a poor inhibitor of monoamine oxidase, was a powerful lathyrogen. These data suggest that lathyrogenic activity is not correlated with monoamine oxidase activity. However, O'Dell et al. (1965) and Miller et al. (1965), on the basis of extensive data, have suggested that β-APN inhibits an enzyme which oxidizes the ε-amino group of lysine to yield the aldehyde intermediate in the formation of the desmosine crosslinkages in elastin.

It is apparent that there is a relation between the elucidation of the mechanism of lathyrogenic activity and the mechanism of crosslinking in collagen. We are currently investigating an hypothesis for the mechanism of crosslink formation. This hypothesis states that hexoses can form N-glycosidic linkages with the ε-amino groups of lysine and/or hydroxylysine in collagen. These glycosides can then undergo the various known steps of the browning reaction to form the Schiff's base of hydroxymethylfurfural (HMF). The crosslinking reaction then involves the condensation of this Schiff's base with the imidazole ring of an histidine residue of a second chain. The chemistry and proof of the feasibility of these reactions have been worked out in this laboratory and will be published.

If the above working hypothesis is a correct one, we must also postulate that the lathyrogenic agents must interfere with crosslink formation by disrupting the Schiff's base with the removal of the HMF. To explore this possibility we set up a model reaction as follows:

$$Bu\text{-}N{=}CH\text{-}F + L\text{-}NH_2 \rightleftarrows L\text{-}N{=}CH\text{-}F + Bu\text{-}NH_2,$$

where Bu-N=CH-F is the Schiff's base of butylamine and furfural and $L\text{-}NH_2$ is the lathyrogen.

The lathyrogen (100 μmole) was dissolved in 1.5 ml of 30% ethanol containing 0.067 M phosphate buffer, pH 7.4 To duplicate samples of lathyrogen we added 25 μl of an alcoholic solution containing 100 μmole of the Schiff's base, prepared according to Emling et al. (1949). The mixture was incubated at room temperature for 6-7 days to insure the attainment of equilibrium. The free butylamine was isolated by the paper chromatographic separation of 30 μl samples, using BuOH:HOAc:H_2O (200: 30: 75) as the developing solvent. Standard amounts of butylamine, covering the entire range of the expected liberation of the base, were chromatographed simultaneously. The butylamine area was cut from the strips. The amine was eluted and determined quantitatively by the ninhydrin method of Rosen (1957).

The results of the experiments, expressed as the per cent disruption of the Schiff's base, are given in the first column of the Table. In the second column, we have expressed the results relative to the value obtained with β-APN, arbitrarily set at 100%. In the third column, we have taken the viscosity data obtained by Levene (1961) as a measure of lathyrogenic activity in vivo and have expressed them relative to his value for β-APN in order that we may have a comparison of the two sets of data.

Considering the fact that we are comparing results from systems which could hardly differ more, the correlation is high. In the two cases where the in vitro results are greater than those in vivo, it is reasonable to suppose that metabolic degradation or the reaction with other tissue components could decrease the effective concentration of the lathyrogen in vivo. If the results had shown greater activity in vivo, the correlation would have been less reasonable.

We have used the Schiff's base of furfural and butylamine because it represents a model of the intermediate of the crosslinking reaction which we propose. There is no reason to believe that the results would have

TABLE I

THE DISRUPTION OF SCHIFF'S BASE BY LATHYROGENS COMPARED WITH LATHYROGENIC ACTIVITY

Lathyrogen	Disruption (%)[c]	Disruption (% of β- APN)	Lathyrogenic Activity[a] (% of β-APN)
β-aminoproprionitrile	40	100	100
hydrazine	85	210	40
semicarbazide	25	65	60
urea	0-5	0-15	0-5
phenylhydrazine	20	50	b
acetone thiosemicarbazone	60	150	70
methylenaminoacetonitrile	40	100	125
cyanoacetohydrazide	15	40	40
β-alanine	0-5	0-15	0-5

[a]From C. I. Levene (1961).

[b]No animals survived.

[c]The standard deviation of the determination was 7% of the values given.

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been different if the Schiff's bases of other aldehydes had been used. Therefore, we can only conclude that there is a good correlation between the ability of the lathyrogens investigated to disrupt a Schiff's base and their lathyrogenic activity. This in turn suggests that the lathyrogens could be active through a common mechanism of Schiff's base disruption, thus preventing the condensation of a Schiff's base on one chain with residues of a second chain of a collagen molecule. The fact that radioactive β-APN is not bound to collagen further suggests that the aldehyde is exogenous, the amine being the integral part of the collagen molecule.

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