Molecular Diseases of Connective and Vascular Tissues. II. Amine Oxidase Inhibition by the Lathyrogen, β-Aminopropionitrile*

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ABSTRACT: Lathyrogens impair the oxidative deamination which precedes covalent interchain cross-linking of collagen and elastin. The enzyme involved in cross-link formation has not been identified; however, the amine oxidase of pig plasma possesses many characteristics expected of the connective tissue enzyme. The activity of this enzyme was examined *in vitro* with respect to the lathyrogen β-aminopropionitrile (BAPN). Using a spectrophotometric assay system, it was shown that the presence of 5 mM BAPN inhibits competitively and reversibly the activity of pig plasma amine oxidase on kynuramine. BAPN was found to combine with the active site of the enzyme. A concentration of lathyrogen which inhibits amine oxidase activity almost completely failed to inhibit the activity of two other copper-containing enzymes, polyphenoloxidase and uricase, and the pyridoxal-containing enzyme glutamic–oxalacetic transaminase. Thus, the binding of the lathyrogen to amine oxidase is relatively specific. It is suggested that lathyrogens may inhibit cross-link formation either by competing with native substrate for the active site of the amine oxidase involved in cross-link production or by binding with strategic lysyl side chains of collagen and elastin.

Lathyrogens induce in experimental animals a disease of connective and vascular tissues manifested by aortic aneurysm, skeletal deformity, and abdominal hernia (Geiger *et al.*, 1933). Underlying these changes is an increased fragility of all connective tissues and a concomitant elevation in the solubility of collagen (Levene and Gross, 1959; Gross *et al.*, 1960). The excess quantity of soluble collagen appears to derive from new synthesis and to result from an inhibition by lathyrogens of covalent interchain cross-link formation (Martin *et al.*, 1961; Smiley *et al.*, 1962; Bhatnagar, 1964; Page and Benditt, 1966a).

Substances considered to be the covalent interchain cross-links of elastin and intramolecular cross-links of collagen derive from lysine and their formation occurs subsequent to oxidative deamination of lysyl side chains presumably by a copper-containing amine oxidase (Thomas *et al.*, 1963; Partridge *et al.*, 1964; Miller *et al.*, 1965; Franzblau *et al.*, 1965; Partridge, 1965; Bornstein *et al.*, 1966). The evidence suggests that this deamination is impaired in lathyritic animals (Bornstein *et al.*, 1966; Miller and Fullmer, 1966; Page and Benditt, 1967). Consequently, we have postulated that lathyrogens may exert their effect on connective tissues by inhibition of the amine oxidase necessary for cross-link formation (Page and Benditt, 1966b).

The enzyme presumably involved in cross-link formation has not yet been identified, but the amine oxidase of pig plasma possesses many of the characteristics which might be expected of the postulated connective tissue oxidase (Blaschko, 1963; Buffoni and Blaschko, 1964; Blaschko *et al.*, 1965). This enzyme was, therefore, chosen for examination with regard to the activity of β-aminopropionitrile.[1]

Methods

Materials. Fresh hog blood was obtained from the Bar-S Packing Co., Seattle, Wash. BAPN fumarate was a gift of the Abbott Co., North Chicago, Ill. L-Tyrosine and uric acid were obtained from the Mann Research Chemical Co., N. Y., and kynuramine HBr from the Regis Chemical Co., Chicago, Ill. Reagent grade catalase (no. 15674, crystalline suspension in water with thymol, sp act. 27,000) was the product of Mannheim Boehringer, N. Y. Lyophylized mushroom polyphenoloxidase (with activity 500 units/mg) and uricase suspension in 10% ammonium sulfate (batch UP 5792, 0.73 unit/mg with 1.4 mg/ml of solution) were obtained from Worthington Biochemical Corp., Freehold, N. J. Glutamic–oxalacetic acid, α-ketoglutaric acid, L-aspartic acid, and L-glutamic acid

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[1] Abbreviation used: BAPN, β-aminopropionitrile.

BAPN-HCl were obtained from California Biochemical Corp., Los Angeles, Calif.

Enzyme Preparation. The method of Yamada and Yasunobu (1962) was followed throughout the collection and preparation of plasma and precipitation of the enzyme. The ammonium sulfate fraction obtained between 0.35 (180.4 g/l.) and 0.55 (293.5 g/l.) saturation of whole citrated plasma was used. Activity was assayed by the spectrophotometric method of Weissbach *et al.* (1960). Incubation mixtures contained 0.5 ml of whole citrated plasma or ammonium sulfate prepared enzyme, 0.15 mmole of sodium phosphate buffer (pH 7.4), 0.3 μmole of kynuramine, and water to 3.0 ml. All solutions were made up in ion-free water. Triplicate incubations were carried out in a circulating water bath at 37° for 1 hr in glass-stoppered, 3-ml cuvets. The change in absorbance at 360 mμ was read in a spectrophotometer at 10-min intervals. Control cuvets contained all of the above ingredients except substrate. One unit of enzyme activity was defined as the amount of enzyme catalyzing an absorbance change of 0.001/min at 37° under the above standard assay conditions. The molar absorptivity of kynuramine of 3520 was used in calculating the amount of substrate used. Protein concentration was determined by the method of Waddell (1956). A purification of four- to sixfold was achieved.

Spectrophotometric Studies Using Amine Oxidase. Inhibition studies were carried out under the standard conditions described except that incubation mixtures contained 1.9 units of enzyme activity and 3–300 μmoles of BAPN-HCl, and the amount of substrate was 0.06–0.6 μmole. Three to four separate incubations were made for each concentration of substrate and inhibitor and the mean of these values was utilized to calculate the slopes and intercepts of reciprocal plots.

Manometric Studies Using Amine Oxidase. Oxygen consumption was measured in the Warburg apparatus at 37°. Each flask contained 8.7 units of enzyme, 5–50 μmoles of BAPN-HCl or 5–30 μmoles of benzylamine or kynuramine substrate, 60 μmoles of potassium phosphate buffer (pH 7.2), and water to 2.9 ml. Some incubations contained 1–100 units of added catalase activity. In order to trap liberated ammonia, the center well contained 0.1 ml of 2% sodium borate (pH 5.1) prepared according to Conway (1957). Ammonia production was measured by microdiffusion and titration with standard HCl (Conway, 1957). Apparent K_m and V_{max} for amine oxidase activity using BAPN or kynuramine as substrate and for uricase activity on uric acid were determined from the statistically calculated slopes and y intercepts of plots of velocity *vs.* velocity/substrate concentration.

Studies Using Polyphenoloxidase, Uricase, and Glutamic–Oxalacetic Transaminase. Assays of the copper-dependent enzymes were carried out in a Perkin-Elmer 350 spectrophotometer equipped with a circulating water bath at 30°, with continuous measurement. In the polyphenoloxidase assay, experimental cuvets contained 25 units of enzyme, 1.0 μmole of L-tyrosine, 500 μmoles of sodium phosphate buffer (pH 6.5), and water to 3.0 ml. To determine the effect of lathyrogen, 300 μmoles of BAPN-HCl previously adjusted to pH 6.5 was added to the test cuvets in addition to the above ingredients. Reference cuvets contained the same ingredients but no enzyme. All solutions were perfused with oxygen for at least 30 min before use. The production of *O*-quinone was followed by measuring the increase in absorbance at 280 mμ. Uricase assay cuvets contained 250 μmoles of sodium borate (pH 8.5), 0.006 unit of enzyme, and water to 3.0 ml. The disappearance of substrate was measured by monitoring absorbance at 290 mμ. Substrate present was varied from 0.06 to 0.5 μmole with and without 300 μmoles of BAPN-HCl previously adjusted to pH 8.5.

Glutamic–oxalacetic transaminase was assayed by the method of Sizer and Jenkins (1965) by following the production of oxalacetate by its absorption at 280 mμ at 37°. Incubation mixtures contained 200 μmoles of Tris-HCl at pH 8.2, 40 μmoles of aspartate, 40 μmoles of α-ketoglutarate, and water to 3 ml. All reagents were adjusted to pH 8.2 before use with NaOH. The linear portion of the curves was used to calculate the initial velocities. In test incubations 40–400 μmoles of BAPN-HCl replaced aspartate as the sole source of amino groups, and in others both 40 μmoles of aspartate and 10–400 μmoles of BAPN were present. Some incubations were run for 15 min to allow the reaction to reach final equilibrium, then the enzyme was precipitated by addition of four volumes of ethanol, and the mixtures were centrifuged. Supernatants were then concentrated under vacuum to 0.5 ml, and 1-μl aliquots were chromatographed on thin layer silica gel H in phenol–water (80:20), following the method of Turner and Redgwell (1966). The chromatograms were then stained with ninhydrin. Separation of all products and reactants was achieved with this chromatographic system, and concentrations of glutamic acid as low as 1 μmole/ml could be seen.

Results

Spectrophotometric Studies Using Amine Oxidase. Data obtained from incubations containing a constant amount of kynuramine as substrate and increasing concentrations of BAPN-HCl are presented in Figure 1. Enzyme, incubated with kynuramine alone as illustrated in the lower curve, showed a rapid turnover of substrate. Incubation mixtures containing the same amount of substrate but with 300 μmoles of BAPN added exhibited no measurable activity. It is apparent that the addition of 5–300 μmoles of BAPN to the incubation mixture inhibits enzyme activity.

Sufficient data were obtained using variable substrate concentrations to determine the kinetic constants for the system. High concentrations of kynuramine caused substrate inhibition and were not used to calculate the reciprocal plot shown in Figure 2. Maximum velocity for the control enzyme on kynuramine was 0.37 μmole/unit of enzyme/min and the apparent K_m was 31.3 mM. Amounts of BAPN up to 15 μmoles did not significantly alter V_{max} but progressively increased the

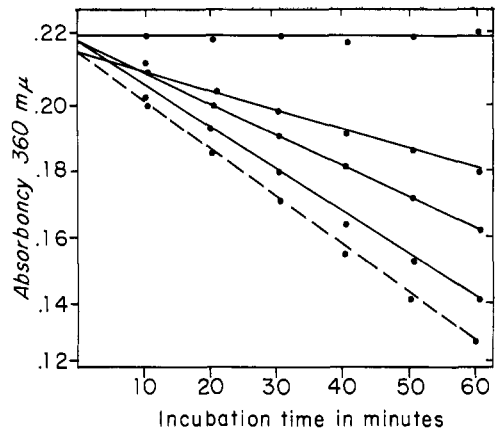

FIGURE 1: Lathyrogen inhibition of the activity of pig plasma amine oxidase on kynuramine. Beginning with the uppermost curve the assays contained 300, 30, 15, and 5 μmoles of BAPN. The control curve is illustrated by the dashed line.

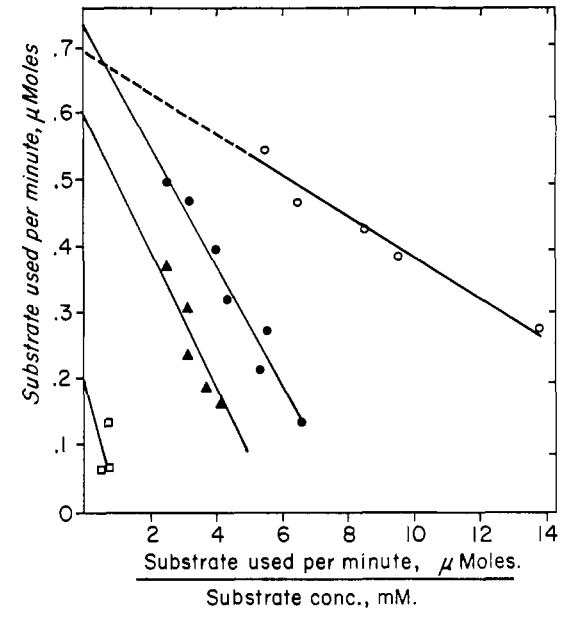

FIGURE 2: The effect of BAPN on the reciprocal plot for the oxidative deamination of kynuramine by amine oxidase. Incubation mixtures contained no BAPN (-O—O-), 15 μmoles of BAPN (-●—●-), 30 μmoles of BAPN (-△—△-), and 300 μmoles of BAPN (-□—□-). Units are micromoles per reaction volume.

apparent K_m. At greater concentrations of BAPN both V_{max} and the apparent K_m were significantly altered. Therefore, the lower concentrations of BAPN competitively inhibit enzyme activity, while the higher concentrations inhibit by some other mechanism.

Since a mixed type of inhibition was noted at high BAPN concentrations, a series of dialysis experiments were done to determine if BAPN is bound irreversibly to the enzyme. Enzyme with kynuramine substrate was incubated for 1 hr in the presence of 10^{-1} M BAPN or, in controls, its ionic equivalent of phosphate buffer. Inhibited and control mixtures were then dialyzed overnight in the cold *vs.* dilute phosphate buffer at pH 7.0 and again assayed. The control enzyme utilized substrate at the rate of 2.1 μmoles/min per 100 mg of enzyme protein present. In the presence of BAPN, no activity on kynuramine could be measured. After dialysis the activities of control and BAPN-incubated enzyme were identical and within the limits of error of the assay procedure were restored to the initial control value. Therefore, BAPN even at this high concentration does not appear to bind irreversibly to the enzyme or produce any permanent alteration in its activity.

Manometric Studies Using Amine Oxidase. BAPN possesses an amino group which one might expect to be susceptible to oxidative removal by amine oxidase. After establishing that BAPN is a competitive, reversible inhibitor, the compound was tested for substrate activity by following oxygen consumption and ammonia production. Initially, the enzyme preparation was examined with several known substrates to establish the stoichiometry of the reaction. Data obtained using benzylamine as substrate show that the oxygen consumed, substrate used, and ammonia produced were stoichiometrically equivalent. Comparable data were obtained using kynuramine as substrate. The presence of catalase did not alter this relationship, indicating that the enzyme preparation contained catalase activity. Therefore, 1 μmole of oxygen consumed is the equivalent to 2 μmoles of substrate used. The absence of any effect by exogenous catalase is not surprising since catalase activity has been found even in highly purified plasma amine oxidase preparations (McEwen, 1965).

Data were then obtained for incubation mixtures containing BAPN as substrate. The curve for oxygen consumption *vs.* time is shown in Figure 3 for mixtures containing 30 μmoles of BAPN substrate. Oxygen was utilized and ammonia was produced; therefore, BAPN is not only a reversible, competitive inhibitor, but it also forms a productive complex with the active site of this amine oxidase. Complete substrate utilization was not achieved even at substrate concentrations as

TABLE 1: Stoichiometry of Amine Oxidase Activity Using BAPN as Substrate.

BAPN Present (μmoles)	No. of Incubn	Oxygen Used (μatoms)	Ammonia Produced (μmoles)
5	4	3.4 ± 0.3[a]	2.5 ± 0.29
10	3	5.3 ± 0.8	4.0 ± 0.12
30	3	9.2 ± 0.6	7.7 ± 0.35

[a] Mean plus or minus standard deviation.

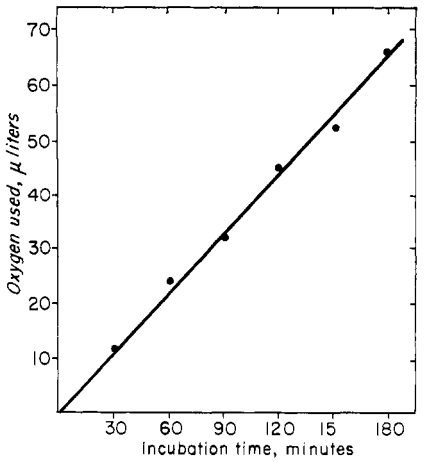

FIGURE 3: Amine oxidase activity using BAPN as substrate.

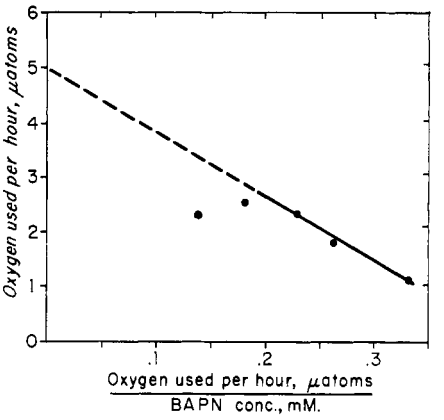

FIGURE 4: Reciprocal plot for the oxidative deamination of BAPN by amine oxidase.

low as 5 μmoles of BAPN. As shown in Table I, the amount of oxygen used was related to substrate present and ammonia produced, but the ammonia measured by microdiffusion and titration was in all cases 15–25% less than the expected value.

Sufficient manometric measurements were made using BAPN as substrate to determine the kinetic constants. The reciprocal plot for this system is presented in Figure 4. Oxygen consumption by incubation mixtures containing less than 10 μmoles of BAPN could not be accurately measured and those containing more than 30 μmoles showed substrate inhibition. The concentration which showed substrate inhibition was in the same range as that which produced a mixed type of inhibition in the spectrophotometric system using kynuramine substrate and BAPN as inhibitor.

The maximum velocity for BAPN oxidation by amine oxidase was 0.57 μmole/hr per unit of enzyme and the apparent K_m was 11.6 mM. The maximum velocity obtained using kynuramine as substrate in the spectrophotometric system was 22.1 μmoles/hr per unit of enzyme and the apparent K_m was 31.3 mM.

Studies Using Polyphenoloxidase, Uricase, and Glutamic–Oxalacetic Transaminase. Since the inhibition of plasma amine oxidase might result from a relatively nonspecific chelation of enzyme copper by BAPN, a series of experiments were performed to determine whether or not BAPN can inhibit the activity of copper-containing enzymes other than amine oxidases. Polyphenoloxidase and uricase were selected as test enzymes.

As shown in Figure 5, the presence of 300 μmoles of BAPN did not alter the activity of polyphenoloxidase on L-tyrosine. The reciprocal plot for the activity of uricase on uric acid is presented in Figure 6. The presence of 300 μmoles of BAPN did not alter the activity over a wide range of substrate concentration. Thus, there is no evidence in either system to indicate that BAPN complexes with enzyme copper.

To test the possibility that BAPN, acting as a carbonyl reagent, may react with enzyme-bound pyridoxal phosphate, and to determine whether or not BAPN can participate in one of the usual types of biologic transamination, we studied the effect of BAPN upon the activity of glutamic–oxalacetic transaminase. The pyridoxal content of this enzyme is well documented and activity is not dependent upon the presence of metals.

The presence in the transaminase system of a molar excess of BAPN over aspartate of up to 500-fold did not appear to decrease the amount of glutamic acid produced. In supernatants of incubation mixtures where BAPN was the only source of amino groups present, no glutamic acid was detected. If BAPN serves as an amino group donor under these conditions, then glutamic acid production would be expected. Therefore, it appears that BAPN does not bind with the active site of the enzyme and does not serve as an amino group donor under these conditions.

Discussion

It now appears reasonably clear that oxidative deamination, presumably by a copper-dependent oxidase, plays a significant role in collagen and elastin cross-linking (Piez *et al.*, 1963; Thomas *et al.*, 1963; Partridge *et al.*, 1964; Miller *et al.*, 1964; Nimni, 1965; Franzblau *et al.*, 1965; Bornstein *et al.*, 1966). Recent evidence indicates that lathyrogens exert their effect on connective tissues and blood vessels by preventing the formation of cross-links and that previously formed cross-links remain intact (Page and Benditt, 1966a). The lathyritic α chains of collagen seem to be intrinsically normal (Nikkari and Kulonen, 1962; Martin *et al.*, 1963; Page and Benditt, 1965, Page and Benditt, 1967), and, therefore, capable of undergoing cross-linking, but the lysyl residues destined to participate in cross-link formation are not oxidatively deaminated in the lathyritic animal (Bornstein *et al.*, 1966; Miller and Fullmer, 1966; Page and Benditt, 1967).

There appear to be two possible mechanisms by

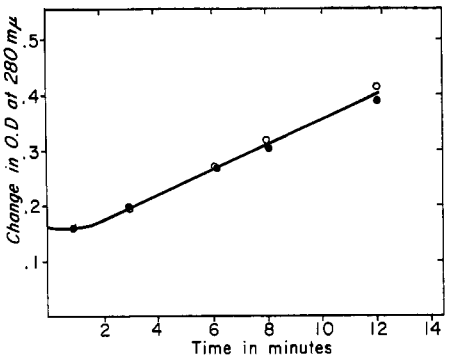

FIGURE 5: Oxidation of L-tyrosine by mushroom polyphenoloxidase. (○) no BAPN. (●) 10^{-1} M BAPN.

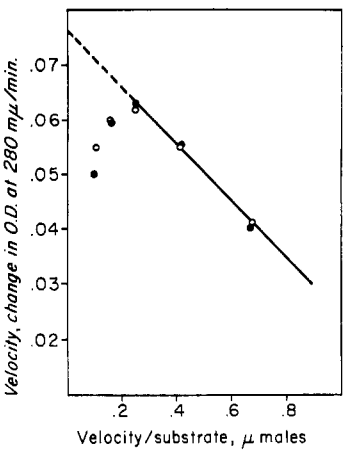

FIGURE 6: Reciprocal plot for the oxidation of uric acid by uricase. Units are micromoles per reaction volume. (○) no BAPN. (●) 10^{-1} M BAPN.

which lathyrogens may selectively block the oxidative deamination of collagen and elastin and thereby prevent subsequent cross-link formation. (a) They may compete with the native substrate molecules for the active site of the enzyme, or (b) they may react with the substrate and block enzyme activity. The latter hypothesis was abandoned when it was shown that significant amounts of lathyrogen could not be found in purified collagen obtained from lathyritic animals (Orloff and Gross, 1963; Ehrhart, 1964), and investigation of the former hypothesis has been hampered by the inaccessibility of the connective tissue enzyme.

The amine oxidase of pig plasma seemed to us to provide a suitable model of the presumed connective tissue enzyme for the following reasons. A plasma enzyme of this character has wide species distribution (Blaschko, 1963; Blaschko *et al.*, 1965); it will efficiently deaminate methyl 6-aminohexanoate (R. C. Page and E. P. Benditt, 1966, unpublished data), a substrate resembling lysyl residues in peptide linkage; and it functions optimally near neutral pH in an ionic and protein environment comparable to that encountered in the connective tissues (Yamada and Yasunobu, 1962; Buffoni and Blaschko, 1964; McEwen, 1965). The enzyme contains copper (Buffoni and Blaschko, 1964) and in the copper-deficient pig, which exhibits defects in cross-link formation in elastin, its activity is dramatically decreased (Blaschko *et al.*, 1965). However, we wish to emphasize that we propose this enzyme only as a model since there is currently no direct evidence available concerning its relationship to the postulated connective tissue oxidase.

Our data show that 5 mM BAPN competitively and reversibly inhibits plasma amine oxidase activity. In higher concentrations BAPN appears to inhibit by other mechanisms and shows substrate inhibition comparable to that observed using another substrate, kynuramine. BAPN forms a productive complex with the active site of the enzyme.

Even though the concentrations of BAPN used in our experiments are higher than those usually encountered for enzyme inhibitors, the results appear to be biologically significant. Some rough estimates of the dose of BAPN necessary to produce lathyrism are available. The dose usually given in the chick embryo is 20 mg/egg (Levene and Gross, 1959; Gross *et al.*, 1960; Tanzer and Gross, 1964). There is some evidence that distribution throughout the egg is uniform (Orloff and Gross, 1963); hence, the concentration would be about 3.6 mM after absorption from the air sac.

Investigators utilizing *in vitro* systems have generally utilized concentrations of this magnitude and higher (Burzynski, 1963; Bickley and Orbison, 1964; Smith and Orbison, 1964; Schryver, 1964). Even though morphologic changes have been shown *in vitro* in the presence of smaller doses of lathyrogen (McCallum and Paul, 1961), it appears that the concentration of BAPN necessary to produce lathyrism *in vivo* is large and in the concentration range which we have shown produces substantial inhibition of amine oxidase activity.

The maximum velocity observed for the activity of plasma amine oxidase on kynuramine is 28 times greater than that for BAPN, while the apparent affinity of the enzyme for kynuramine is only about one-third that observed for BAPN. These values for BAPN are in the direction and order of magnitude expected of a compound which functions as an enzyme inhibitor; however, the immensely complex mechanism of amine oxidase activity (Zeller, 1963) and the lack of better mathematical models applicable to the system (Reiner, 1959) preclude detailed interpretation of the kinetic data.

Our data do permit some comments concerning the specificity of BAPN binding. Dasler and Stoner (1959) have proposed that lathyrogens may serve as metal chelators, and evidence has been presented suggesting that the administration of copper partially reverses the effect of lathyrogens on connective tissue (Shields and Duffy, 1965). Administration of the copper chelator penicillamine inhibits covalent cross-link formation in elastin and collagen (Miller *et al.*, 1965; Nimni, 1965).

Pig plasma amine oxidase contains copper, and enzyme activity decreases remarkably in the copper-deficient pig (Buffoni and Blaschko, 1964; Blaschko *et al.*, 1965).

These considerations raise the possibility that BAPN could function *in vivo* as a chelator of copper and that the inhibition of plasma amine oxidase noted by us could indicate a relatively nonspecific binding of BAPN to enzyme copper. This seems unlikely in view of the results obtained with polyphenoloxidase and uricase. Even though these enzymes represent diverse types of copper oxidase activity and both are inhibited by copper chelators (Dressler and Dawson, 1960; Mahler *et al.*, 1955), the presence of 100 mM BAPN does not alter the activity of either enzyme. These data indicate that BAPN is not an avid chelator of enzyme copper and suggest that additional factors are involved in the binding of BAPN to the active site of amine oxidase.

In addition to copper, the plasma amine oxidases are reported to contain pyridoxal phosphate at their active site (Yasunobu and Yamada, 1963; Buffoni and Blaschko, 1964). Indeed, sensitivity to carbonyl reagents appears to be a dependable criterion upon which the intracellular and intercellular amine oxidases may be differentiated (Blaschko, 1963). Lathyrogens such as semicarbazide are carbonyl reagents, and carbonyl activity is suspected of BAPN. This raises the possibility that BAPN, acting as a nonspecific carbonyl reagent, might inhibit plasma amine oxidase. For this reason we desired to test the effect of BAPN upon the activity of a pyridoxal-containing enzyme which has an affinity for amino groups, but no dependence upon the presence of metals.

Since some amines such as spermine and putrescine appear to be susceptible either to oxidative deamination or transamination (Meister, 1965), the selection of a transaminase with a broad substrate spectrum for testing appeared to be reasonable. The activity of glutamic–oxalacetic transaminase on its normal substrate, aspartate, in the presence of relatively large amounts of BAPN was not inhibited, indicating that BAPN does not bind with pyridoxal phosphate of the enzyme under these conditions. Moreover, BAPN does not serve as an amino group donor in this transaminase system.

Thus, the concentration of BAPN which inhibits plasma amine oxidase almost completely does not appear to interfere with the activity of the copper-dependent enzymes or the pyridoxal-containing enzyme tested. These results indicate some degree of specificity for the observed binding of BAPN to amine oxidase and suggest that the active site of this class of enzymes possesses characteristics of "fit" resulting in a peculiar affinity for BAPN.

Even though our data show that lathyrogens may competitively inhibit the amine oxidase functional in cross-linking, it does not rule out the alternative mechanism, that BAPN or one of its metabolites may bind to specific lysyl side chains in collagen and elastin and block oxidative deamination. BAPN *per se* would not be expected to react chemically with free amino groups, but its reaction with previously produced aldehyde within collagen has been proposed (Levene, 1962). This does not seem likely. Even though lathyritic collagen is deficient in aldehyde (Page and Benditt, 1967), the defect appears at the present time to be lack of oxidative deamination rather than blocking of produced aldehyde (Bornstein *et al.*, 1966).

The reaction of possible metabolic intermediates with collagen and elastin has not been previously considered. BAPN is converted *in vivo* to cyanoacetic acid presumably by oxidative deamination (Lalich, 1958; Sievert *et al.*, 1960; Orloff and Gross, 1963). Therefore, an expected metabolic intermediate is cyanoacetaldehyde. Recent studies *in vitro* provide evidence that cyanoacetaldehyde is an intermediate and that it is a highly reactive compound under conditions of pH and ionic strength encountered in the connective tissues (Page and Benditt, 1967). If cyanoacetaldehyde is produced *in vivo*, it may undergo Schiff base formation with strategic amino groups of collagen and elastin and block subsequent oxidative deamination.

As noted previously, significant amounts of lathyrogen have not been found in purified collagen obtained from lathyritic animals (Orloff and Gross, 1963; Ehrhart, 1964). However, recent data (Holmquist and Schroeder, 1966) indicate that Schiff base linkages of the type proposed above may not be stable under the conditions which were used for isolation and purification of collagen in these experiments. Therefore, there is as yet no compelling reason to abandon the lathyrogen-binding hypothesis.

Consequently, we wish to suggest two possible mechanisms by which BAPN may cause inhibition of oxidative deamination and cross-linking of collagen and elastin. (a) BAPN may compete with native substrate molecules for the active site of the amine oxidase functional in cross-linking, or (b) a highly reactive aldehyde derived from BAPN by the action of amine oxidase may react with the amino groups of strategic lysyl side chains of collagen and elastin and block enzyme activity. It is not presently possible to select between these alternatives nor are they mutually exclusive.

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