sites might be part of the same macromolecule. If this were so, then the solubilized molecule should be an agglutinin and therefore relatively easy to assay. However, if an agglutinin is obtained which causes aggregation of like cell types, the specificity of this agglutination reaction must be demonstrated because substances like DNA (*17*) or basic proteins (*18*) cause cells to aggregate nonphysiologically. In the yeast mating system, we could define specificity in terms of adsorption of an activity (be it inhibitory or agglutinating) to the opposite cell type from which it was prepared. Perhaps a similar criterion for specificity could be established if it were possible to chemically treat cells from one tissue with a reagent which destroys only one of the two complementary combining sites.

Now that the specific cell-surface components have been isolated and purified from both mating types, the original hypothesis (*14*), that agglutination was due to the association of complementary macromolecules, seems well established. The two substances interact with each other, or interact with cells of opposite type, in a manner precisely analogous to antibodies and antigens. Indeed, the agglutination factors of *H. wingei* provide one of the clearest cases for the hypothesis that cell-to-cell and virus-cell interactions resemble immunological reactions. Since the substances from this yeast system are of relatively low molecular weight, a molecular analysis of their complementary association may be possible. The genetics of the two substances can be analyzed (*7*), and a procedure is available for isolating nonagglutinating mutants (*12*). This system is, thus, an attractive one for further analysis.

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Desmosine Biosynthesis: Nature of Inhibition by D-Penicillamine

Abstract. *Administration of* D-*penicillamine and lathyrogens such as β-aminopropionitrile to animals markedly alters connective tissue by preventing the normal cross-linkage of elastin and collagen. It had been shown that β-aminopropionitrile blocks the cross-linkage of elastin and collagen by preventing the initial step in cross-linkage: the conversion of lysine in peptide linkage to α-amino adipic-δ-semialdehyde. We show that penicillamine acts after the initial step, causing the accumulation of an elastin rich in α-amino adipic-δ-semialdehyde.*

The desmosine cross-links in elastin arise from condensation of the side chains of four lysine residues in peptide linkage to form a pyridinium ring (*1*). After the synthesis of the protein, the initial step in biosynthesis of desmosine appears to be deamination of a lysine ε-amino group to form the δ-semialdehyde of α-amino adipic acid (*2, 3*). After performic acid oxidation of elastin, α-amino adipic acid, the acid-stable derivative of α-amino adipic-δ-semialdehyde, can be readily detected in elastin hydrolyzates (*3*).

The lathyrogen β-aminopropionitrile blocks biosynthesis of desmosine by interfering with the initial deamination of elastin lysyl residues to α-amino adipic-δ-semialdehyde (*3*). Penicillamine also has been shown to interfere with biosynthesis of desmosine (*4*), but its mode of action has remained unknown. We now present evidence that the inhibition follows the deamination of elastin lysyl residues to α-amino adipic-δ-semialdehyde, perhaps by chemical blockage of the reactive aldehyde group.

D-Penicillamine, β-aminopropionitrile fumarate (BAPN), or saline was injected into the yolk sacs of 10- or 12-day-old fertile eggs. Elastin was isolated as the insoluble residue from minced aortas by these successive extractions in nonhydrolytic solvents: 24 hours in 3 percent Na_2HPO_4 at 5°C; 72 hours in a mixture of 0.5*M* penicillamine, 0.5*M* NaCl, and 0.025*M* tris at *p*H 7.4 and 5°C; and 24 hours in a mixture of 1-percent sodium lauryl sulfate and 0.05*M* tris at *p*H 7.4 and 25°C. After these extractions the residues were washed repeatedly with H_2O and lyophilized. Penicillamine has been included in the solvent-extraction preparation of elastin because of its efficacy as a solvent of collagen (*5*).

After overnight oxidation with performic acid (*6*) the insoluble elastin residues were hydrolyzed in 6*N* HCl for 72 hours and analyzed with an automatic amino acid analyzer equipped for accelerated analysis (*7*). Amino acid analysis of unoxidized elastin residues revealed less than 0.5 residue per 1000 total residues of α-amino adipic acid.

Elastin was prepared by solvent extraction rather than by the more usual hot-alkali extraction (*8*) because amino acid analyses of the oxidized alkali extract revealed that appreciable amounts of α-amino adipic acid had been solubilized. Elastin prepared by this method of extraction with nonhydrolytic solvent is similar in amino acid composition to alkali-extracted elastin except for increases in contents of threonine, serine, and the polar amino acids. Amino acid analysis after oxidation revealed less than 0.5 residue per 1000 total residues of α-amino adipic acid in each of the solvent extracts.

Figure 1 (left) shows α-amino adipic acid, desmosine, and lysine contents of insoluble elastin isolated from aortas of

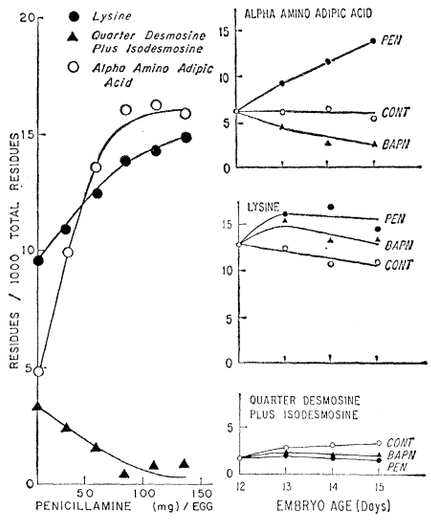

Fig. 1. (Left) Influence of penicillamine on the contents of selected amino acids in aortic elastin from embryonic chicks. (Right) Comparison of the effects of BAPN and penicillamine on the contents of selected amino acids in aortic elastin from embryonic chicks.

15-day-old embryonic chicks receiving varying does of penicillamine on day 10; no significant changes were noted in the other amino acids. The dose curve demonstrates a marked rise in content of α-amino adipic acid as desmosine content diminishes. Lysine progressively increases with higher concentrations of penicillamine.

Figure 1 (right) compares analyses of aortic elastin from embryos 12, 13, 14, and 15 days of age injected at 12 days of age with 100 mg of penicillamine, 5 mg of BAPN, or saline. Elastin from embryos treated with BAPN or penicillamine shows a loss in desmosine content when compared to controls. While elastin from the aortas of embryos treated with BAPN shows a lower content of α-amino adipic acid than does that from controls, the α-amino adipic acid content of elastin from embryos treated with penicillamine is elevated. The lysine content of elastin from embryos treated with either BAPN or penicillamine is increased over the levels in control elastins.

The striking increase in content of α-amino adipic-δ-semialdehyde (measured after oxidation as α-amino adipic acid), in aortic elastin isolated from embryos treated with penicillamine, reveals that the biosynthesis of desmosine is blocked after the oxidative deamination of lysyl residues (Fig. 2). In contrast, the blockage in biosynthesis of desmosine, produced by BAPN, results in reduction in content of α-amino adipic-δ-semialdehyde in comparison with controls. In accordance with an earlier suggestion (*3*) the evidence supports the idea that the blockage in biosynthesis of desmosine, resulting from administration of BAPN, occurs before formation of α-amino adipic-δ-semialdehyde (Fig. 2).

Under the influence of the highest levels of penicillamine, accumulations are greater by five residues of lysine and about 11 residues of α-amino adipic-δ-semialdehyde than in controls. These accumulations, accompanied by decrease in desmosine content, represent an estimate of the number of lysine residues that would have been normally converted to the desmosines, or intermediates in biosynthesis of desmosine.

The rise in level of lysine with increasing dosage of penicillamine may result from failure to utilize unaltered lysyl residues in formation of desmosine. The normal biosynthetic route utilizes one lysyl residue and three residues of the aldehyde for every desmosine cross-link.

An alternative explanation is possible for the increased content of lysine after administration of penicillamine. Similar accumulations of lysine residues are seen in lathyrism and copper deficiency (*4, 9*); both conditions are thought to result from inactivation of the enzyme that deaminates lysyl residues (*10*). Penicillamine chelates copper (*11*) and binds to pyridoxal covalently (*12*). Both copper (*4, 13*) and pyridoxal (*14*) deficiencies have been shown to affect biosynthesis of desmosine; if either is a cofactor for the enzyme necessary for deamination of lysyl residues, the enzyme may be inactivated at higher levels of penicillamine. Were penicillamine to have a dual effect, producing relative deficiency in deamination of lysyl residues and yet trapping the α-amino adipic-δ-semialdehyde that was produced, one might find an accumulation of lysine as well as α-amino adipic acid.

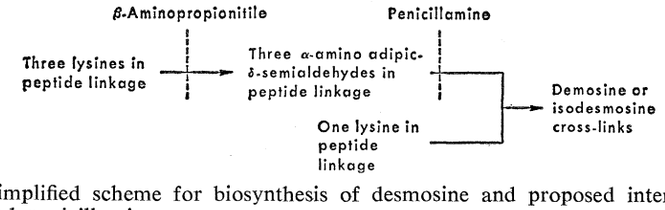

Fig. 2. Simplified scheme for biosynthesis of desmosine and proposed interference by BAPN and penicillamine.

The mechanism whereby penicillamine inhibits assembly of the desmosines after deamination of lysyl residues is unknown. Penicillamine might react with α-amino adipic-δ-semialdehyde to form a thiazolidine ring that would remove this intermediate from further reactions leading to biosynthesis of desmosine (*12*).

Cross-linkage of collagen similarly involves α-amino adipic-δ-semialdehyde (*15*). Moreover, administration of penicillamine results in accumulation of soluble collagen consisting predominantly of components not cross-linked (*16*). The elevation of the aldehyde content of this collagen (*17*) indicates that penicillamine may by the same mechanism block cross-linkage of collagen and elastin.

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