Meetings

Lathyrism

Osteolathyrism has been defined as an experimental disease produced by a variety of organic compounds which results in effects directly related to changes in the connective tissue proteins, collagen, and elastin. On the molecular level, the resulting bone deformities and aortic aneurysms appear to be related to the lack of crosslinkages in the connective tissue fibers.

On 18 and 19 March 1966, several American scientists interested in the problem of lathyrism met at Western Reserve University to discuss their latest work and to exchange ideas through a series of informal talks and discussions.

W. Dasler (University of Chicago), in presenting the results of screening various derivatives of known lathyrogens for lathyrogenic activity, gave a preview of the complexity of the problem because of the varying pathology with different lathyritic agents in the albino rat. *N*-alkyl derivatives of β-amino proprionitrile (BAPN) resulted in the skeletal exostosis of the parent compound but no exostosis was found with β-methyl-BAPN. Instead, a severe necrosis was found in areas of new bone formation. $\underline{N}^2$-phenyl-β-hydrazinoproprionitrile, on the other hand, resulted in questionable skeletal lesions but caused 100 percent cataract formation in weanling rats. Thiosemicarbazide resulted in minor skeletal lesions, aortic damage, and a severe muscle atrophy. Mercaptoethylamine and cystamine appeared to be unique in not showing an effect on the collagen in sponge biopsies of adult rats although they caused an increase in soluble collagen in sponge biopsies of young rats.

E. J. Miller and G. R. Martin (National Institutes of Health) outlined previously published results [*J. Biol. Chem.* **240**, 3623 (1965)] showing that peptide-bound lysine is a precursor of desmosine and isodesmosine, compounds which are presumed to serve as crosslinks between polypeptide chains of elastin. They suggest that lathyrogens inhibit the enzyme or enzymes responsible for the oxidation of lysine residues which normally results in aldehydic precursors of the desmosines. Chemical evidence showed that the content of aldehyde of the elastin from aortas of normal and lathyritic animals varied inversely with the content of lysine. However, Z. T. Wirtschafter (Veterans Administration Hospital, Oregon) presented evidence that the aortas of pigs fed lathyritic agents showed no changes in the content of the desmosines or compound X_4 or in the content of collagen or elastin. On the other hand, there was a decrease in the retraction of the aortas of the pigs in all segments and an increase in the lysine content of the isolated elastin. Such data suggested that there is a lysine crosslinking compound that has not yet been described.

P. Bornstein (National Institutes of Health) reviewed his recently published work [*Proc. Nat. Acad. Sci. U.S.* **55**, 417 (1966)]. He showed that a certain residue of lysine, located five residues from the N-terminus of each α-chain in acid-soluble collagen, is converted to an aldehyde, presumably by the oxidative deamination of the ϵ-amino group by enzymes. This aldehyde also participates in the intramolecular crosslink. It is suggested that the intramolecular crosslink results from the aldol condensation of two of the aldehyde groups. These results further suggest that lathyritic collagen, which was shown to contain very little of the aldehyde, is the result of the inactivation of the oxidizing enzyme by the lathyrogen. The results presented by M. Tanzer (Massachusetts General Hospital) were in general agreement with Bornstein's results. Tanzer treated acid-soluble collagen with thiosemicarbazide (TSC) and found that there were 2 moles bound per mole of collagen. Spectroscopic evidence indicated that the TSC was bound as the thiosemicarbazone derivative of both saturated and unsaturated aldehydes. However, contrary to the postulated aldol-type crosslink of Bornstein, Tanzer found that β-chains contained 2 moles of TSC while the α-chains contained only one. An interesting finding by Tanzer was that collagen treated with TSC behaves like lathyritic collagen with respect to its ability to redissolve on cooling after fibril formation.

Data of H. B. Bensusan (Western Reserve University) reveal good correlation between lathyrogenic activity and the ability of the lathyrogens to disrupt the Schiff's base, furfurylidenebutylamine. These results are in agreement with the hypothesis that exogenous aldehydes could be responsible for intermolecular crosslinking. However, it was pointed out that these results could also be interpreted to include Schiff's bases of any aldehyde, including those related to enzymes.

To add further questions related to the elucidation of the intramolecular crosslink, J. H. Fessler and A. J. Bailey (California Institute of Technology) demonstrated that the in vitro incubation of BAPN with gelatin derived by mild denaturation of acid-soluble tendon from a rat tail, cleaved β and γ units into α units by an apparent first-order reaction at neutral *p*H. This proves that direct interaction between BAPN and protein can occur although they did not claim it to be a mechanism of lathyrogenesis since the concentrations used were far greater than those required in vivo. However, it does indicate that a relatively reversible bond is formed by an aldehydic component. M. E. Nimni (University of Southern California, Los Angeles) was also able to demonstrate a partial in vitro degradation of β-gelatin to α-gelatin when denatured acid-soluble collagen from the skin of a rat was incubated with the lathyrogen, penicillamine. He also showed evidence that the amount of insoluble collagen decreases in lathyrism produced by penicillamine but the extent of this decrease diminishes with increasing age of the animal. In this type of lathyrism the soluble collagens show a diminished rate of turnover.

An entirely different approach was taken by J. J. Clemmons (University of Vermont) based on his studies of the effect of lathyrogens on the oxygen uptake of developing chick embryos. He showed that BAPN, amino acetonitrile (AAN), and semicarbazide markedly inhibited the consumption of oxygen, but that the pattern of the inhibition differed from that produced by nonlethal amounts of cyanide and

azide. Clemmons postulated that the depression of aerobic metabolism would limit the availability of essentials in collagen synthesis that may be derived from the citric acid cycle. Also considered were glutamic semialdehyde, aspartic semialdehyde, and the aldehyde derived from the oxidative deamination of lysine. These unusual amino acids, according to Clemmons, may be part of the crosslinking mechanism, pyrrolidine structures, or desmosine-like structures. This inhibition of the cytochrome oxidases must be considered in terms of the complex biochemical alterations and their effects on collagen synthesis. R. S. Bhatnagar (Philadelphia General Hospital), in describing the effects of AAN on collagen synthesis of the tibiae from chick embryos in a synthetic medium, found an increase in both protein synthesis and the utilization of glucose. He also demonstrated an increase in collagen synthesis and an increased specific activity of newly synthesized collagen in the lathyritic bones, by using proline-C^{14} in the medium. The increase in soluble collagens and the increase in free and peptide-bound hydroxyproline liberated into the medium were proportional to the AAN concentration. AAN failed to solubilize prelabeled collagen, a finding which differs from that of Nimni with penicillamine.

V. Matukas and J. L. Orbison (University of Rochester) studied the dense granules found in cartilage matrix of normal animals. These granules disappear from the matrix of 14- to 16-day-old chick embryos 48 hours after injection of BAPN. Modification of histochemical techniques for electron microscopy showed the granules to react with colloidal iron, to be removed by hyaluronidase and trypsin, and not to react with methenamine silver. Loss of sulfate in the areas containing the granules followed the loss of these granules. These results are consistent with the interpretation of the granules as protein-polysaccharide complexes. Their rapid disappearance following BAPN is thought to add new evidence for a defect in protein-polysaccharide metabolism as a major alteration in lathyrism.

I feel that the workshop was an unqualified success in accomplishing its aim, namely, to promote the exchange of ideas and to examine the various hypotheses in the light of a variety of experimental results. As the reader must realize from this summary, no single hypothesis emerged as being the most reasonable one. This reflects the complexity of the problem. The many approaches taken in investigating the phenomenon of lathyrism are a credit to the independent thinking of the investigators.

The questions raised by such a workshop are as important as the answers which were obtained on the basis of the data. Below are listed some of the questions which came to my mind as a result of this conference:

1) What is the nature of the relationship between lathyrogenic activity and the lathyrogens as carbonyl-reacting reagents?

2) Do the lathyrogens affect specific enzyme systems and, if so, which ones and by what mechanisms?

3) Is there any direct effect of the altered carbohydrate metabolism on the chemistry of the collagen?

4) What is the nature of the aldehydes in acid-soluble collagen?

5) Are both neutral-salt-soluble and acid-soluble collagens precursors of insoluble collagen?

6) Why do the different lathyrogens produce different pathologies in the same or different in vivo systems?

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Behavioral Sciences: Vocabulary

The breakdown of precise boundaries between scientific disciplines is reflected in the need for indexing and retrieval vocabularies that describe new conceptual frontiers. The behavioral sciences in particular are hard pressed to intercommunicate with impinging disciplines, as well as among themselves. The development of a biomedical thesaurus of behavioral sciences was discussed by psychiatrists, psychologists, sociologists, and anthropologists meeting with representatives of groups concerned with information exchange in these areas. Held at the National Library of Medicine, the meeting was the first step of a task force to update the Library's medical subject headings (MeSH) in behavioral sciences on which the Medical Literature Analysis and Retrieval System (MEDLARS) and Index Medicus are based.

Martin Cummings (National Library of Medicine) emphasized that only scientists with specialized knowledge can develop vocabularies capable of giving access to information of direct importance as well as providing serendipitous exposure to peripheral material. Paul T. Wilson (American Psychiatric Association) described dangers besetting the development of vocabularies, especially the human factor in term selection. Jonathan Cole (National Institutes of Health) noted that the major problem in interdisciplinary communication is lack of understanding of concepts. The importance of conceptual control, mediated by scientists, rather than formal vocabulary control was emphasized by David Hersey (Smithsonian Institution); he pointed out that term compatibility of itself does not guarantee consistency in usage. Discussion brought out an unanswered educational question, whether it is more efficient to train the individual scientist to communicate effectively outside his immediate field, or to educate a cadre of scientist-communicators within specialties to "translate" from one field to another.

Formulation and use of vocabularies were briefly described by some of the organizations that process behavioral science information. The vocabulary needs of the indexing and search sections of the National Library of Medicine served as a mandate for the task force. George Saslow (University of Oregon Medical School) summarized three points to be kept in mind: (i) It is impossible to eliminate variability in indexing and retrieval due to human differences in understanding; (ii) a means of spot checking how well a vocabulary is being used must be devised for quality control; and (iii) scientists at the frontier of concept development must be able to maintain contact with vocabulary users. Thomas A. Sebeok (Indiana University, Bloomington) pointed out that whereas selection of terms might be done easily, their satisfactory definition would be a time-consuming problem of consensus where, in fact, none may exist. The necessity of relating vocabulary selection (for example, in anthropology) to the kind of literature described (for example, biomedical) was stressed by Clellan Ford (Yale). Indexers tend toward generalization when they are uncertain, and Clifford T. Morgan (University of California, Santa Barbara) emphasized that term