pounds with their carcinogenicity and presented data on the photodynamic toxicity of atmospheric pollutant samples. Photodynamic toxicity is studied by dark incubating, motile ciliates, paramecia, or tetrahymena, with polynuclear compounds to which they are resistant in the absence of light. Subsequent long-wave ultraviolet irradiation, however, leads to severe toxic effects on the organism. Crocker *et al.* (San Francisco) presented data on the effects of carcinogenic hydrocarbons on cell multiplication in organ cultures from trachea of suckling rats. Hydrocarbons, such as 7,12-dimethylbenz[α]-anthracene, benz[α]pyrene, and 3-methylcholanthrene were studied at different concentrations. DNA synthesis in basal cells increased before columnar cells had changed in appearance. Metaplastic epithelium replaced differentiated epithelium in a pattern dependent on the hydrocarbon used. Withdrawal of hydrocarbon permitted differentiated cells to reappear while basal cell hyperplasia persisted. Mueller (Berkeley) studied the effects of gas and particulate matter in automobile exhaust on tissue culture cells in vitro. The growth-stimulating property appeared to be associated primarily with the particle phase and was independent of changes resulting from ultraviolet irradiation. Rounds and Bils (Pasadena), studying air pollutant effects on tissue cultures, concluded that all cell types tested showed partial but reversible inhibition in oxidative activity during treatment with sodium nitrate (simulating NO_2 exposures). Morphologic changes, including reversible changes in the shape of nucleus, the cytoplasm, and ultrastructure of mitochondria, occurred in alveolar wall cells.

Nusbaum *et al.* (Los Angeles) reported on the trace metal studies in bone in relation to air pollution exposure. Calvarium and rib samples revealed no correlation between lead levels and the duration of previous residence in Los Angeles. Specimens from males had significantly greater lead concentrations than those from females. Twelve other metals were examined with emission spectroscopy. Arsenic levels were obtained with wet chemical methods. Curphey and Perkins (Los Angeles and Berkeley) reported on the carboxyhemoglobin levels in individuals examined by the Los Angeles County Coroner's Office. A smoking history was obtained from the next-of-kin, and the distribution of carboxyhemoglobin levels in individuals dead from cardiac disease resembled very closely the comparable distribution obtained by expired air analysis in the studies of living populations.

Pernis (Milano, Italy) described the role of lymphocytes in infiltrative lung disease. Experimental studies with guinea pigs, immunized against ovalbumin, involved inhalation of aerosols of extremely minute amounts. Despite the absence of immediate anaphylactic reaction, induration, which was largely lymphocytic, was observed. Pernis then considered whether haptens might be capable of producing similar reactions in occupational exposure. The implication of these mechanisms for effects of complete antigens thought to be involved in the farmer's lung syndrome were discussed. Similar effects were thought to possibly play a role in silicosis or coal worker's pneumoconiosis. Rankin *et al.* (Madison) reported on agricultural dusts as an agent producing infiltrative lung disease. They outlined the experimental, immunologic, and epidemiologic evidence relating thermophilic actinomyces as an agent causing farmer's lung. Rankin also reported that certain industrial exposures to polymers could lead to changes similar to those predicted by Pernis.

Epidemiologic methods suitable for air pollution studies were reported by Blackburn *et al.* (Minneapolis) who compared ventilatory function and respiratory conditions in active and sedentary railway employees. When corrections were made for age and smoking, clinical chest findings were used to test the hypothesis that regular exercise of the ventilatory apparatus may enhance its efficiency and protect against the development of chronic bronchopulmonary disease. Only small and inconsistent differences in ventilatory function and respiratory complaints existed between sedentary and active workers. Stone (New York) studied the frequency of respiratory complaints among volunteers in a telephone company. The volunteers had significantly less frequent evidence of disease than did a population in which all of the subjects were available for study. Gocke *et al.* (Jersey City) reported on a respiratory survey in an industrial population of men 40 to 64 years of age and compared the results with the absence of respiratory disease absenteeism. Cough, exacerbations of cough and sputum, shortness of breath, and the forced expiratory volume in one second, were best predictors of the absence of respiratory disease lasting one week or more. Ury (Berkeley) presented a rank order test for evaluating interviewer agreement.

Two major new directions of research were highlighted by the conference. One concerned the immunologic mechanisms involved in air pollution exposure. This includes the relationship of air pollution with epidemic asthma on the one hand, and infiltrative lung disease on the other. A second was the use of reactions of biochemical, tissue, or organ culture, and protozoa to pollutants, especially to potential carcinogens, as biological pollution monitors.

The meetings were held at the Los Angeles County Medical Association with the assistance of the Engineering and Sciences Extension of the University of California.

The conference was supported in part by a grant from the Division of Air Pollution, Public Health Service, and sponsored by the California State Department of Health. It is planned that the complete proceedings will be published in early 1965 in the *Archives of Environmental Health.*

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Cross-Linkages in Collagen

Collagen is the major structural protein of vertebrates and comprises approximately one-third of the total body protein. It undergoes conspicuous changes with age and plays an important role in the healing of wounds and other pathologic processes. It is also the basis of the gelatin, glue, and leather industries. Important questions in the chemistry of collagen deal with the type of bonds which hold three polypeptide chains together in the collagen molecule and the nature of intermolecular cross-links responsible for fibril and fiber formation. Ten researchers in this field discussed these problems at a workshop on cross-linkages in collagen, held at Western Reserve University School of Medicine, 28–29 February 1964.

K. A. Piez (NIH) reported that when skin from a human infant was extracted with guanidine, two-chain components (β-components) were obtained, some of which arose from intermolecular links. Isotope incorporation

showed that the material extracted was not necessarily synthesized later than acid-soluble collagen. It was tentatively concluded that both intra- and intermolecular cross-links might form by a single continuous process. Collagen molecules from calfskin were sonicated and bisected by M. P. Drake (M.I.T.). Subsequent denaturation, renaturation, enzymatic, and electron microscopic studies of the fragments indicated that one-half of the molecule contained at least two α_1-α_2 links and that the other half contained at least one. The latter half also contained at least one α_1-α_1 link.

The possible role of aldehydes in cross-linking of icthyocol monomers was studied by A. Veis (Northwestern). Introduction of formaldehyde links caused the formation of β-components with four aldehyde molecules per 1000 amino acid residues before the appearance of γ-components. Degradation of the synthetic γ's resulted in the reappearance of β's. These observations indicated different sites for β and γ cross-links. Veis also showed by his measurements of swelling and solubility how dentine collagen is very closely cross-linked. Degradation with periodate yielded peptides rich in phosphate and led to the conclusion that phosphate-mediated, di- or tri-ester intermolecular cross-links might occur in dentine collagen. From the action of periodate on corium and dentine collagen, it was indicated that both collagens contained intermolecular cross-links involving tyrosine-rich regions of the monomers. Tryosine was also implicated in cross-linking of calfskin tropocollagen by H. B. Bensusan (Western Reserve). Iodination resulted in the formation of fibers insoluble in acid. In addition, more γ-components were formed on the renaturation of iodinated, denatured tropocollagen. Fiber formation from iodinated tropocollagen was inhibited by aldehyde-blocking agents. The data suggested that tyrosine is a site of both intra- and intermolecular cross-links which are mediated by aldehyde.

Aldehydes were also discussed by J. Bello (Roswell Park) who found that borohydride treatment of tendon collagen inhibited cross-linking. Cross-linking was inhibited by histidine but not by other amino acids. Thus, histidine may serve as a binding site. Bello is also using hydrazines of varying structure to break ester-like bonds and distinguish between intra- and intermolecular bonds. Methylated hydrazines yielded products of higher molecular weight than those resulting from hydrazine treatment. An amide bond between the ϵ amino group of lysine and α carboxyl of glutamic acid was identified in tendon collagen by M. Levy (New York University). After hydrolysis, which may have destroyed some of the bonds, there was approximately one per 1000 amino acid residues. Levy pointed out that 1 to 2 percent of such amide bonds would double the particle weight of collagen.

P. M. Gallop (Albert Einstein) reported on the treatment of ichthyocol tropocollagen with hydroxylamine and on the release of subunits after treatment with nucleophilic reagents. He concluded that each α-component consisted of four subunits of about 27,000 molecular weight, held in linear array by three pairs of aspartyl ester bonds close to the carboxyl end of each subunit. It was also most likely that each subunit contains on the average either one glucose or galactose residue; these hexoses are probably not directly involved in the subunit attachments. Another component, aldehydic in nature, is most likely associated directly at the subunit attachment sites; on the basis of various chemical procedures it is felt that this material is similar to a 2-hydroxy, 1,5-dialdehyde.

The defect in collagen in lathyrism was studied in order to gain information on the types of cross-links involved. J. Gross (Harvard) discussed collagen turnover in lathyritic chick embryos. On the basis of work by him and M. L. Tanzer, they concluded there was no complete block in the formation of insoluble collagen from soluble collagen. The extractable, lathyritic collagen pool appeared to be heterogeneous and to be a mixture of newly synthesized and older collagen. It thus appeared that in lathyrism the whole spectrum of states of collagen aggregation is affected. There is no evidence that lathyrogens directly affect collagen. They favor the hypothesis that stimulation of the cell by the lathyrogen produces an enzyme which can sever intra- and intermolecular cross-links. C. I. Levene (Chicago) reported that lathyrism in chick embryos is caused by carbonyl-blocking agents. Lathyritic collagen was found to bind less 2,4-dinitrophenylhydrazine. This indicates that the binding sites were either blocked or absent. Gallop suggested that incomplete 27,000-molecular weight subunits are formed in lathyrism.

Cross-links and other factors in the elastic properties of collagen were studied by H. R. Elden (University of Miami) who measured physical properties of tendon as a function of cross-linking, hydration, and temperature. The elastic behavior of melted collagen obeys the theoretical expression for an amorphous network held together by cross-links at certain points. Stress relaxation of melted tendon shows that cross-links are added with age. The elastic properties of intact tendon appeared to be governed by glass state, crystalline structure, and state of hydration.

It was apparent from the proceedings that considerable progress is being made in cataloging probable cross-links and, especially, in developing techniques. This should result in a more rapid accumulation of information in the future. It was also apparent, however, that only a start has been made in describing the differences in collagens from different sources and in understanding the role of various cross-links in biologic processes.

A general discussion revealed that the present nomenclature for the components which arise from denatured collagen was confusing. There was unanimous agreement regarding certain changes and additions, which have been summarized by K. A. Piez as follows:

The original meanings of the terms α-component, β-component and γ-component, which are now known to have a monomer, dimer, and trimer relationship, are retained, but since it is recognized that they may be mixtures, they are to be used in a generic sense. The term α chain as an alternate to α-component is tentatively acceptable, although the α chain probably contains some nonpeptide bonds that join polypeptide segments. The designations $\alpha 1$ and $\alpha 2$ currently applied to the two types of α chain are also retained. They are distinguished by the fact that, according to present evidence, there are two $\alpha 1$ chains and one $\alpha 2$ chain in each molecule. The numbers 1 and 2 are to be written full size, not as subscripts. The subscript position is reserved for future use to designate minor variations (such as genetic variants) or nonpeptide-linked segments within an α chain. The designations $\beta 1$ and $\beta 2$ which were assigned arbitrarily to the covalently linked dimers $\alpha 1$-$\alpha 2$ and $\alpha 1$-$\alpha 1$ are replaced by the self-explanatory terms β_{12} and β_{11}. The γ-component $\alpha 2$-$\alpha 2$ arising from an intermolecular cross link is then β_{22}. The β-component arising from interchain, intramolecular covalent cross links would be designated γ_{112}. Conceiv-

ably, other types of γ can arise by a combination of inter- and intramolecular cross links. Since β and γ can also be made synthetically from noncross-linked collagen, the products are assigned modified terms, S-β and S-γ, with appropriate subscripts where the chain composition is known.

It is hoped that this nomenclature will be generally useful and will provide the clarity and flexibility needed as research in this area progresses. It is strongly urged that it be employed by everyone in the field.

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Forthcoming Events

July

20–22. **Magnetic Resonance** in Biological Systems, Boston, Mass. (R. G. Shulman, Bell Telephone Laboratories, Murray Hill, N.J.)

20–23. New Mexico Acad. of **General Practice**, Ruidoso. (H. L. Douglas, Box 767, Tatum, N.M.)

20–24. International **Diabetes** Federation, 5th congr., Toronto, Ont., Canada. (H. Best, Organizing Council, 477 Mt. Pleasant Rd., Toronto 7)

20–24. **Nuclear Radiation** Effects, technical conf., Seattle, Wash. (Inst. of Electrical and Electronics Engineers, Box A, Lenox Hill Station, New York, N.Y.)

20–24. **Semiconductor Physics**, intern. conf., Paris, France. (M. Balkanski, Laboratoire de Physique, Ecole Normale Supérieure, 24, rue Lhomond, Paris 5e)

20–25. **Catalysis**, 3rd intern. conf., Amsterdam, Netherlands. (D. M. Brouwer, c/o Badhuisweg 3, P.O. Box 3003, Amsterdam-N, Netherlands)

21–23. Physiology and Experimental Psychology of **Color Vision**, Ciba Foundation symp., London, England. (Ciba Foundation, 41 Portland Pl., London, W.1)

21–24. American **Malacological** Union, New Orleans, La. (M. C. Teskey, Rt. 2, Box 318, Marinette, Wis.)

21–28. International **Geographical** Union, 20th intern. congr., London, England. (T. H. Elkins, Royal Geographical Soc., Kensington Gore, London, S.W.7)

25–1. **Religion and Science**, 11th conf., Star Island, Portsmouth, N.H. (Religion and Science, 280 Newton St., Brookline, Mass. 02146)

26–29. **Photobiology**, 4th intern. congr., Oxford, England. (Blandford Site, Whiteknights Park, Reading, England)

26–31. American **Crystallographic** As-