Remarks made on acceptance of the Proctor Medal Award, June 6, 1965

I should like to express my deep gratitude to the Proctor Medal Committee for making me the recipient of the Proctor Medal for the year 1965. I consider it a great honor, and I am particularly gratified because I receive it being not an ophthalmologist, but a biochemist who was carrying on basic research in biochemistry using the eye as a model for certain properties of living systems which were of interest to me as a biochemist. The basic scientist who works only in his laboratory is in a somewhat privileged position inasmuch as the work that he is doing primarily satisfies his curiosity and investigative instincts and thus at the outset impractical endeavor is a source of profound intellectual pleasure. I believe, therefore, that it is a kind of reward in itself when he finds that those of his colleagues in the scientific community, who are by their profession much closer to practical situations in which the necessity arises to help people, find the results of his investigations interesting and useful to them as theoretical basis in their pursuits. It is customary in acknowledging a scientific award of such high standing as the Proctor Medal to pay tribute to the teachers of the recipient who introduced him into the field of his scientific endeavor. I am in a somewhat embarrassing situation in this regard as I must consider myself essentially an autodidact in biochemistry. I received my stimulation in my investigations, apart from the reading of scientific literature, from occasional conversations with my colleagues in the department at which I was working and on visits to scientific laboratories in various parts of the world. But I believe that I owe a debt of gratitude to a certain number of scientists who during my career helped me greatly by providing me with opportunities and facilities for carrying on my research. First of all, I must acknowledge my debt to the late Professor Otto von Fürth who gave me the opportunity of starting my scientific work in his laboratory in 1924, although I spent only a very short time in collaboration with him, and after that immediately started as an independent investigator. Fürth had the gift of recognizing the capacity for successful scientific work in young people and giving them unlimited opportunities for successful efforts without any direct advantage to himself. He also stimulated the scientific imagination of those who worked in his laboratory by benevolent but sharp criticism.

After I left Vienna and had to find a new place to continue my investigations, I was greatly helped during the first two years of my sojourn in Paris by Dr. Andre Tsanck who was the Director of the Institut de la Transfusion Sanguine à l'Hôpital St. Antoine in Paris and who, after the outbreak of the Second World War, when I was no longer able to support myself, took care of providing me with an adequate means of livelihood. Later, in Marseille, it was Professor Jean Roche who was the Chairman of the Department of Biochemistry at the Medical School of the University of Marseille, and is at present Rector of the University of Paris who, on the basis of a recommendation by Otto Meyerhof, helped me greatly in my inves-

tigations by giving me adequate laboratory space in his department and protecting me against numerous bureaucratic difficulties in my life as a refugee in World War II. Later, in this country, when I was finally able to begin a regular scientific career after many years of delay, it was Professor Hans T. Clark who made it possible for me to start my life at Columbia University, and finally I could embark upon the final stage in my investigations in 1948, when Dr. Ludwig von Sallmann offered me the position of a chemist in the research department of ophthalmology. I should also like to mention that the work in ophthalmic biochemistry for which I am receiving the Proctor Award today could proceed successfully only due to the cooperation and dedication to the work of several of my assistants active in my laboratory research. First for several years, Dr. Ellen Borenfreund, now at the Sloan-Kettering Institute for Cancer Research, assisted me in my research, and later on Mrs. Ginevra Zelmenis, Mrs. Antonina Danilchenko, Mrs. Nina Larys, and Miss Dagmara Igals. Finally, I think I should tell you in a few words why I decided 17 years ago to go into the field of opthalmic biochemistry.

The living systems are highly organized spatially or functionally integrated complexes of elementary units which themselves can represent organized aggregates of many molecules of very different kinds. If we consider, for instance, the metabolic machinery of the living cells, we see that the turnover of energy is mediated by several systems of chain reactions catalyzed by integrated groups of enzymes. First we have, for instance, the system of anaerobic glycolysis which comprises about 20 different enzymes which are coordinated in their activity in such a way that they can maintain, at varying levels required by the situation of the living cell, a steady flow of energy and reaction products of the anaerobic breakdown of glucose. In living cells, however, this system is integrated into a more comprehensive and complex metabolic machinery, inasmuch as certain metabolic intermediates or end products of glycolysis are immediately utilized in a highly organized way in other reactions which achieve an aerobic breakdown of glucose. The aerobic and anaerobic system is integrated in such a way that whenever the first one is operating, the second one is suppressed by the so-called Pasteur reaction. We know, in addition, that the course of the aerobic, as well as the anaerobic, breakdown of glucose is dependent on the presence of ATP not only as donor but also as acceptor for inorganic phosphate and, therefore, the enzymic breakdown of ATP in the cells must be strictly coordinated with the rate of turnover of the reactions of the energy producing glucose breakdown. The same is true also of numerous phosphotases which are able to interfere with the course of energy-producing reactions. As far as the structure of the framework of the living systems is concerned, here again, we observe that certain structural units are utilized as elements of ever more complex structural units. This organization of complex units is often accompanied by elaborate structural transformations for the purpose of adaptation to the specific function of the more complex system. The equivalence of such compositional elementary units of the metabolic machinery of the cell and of chemical composition of elementary structural units incorporated into larger structural assemblies can be formulated in the concept of chemical homology. We can see an example of it in the structure of the outer limbs of the rods in the retina which the electron microscope reveals to be a transformation product of cilia. The eye is a highly organized system, not only of tissues, but also of different organs in which these subunits derived from homologous organs or tissues had to be highly specialized to adapt them to spatial and functional requirements inherent in the function of the eye. For this purpose, the elementary functional units of living cells appear very often dissoci-

ated from units which in other differently or less specialized tissues have to be integrated with others into units of higher order, and this sometimes produces a unique opportunity to study such elementary units in isolation from other constituents of the more complex system. The lens fibers, for instance, offer an opportunity to study the metabolic machinery of the cell completely separated from the tricarboxylic acid cycle. A similar situation appears also in the nonnucleated red cells of the blood, and these cells were repeatedly used by myself for studies on the glycolytic system and the pentose phosphate pathway and by others for the study of the direct oxidation of glucose. Although the lens, in this respect, resembles the red cells, the study of metabolic reactions of this system offers an additional advantage. Whereas the nonnucleated red cells are rapidly decaying, the life of the lens fibers extends over the life-span of the individual, and the phenomena of age and differentiation can be studied in their relation to the metabolic systems of the living cells. This cannot be done in the case of nucleated red cells.

If we now turn from the metabolic machinery of living cells to their structural foundations, we see again a possibility of applying the principle of chemical homology by considering the lens as homologous to another epidermal structure, namely, the skin, and considering the continuous production of the albuminoid as a differentiation event homologous to the production of keratin, in which this chemical process is arrested at a certain stage, and, therefore, permits the study of certain parts of this chemical process isolated by completely natural means in its relation to various biological factors. Finally, as an example of the utilization of this principle of homology on a larger scale in our laboratory, I should like to mention a series of investigations which have been carried out by our group during the last ten years, and which were concerned with certain chemical aspects of the organization of collagen. Collagen in the connective tissue of the adult animal is present in the form of thinner or thicker bundles, consisting of collagen fibrils or smaller bundles of fibrils interconnected to a greater or lesser degree by molecules of acid mucopolysaccharides. The elementary unit of collagen observable by the electron microscope is a fibril which consists of an array of tropocollagen molecules. These latter are arranged in the mature collagen in such a way that a spatial periodicity of spots of greater electron density results. This spacing is, therefore, a sign of a high degree of organization of sets of individual tropocollagen molecules. It has been noted for some time that this stage of organization is preceded in the embryo by a stage in which tropocollagen molecules are assembled in long thin fibrils without any periodicity. It has also been known for some time that there exists an intermediate stage between the embryonal and the mature collagen which we find in the so-called reticulin formations. They contain fibrils of approximately the same diameter as the embryonal collagen, but already show characteristic spacings. Finally, it had been shown by A. Pirie in 1953 that the lens capsule which, even in the electron microscope, does not show any marked fibrillary structures contains a large amount of the amino acid hydroxyproline which is considered as a characteristic constituent of collagen. Furthermore it has been known for a long time that the lens capsule contains a high percentage of carbohydrate which produces reducing sugars after acid hydrolysis. In 1954 when we started the investigation of the composition of this carbohydrate of the lens capsule, we found that it represents a sugar polymer, so far unknown in animal tissues because it consists only of the two hexoses, galactose and glucose, and is stable against heating with alkali, which indicates that aldehydic groups of the sugars must be substituted. This suggested that this carbohydrate may have something to do with the lack of the organization of the lens

fibrils and made it appear interesting to see whether there is a progressive change in the content in this carbohydrate with the progressing organization of the collagen. The eye offered an extraordinary opportunity to do such a systematic study because, in addition to the lens capsule with practically complete lack of fibrillary structures, and in addition to the normal adult connective tissue as can be found in the sclera and the chorioid, it contains two intermediate systems, namely, the vitreous body and the corneal stroma. In the vitreous body, we have a network of very thin collagen fibrils of no more than 250 Å diameter which are devoid of any striation, but are immersed in an interfibrillary matrix which can be considered as an equivalent of the ground substance of connective tissues. In the corneal stroma, on the other hand, these fibrils still have the very small diameter characteristic of embryonal collagen, but are already striated and in this respect resemble the reticulin fibrils. After having completed, therefore, the investigation of the collagen-linked carbohydrate of the lens capsule, we turned to the investigation of the carbohydrate linked to fibrils of the vitreous body and to the carbohydrate present in the interfibrillary system. This investigation again showed the presence of the same polymer, consisting of glucose and galactose attached to the fibrils but in significantly smaller amounts than in the lens capsule, and the presence in the interfibrillary matrix, in addition to the hyaluronic acid discovered long ago, of a glycoprotein the carbohydrate moiety of which was a fucohexosaminoglycan of a type since then found as a constituent of practically all connective tissues and of the blood plasma. Finally, in the investigation of the carbohydrate in the corneal stroma, we found again that fibrils of the collagen in this formation are linked to a polymer of glucose and galactose. But corresponding to the higher organization of these fibrils, the amounts found in collagen were only about one-third of the amounts found in the lens capsule and about one-half of that found in the fibrils of vitreous. On the other hand, the corneal stroma was also shown to contain, in addition to the well-known acid mucopolysaccharide, a glycoprotein of the type found in other connective tissues which could be separated from the collagen and, therefore, can be presumed to be a constituent of the ground substance. These findings are highly suggestive of the possibility that the presence of this carbohydrate on the collagen molecule prevents the high organization of the collagen proteins and is in an inverse relation to the degree of this organization. Thus, the structural simplicity of the tissues of the eye offered, in this case, an opportunity to study the relation of a highly specific carbohydrate with the degree of organization of the collagen.

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