Present state and perspectives in research of the lens

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Two important aspects of the lens research are reviewed: the morphology and the physiologic chemistry. In this last part the scope of the study is limited to cations, carbohydrates, nucleotides, nucleic acids, and proteins. A great number of gaps have still to be filled in these fields, and even more in the study of the pathogenesis of the different forms of cataract. Our objective for the next time should be to increase the certitudes. After this first step at least three directions of research can be foreseen: the chemists will penetrate deeper into the knowledge of cytochemical mechanisms, determine the cause of metabolic deficiencies, and localize the anomalies of the enzyme synthesis. The geneticists knowing the biochemical nature of the genes will apply the one gene-one enzyme hypothesis to the great problem of the hereditary cataracts. The morphologists will establish the basic reactions of the protein molecules in the normal and abnormal lens.

I would like, first of all, to thank the organizers of this symposium. Not only have they invited me to participate but they have also asked me to deliver a sort of keynote address at the opening of the meeting. This is a great honor for me, which I attribute to the fact that for a very long time I have been interested in the crystalline lens. Indeed, it is now almost forty years since I published my first clinical report on a special tetanic cataract. Shortly afterward I asked my chief to give me a subject for a thesis. His answer was: "Study the pathogenesis of senile cataract and try to find a nonsurgical cure for it." He did not suspect then that nearly forty years later our knowledge of these problems would still be very fragmentary and that specialists representing many different branches of science would be meeting in Minneapolis in order to compare their results and to try better to coordinate their efforts in the future.

We have here with us morphologists, physicists, chemists, immunologists, physiologists, and clinicians. For all of us, the crystalline lens constitutes an ideal field of work. Some appreciate especially its transparency, which makes it possible to carry out precise measurements and thorough biomicroscopic examinations. Others attach more importance to its structural peculiarities. In fact, the arrangement of epithelial cells in a single layer greatly facilitates the study of mitoses and differentiation into fibers. These are constantly pushed into the depth of the lens by younger fibers and ultimately gather round the central nucleus of primary fibers which has been in place since the embryonic period. Thus the adult crystalline lens contains fibers dating from all periods of life. The absence of vessels and nerves makes it possible to compare it to a culture of epithelial cells whose aging can be followed with remarkable clearness from the morphologic and physiologic point of view.

The characteristics which in themselves

Most of the references before 1961, alluded to in this paper, are not included in the bibliography but can be found in previous reviews of the author.92 93

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make the crystalline lens a choice field of research are further enhanced by two facts, the importance of which has perhaps not been sufficiently emphasized, namely, first, the similarity of the transparency, structure, and evolution throughout the whole series of vertebrates, and, second, from the immunologic point of view, a certain degree of "organ specificity" which, in spite of all, seems more pronounced than the species specificity. Hence, much more than in other fields, observations made on the crystalline lens of one species are also valid for a neighboring species; they can usually be transposed from a laboratory animal to man.

Let us now look at some of these data, beginning with morphology.

Morphology

It is true that Rabl in 1900 in his basic studies of comparative anatomy, had already noted many important facts, but new precise observations are now available. This modern research on the normal and abnormal structure of the crystalline lens may be classified in three series. These were initiated and continued by Dr. von Sallmann, to whom I would like to express my profound admiration.

A first series which started in 1951 was centered on mitoses. For a long time these were examined by the improved technique for preparing flat mounts of the lens epithelium and remarkable results were obtained. Then, Harding and his co-workers10, 17 and Hanna and O'Brien42, 43 applied to the crystalline lens the method of autoradiography after an injection of tritiated thymidine. Cotlier23, 2* rounded off the process by introducing the administration of colchicine.

By a second series of investigations we have been provided with observations made with the electron microscope and concerning the constitution of the capsule and the fine structure of epithelial cells and fibers.

The third series deals with the abnormal crystalline lens. Thanks to von Sallmann12, 14 we are familiar today with the rhythm of mitoses in many experimental opacities, and we know the localization of the first lesions. Two groups of cataracts can thus be distinguished, the initial epithelial insult being characteristic of opacity caused by radiations, Myleran, and mimosine; on the other hand, the initial cortical change is found in cataracts caused by galactose, alloxan, and by a tryptophan-free diet.

In this connection I would like to mention the beginning of a galactosic cataract I observed in two young children. As in the case of animals, the initial lesion was found in the deep cortex.

The results obtained in the three series of researches, of which I have just given a brief outline, make up the gist of the knowledge recently acquired on the morphology of the crystalline lens. There are, however, two more investigations in progress which, though in their infancy, seem promising.

The first is based on observations made on capsular wounds of the anterior polar region. Brini and co-workers13, 14 have shown evidence of an intense epithelial and capsular regeneration. The main emphasis should be placed on the neoformation of the capsule; and this for several reasons:

1. Its role in the limitation and resorption of necrotic lens material is clear.
2. It is found identically in senile cataract and does not, in general, seem at all exceptional.
3. It sheds a new light on reduplicated cataract and also makes it possible to interpret the very special aspects described by Cogan and Kuwabara21 in the first mongoloid cataract examined histologically.

It is important, however, to remember that the process of capsular neoformation has never been observed in the posterior capsule where any gap is filled up simply by epithelial cells from the equator and by fiber formations which issue from it.

The second investigation is based on slit-lamp examination. Its point of depar-
ture is the classical observations on the pathologic thinning or thickening of the crystalline lens and the anomalies of form, number, and clearness of the zones of discontinuity.

The new Goldmann and Niesel measurements relate to the disjunction zone. These workers have found a thinning with age, a thinning and sometimes even temporary disappearance after a recent concussion, as opposed to thickening, especially in juvenile diabetes. It may be concluded from these findings that the disjunction zone seems to be made up of young fibers which have reached a certain stage of evolution. If their formation is slowed down or stopped, the zone shrinks. Conversely, if there is a hyperproduction of fibers, the zone thickens.

Thus we now have a real test for the formation of fibers, which makes it possible to study, among other things, hyperactivity in new fiber formation, of which we still know nothing. Can it be durable or is it only transient following and compensating for a slowing down or, on the contrary, preceding a breakdown? Whatever the answer to these questions, the work of the Berne Clinic has already been beneficial in that it has clearly brought out the importance of clinical work at a time when there is a tendency in some quarters to neglect it and to be content with examining the transparency of the crystalline lens through the magnifying glass.

Chemistry and physiology

We now come to the chemical and physiologic aspects. The first problem arising is one of choice. Our time is limited and we do not intend to give a complete report. We have thus decided to pass over in silence all research work that does not seem so far to give information on the genesis of cataract and to limit the scope of this study to cations, carbohydrates, nucleotides, nucleic acids, and proteins.

Cations. As regards cations we should like briefly to recall the successful studies carried out by Harris and co-workers on active transport. They have been completed by observations on ouabain, sodium-potassium-adenosine-triphosphatase, and studies with rubidium-86.

The results obtained are well known, so I can limit myself to very recent publications.

The first concerns the relationship between active transport and passive diffusion, which has so far never been clearly defined. Kinsey and Reddy were able to show that the difference between the anterior and posterior capsule is of a 2:1 ratio and that only the epithelium is able to transport against a concentration gradient a certain amount of potassium and rubidium into the lens and cause sodium to leave it. Where there is no epithelium the exchange simply occurs by diffusion.

This superficial localization of the cation "pump," already seen by Harris and Nordquist, has been confirmed by two searches on cataracts. The first deals with galactose opacity, which, as we have just mentioned, begins in the deeper layers. At this first period Kinoshita and co-workers have found the total cation concentration unchanged and the pump functioning still rather well. It is only with the maturation of the cataract that the sum of these cations increases and that there is a new afflux of water. The second even more convincing investigation concerns human senile cataract. Pau and Leithäuser noticed a normal potassium and sodium content in the deep supranuclear and nuclear forms, whereas the amounts seem to be close to those of the aqueous humor in the subcapsular, complicated, intumescent, and mature varieties.

So it can be concluded that the cations remain unchanged as long as the superficial layers are not affected, and that the rapid decrease of active transport in the cataract is a secondary phenomenon.

This is precisely what we have been saying for a number of years and this is why we have always considered as inade-
quate the term, *Permeabilitätskatarakt*, which seems wrongly to regard the change in the mode of permeability, that is to say, the suppression of active transport, as one of the factors determining the appearance of cataract.

When the capsule is crossed the cations gather in the superficial layers. All the authors agree on this point. However, the question of the deeper penetration remains and we may wonder whether a certain active transport occurs in every fiber. The studies by Sperelakis and Potts, on resting potential, and the calculations of Kinoshita, regarding the presence of Na-K-ATPase, seem to allow an answer in the affirmative, but further indication on these interfiber exchanges would be highly welcome.

**Carbohydrates.** Here are the points which seem well established in the field of glucidic chemistry. The presence of all the intermediary and terminal compounds, enzymes, and coenzymes necessary for a normal glucidic catabolism has been demonstrated in the lens. This is true even for glycogen, whose existence until recently was only suspected. Remarkable is the reduced level of glucose and the importance of free fructose. In fact the ratio fructose:glucose reaches very high figures, especially in the cattle lens which contains at the most 1 to 2 mg. per 100 Gm. of glucose. In regard to polyols, it must be emphasized that the amount found in normal conditions is small.

The modifications due to the age of the subject or of the fiber generally tend to a diminution of the different substances and of the enzymatic activities, but the reductions are of varying proportions and the evolution is, according to Hockwin and co-workers, far from regular. So here, as in other fields, aging is not only a quantitative but also a qualitative phenomenon, the more so as certain substances are present in even greater proportion in old subjects.

From the physiologic point of view I would recall that the passage of glucose into the lens takes place partly by active transport and that this also applies to the phosphorus required for the glucidic metabolism. But part of the entered glucose disappears very rapidly and great proportions of the phosphorus are found in the different organic compounds.

On the other hand, we know the importance of the Embden-Meyerhof pathway. Already observed by Kronfeld and Bothman in 1928, this point has since been confirmed many times. With Kinoshita we can say that in the young cattle lens three fourths of the catabolized glucose is transformed to lactic acid. In spite of a striking absolute and relative reduction with age, the glycolysis remains predominant.

After these certitudes, there are three main questions still to be discussed. The first is that of the sorbitol pathway. Thanks to Kuck and van Heyningen we know the different stages, the enzymes, and the coenzymes, but the question remains what is the part played by this pathway and what is the signification of the high fructose concentration.

The second point deals with the metabolic value of the hexosemonophosphate shunt and of the Krebs cycle. The relative importance of the latter for the glucose consumption is estimated by Kleinfeld and Hockwin to be about 5 per cent, and Sippel gives even a maximum value of 3.8 per cent. With labeled glucose the shunt seems 2½ to 6 times more active than the Krebs cycle. This proportion further increases with age and yet we do not think that the metabolic part of the citric cycle is negligible. We must indeed remember the many experiments carried out after inhibition of respiration as well as the enzymatic activities pointed out by Wortman and Becker; all bear witness to a definite importance of the cycle, especially in the zones with mitochondrias. New investigations on these zones and also on the deeper cortex where the mitochondrias disappear and many senile cataracts begin are necessary.

The third question is that of the real energetic value of the three pathways.
Like Kinoshita we did not find the calculations reliable, and preferred therefore a direct experimental approach. Examining the net synthesis of ATP by a dialyzed lens homogenate in the presence of ADP and a substrate, we observed that neither the inhibition of the respiration nor the stimulation of the shunt markedly modified the incorporation of labeled phosphorus into ATP.

On the other hand this incorporation was stopped as soon as glycolysis was blocked and was strongly stimulated by an addition of fructose-diphosphate or another intermediary product of glycolysis.

Unfortunately it has not been possible to repeat these experiments with whole lenses, but the problem has also been studied by an indirect method. Since active transport requires energy and since this energy can only be provided by the glucidic catabolism, we can learn more about the energetic value of a pathway by inhibiting the others. Thus different groups conclude that in the transport of cations the necessary energy originates mainly in glycolysis. Kern observes the same facts in the case of amino acids. Kinsey and Reddy, however, reported that the transport of α-AIB in rabbit lenses was decreased by about one-third under anaerobic conditions or in the presence of dinitrophenol under aerobic conditions. Recently these investigators repeated their experiments but could find no effect of either anaerobiosis or dinitrophenol on α-AIB transport. The results are reported in detail in the present symposium. They conclude that sufficient energy for amino acid transport can also be provided from anaerobic metabolism in rabbit lenses, although it is probable that ATP derived from respiration also contributes to the energy pool involved in transport.

Nucleotides. Our knowledge of nucleotides has been increased by the technique of column chromatography with ion exchangers. Mandel and Klethi published the first results in 1956. They were followed by others, especially by the research department of Bonn. The wide measure of agreement between these authors will allow us to be brief. The lens contains all the four nucleotides as mono-, di-, and triphosphate, and during aging of the subject or of the fiber there is a reduction of all the triphosphates.

Moreover, the incorporation of labeled phosphorus into the triphosphates diminishes with age and it seems probable that the penetration into the nonadenylic nucleotides takes place after transphosphorylation with ATP.

For further information on this subject I can refer you to the exhaustive thesis of Klethi.

Nucleic acids. As opposed to the question of nucleotides, the problem of lenticular nucleic acids is just now in full evolution. Things began in Strasbourg with determinations of total RNA and DNA in the whole lens, then in different zones with several species.

Within this group of studies on total RNA we must also include research work on the incorporation of 32P, of uridine-14C, and of labeled amino acids.

In fact, this stage of research is out of date since Dische and co-workers initiated a second series of investigations by showing the heterogeneity of lens RNA and by studying the modification with age of the three fractions. At the same time van Heyningen and Waley made similar observations, which were confirmed and completed by Lerman and co-workers as well as by Antoni and co-workers. For their part Zigman and co-workers observed the incorporation of adenine-14C into the fractions.

The third series takes us back to Strasbourg, where Virmaux and co-workers struck by the abundance of the soluble fraction, closely studied it and were able to show its heterogeneity by chromatography on Sephadex G-100. They obtained two peaks in the 2,600 Å ultraviolet range. The second is one of traditional transfer RNA 4 S, whereas the first—approximately 40 per cent of the soluble RNA in young
cattle—is one of a new RNA 7S, the significance of which is being determined.

Bringing together soluble RNA and the pH 5 fraction produces evidence for the amino-acyl RNA synthetase activity of the lens, i.e., the faculty of binding amino acids with RNA. In fact the incorporation takes place only with the second traditional RNA 4 S. With old cattle it is 40 per cent less than with young animals.

While the investigation on this special subject is still in progress, interest begins to center on messenger RNA of the lens. There is no doubt that this field of research will in the near future be more and more important.

**Protides.** Before we undertake the study of lens protides we would like to pay tribute to the names of Moerner (1894) and Jess (1913-1930). They provided us with the basic knowledge on which our work today still rests. Among many other observations, Jess published the first analysis of the lens amino acids. Since then others have followed using different methods and many technical improvements. The last results obtained by ion exchange resin come from Reddy and Kinsey and Dardenne and Kirsten. In spite of differences in species and methods of preparation, both groups found the amount of free amino acids higher in the lens than in the aqueous humor. Later on it could be demonstrated that the amino acid active transport has the same characteristics as those of K and Rb. Since Dardenne uses the same species and the same technique as the Detroit group, general agreement is reached and the only point still to be discussed concerns the presence or the absence of free cystine.

For the tripeptides I would recall that in spite of extensive work on glutathione, on ophthalmic acid, etc., we still do not know why the lens contains more glutathione than any other tissue in the organism and what is the part played by these substances. The recent review of Kinoshita shows clearly that here there are large perspectives for research work.

Among the proteins, let us first consider the insoluble fraction and emphasize the importance of the studies by Dische and his co-workers.

The first question arising is that of the mechanism of insolubilization and aging. A few years ago we all agreed that they were due to the oxidation of SH groups and to the formation of S-S bonds. But two observations have somewhat cast doubt upon this opinion. First, Kinoshita and co-workers noted the difficulty of this oxidation; second, Thomann showed the presence in albumin of as much cysteine as in $\alpha$-crystallin. By submitting later the insoluble protein to the action of increasing urea concentrations, he succeeded in practically fully transforming it into $\alpha$-crystallin. So it seems that insolubilization would be due to an increase in the number of intermolecular hydrogen bonds, which would result in rendering the groups inaccessible to certain reagents. I hope the solution of this problem will be found rapidly.

The second question concerns the origin of albuminoid. According to Hockwin and co-workers and François, it appears to be double, (1) direct synthesis and (2) transformation of one of the crystallins. Many arguments point to the conclusion that $\alpha$-crystallin and albuminoid are near relatives, so that a transformation from the one into the other seems easily possible. The aggregates of $\alpha$-molecules observed by Bon in old animals as well as the less soluble $\alpha$-protein found by Rabaei could then constitute the intermediary stages in this evolution. For the $\beta$-crystallin the situation is quite different; in spite of a few experiments it is nowadays impossible to believe in a physiologic insolubilization of this protein. So we have in the field of insoluble protein two problems to be studied further.

As starting points for our discussion on soluble proteins we should like to take on the one hand the observations of Dische and co-workers, made with traditional methods, on the other hand, the results
of paper electrophoresis found simultaneously at Bonn and Ghent.

These studies have reached numerous and important conclusions; but, with the exception of α-crystallin, the two series were not confronted. In general the authors preferred to have recourse to more and more perfected methods and to try to increase the number of fractions. François and Rabae have thus identified as many as fourteen components in the suckling.

Here we must ask if all these accidents really correspond to distinct proteins. Now, the immunologic techniques have never shown more than ten antigenic determinants and in a great number of very valid experiments the number of five was not exceeded.

Moreover, we know that one protein can have several antigenic determinants so we come back more and more to the old conception of the three crystallins, which we are trying to obtain as pure as possible in order to determine their characteristics.

In the case of α-crystallin, Bloemendal and co-workers7,8 and Björk9 and Spector135 have already reached satisfactory conclusions. For the γ-crystallin, mainly studied by Björk5,6 and Spector,130 things are very much the same, but in the case of the β-crystallin more results would be highly welcome. Nevertheless it seems probable that we are dealing here with a group of proteins. The isolation and the characterization of its various components should enable us to compare it to the electrophoretic fractions as well as to the three β-proteins of Dische. This would be an important step forward.

Further progress will be made when we know the relationship between constitutive proteins and enzymes of proteic nature. This problem has already been studied by Fowlks and co-workers,37 in the case of lactic dehydrogenase, and by Bloemendal and co-workers9 for the neutral protease of van Heyningen and Waley.57 But the same questions arise with other enzymes, in particular with the two that are part of the pH 5 fraction together with the protease; we are referring to the RNA-synthetase and the leucinaminopeptidase.

As a third line of research it would be interesting to have more information about amino acid incorporation into proteins. These studies were initiated by Kinsey and his group and continued at Strasbourg and Bonn on the different lens zones of young and adult animals.

For their part, Lerman and co-workers8 followed the incorporation separately into albumoid and into crystallins. But Spector and Kinoshita135,136 go further and submit the soluble proteins to DEAE chromatography. They are thus able to observe that single protein representing no more than 11 per cent of the total protein actually incorporates 34 per cent of all amino acids. This last observation seems to open many new prospects.

We should like to add some remarks on the catabolism of proteins, especially as regards enzymes, where basic questions have to be discussed. But it is not possible to do it within the limits of this lecture. Things are very much the same for the important chapter of immunology, on which we cannot dwell at greater length. On the contrary, we have to tackle immediately the problem of cataract.

Cataract. What are the modifications noticed during the evolution of a cataract? We will devote our attention above all to abnormal manifestations preceding or accompanying the initial stages of the opacification, which should enable us to detect the factors responsible for the disorder. Our particular interest will be retained by the diabetic and galactose cataracts, which are today the more thoroughly studied forms; only a few words will be added concerning x-irradiation and senile cataracts.

In diabetic cataract caused by alloxan or by dithizone, several important facts seem well established: the accumulation of glucose, sorbitol, and fructose and the reduction of the amino acid incorporation
of the ratio of concentration in lens water to that in aqueous humor of all but four amino acids. As regards the loss of available energy, Kuck\textsuperscript{72} estimates its value at 40 per cent, but the decrease of ATP and creatinephosphate is—at least in the first days—much more questionable than had been thought until recently.

We still have to localize the disorder. It has successively been thought to take place in the three pathways of glucidic metabolism. Personally we have reason to point to the existence of an anomaly in glycolysis without ruling out the possibility of another pathway being affected. Further data concerning these matters would undoubtedly contribute to a better understanding of the genesis of diabetic cataract.

In galactosemia of the young rat or rabbit the first clinical signs in the lens already are accompanied by three kinds of modifications. Some are related to the glucidic metabolism and consist in an accumulation of galactose-1-phosphate and of UDP-galactose, together with an important diminution of UDP-glucose and glycogen. At the same time the amount of dulcitol increases to a point where the polyol reaches the level of potassium, whereas fructose remains unchanged.

In the later stages of catabolism we noticed at the same time a reduction of aldolase activity,\textsuperscript{131} a 22 per cent diminution in lactic acid and one of 18 per cent in pyruvic acid.\textsuperscript{70}

All these facts point to a serious and immediate perturbation in the Embden-Meyerhof pathway.

The second series of disorders is related to proteins. Indeed, the active transport and the incorporation of amino acids are at an early stage markedly diminished. Moreover the electropherogram is modified, the amount of glutathione decreases, and above all the crystallin synthesis is inhibited by 60 to 100 per cent.

Finally, reaching the third series of precocious anomalies, we should notice the fact that the energy reserves undergo relatively small modifications. In fact, the decrease of ATP does not exceed 10 to 20 per cent, the amount of creatinephosphate remains unchanged,\textsuperscript{79} and the ATP-Cr transphosphorylase retains its normal activity.\textsuperscript{79} Thus the important disturbances of the glucidic metabolism apparently do not produce an immediate decrease in high energy bonds, probably because the consumption by the protein anabolism drops at the same moment. We observe later only a diminution of the ATP.

We must then ask ourselves whether the accumulation of the dulcitol and the resulting hydration do in fact play as important a part as was thought by Kinoshita.\textsuperscript{69} It is certain that hydration occurs in galactosemia, and Kinoshita has been able to show the influence of osmotic changes of the lens on the amino acid transport mechanism. But Dische noted that the inhibition of the protein synthesis was immediate, instantly reaching its maximum, thus appearing as a primary phenomenon. Moreover, here are examples of very early galactosisc opacities obtained with rabbits in which the hydration level is relatively low. The same is true of certain diabetic cataracts and of the xylosic opacity. It thus appears that this hydration may well play a secondary part in protein alteration, but it cannot be considered as the decisive factor. Under these conditions our actual opinion points to the presence of a block between the triphosphates, ATP and GTP, and RNA protein synthesis. Our aim is to demonstrate it, but as yet we have not succeeded.

We will be brief in our survey of x-ray cataract. In spite of the many studies on the subject, carried out in particular by Pirie and co-workers, we so far know but one really constant modification: the decrease of reduced glutathione. It was noticed by Hockwin\textsuperscript{53} immediately after irradiation, and was found by the Oxford group 20 hours later, during the period of latency and in the course of the evolution of the cataract. Other anomalies either appear early—they must be considered for
the time being as temporary epiphenomena due to the irradiation—or they appear late, only a short time before the beginning of the cataract, and their relationship with the genesis of the opacity is not really established.

Let us add a few remarks on senile cataract, which we persist in considering as a hereditary exaggeration of physiologic aging. However, we should not forget the possibility of extralenticular factors. Indeed, following LINNER, several authors have measured the plasma and the aqueous flow of the subject with senile cataract and have observed definite anomalies. The question is to know whether circulation disturbances really contribute to the genesis of senile cataract. At present we are studying this problem along those lines.

Is it possible to modify the evolution of a cataract? The answer is yes in the three cases of experimental opacities with which we have just dealt. Let us recall in this regard that Patterson succeeded in avoiding or delaying the appearance of diabetic cataracts in spite of a persistently high glycemia with a diet rich in fructose, fat, and proteins. Proteins also prove effective in galactose cataract, whereas in x-ray cataract the beneficial action of cysteine has been established by von SALLMANN and coworkers and confirmed by PIRIE and others.

But what about senile cataract? We are still looking for a means of preventing it or at least arresting its evolution. This is the main concern of our research work on the lens, but so far our efforts are of no avail. Every year new, "successful" methods are described—the latest being from Russia and Japan—but to our knowledge none answers the three criteria we have been advocating for many years: strictly identical conditions for the successive observations, elimination of all psychological factors in the physician as well as the patient, and a great number of cases to enable us to draw up reliable statistics. For the moment we are pleased to see that the theoretical possibility of influencing a hereditary disorder is more and more accepted, whereas this conception met with considerable opposition when we first undertook to advance it.

This brings us to the end of our necessarily incomplete survey.

We have seen that in morphology the basic work is already achieved. However, in chemistry and physiology the situation is quite different. A great number of gaps have still to be filled and it seems to me that in these fields our objective for the next time should be to increase the certitudes. In fact, as long as we do not have quite solid notions about the penetration into the cells and the fibers, about the shunt and Krebs cycle in the different zones, about the synthesis of the specific proteins, and about the physicochemical changes leading to the opacification, it appears premature to anticipate what lens research may be in ten or twenty years.

Nevertheless we may imagine that future investigators will follow the lines established in general biology and, owing to the special conditions in the lens, will remain in the advanced guard. At least three directions of research can be foreseen.

The chemists will penetrate deeper and deeper into the knowledge of cytochemical mechanisms. Today we consider the metabolic pathways as a whole or are especially interested in one or another of their steps. Sometimes we can assume the existence of a block and try to make precise its characteristics by examining the specific and enzymatic activities. For our successors it will be possible to determine the reason for the metabolic deficiencies by better localizing the anomalies of the enzyme synthesis and to act on this special point in order to re-establish the normal situation.

On the other hand, the research on nucleic acids already has established the biochemical nature of the genes. So the question arises, whether the one gene—one enzyme hypothesis will help us to clarify the great problem of hereditary cataracts and...
actually to obtain their prevention or their arrest.

Finally, we will not forget that not only the geneticists, but also the morphologists, collaborate more and more with biochemists in using chemical methods. Since we see the molecules, we analyze the effective amino acids and the terminal groups. Under these conditions we can hope to establish the basic reactions of the protein molecules in the normal and abnormal lens and to explain the metabolic disturbances as well as the genesis of certain cataracts. For example, it may be possible to follow the reactions of an epithelial cell molecule in the presence of a hormone whose activity on the molecular level will be known.

Certainly there are already now more perspectives, and new ones will arise and develop in directions which will probably not be the same as those we have outlined. But as unfortunately I am a very poor prophet, I prefer to stick to the present, to my keynote, where I tried to be objective and give to every fact the place it deserves. If we adopt this general line throughout—even for the references in our publications—all lens research workers will soon form a great international team. At Minneapolis, which follows Brussels and Oxford and precedes Munich—already scheduled for 1966—I hope that a new step will be taken along this road. In my opinion two or three of our groups in close contact should attack the same problem from all possible sides. There is no doubt that by this method very important advances will be realized, quicker and better, than in an isolated laboratory.

By a large discussion we shall during the next days prepare the subsequent evolution. So this symposium will be useful and satisfactory to all participants and a great success for the organizers, to whom I would like, in closing, to present my best wishes and our sincere thanks.

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