Enzyme activities in relationship to age and phosphorylated intermediates in energy metabolism

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A brief review of the presence and the age-associated changes of enzymatic activities of the lens metabolism is given. A consideration of the data on the activities of some enzymes of different animal species indicates that changes in lens metabolism related to age should be controlled by multiple sampling at intervals of aging over a wide age span rather than at just two ages. Changes in the concentration of many phosphorylated intermediates of carbohydrate metabolism in the lens with increasing age are well known. In some cases the results may be interpreted to indicate that there might be a “partial block” of glycolysis. The causes of such “partial block” and the experimental advance are discussed.

Enzymes of glycolysis have been investigated in lenses of animal species. It is possible to discuss briefly only a few of the numerous results. The hexokinase found in lenses has a much greater affinity for glucose than for fructose. Mandel and Israelwicz found that the activity of this enzyme decreases with the age of the animals. These investigators also measured the activity of 6-phosphofructo-1-kinase. Green, Bocher, and Leopold found a phosphoglucomutase and observed that its activity was greater in the lens cortex than in the capsule. Many results are available on the behavior of fructoaldolase, the activity of which is highest within the superficial cortical layer and decreases with age. In addition, the presence of the following enzymes should be mentioned: glyceraldehyde-3-phosphate-dehydrogenase, glyceraldehydehydrogenase, phosphoglyceric mutase, enolase, pyruvate kinase, and lactate dehydrogenase. The highest activities of these enzymes also are located in the lens cortex, and a decrease in activity is found in lenses of older animals.

Phosphorylases also are present in the lens. It has been shown that the activity of fructose-1, 6-diphosphatase is higher in older lenses than in the lenses of young animals. The results obtained on the behavior of phosphomonoesterase are not uniform. There have been reports of both increased and decreased activities with aging. In addition, phosphate-splitting enzymes, such as pyrophosphatase, adenosinetriphosphatase, and glycerophosphatase, have been found.

The enzymes of the citric acid cycle have been found by many investigators. Condensing enzyme, succinohydrogenase, fumarase, malic enzyme, and malate dehydrogenase are known to occur in
lenses of various species of animals. Present also are enzymes of the hexose-monophosphate shunt and the sorbitol pathway.

As a summary one can say that to date no enzyme which has been searched for has remained undetected. As far as our present knowledge permits an assessment of metabolism, all of the enzymatic catalysts necessary for energy formation are present in the lens. One may thus be led to the conclusion that all important problems about enzyme activities and energy metabolism in relation to age are solved.

However, most of the investigations on the influence of age on a particular metabolic process have been conducted with the use of only two age-groups, namely, "young" and "old." Frequently the age was determined by the lens weight. The failure to obtain sampling at various age intervals over a very long age span has occurred not only in experiments on enzyme activities, but also with other investigations on the lens. Indeed, it may be possible that no truly old animals have been investigated in some animal species for it is not easy to find or obtain aged animals. Probably many animals have been labeled as "old" when they were only "mature." The necessity of carrying out investigations over a longer life span with many intermediate ages for different species can be illustrated by the following examples, which were presented recently to the meeting of the Research Association.4

It has been shown that the weight of the lens of different species is related to the age of the animal. Fig. 1 shows the lens weights of chicken, bovine, and guinea pig lenses and their relationship to the age of the individual. The findings are similar in the three animal species. That lens weight is an exponential function of age has been shown for the bovine lenses. The lens weight is proportional to the log of the age with a correlation coefficient of $r = 0.93$.5 In order to study the metabolism of the lens and its changes during aging over a longer life span, many enzyme activities in bovine, guinea pig, and chicken lenses were investigated. Two examples may be used as illustration. Fig. 2 gives the results obtained in these experiments for fructosealdolase. The enzyme activity has been expressed in terms of activity per lens and activity per gram lens fresh weight.
Fig. 2. Activity of fructoaldolase of bovine, chicken, and guinea pig lenses.

The activity is represented in arbitrary or laboratory units and a direct comparison between different species is not possible. The age is plotted in months on a log scale. Bovine lenses were available up to an age of about 90, guinea pigs up to 36, and chickens up to 12 months. Each point represents averages of 8 or more lenses. The term activity per lens, as originally used by Green and Solomon, is preferred as it gives a better understanding of the absolute amounts.

The fructoaldolase activity in relation to aging shows a similar behavior for all animal species investigated. The activity per lens increases after birth, reaches a maximum, and decreases again. It is apparent that if only two age groups had been used quite different results would have been obtained. If the activity is related to the unit of fresh weight of lenses, an almost linear decrease results. An entirely different relationship is obtained when the lactate dehydrogenase activity of the lenses of the three animal species is determined (Fig. 3). LDH activity of bovine lenses decreases but the LDH activity of chicken lenses increases with increasing age. Similar findings in chicken lenses were reported by Maisel. His results were obtained by agar-gel electrophoresis. The activity of LDH in guinea pig lenses also shows an increase with age with changes in the rate of increase. If one takes the lens weight into consideration, the shape of the activity curve obtained from lenses of guinea pigs again is remarkable.

Enzyme activities are associated with the presence of water-soluble proteins. Since it is known that the composition of water-soluble proteins of the various parts of the lens relates to the age of the individual when the lens fibers were formed, we tried to determine if these changes in protein composition could be related to the changes in enzyme activities. Therefore, using a DEAE-cellulose column, we separated the water-soluble proteins of the
Fig. 3. Activity of lactate dehydrogenase of bovine, chicken, and guinea pig lenses.

Fig. 4. Water-soluble protein content and enzyme activities of the lens nucleus of calf in comparison to the bovine lens cortex. Enzyme activities are expressed in terms of activity per gram protein. The single fractions were separated by the DEAE-cellulose method. The white columns represent the protein content in percentage of the total water-soluble protein. The cross-hatched columns represent the specific enzyme activity (activity per gram protein).
nucleus of calf lenses which represent the embryonic lens. The outer cortex of old bovine lenses (a part of the lens which has grown in a late period of animal life) also was fractionated by a similar procedure. Fig. 4 shows part of the results of these experiments. Five different protein fractions are shown. Of special interest are fractions I and III. Both fractions III (calf lens nucleus and bovine cortex) show a remarkably high specific activity of LDH with almost no difference between them. Fraction I behaves differently. The percentage of protein in fraction I is equal or even higher in the cortex of the bovine lenses compared to the nucleus of calf lenses. However, LDH specific activity of the cortical protein fraction I is much decreased. Since it has been shown that the LDH activity in whole bovine lenses decreases with increasing age, this decrease may be related to the decrease in activity within protein fraction I of the cortex. One interpretation of these observations may be that fibers produced in later life are of different quantity as regards their enzymatic activity.

In addition to the above-mentioned problems relating to age sampling, similar problems exist concerning the sampling of different parts of the lens. Thus the many different expressions like “outer cortex,” “superficial layer,” etc., do not mean the same thing to different investigators. If results are to be compared, it seems necessary that the same method of separation be used to obtain the capsule, epithelium, outer cortex, equatorial zone, and nucleus. Perhaps it is possible that the participants in this symposium can make a proposal for standardization of techniques for the future studies. Most of the above studies have been made on homogenates of the various areas of lens. It is to be hoped that the progress of histochemistry of the lens will bring new information about the topographical distribution of these metabolic processes.

Because of the importance of energy metabolism, most efforts during the past years have been concerned with the investigation of carbohydrate metabolism. The greatest difficulties encountered during these analyses were methodological ones; only isolations by paper chromatography and ion exchange techniques have resulted in truer values. The application of enzyme analysis for substrate determination as carried out by van Heyningen and Pirie showed a considerable improvement compared to the usual group analysis.

In 1962 at Oxford, Prof. Mandel reported the “Strasbourg results” on phosphorylated intermediates and on the localization of these compounds in various areas of the lens. It was noted that the high energy phosphate compounds, including the triphosphates of adenine, cytosine, guanine, uracil, and urapidil, are diminished with age, and are located in the outer layers of the cortex. However, these experiments still showed the presence of triphosphate nucleotides in the nucleus even of old lenses.

Our experiments on the phosphorylated intermediates of carbohydrate breakdown have been reported in more detail elsewhere. Older lenses showed a higher concentration of hexose monophosphates. This we believe is due to a “partial block” of glycolysis. Another explanation of the increase of hexose monophosphates in the aging lens may be related to the larger amounts of glycogen in these lenses. This “partial block” in the glycolysis can have several causes: (1) decrease in enzyme quantity; (2) decrease in coenzyme concentration; (3) decrease in substrate concentration; (4) increase in competitive inhibitors; (5) decrease in flow-equilibrium constant as a result of hormone influence; (6) negative autocatalysis; (7) accumulation of reaction products due to decreased permeability of the membrane surrounding the field of reaction.

Further experiments are being undertaken to determine which of these points may be responsible for the “partial block”
of carbohydrate breakdown in the lens. The simple determination of concentrations of nucleotides or other phosphorylated intermediates of carbohydrate breakdown does not always give very significant results. The determination of the various concentrations can be valuable only in those cases which indicate that synthesis or breakdown is disturbed. Only the knowledge of the kinetics of single substances gives a survey on the reaction. In this regard the determination of specific activity with labeled isotopes, as e.g., $^{32}$P, is very suitable.\textsuperscript{13}

Unfortunately, the achievements of the experiments are limited by the method. The separation of phosphorylated intermediates in microquantities is not absolutely reproducible. Our institute is occupied with this problem.

The figures in the present text are used primarily as examples, for the values cannot be compared with results of others because the methods and materials are different. It has been my wish to show that the investigation of alterations in energy metabolism of the lens with aging is not only of ophthalmological, but also of natural science interest, although the stimulation of these studies from the hospital was always evident.

The most detailed research on aging of a tissue probably has been done with lenses. But, of course, in this case also we seem to be restricted in our progress. So I should like to cite Prof. Müller,\textsuperscript{14} who said in 1957 at Vienna in a festival lecture, "The process of aging itself probably never will be absolutely clear; we only can determine such reactions as accompany the aging."

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\section*{References}