Fluorescent globulin of the lens*

In the study of the optical properties of structural proteins the lens, as a material, deserves special attention since, in contrast to other organs, it contains no colouring matter (such as cytochromes) which would interfere with observation.

If a lens, freshly isolated from a bullock's eye, is illuminated with near UV light it shows a brilliant blue fluorescence. If the incident light is polarised, the fluorescent emission has a high degree of polarisation indicating that the emitting molecules have no free mobility. The fluorescent material can be extracted and shows the reactions of an euglobulin. It is possible that fluorescence is not a specific property of this lentoglobulin but is a general property of its group and is not observable in other organs because of optical heterogeneity. Such a possibility is supported by the fact that another optically homogeneous tissue, the corneal epithelium, also shows a vivid fluorescence. The fluorescence of nails or the skin epithelium may be connected with their anhydrous solid state.

The isolated lentoglobulin shows a strong absorption maximum at 2800 A; consequently one may expect that most of the fluorescent light is emitted in the UV and escapes visual observation. It seems to involve the protein molecule as a whole since it is readily lost on heat denaturation. The lentoglobulin is easily denatured and becomes hereby increasingly opalescent or turbid. It seems possible that some such change is involved in the genesis of cataracts.

Experimental. 86 g of bullock's lenses were kept at —20° C for a month, and then placed in a 500 ml jar. The jar was filled with water and the material, after thawing, blended for two minutes in the Servall Omnimixer. After storing the liquid for two hours in the refrigerator, undissolved particles were eliminated in the Sharples centrifuge, using its closed bowl. About one tenth of the supernatant was set aside, while the rest, 360 ml, was mixed with 210 ml saturated ammonium sulphate which brought the saturation to 40%. Twenty minutes later the precipitate was separated in the Sharples, then dissolved in 150 ml water and freed from undissolved material in the same centrifuge. To the supernatant was added 100 ml saturated ammonium sulphate solution, then the precipitate was separated by centrifugation and dissolved in a small volume of water and dialysed overnight against 0° distilled water. The salt-free solution was diluted with 50 ml water to a volume of 150 ml. 1% acetic acid was added till a granular precipitate was formed at pH 6. The precipitate was dissolved in water, $NaHCO_3$ being added to bring the pH to 7.5. The 80 ml final solution contained 10 g protein. It was opalescent and showed a strong fluorescence in the UV.

The solution of the globulin could be lyophylised. Ammonium sulpahte caused gradual denaturation which rendered the protein insoluble. Ammonium sulphate precipitates the lentoglobulin at a fairly sharp limit: at 0.33 saturation there is no precipitation while at 0.37 saturation the precipitation is almost complete. Acetic acid precipitates the protein only in absence of salts. The extinction of a 1 cm layer of a solution containing 0.02% lentoglobulin was 0.3.

The lens also contains a considerable amount of albumin which is left in solution at 0.4 saturation with ammonium sulphate or on acidification. This dissolved albumin showed only a weak fluorescence which might have been due to an admixture of globulin.

At neutral reaction and in absence of salts the solution of the lentoglobulin can be boiled without any apparent change in dispersity, but its fluorescence is lost. The fluorescence cannot be detached from the protein by hot alcohol. The lability of the globulin makes it necessary to carry out its preparation as quickly as possible and at low temperature. The final product showed in the ultracentrifuge, in addition to the main fraction, a more rapidly settling component, which was probably denatured globulin.

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