Observations on a Cytoplasmic Component in Lens Fibers. By Robert A. Resnik, Theodor Wanko, and Mary Ann Gavin. (*From the Ophthalmology Branch, National Institute of Neurological Diseases and Blindness, National Institutes of Health, Public Health Service, United States Department of Health, Education, and Welfare, Bethesda.*)*

INTRODUCTION

Electron microscope studies of adult animal lenses have revealed the presence of a cytoplasmic substance in the form of filamentous and possibly spherical structures (1). It has been designated as low density material with reference to its opacity to the electron beam. Since this system bore no resemblance to any cytoplasmic elements previously described in other tissues, an identification of the low density matter was attempted. The present report deals with the isolation and general properties of a cell fraction which appears identical with the low density material in the osmium-fixed and embedded state.

Materials and Methods

Both rabbit and calf lenses were used. All procedures were carried out in a cold room or in an ice bath. First, the decapsulated lenses were homogenized in 0.88 M sucrose in an all glass homogenizer or in a Waring blendor for 10 seconds. The homogenate was then fractionated into five successive pellets (P1 to P5) and a final supernatant by differential centrifugation (2). When it was found that the low density material was the exclusive component of the final supernatant (P5S) obtained after centrifuging at 105,000 *g* for 16 hours, the intermediate steps were omitted. Further experiments showed that homogenization in 0.15 M sucrose followed by centrifugation for 2½ hours at 105,000 *g* yielded an identical supernatant preparation. Because of its convenience, the second procedure was adopted for subsequent experiments but the original designation P5S was retained.

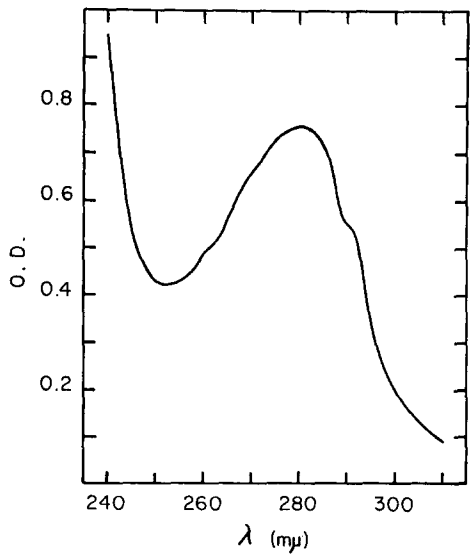
For electron microscopy, the five residues P1 to P5 obtained initially were resuspended in 0.09 M pH 7.3 veronal-acetate buffer and fixed by adding an equal quantity of buffered 2 per cent osmium tetroxide (3). To fix the material in the final supernatant (P5S), an equal volume of 2 per cent osmium tetroxide was added to the solution. The material in each instance was then centrifuged at 105,000 *g* for 60 to 90 minutes. The pellets obtained were dehydrated in a graded series of concentrations of ethyl alcohol. If their size permitted they were then separated into superficial and bottom portions. Small fragments were embedded in a mixture of prepolymerized butyl and methyl methacrylates in the ratios of either 9:1 or 4:1. Polymerization was carried out at 65°C., with 0.25 per cent of the initiator, benzoyl peroxide, added before prepolymerization. Sections were cut on a Servall, Porter-Blum microtome and transferred to collodion-coated grids upon which a layer of carbon had been evaporated. Sections of normal lens fibers were prepared as described previously (1). All specimens were observed with an RCA model EMU-3C electron microscope. Photographs were taken at a magnification of 21,500 and enlarged ×4.

For chemical identification, boundary electrophoresis was done in a Spinco model H apparatus at 0.9°C. Absorption spectra were obtained using a Cary model 14 recording spectrophotometer. Nucleic acid and protein concentrations were estimated by the method of Warburg and Christian (4).

RESULTS

Morphology.—Examination of pellets P1 to P5 and P5S in the electron microscope established the fact that P5S was a preparation of the low density material. No qualitative differences were noted between the superficial and deep portions of the pellets obtained from P5S.

The elements in this fraction (Fig. 1) appeared to be very similar to those seen in sections of lens fibers (Fig. 2). Filamentous structures, about



Text-Fig. 1. Absorption spectrum of an aqueous solution of P5S.

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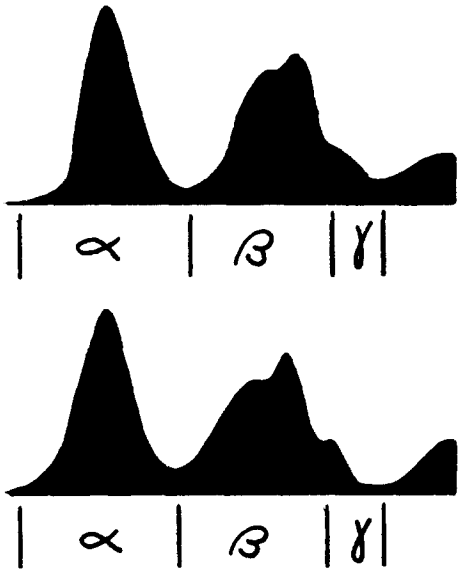

TEXT-FIG. 2. Electrophoresis patterns of P5S (upper) and whole lens homogenate (lower) in pH 8.3, veronal buffer μ-0.1. Direction of migration is from right to left. At the extreme right is the starting boundary anomaly.

100 A in diameter were present in both preparations, together with round profiles, about 100 to 120 A in diameter. These components, when in close association, resembled short, chain-like structures with alternating filamentous and spherical portions. Additional details of these structures were not revealed by counterstaining with 10 per cent phosphotungstic acid (5), or shadowing after partial evaporation under a medium intensity electron beam.

Chemistry.—The ultraviolet absorption spectrum of P5S had a maximum at 280 mμ and inflection points at 260 and 290 mμ (Text-fig. 1). The absorption at 280 mμ indicated the presence of protein. The inflection at 260 mμ might be due to phenylalanine or the nitrogenous bases of nucleic acid while that at 290 mμ might be caused by tryptophan and phenylalanine (6). Identical results were obtained with whole lens homogenate.

The amount of nucleic acid in P5S was lower than in whole lens homogenates. For example, preparations of P5S and whole lens homogenate with protein concentrations of 5 per cent contained 0.04 per cent, and 0.1 per cent, nucleic acid respectively. Thus, nucleic acids did not represent a significant portion of this material.

Boundary electrophoresis indicated that the same groups of soluble proteins were present in P5S and in whole lens homogenate (Text-fig. 2). The fastest component was alpha crystallin. The remainder of the electrophoresis pattern, but for the starting boundary anomaly, represented the rest of the soluble lens proteins.[1]

DISCUSSION

The purpose of this presentation is to report the isolation of a cytoplasmic substance from the crystallin lens which appears as low density material under the electron beam. The correlation of this material in the sectioned lens with the fraction P5S obtained in this study, is based, as indicated earlier, solely upon its morphological properties as osmium-fixed and methacrylate-embedded material. Therefore, it is not possible to define these structures in any greater detail at this time other than as the product of the interaction between osmium tetroxide and the soluble lens proteins. Recent observations in this laboratory confirm those of other investigators (8, 9) that the soluble lens proteins constitute a complex and as yet, incompletely resolved system. Further electron microscopic studies on lens proteins, therefore, appear unprofitable at the present time.

Since the lens is avascular, a preparation such as P5S is devoid of blood proteins which might be a source of contamination in an analogous fraction obtained from most tissues.

SUMMARY

Homogenates of rabbit and calf lenses were fractionated by differential centrifugation in 0.88 M sucrose. The pellets and final supernatant were examined by electron microscopy to determine which fraction contained the low density material seen in sectioned lens fibers. It was present in the final supernatant after centrifuging for 16 hours at 105,000 *g*. The soluble lens proteins were the principal constituents of this fraction.

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[1] The terms alpha and beta crystallin, and albumin were introduced by Mörner (7) to denote the soluble lens proteins. In some instances the term gamma crystallin is used instead of albumin (8). In the light of recent data (8, 9) each of these terms, alpha, beta, and gamma crystallin is in reality, generic. Their use, therefore, should not be construed as indicating a single protein.

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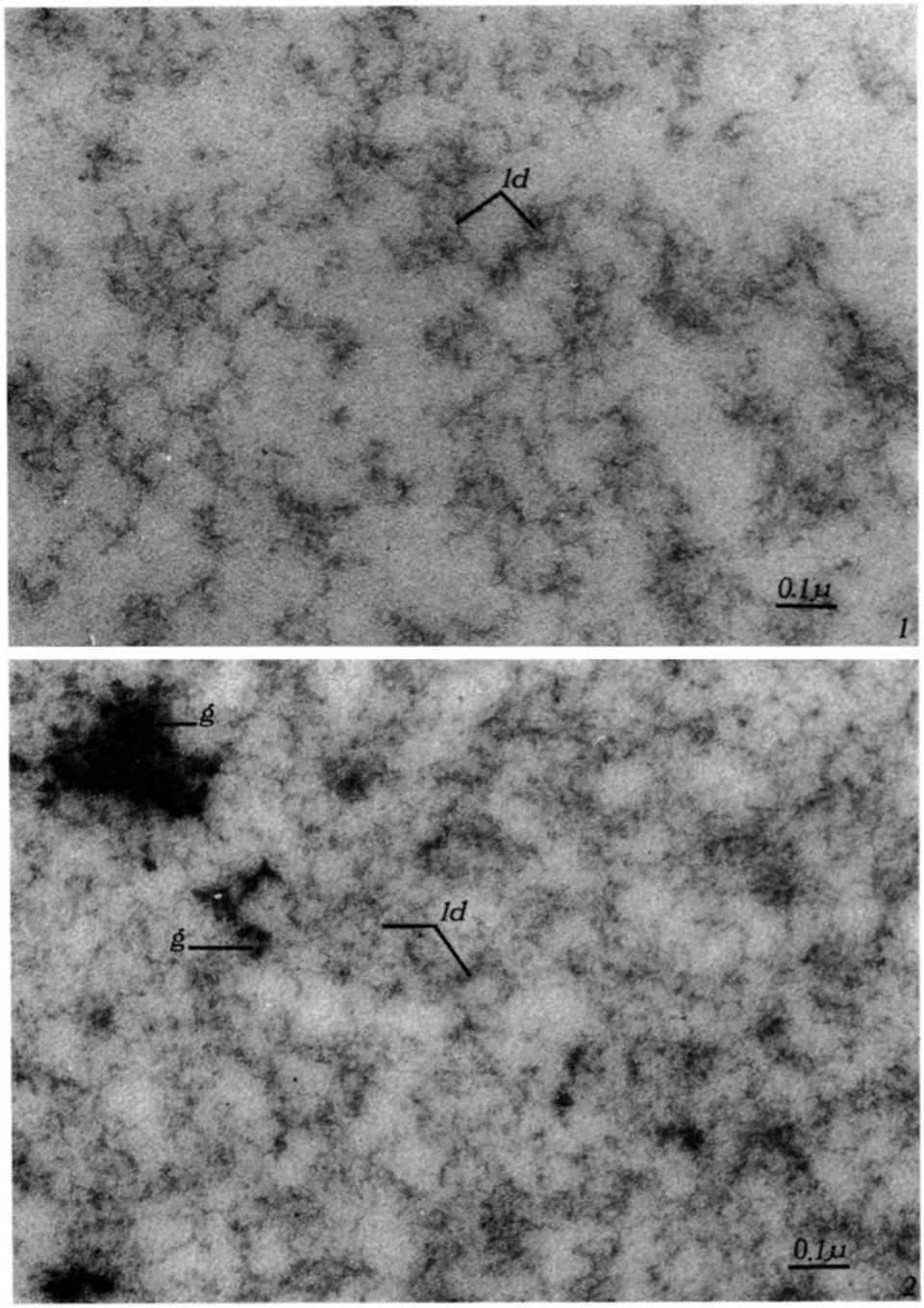
EXPLANATION OF PLATE 222

FIG. 1. Fixed and sectioned supernatant of P5 (P5*S*). This fraction contains elements of low density (*1d*) comparable to those seen in lens fibers (Fig. 2). Approximately × 86,000.

FIG. 2. Sectioned lens fiber. Filamentous and circular elements of low density (*1d*) together with clusters of dense granules (*g*) represent the main cytoplasmic constituents in lens fibers. Approximately × 86,000.



(Resnik *et al.*: Cytoplasmic component in lens fibers)