APPENDIX

Structural Protein Contamination in Lens Polyribosome Preparations

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We now have definite proof that the material that can be extracted from lens polyribosomes by treatment with 5·5M-urea and reconstituted by subsequent exhaustive dialysis after removal of the ribosomes is identical with lens protein. To demonstrate the relationship between native crystallins and this protein the immunological technique of Ouchterlony (1949) in agar gel was used. The result of the double-diffusion test is shown in Plate 1.

Antiserum was prepared by injection of a mixture of lens protein and complete Freund's adjuvant into rabbits. The central hole (hole AL) of the agar plate was filled with this antiserum, and the holes S₁, S₂ and B were filled with extracted protein from polyribosomes prepared by the methods of Spector & Travis (1966) (holes S₁ and S₂) and Bloemendal, Schoenmakers, Zweers, Matze & Benedetti (1966) (hole B). Hole L contained water-soluble lens protein. It is clearly shown that the antiserum

reacted specifically with all the protein solutions. Strong precipitation arcs that have already, after 8 hr., started developing between holes S₁ and S₂ and hole AL pass over continuously into the precipitation arcs obtained with the water-soluble lens protein in hole L. A very weak precipitation arc that becomes visible only after 48 hr. between antiserum against lens protein and protein extracted from polyribosomal preparations by the method of Bloemendal *et al.* (1966) is in agreement with the minute amount of lens protein that was also observed in these highly purified preparations under the electron microscope.

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EXPLANATION OF PLATE 1

Double-diffusion analysis of non-ribosomal protein extracted from lens polyribosomes. The central hole (AL) contains 100 μ l. of antiserum against water-soluble bovine lens protein (titre 1:8). Hole S₁ contains 60 μ l. of a 2% (w/v) solution of protein extracted from polyribosomes obtained from seven eye lenses and prepared by the method of Spector & Travis (1966). Hole S₂ contains 30 μ l. of the same preparation. The precipitation arcs appear after 8 hr. at 37°. The hole designated B contains 30 μ l. of protein solution extracted from polyribosomes obtained from seven eye lenses and prepared by the method of Bloemendal *et al.* (1966). The precipitation arc appeared after 48 hr. at 37°. Hole L contains 25 μ l. of a 10% (w/v) solution of bovine lens protein. The photograph was taken after 50 hr.