precursor has been demonstrated previously[1]. The possibility of a conversion of pregnenolone to dehydroepiandrosterone has been suggested by LIEBERMAN[2] but this reaction has not been demonstrated previously. A likely pathway for the conversion might involve 17α-hydroxypregnenolone.

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[1] F. UNGAR AND R. I. DORFMAN, *J. Biol. Chem.*, 205 (1953) 125.
[2] S. LIEBERMAN, in *Adrenal Function in Infants and Children: A Symposium*, edited by L. I. GARDNER, Grune and Stratton, New York, 1956, p. 62.

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Fractionation of calf-lens protein

Since 1894 when MÖRNER[1] reported that lens contains three soluble proteins, α-, β- and γ-crystallins, attempts have been made to obtain a definitive fractionation of soluble lens protein. Different results have been obtained by different methods of fractionation. Thus conventional electrophoretic techniques[2,3] have indicated the presence of two to three components; continuous-flow electrophoresis[4], 5 components; immunological studies[5,6] from five to eight components. Furthermore FRANÇOIS[7] has reported the fractionation of embryonic lens into ten fractions by means of ultramicro electrophoresis in agar gel.

A method has now been developed for the chromatographic fractionation on DEAE-cellulose* of the soluble protein of calf lens. By this procedure, 10 components have been isolated.

DEAE-cellulose (capacity, 0.8 mequiv./g) was prepared by washing with 0.5 *M* NaOH and water. The fine particles were decanted and the cellulose was suspended in 0.001 *M* phosphate buffer, pH 7.0. All further operations were performed in a cold room at 4°. The DEAE-cellulose column, prepared by the procedure of PETERSON AND SOBER[8], contained 16 g of adsorbant.

In a Potter-Elvehjem homogenizer, 1 g of calf lens was homogenized in 10 ml water. The lens homogenate was then centrifuged for 2 min at 11,000 × *g* and the insoluble material was discarded. The soluble lens protein was dialyzed against distilled water for 18 h, after which an equivalent of 250 mg (dry wt.) was added to the column.

The components were fractionated by stepwise elution. All buffers were prepared by appropriate dilution of 0.500 *M* (NaH_2PO_4, K_2HPO_4), pH 6.80 ± 0.01. Fractions were collected in 10-ml aliquots at the average rate of 40 ml/h. Only slight modification of the elution pattern was found when the flow rate was 100 ml/h. Regeneration was accomplished by washing the column with the following solutions: 180 ml

* Purchased from Brown Co., Berlin, New Hampshire.
Abbreviation, DEAE-, diethylaminoethyl-.

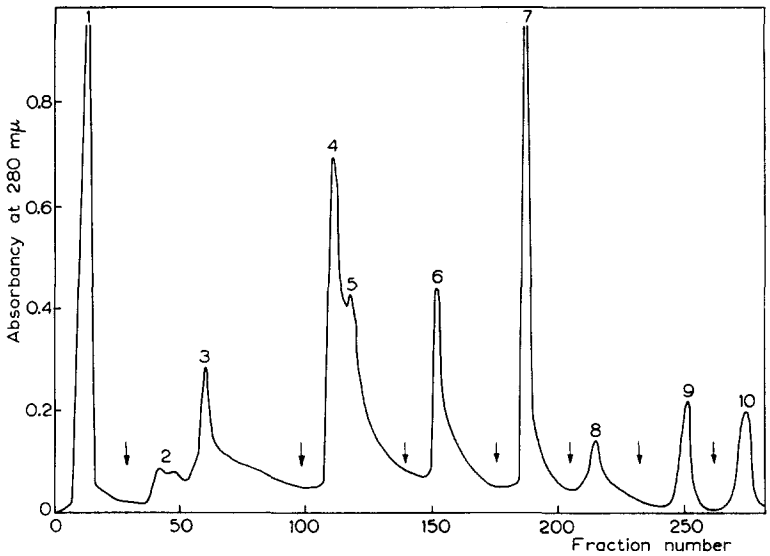

Fig. 1. Chromatographic pattern of 250 mg of soluble calf-lens protein applied to 16 g of DEAE cellulose. Stepwise elution was performed by successive application of the following buffers prepared from 0.500 *M* phosphate, pH 6.80: 0.001 *M*, 0.015 *M*, 0.030 *M*, 0.050 *M*, 0.080 *M*, 0.10 *M*, 0.40 *M*. The final eluting solution contained 0.20 *M* NaH_2PO_4 and 0.20 *M* NaCl. Arrows indicate the change in eluting buffer.

of 0.5 *M* NaOH, 160 ml of the 0.500 *M* phosphate buffer, 80 ml of 0.1 *M* phosphate buffer, and finally 500 ml of 0.001 *M* phosphate buffer.

Fig. 1 illustrates a typical fractionation of 250 mg (dry wt.) of calf lens. The fractionation was followed by the absorbancy at 280 mμ and by reaction with ninhydrin. Recoveries of 90 % ± 5 % have been obtained. The six major components represented by peaks 1,3,4,5,6 and 7 comprise 85.6 % of the total protein recovered.

With this method lens protein can be fractionated at neutral pH in 65 h. Characterization of the protein components is in progress.

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[1] C. T. MÖRNER, *Z. physiol. Chem.*, 18 (1894) 61.
[2] L. HESSELVIK, *Skand. Arch. Physiol.*, 82 (1939) 151.
[3] J. FRANÇOIS, R. WIEME, M. RABAEY AND A. NEETENS, *Experientia*, 10 (1954) 79.
[4] D. C. WOOD, L. MASSI AND E. L. SOLOMON, *J. Biol. Chem.*, 234 (1959) 329.
[5] S. P. HALBERT, D. LOCATCHER-KHORAZO, L. SWICK, R. WITMER, B. SEGAL AND A. FITZGERALD, *J. Exptl. Med.*, 105 (1957) 439.
[6] J. FRANÇOIS, M. RABAEY AND R. J. WIEME, *A.M.A. Arch. ophthalmol.*, 53 (1955) 481.
[7] J. FRANÇOIS AND M. RABAEY, *Am. J. Ophthalmol.*, 44 (1957) 347.
[8] E. A. PETERSON AND H. A. SOBER, *J. Am. Chem. Soc.*, 78 (1956) 751.

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