example, of mouse sera the number of relatively slow-migrating components seems larger than in human sera and an additional zone, β_3, has been envisaged to designate some of them[3,10].

It would certainly be desirable that the somewhat complicated nomenclature which we have been obliged to introduce, should be replaced by special names given to every detected component, as soon as its role and physical-chemical properties become known, as, for example, haptoglobin, cæruloplasmin, transferrin, etc.

PIERRE GRABAR

Institut Pasteur,
25 Rue du Docteur Roux,
Paris.

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Isolation of Two Permeability Globulins from Human Serum

A PERMEABILITY factor, activated by dilution or surface contact, has been described in the β-globulin fraction of human serum[1]. This substance, designated *PF/dil*, seems to have much in common with the enzyme kallikrein first noted by Frey[2]. Whether the serum of any given species contains one, or more, permeability globulin has so far not been clear. Based on similarities of hypotensive and permeability properties, it has been suggested that *PF/dil* and serum kallikrein may be the same[3]. Indeed, di-isopropyl phosphofluoridate (DFP) and soy-bean trypsin inhibitor seem to inactivate both[4-6]. Permeability globulins from guinea pig serum have been reported with the electrophoretic mobility of α_2 (ref. 1) as well as γ-globulin[7]; whether this indicates two molecular species, or is an artefact of preparative procedures, is not certain. Early chromatographic work begun by one of us (J. P. L.) on certain lots of Cohn Fraction III, obtained commercially from human plasma, had suggested the presence of more than one non-dialysable permeability active substance. We wish to report the isolation of two permeability globulins, differing in their electrophoretic mobility and chromatographic behaviour, from sera of normal young men.

Permeability activity was assayed by its bluing action in the guinea pig skin, essentially as described by Miles and Wilhelm[8].

Samples of normal human serum, when subjected to preparative starch-block electrophoresis, demonstrated broad, merging peaks of activity in the γ- and β-globulin regions. Although these findings suggested the presence of two factors with differences in electrophoretic mobility, it was not possible to separate them completely from one another. We therefore turned to ion-exchange chromatography on DEAE cellulose.

As seen in Fig. 1, the proteins of whole serum were separated with the use of a variable ionic gradient, and two, non-dialysable, discrete peaks of permeability-inducing substances were found. The first of these (*A*) was eluted with a buffer conductivity of 0·0066 mhos, principally with the serum γ-globulins. The second peak (*B*) desorbed from the column at a higher ionic strength (between 0·0200 and 0·0351 mhos), along with a mixture

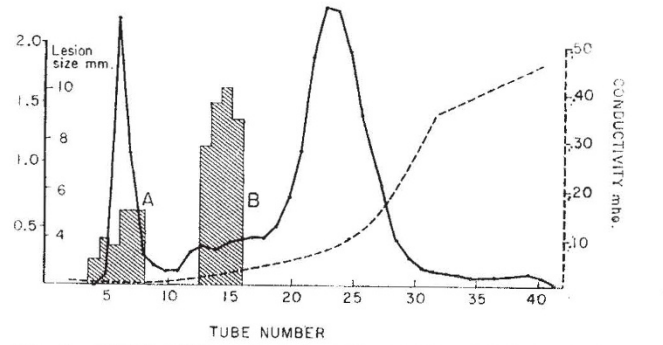

Fig. 1. DEAE cellulose chromatography of 4 c.c. of whole serum. Starting buffer: 0·005 M phosphate—conductivity 0·0069, *p*H 8·10; final buffer: 0·0125 M phosphate + 1 M sodium chloride—conductivity 0·459, *p*H 8·10. 10 c.c. collected in each tube. Column 20 cm × 2 cm packed with DEAE cellulose (approx. 9 g, 0·92 m.equiv./g) under 6 lb./in.² pressure. Bioassays were carried out in dilutions of 0·15 M saline. ——, Protein concentration; – – –, conductivity; shaded, bioassay of 1 : 3 dilution of content of each tube

of γ- and β-globulins. Because of the small amounts of active material present, attempts at starch-block electrophoresis of these fractions failed to recover activity, so that preliminary fractionation of larger amounts of serum was undertaken.

247 ml. of pooled human serum were fractionated with ethanol, according to the method 10 of Cohn *et al.*[9]. Permeability increasing activity was present mainly in fractions I + III–3, and III–1, 2, with trace amounts in fraction III–0. The first two of these fractions were separately chromatographed on DEAE cellulose, under the same conditions as for whole serum. From each fraction, two peaks of activity were again recovered in the same positions as shown in the elution diagram for whole serum (Fig. 1).

Samples of fraction *A* obtained from the chromatography of the two ethanol fractions were pooled, as were those of fraction *B*, and each was run in starch-block electrophoresis, with good recovery of activity. The permeability activity in fraction *A* migrated towards the cathode in the manner of the serum γ-globulins. Activity from *B* moved towards the anode with the mobility of β-globulin.

Active samples of *A* and *B* were recovered after electrophoresis and each was rechromatographed on DEAE cellulose, with no change in chromatographic behaviour.

The active fractions *A* and *B* were both inhibited by 50 μg/ml. of soy-bean trypsin inhibitor, or 5×10^{-4} M DFP after incubation at 4° C for 1 h.

Treatment of guinea pigs with the antihistamine diphenhydramine (20 mg/kg intraperitoneally, 2 h before testing) did not prevent the action of either permeability globulin.

In view of the report[10] of little affinity of serum kallikrein preparations for DEAE cellulose, the identity of the γ-globulin factor with serum kallikrein, and of the β-factor with *PF/dil* of Miles may be suggested.

LAWRENCE J. KAGEN
JOHN P. LEDDY
ELMER L. BECKER

Walter Reed Army Institute of Research,
Washington, 12, D.C.

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