# THE EXAMINATION OF SOME PLASMA PROTEINS BY ELECTRON MICROSCOPY

by

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The investigation of the physical properties of proteins has reached a point where the molecular weights of many proteins have been determined with a considerable degree of accuracy, but although several methods have been developed to determine the molecular shape, there has not been complete agreement between the results. A common weakness of most of these methods is that it is necessary to interpret the experimental results in terms of a model which is amenable to mathematical treatment. The resolution of the electron microscope is now such that one may hope by its use to confirm or disprove the results obtained with other methods on macromolecules of regular shape. At present direct observation with the electron microscope offers the only possibility of obtaining full information about molecules of irregular shape, and the prospect is also held out of following the course of macromolecular reactions. The usefulness of the instrument for these purposes will, however, depend on the extent to which it is able to give a true representation of the molecules as they exist under specified conditions in solution. The fact that one is unable to maintain constant such factors as protein concentration and ionic strength in the preparation for microscopy, together with the necessity for drying the material, introduces possibilities of producing artefacts.

The results of an electron microscopical examination of a number of proteins from normal and pathological human plasma are presented here.

## METHOD AND MATERIALS

It was found that the most satisfactory method of specimen preparation was that employed by BACKUS AND WILLIAMS[1]. The protein in dilute salt solution was sprayed on to nitrocellulose supporting films and shadowed with alloys of gold-manganin (50–50) or platinum-palladium (80–20). The shadowing angle was 1:4 and the calculated film thickness was 3 A (platinum-palladium) and 4 A (gold-manganin). The dry residue of each droplet was fairly uniformly dispersed and was taken to be a representative sample of the bulk material. The presence of an area of blank supporting film around each droplet provides a check on the structure of the shadow layer which is essential for correct interpretation.

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*Pathological ($S_{w,20} = 18$) globulin*

This blood plasma globulin came from a patient suffering from an obscure disease characterized by abnormally high blood viscosity (McFARLANE *et al*[2]).

The globulin was shown electrophoretically to constitute about 80% of the total protein of the serum, the remaining 20% being albumin. The fraction (A) used in this investigation was rendered albumin-free by sodium sulphate precipitation, and shown by ultracentrifugal analysis* to contain three components. The main one, having a sedimentation constant $S_{w,20} = 18$, constituted approximately 90% of the whole. The remaining 10% was partly normal $\gamma$ globulin and partly molecules having a very high sedimentation constant (25–30). Drs. EDSALL AND DANDLIKER[3] kindly carried out light scattering observations on another fraction (B) which differed somewhat in composition, comprising 70% material with $S_{w,20} = 18$, 15% normal $\gamma$ globulin and 15% with $S_{w,20} = 25$–30. The globulin was suspended in 0.2 *M* NaCl at pH 6.7 for the scattering observations.

The patient's serum possessed the unusual property of forming two layers when allowed to stand in the cold, the bottom layer being a gel. The purified globulin fractions showed a similar behaviour at low salt concentrations provided the concentration of protein was sufficiently high. For example, a 1.5% solution of protein, when dialysed against distilled water in the cold, gave upper and lower layers in which the globulin concentrations were 1.2% and 13% respectively. Warming to room temperature for 36 hrs. and shaking up with water did not disperse the gel, but the addition of a trace of salt was immediately effective.

*Normal human $\gamma$ globulin*

Two samples were used with similar results. One had been stored in the freeze-dried condition, while the other was freshly prepared by three-fold precipitation with 12% sodium sulphate. No direct check was made on this material either with the ultracentrifuge or by light scattering, but it was shown to be electrophoretically homogeneous, having the mobility of $\gamma$ globulin.

*Pathological ($S_{w,20} = 10$) globulin*

This was a globulin from a patient suffering from typical myelomatosis. The sodium sulphate fraction used in the electron microscope investigations was shown by the ultracentrifuge to consist almost entirely of material of $S_{w,20} = 10$, with a trace of a slightly faster component.

*Human fibrinogen*

The fibrinogen preparation used was supplied by Dr R. A. KEKWICK as a freeze-dried residue from a solution of the material in citrate saline. The material was 90% clottable and was shown by the ultracentrifuge to contain only one main component having $S_{w,20} = 8$, together with a small amount (5% of the total protein) of heavier material.

For electron microscopic observation, the globulins were usually suspended in a 2% solution of ammonium carbonate or ammonium acetate. However, to verify that the results were independent of salt effects, the concentration was varied through wide limits and the salts were also used together in varying proportions. Distilled water, to

* Kindly carried out by Dr R. A. KEKWICK of the Lister Institute of Preventive Medicine.

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which had been added just sufficient potassium phosphate buffer of pH 7.8 to keep the molecules in solution, was also used. The fibrinogen was dissolved in distilled water, or after the initial reconstitution with water, in citrate saline. In the latter case the specimens were washed on the grids after drying to remove excess salt. This washing tended to destroy the clear-cut structure of the droplets.

Treatment with osmium tetroxide and phosphotungstic acid did not produce any apparent changes in the size and shape of any of the molecules.

A Siemens electron microscope was used for the investigation, the high tension of 45 KV. being supplied from a high frequency source. A 70 $\mu$ objective aperture was used in all cases.

## RESULTS

### *Pathological* ($S_{w,\,20} = 18$) *globulin*

A representative field from a droplet is shown in Fig. 1. It consists predominantly of particles which may be visualised as oblate ellipsoids of revolution with semiaxes of 90 A and 45 A. Assuming a partial specific volume of 0.75, such particles would have a molecular weight of $1.2 \cdot 10^6$.

Such an estimate is necessarily crude, but we were unable to determine it more accurately (*e.g.* by using the particle counting technique of WILLIAMS AND BACKUS[4]) because of the presence in each dried droplet of a deposit which could not be eliminated. Fig. 2 (a) shows such a deposit in a preparation having a rather high protein concentration. As can be seen from Fig. 2 (b) the material appears to be the remnant of a monolayer of the protein, presumably formed on the surface of the drop. No similar effect was observed with the other less soluble and smaller molecules, and it may be that surface denaturation of the kind encountered is a peculiarity of this particular globulin.

A particle of the above dimensions would have a sedimentation constant of 38.5*, so there is serious disagreement with the experimental value of 18.

Light scattering investigations carried out on fraction B gave a molecular weight estimate of $1.16 \cdot 10^6$, and data for the angular dependence of scattering were shown to be accounted for by the assumption that the molecules are thin rods 560 A long. It is more convenient, however, to choose as models ellipsoids of revolution having semiaxes a, a, c, and we find that the observations are consistent with the assumption that the particles are oblate ellipsoids having a = 250 A, c = 5 A, or prolate ellipsoids in which a = 30 A and c = 360 A. The frictional ratios $(f/fo)_a$ for such particles would be 2.4 and 1.6, respectively. Taking hydration into account the frictional ratios $(f/fo)_{obs.}$ become 2.7 and 1.8, the corresponding sedimentation constants being 14 and 20.

Thus light scattering and ultracentrifuge results favour the assumption that the molecules are prolate ellipsoids of revolution having a molecular weight of approximately $1.2 \cdot 10^6$ and an axial ratio of 12 : 1. This conclusion is at variance with the electron microscope observations which show particles having this order of molecular weight, but fail to reveal the high degree of asymmetry predicted.

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* In the absence of data on the hydration factor of the material it has been assumed to be 0.3 g water/g protein, being the value obtained by BOYES-WATSON, DAVIDSON AND PERUTZ[5] for horse methaemoglobin.

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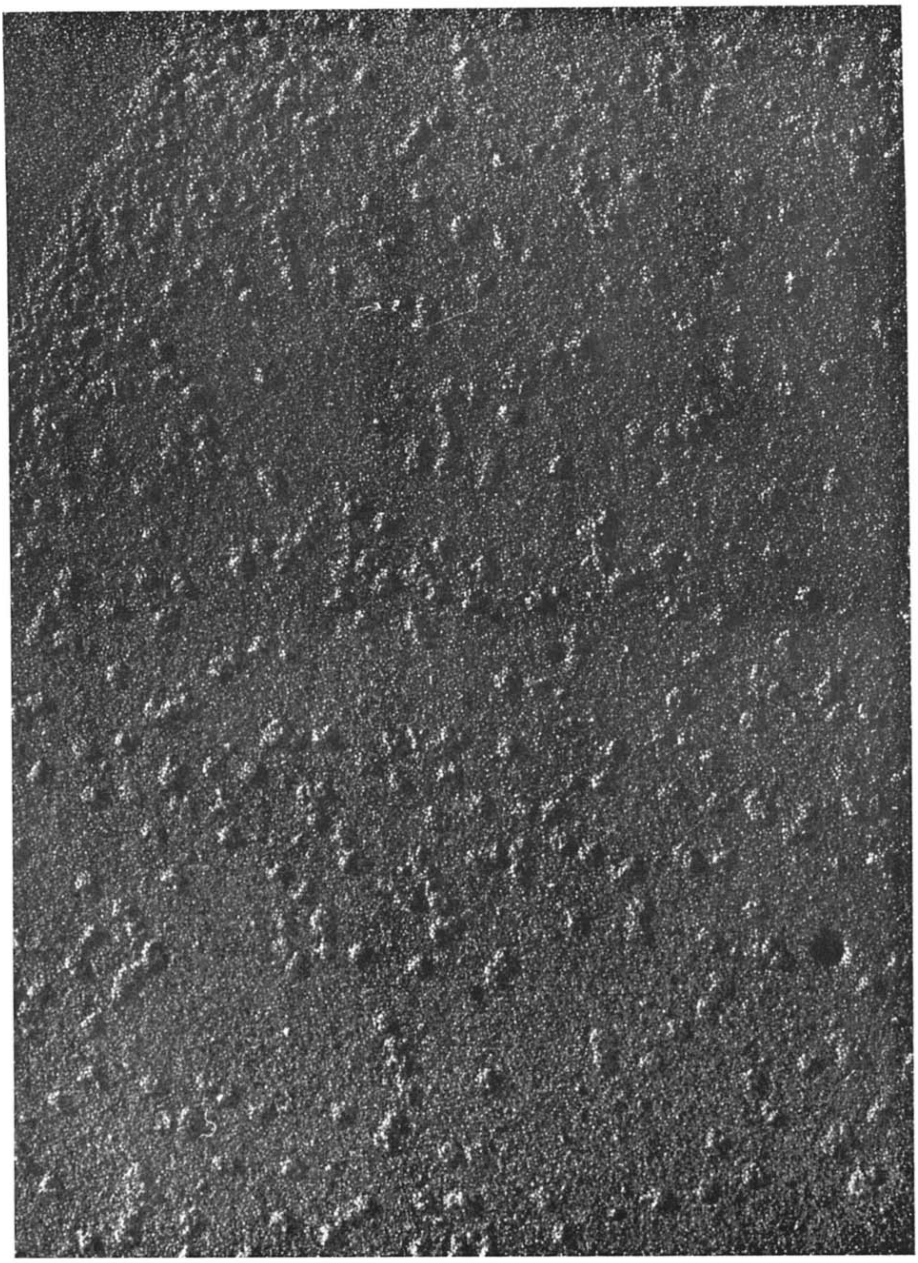

Fig. 1. Portion of a droplet of a high molecular human globulin. Shadowcast with gold-manganin. Mag. × 130,000

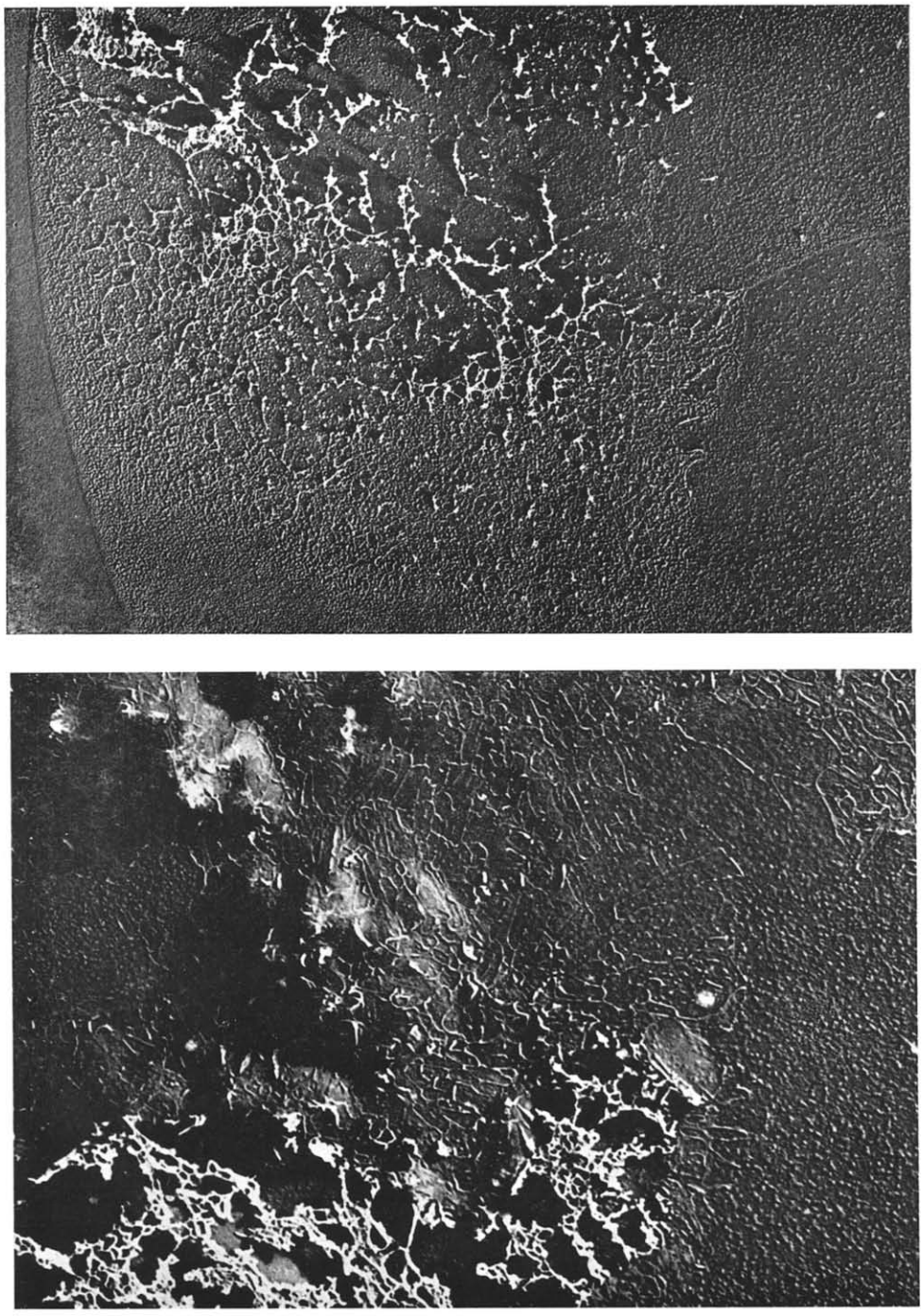

Fig. 2. (a) Typical deposit in a droplet of relatively concentrated high molecular human globulin. (b) Micrograph showing thin film of material which collapses to form the deposit shown in (a). Both (a), (b) are shadowed with gold-manganin. Mag. × 25,000

*Normal γ globulin*

A portion of a droplet of this material is shown in Fig. 3. The effective resolution is insufficient to enable one to say more than that the material consists of more or less globular particles having an average cross sectional diameter approximately 75 A.

The generally accepted model for a γ globulin molecule in solution is a prolate ellipsoid of revolution with semiaxes 25 A and 125 A, and having a molecular weight of 156,000 (ONCLEY, SCATCHARD AND BROWN[6]). A spherical particle of this molecular weight has a diameter of approximately 65 A. In view of the inaccuracy associated with our estimate of diameter and of the possibility that the particles we observe are somewhat flattened spheres, our results are not inconsistent with such a molecular weight, but once again fail to reveal the expected asymmetry.

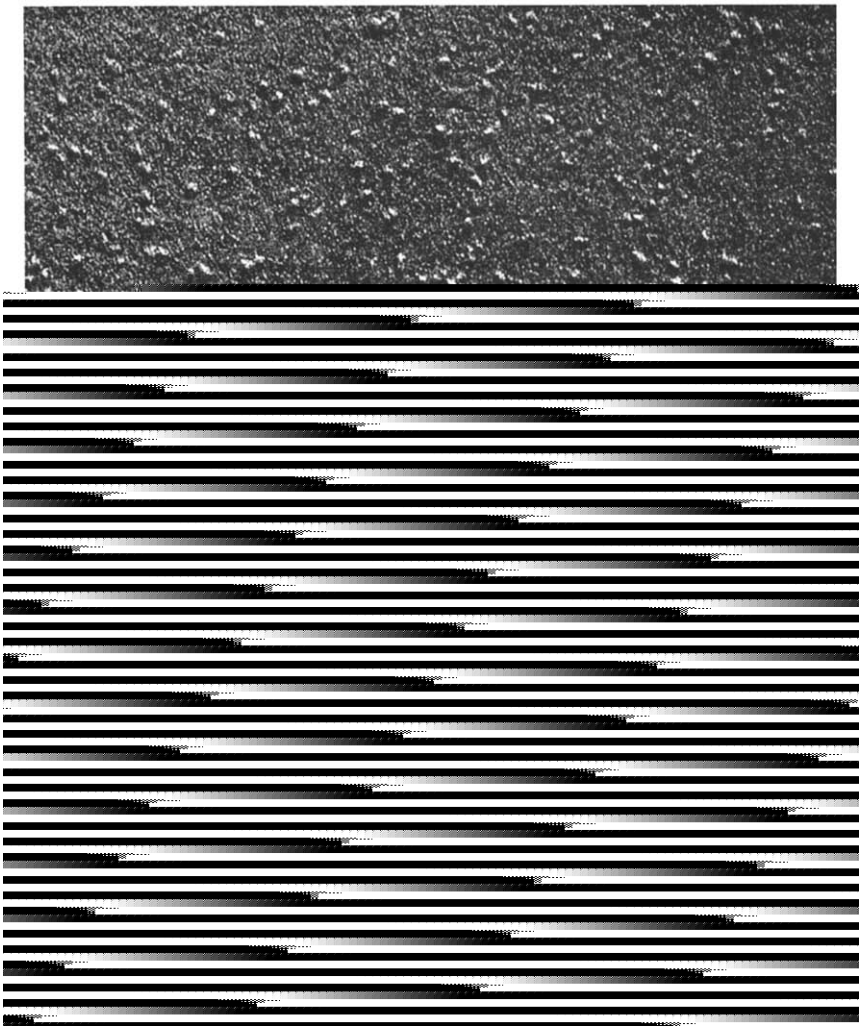

Fig. 3. Normal γ globulin molecules. Platinum-palladium shadowed. Mag. × 130,000

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Beobachtungen an gewissen Globulinen weisen auf Molekulargewichte hin, welche mit den durch weniger direkte Methoden ermittelten Werten übereinstimmen; doch beweisen sie nicht die auf Grund jener Methoden zu erwartende Asymmetrie der Moleküle. Gewisse Tatsachen aus der Literatur, welche eine ähnliche Abweichung im Falle des Edestins erkennen lassen, werden angeführt.

Im Falle des Fibrinogens dagegen ist, weder was die Grösse noch was die Form der Moleküle betrifft, Übereinstimmung zwischen den verschiedenen Methoden; im Elektronenmikroskop werden kugelförmige Teilchen beobachtet, welche viel kleiner sind, als auf Grund der indirekten Methoden zu erwarten war.

Die Faktoren, welche die Auslegung der Ergebnisse der direkten und indirekten Methoden beeinflussen und daher der Grund dieser Abweichungen sein könnten, werden erörtert.

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