

Further work is in progress on the non-galactosic portion and also on the polysaccharides extracted by cold water.

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<sup>1</sup> Dillon and O'Colla, *NATURE*, **145**, 749 (1940).

<sup>2</sup> Haas, *Biochem. J.*, **15**, 469 (1921).

## Native State of Proteins in Egg-White

THERE are two opposing conceptions of the native state of proteins in biological fluids. One holds that the native particle is a complex of the various species of proteins present and is altered in the process of isolation. The other supports the independent existence of these species in a state of homogeneous dispersion. Difficulties of separation and inconstancy in amino-acid analyses support the former, and these were encountered<sup>1</sup> in efforts to separate and characterize the proteins in hen's egg-white. Block<sup>2</sup> has suggested the existence of an 'orosin' in egg-white on the basis of the ratio of arginine, histidine and lysine. The evidence from physical methods with blood serum supports the second hypothesis, but these have not been applied to egg-white except for a brief note by Svedberg<sup>3</sup> relative to irregularities in the sedimentation constant of native and 'purified' ovalbumin.

Egg-white has been examined in the ultra-centrifuge at 60,000 ( $\pm 300$ ) r.p.m. in a field strength of 270,000 times gravity using the 'diagonal schlieren' method of Philpot<sup>4</sup> in the optical system and a high-pressure mercury vapour lamp. The temperature of the rotor was 25–27° and the period of spinning up to five hours. Native egg-white and that diluted with 1 per cent sodium chloride from one to three times exhibited a single sedimenting boundary. The concentration of total protein varied from 9.8 to 2.6 per cent, measured by the Zeiss dipping refractometer and light of wave-length 546 m $\mu$ .

The differentiation of the native proteins in the white was attempted on the basis of ionic mobilities by the method of Tiselius<sup>5</sup>. The migration of the boundaries was followed optically by the 'schlieren' method using a sodium vapour lamp. The native or diluted egg-white was dialysed previously at 2° against a phosphate buffer mixture of pH 7.2 and 0.1  $\mu$  ionic strength until equilibrium was attained and chloride removed. Egg-white diluted three times, twice and once with buffer solution and exhibiting concentrations of total protein of 1.95, 2.75 and 4.00 per cent, separated into five and sometimes six boundaries. The electrophoresis was continued for three hours at a potential gradient of 120 v. and 10 mA. and a temperature of 1° C. In the most concentrated preparation three of the bands were heavy and three relatively light. A fresh preparation, with a protein concentration of 5.57 per cent, made with a phosphate buffer of pH 8.0 and 0.1  $\mu$  ionic strength gave a similar result in five hours of migration. The concentration of the original egg-white was 9.77 per cent. It has not been possible to study the migration of the native white without some degree of dilution by phosphate buffer. However, this is in remarkable agreement with the conclusion of five distinct protein components reached by

Hektoen and Cole<sup>6</sup> on the basis of immunological reactions. The chemical methods of separation would, therefore, seem to have a degree of justification.

The chemical individuality of the products thus separated was tested. Using methods previously described<sup>1</sup>, ovomucin and ovomucoid were very carefully fractionated and obtained in solution after dialysis against phosphate buffer of pH 7.2. Such solutions were never homogeneous. The best preparation of ovomucin showed a single boundary by electrophoresis but two by centrifugation in a concentration of 1.33 per cent. A specially purified preparation of ovomucoid showed three migrating boundaries in the Tiselius apparatus at 2.7 per cent concentration but only one sedimenting boundary in the centrifuge. The results thus appear to justify the further conclusion that our chemical methods are not yet adequate for the separation of the five or six species of protein in native egg-white.

The present note is published at this time because no further work appears possible in the immediate future. I wish to acknowledge the kind hospitality of Sir John Ledingham and Dr. A. S. McFarlane at the Lister Institute during the summer months of 1938, and the sympathetic assistance of Mr. R. A. Kekwick.

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<sup>1</sup> Young, *J. Biol. Chem.*, **120**, 1 (1937).

<sup>2</sup> Block, *J. Biol. Chem.*, **105**, 455 (1934).

<sup>3</sup> Svedberg, *NATURE*, **128**, 999 (1931).

<sup>4</sup> Philpot, *NATURE*, **141**, 283 (1938).

<sup>5</sup> Tiselius, *Trans. Faraday Soc.*, **33**, 524 (1937).

<sup>6</sup> Hektoen and Cole, *J. Infec. Dis.*, **42**, 1 (1928).

## Chemistry of Urea Stibamine

IN his article on the "Antimony Treatment of Kala-azar"<sup>1</sup>, Sir Leonard Rogers referred to the findings of an early worker on the constitution of urea stibamine; reference was not made to later work on this subject, notably that of Gray *et al.*<sup>2</sup>

To some extent the controversy about the constitution of urea stibamine is comparable to that about atoxyl when it was discovered by Ehrlich. Gray *et al.* have noted that the most interesting of the more important derivatives of *p*-amino-phenyl stibinic acid is a material prepared by Brahmachari (1922) under the name 'urea stibamine' by heating *p*-amino-phenyl stibinic acid with urea solution. They have shown that the 'essential active principle' in urea stibamine is *S*-diphenyl-carbamide-4:4 distibinic acid



which is rendered water-soluble in the presence of protective colloids, and that this active principle is responsible for the remarkable therapeutic properties of urea stibamine. So far as I am aware, these findings have not been contradicted by any subsequent observer.