- Ellis, R. J. (1969). *Science, N.Y.*, **163**, 477.
 Ellis, R. J. (1970). *Planta*, **91**, 329.
 Ellis, R. J. & Hartley, M. R. (1971). *Biochem. J.* (in the Press).
 Kirk, J. T. O. (1970). *A. Rev. Pl. Physiol.* **21**, 11.
 Kirk, J. T. O. & Tilney-Bassett, R. A. E. (1967). *The Plastids*. London and San Francisco: Freeman.

neutral aqueous buffers. In the absence of rabbit plasmin the fragmentation can be achieved by the addition of human plasmin (Kabi Pharmaceuticals Ltd., Stockholm, Sweden) after acid treatment of the IgG.

- Utsumi, S. & Karush, F. (1965). *Biochemistry, Easton*, **4**, 1766.

A New Enzymic Fragment (Facb) of Rabbit Immunoglobulin G

By G. E. CONNELL and R. R. PORTER. (*Medical Research Council Immunochemistry Unit, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, U.K.*)

IgG* prepared from rabbit serum by repeated ammonium sulphate precipitation and chromatography on DEAE-cellulose contains traces of a proteolytic enzyme that is probably plasmin. The immunoglobulin is not digested by the enzyme even during prolonged incubation in neutral solutions, but it can be made susceptible to the enzyme by preliminary incubation at low pH.

A solution of rabbit IgG (20mg/ml in phosphate buffer, pH 7.0) was adjusted to pH 2.5 with 1.0M-hydrochloric acid. The solution was incubated at 30°C for 60min and then adjusted to pH 7.0 with 1.0M-sodium hydroxide. On further incubation at 30°C for 30–120min the immunoglobulin was quantitatively hydrolysed, yielding two fragments that were detected by starch-gel electrophoresis. These fragments differed in size and could be separated by gel filtration on Sephadex G-150 in 6M-urea–0.1M-sodium acetate buffer, pH 4.7. Characterization of the fragments showed that the point of cleavage was between lysine-326 and alanine-327 in the heavy chains, 108 residues from the C-terminus. The smaller fragment therefore resembles the Pep III fragment described by Utsumi & Karush (1965). The remainder of the immunoglobulin molecule makes up the larger fragment, which will be designated Facb (fragment antigen- and complement-binding).

The Facb fragment was prepared from IgG from rabbits hyperimmunized with ovalbumin. The fragment formed an immune precipitate with ovalbumin and the quantitative precipitin curve was almost indistinguishable from that of the original IgG. The ovalbumin-fragment complex was found to have complement-fixing activity that was approx. 60% of that of the corresponding ovalbumin-antiovalbumin complex.

Rabbit IgG can be freed of plasmin activity by repeated passage through Sephadex G-200 in

Antigenicity of Sheep Cortical-Bone Matrix Proteins

By I. R. DICKSON. (*Institute of Orthopaedics, Stanmore, Middx., U.K.*)

The antigenicity of sheep cortical-bone proteins was studied as a preliminary to evaluation of the feasibility of using monospecific antibodies, labelled with radioactive isotopes or fluorescent reagents, to study bone protein metabolism and also to estimate new bone formation in grafts and implants. Sheep cortical-bone powder was extracted with EDTA, pH 8.0, by the method of Herring (1964). Chromatography of the concentrated extract (fraction I) on CM-cellulose columns (10mm-potassium acetate buffer, pH 5.0) yielded a fraction (fraction II) that was retained on the column and one that was not adsorbed. The latter was ultra-centrifuged (80000g for 48h at 4°C) in a caesium chloride solution ($\rho = 1.5$); fraction III was recovered as a brown gel from the supernatant solution, and the remaining fractions were pooled (fraction IV). Fraction II consisted mainly of soluble collagen and a glycoprotein with the mobility of serum albumin on cellulose acetate electrophoresis (0.12M-sodium barbiturate buffer, pH 8.6). Fraction III had an electrophoretic mobility of 0.9 relative to serum albumin and contained 0.3% hydroxyproline, 0.7% uronic acid and 2.8% sialic acid. Fraction IV contained 8.7% uronic acid, 7.3% sialic acid and 0.4% hydroxyproline; it stained with Alcian Blue but not protein stains and it had an electrophoretic mobility of 2.0 relative to serum albumin.

Antisera, raised in rabbits to fraction I, sheep serum, sheep γ -globulin and a 3M-magnesium chloride extract of powdered sheep articular cartilage, were used to study the bone fractions by immunoelectrophoresis, double diffusion and 'tanned' erythrocyte haemagglutination techniques. Serum albumin was present in fractions I, II and III; fractions I and II also contained immunoglobulin G. Precipitin lines were obtained when antisera to fraction I were double-diffused against fraction I and its fractions: this was due to anti-(serum protein) activity. When this activity was adsorbed out with sheep serum no precipitin lines were

* Abbreviation: IgG, immunoglobulin G.

obtained. However, the adsorbed antisera to fraction I agglutinated 'tanned' sheep erythrocytes coated with fraction I or its fractions giving titres [$\log_2$ (maximum dilution for observable agglutination)] of 7–10. Antisera to sheep articular cartilage extract gave a very low titre against cells coated with sheep serum; the serum-adsorbed antisera had little reactivity with cells coated with cortical-bone fractions although, with articular-cartilage extract, they gave haemagglutination titres up to 14 and precipitin lines were obtained by double diffusion.

Herring, G. M. (1964). *Proc. 1st Eur. Bone and Tooth Symp., Oxford*, p. 263. Ed. by Blackwood, H. J. J. Oxford: Pergamon Press Ltd.

Some Properties of Embden-Goose Egg-White Albumin

By B. M. AUSTEN, ELZBIETA J. ROMANOWSKA and R. D. MARSHALL. (*Department of Chemical Pathology, St Mary's Hospital Medical School, London W.2, U.K.*)

Goose egg-white albumin was prepared by a procedure closely related to that (Warner, 1954) used in the isolation of the similar protein from the hen's egg. Final purification of the monomeric form was achieved by chromatography on 0.1M-sodium chloride on a Sephadex G-100 column. The protein exhibits three closely running bands on polyacrylamide-gel disc electrophoresis (Davis, 1964). A single band was observed on electrophoresis in polyacrylamide gels containing sodium dodecyl sulphate, and the molecular weight was found to be 47500 by this technique (Marshall & Zamecnik, 1969) as well as by chromatography on Sephadex G-100. The glycoprotein contains 24 alanine, 21 glycine, 30 valine, 30 leucine, 21 iso-leucine, 13 proline, 23 phenylalanine, 12 tyrosine, 7 tryptophan, 39 serine, 27 threonine, 8 half-cystine, 19 methionine, 12 arginine, 6 histidine, 23 lysine, 36 aspartic acid and 51 glutamic acid residues as well as 8 glucosamine and 12 mannose moieties/molecule. The main differences from hen egg-white albumin are higher threonine (11 additional residues/molecule), lower alanine (10 residues less/molecule) and greater carbohydrate contents.

An enzymic digest (pepsin at pH 2.8 followed by trypsin and chymotrypsin at pH 7.8) of 660mg of heat-denatured albumin was neutralized (to pH 5.4) and evaporated under reduced pressure to a volume of about 3ml. The portion of the residue that was insoluble in ethanol was chromatographed in 0.1M-acetic acid on a Sephadex G-50 column. Three fractions were obtained: the first (fraction I) (at the

void volume of the column) accounted for 22% of the original mannose of the protein, the second (fraction II) accounted for 20% of the mannose and the third (fraction III) accounted for about 50% of the mannose. The mannose/glucosamine molar ratios decreased in the order fraction I > fraction II > fraction III. Fraction III had a molecular weight, by gel-filtration methods, of about 1900 and contained 1.8mol of glucosamine, 1.7mol of mannose, 0.69mol of threonine, 0.55mol of serine and 0.35mol of glycine/mol of aspartic acid. Smaller amounts of other amino acids were also present.

Goose egg-white albumin therefore contains more than one carbohydrate prosthetic group/molecule. It is reasonable to deduce from this observation, and from the amino acid composition of the whole protein, that the necessary amino acid sequence for the formation of 4-*N*-(2-acetamido-2-deoxy- β -D-glucopyranosyl)-L-asparagine (see Marshall & Neuberger, 1970) occurs more frequently in this glycoprotein than it does in hen egg-white albumin.

E.J.R. is a British Council Visitor from the Polish Academy of Sciences, Wrocław, Poland.

Davis, B. J. (1964). *Ann. N.Y. Acad. Sci.* **121**, 404.
Marshall, R. D. & Neuberger, A. (1970). *Adv. Carbohydr. Chem. Biochem.* **25**, 407.
Marshall, R. D. & Zamecnik, P. C. (1969). *Biochim. biophys. Acta*, **181**, 454.
Warner, R. C. (1954). In *The Proteins*, 1st ed., vol. 2A, p. 435. Ed. by Neurath, H. & Bailey, K. New York: Academic Press Inc.

Cross-links in Protein from Cataractous Lenses

By R. H. BUCKINGHAM. (*Nuffield Laboratory of Ophthalmology, University of Oxford, Walton Street, Oxford OX2 6AW, U.K.*)

Human cataractous lenses removed at operation vary widely both in opacity and depth of colour when examined after extraction. Cataractous lenses with deep-brown or black nuclei contain about 15% of their total protein in a form that is insoluble in 6M-guanidinium chloride–50mM-dithiothreitol, a medium that dissolves the normal lens completely (Pirie, 1968). Cataractous lenses that are lighter in colour, but still with a golden-yellow nuclear core, yield little or no insoluble protein under such conditions. The proteins from these lenses, classed by Pirie (1968) as group III cataracts, have been studied in an attempt to account for the lens pathology and for the insolubility of proteins from darker lenses.

Group III cataractous lenses were decapsulated