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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.

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A New Enzymic Fragment (Facb) of Rabbit Immunoglobulin G

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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

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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.