chondromucoprotein used to prepare Sample 9 showed the incorporation of only 7 μmoles of bound hydrazine per gram. If this is corrected for protein content, it would represent at the most 10 μmoles of hydrazine per gram of chondroitin sulfate. These results are incompatible with an ester bond.

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PN 1243

Further investigations on the carbohydrate moiety of egg albumin*

It has been suggested that the bond linking carbohydrate to protein in egg albumin is that of an *N*-(β-aspartyl)glycosylamine[1,2] of *N*-acetylglucosamine[3–5]. Partial acid hydrolysis of a purified glycopeptide from the protein gave rise to small amounts of a substance, containing glucosamine and aspartic acid but no mannose, which behaved electrophoretically and chromatographically like 2-acetamido-1-β-(L-β-aspartamido)-1,2-dideoxy-D-glucose[6]. Further high-tension paper-electrophoretic studies (Whatman 3 MM, 40 V/cm, 60 min, pH 1.87) of partial acid hydrolysates (2 N HCl, 12 min, 100°) of the latter compound and of the glycopeptide from egg albumin have revealed a great similarity in the "fingerprint" obtained by staining the paper strip with the ninhydrin reagent described previously[7] (Fig. 1). In this Figure, Spot 2 in each case (brown with ninhydrin) was given by a substance which had the same mobility as 2-acetamido-1-β-(L-β-aspartamido)-1,2-dideoxy-D-glucose; Spot 3 (blue-purple) the same mobility as aspartic acid and Spot 8 (purple) the same mobility as glucosamine. Spot 8 was also Elson–Morgan positive in each case. The identity of the remainder

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of the spots is at present unknown but it is possible that Spot 7 was produced by 2-amino-1-β-(L-β-aspartamido)-1,2-dideoxy-D-glucose. Spot 9, which occurs solely in that electropherogram from the egg-albumin glycopeptide, might be produced by a similar deacetylated derivative but containing a further residue of glucosamine with a free amino group. Paper chromatography in phenol, of similar partial acid hydrolysates of both substances, has revealed in each case the presence of a substance staining brown with ninhydrin with an R_F value of 0.37, the same as that of the unhydrolysed model compound.

Similar electrophoretic experiments with partial acid hydrolysates of 2-(L-β-aspartamido)-2-deoxy-D-glucose[8] have indicated the presence of glucosamine, aspartic acid, a compound staining purple with ninhydrin with minimal mobility, and some of the original substance. Paper chromatography, in phenol, of a partial acid hydrolysate of 2-(L-β-aspartamido)-2-deoxy-D-glucose gave rise to a substance (R_F = 0.04) staining brown with ninhydrin, similar to the original substance.

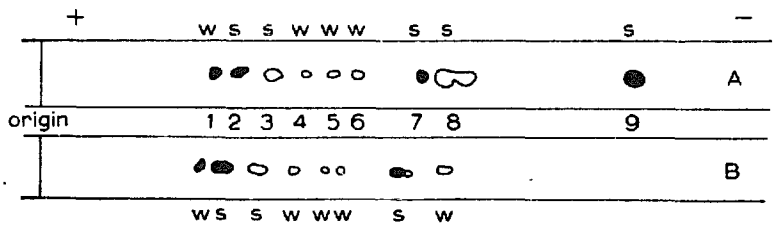

Fig. 1. "Fingerprints" obtained by paper electrophoresis (Whatman 3 MM, 40 V/cm, 60 min, pH 1.87) of partial acid hydrolysates (2 N HCl, 12 min, 100°) of (A) egg-albumin glycopeptide and (B) 2-acetamido-1-β-(L-β-aspartamido)-1,2-dideoxy-D-glucose. The strips were stained with ninhydrin[7]. A purple colour is represented by an open area, and brown by a black area: W = weak, S = strong.

By comparing ultraviolet-absorption measurements in water at 205 mμ with those of *N*-acetylglucosamine, we have found that the glycopeptide with aspartic acid as the only amino acid constituent contains 3.85 peptide bonds. This value was based on weight measurements of the lyophilised glycopeptide after correction for moisture content and absorption due to carboxylate ion[9]. This value would be expected if there were one amide bond in the carbohydrate–protein linkage, together with the three *N*-acetylglucosamine bonds[7]. We do not agree with the recent suggestion[10] of the presence of four glucosamine residues in the protein.

The infrared spectrum of the glycopeptide (solid phase, NaCl) revealed the presence of absorption bands due to amide bonds and the anticipated absence of bands due to esters.

Molecular-weight estimations of the pure egg-albumin glycopeptide have been carried out by ultraviolet-absorption measurements in ethanol–water (30:70, v/v) after substitution of the free α-amino group (of the asparagine moiety) with the benzyloxycarbonyl group[7]. The spectrum of this substance is represented in Fig. 2, showing three peaks, a, b and c. The molar extinction coefficients for the comparable three peaks exhibited by 1-β-(*N*-benzyloxycarbonyl-L-β-aspartamido)-1-deoxy-D-glucose[8] were 167 for Peak a, 205 for Peak b and 119 for Peak c; for *N*-benzyloxycarbonylglucosamine 166 (a), 213 (b) and 115 (c); for *N*-benzyloxycarbonyl-α-methylglucosaminide 168 (a), 215 (b) and 114 (c); for *N*-benzyloxycarbonyl-α-amino-β-hydroxyadipic acid[11] 173 (a), 206 (b) and 115 (c); for *N*-benzyloxycarbonylaspartic-β semialdehyde[12] 168 (a), 209 (b) and 119 (c). (The last compound was kindly given

by Dr. G. H. TAIT.) The molecular weight of the *N*-benzyloxycarbonyl glycopeptide was calculated from these data and from measurements of the absorbancy of a weighed quantity in a known volume of solution. Corrections were applied for the moisture content of the material and for the small contribution made to the absorption by the glycopeptide itself. Values of 1710, 1750 and 1660 were obtained for Peaks a, b and c, respectively. The mean value for the glycopeptide unsubstituted by the benzyloxycarbonyl group is thus 1570, in good agreement with the value of

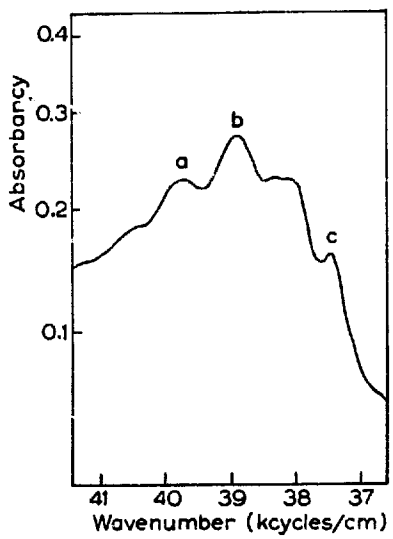

Fig. 2. Spectrum of the *N*-benzyloxycarbonyl derivative of egg-albumin glycopeptide (2.31 mg/ml in 1 cm cell) in ethanol–water (30:70, v/v). The results were obtained using a Unicam SP-700 recording spectrophotometer.

1580 found by KAVERZNEVA AND BOGDANOV[13] by titration methods. The molecular weight required for a glycopeptide consisting of 5 mannose, 3 *N*-acetylglucosamine and 1 asparagine residues is 1551. A radioisotope-dilution method indicated the presence of 5 moles of mannose in the whole protein[14] in agreement with most estimates obtained by colorimetric assay. However, other workers, using colorimetric procedures, have suggested the presence of 6 residues[10,15] which might be explained in one of two ways: either the protein contains one single mannose residue not bound to the main heterosaccharide, which is very unlikely[1], or egg albumin from different strains of hen contains different amounts of mannose. Moreover, colorimetric procedures, which may be non-specific, have to be interpreted with caution.

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An intermolecular defect of collagen in experimental lathyrism*

The experimental disease, osteolathyrism, induced in a variety of animals by agents such as β-aminopropionitrile is characterized by mesenchymal deformities[1], loss of tensile strength, and dramatic increases in collagen solubility[2]. It has been proposed that the large pool of extractable collagen is derived from old insoluble fibrils transformed to an extractable state[2]. A contrary view holds that newly synthesized molecules are prevented from polymerizing to fibrils[3]. While the molecular dimensions, conformation and fibril-forming ability of lathyritic collagen seems not to be grossly altered[2] there is evidence for a failure of intramolecular cross-linking characteristic of the maturation process[4,5].

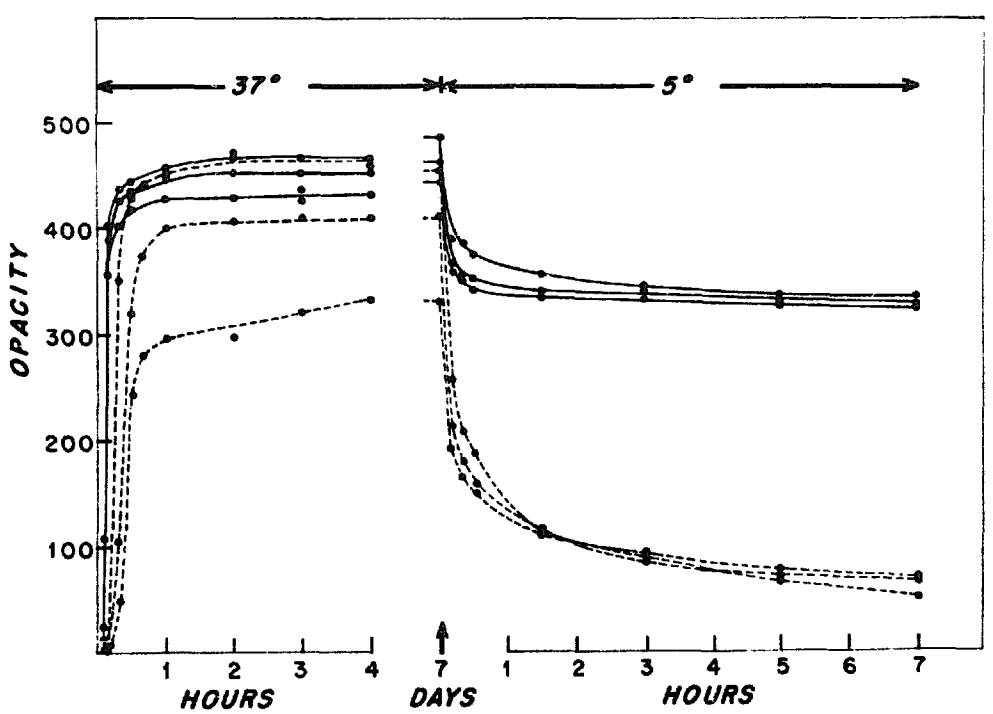

Fig. 1. Thermal gelation of NaCl purified normal (———) and lathyritic (------) collagen (7 days at 37°).

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