pregnancy, and the foetal plasma concentration is considerably higher than the maternal. In contrast, the pregnant rhesus monkey was found to increase its plasma concentration of cystine from 4.7 ± 0.5 to $8.1 \pm 0.5 \mu\text{mol}/100\text{ml}$; the foetal plasma cystine concentration was intermediate.

Four pregnant rhesus monkeys were injected with 5mCi (0.25mmol) of L-[^{35}S]methionine or L-[^{35}S]cystine into the cubital veins of the mothers or into the umbilical veins for the foetuses, under Sernylan anaesthesia, and blood samples were withdrawn from indwelling catheters in the femoral veins of mother and foetus at 0, 15, 30, 45 and 60 min after injection. Amino acids and radioactive compounds were measured with an automatic amino acid analyser coupled with a flow-cell scintillation counter (Sturman, Cohen & Gaull, 1970).

When methionine was injected into the mother, it was accumulated by the foetus, which maintained its usual threefold higher concentration. The specific radioactivity of methionine in foetal plasma exceeded that in maternal plasma for much of the experiment. When methionine was injected into the foetus, the concentration of methionine in foetal plasma, and its specific radioactivity, remained higher than in maternal plasma.

When cystine was injected, in each case the circulation injected had the highest concentrations and highest specific radioactivities of cystine for the duration of the experiments, the concentration of cystine in the secondary circulation being increased relatively little.

These results indicate that the foetus can quickly accumulate methionine from the maternal circulation against a concentration gradient, as it can for other amino acids; however, it cannot similarly accumulate cystine from the maternal circulation. A load of methionine or cystine in the foetal circulation was dissipated into the maternal circulation very slowly. This inability of the foetal placenta to concentrate cystine against a gradient may account for the anomalous behaviour of cystine during pregnancy in the rhesus monkey.

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Peptides from Human Blood-Group-Specific Glycoproteins

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Human blood-group-specific glycoproteins isolated from ovarian-cyst fluids were subjected to degradation with 0.25M-sulphuric acid in acetic acid (Donald, Creeth, Morgan & Watkins, 1969). The products were considerably enriched in peptide compared with the original glycoprotein, and the amino acid composition was largely unchanged. Most of the remaining sugar was *N*-acetylgalactosamine, but small amounts of galactose and *N*-acetylglucosamine were also present even after 24 h treatment. Periodate oxidation, followed by reduction with sodium borohydride and acid hydrolysis of the resulting polyalcohols (Abdel-Akher, Hamilton, Montgomery & Smith, 1952), was used in attempts to remove the residual carbohydrate. The oxidized and reduced fragment of *N*-acetylgalactosamine glycosidically linked to the hydroxy amino acids in the peptide was resistant to hydrolysis under the mild acid conditions normally employed in the Smith degradation; this fragment was liberated by treatment with 0.2M-hydrochloric acid for 1.5 h at 100°C. After three cycles of oxidation, reduction and hydrolysis, small yields of non-diffusible peptide were obtained that were free from sugar. The amino acid compositions of these materials were closely similar to that of the parent glycoproteins, but paper electrophoresis at pH 1.9 revealed them to be mixtures of many different peptides.

The peptides from the degradation products of a blood-group A substance (no. 401) were separated by column chromatography on Sephadex G-100, G-50 and G-25 and by preparative electrophoresis at pH 1.9. Nineteen peptides, judged to be pure on the basis of (1) their electrophoretic behaviour, (2) the molar proportions of the component amino acids and (3) the fact that they gave only one *N*-terminal acid by the dansyl procedure (Gray, 1967), were isolated in sufficient yield for partial sequence analysis by the dansyl-Edman method (Gray, 1967). From their behaviour on Sephadex columns and their amino acid composition these peptides contained 10–20 amino acid residues. Four peptides, differing in overall composition, had the *N*-terminal sequence Ala-Gly-Ser, three had Ala-Gly-Gly, two had Asp-Gly-Gly, two had Ser-Gly-Gly and two had Thr-Pro-Gly. The sequences of the other six peptides were Gly-Ala-Gly, Gly-Gly-Ser, Asp-Asp-Gly, Glu-Gly-Ala, Ala-Gly-Ala and Gly-Pro-Ala-Gly. Three peptides obtained from a blood-group B glycoprotein (no. 531) had the

sequences Ala-Gly-Ser, Ala-Gly-Gly and Ser-Gly-Ala. These preliminary experiments indicate that similar degradation procedures, carried out on a larger scale, should yield peptides from blood-group-specific glycoproteins suitable for detailed sequence analysis.

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Isolation, Purification and Characterization of a 0.6S γ_2 -Globulin from Normal Human Plasma

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Of the large number of proteins that have been isolated from normal human plasma, most are non-dialyzeable and of relatively high molecular weight. In contrast, in this communication, we now report the isolation of a diffusible low-molecular-weight protein fraction and the purification and partial characterization of one of its constituents.

Pooled normal human plasma was fractionated by Cohn method 6 (Cohn *et al.* 1946). The proteins present in the supernatant solution of fraction V were rendered insoluble by a previously described procedure (Bürgi & Schmid, 1961), dissolved at -5°C with EDTA and then separated on a DEAE-cellulose column at pH 5.5 into two fractions (Iwasaki & Schmid, 1967). The main fraction was then separated into Zn^{2+} -soluble and Zn^{2+} -precipitable proteins (Schmid, 1953), and the precipitate was subsequently solubilized and further purified by column chromatography on DEAE-cellulose at pH 5.4. The bulk of the proteins was retained, and the effluent fraction contained γ_2 -, γ_1 - and β -globulins as judged by paper electrophoresis at pH 8.6. On ultracentrifugal analysis this mixture revealed a single symmetrical refractive-index gradient, sedimenting with a coefficient of 0.6S. Purification of one of these components, a γ_2 -globulin, was subsequently achieved by ion-exchange chromatography on TEAE- (triethylaminoethyl-) and CM-cellulose. Homogeneity of this protein was established by polyacrylamide-gel electrophoresis at pH 8.3 and 4.5 and N- and C-terminal amino acid analyses.

Characterization of the homogeneous 0.6S γ_2 -globulin indicated that this protein was devoid

of neutral hexoses and sialic acid. The molecular weight determined by the sedimentation-equilibrium technique of Yphantis (1964) was found to be 5100. With regard to its conformation, preliminary analysis of circular-dichroism spectra suggest that this protein possesses a small percentage of α -helix and contains considerable amounts of β -structure and random coil.

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The Complete Hydrolysis of Peptides by a Mixture of Sepharose-Bound Peptidases

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Hill & Schmidt (1962) reported that incubation of polypeptides with a series of peptidases resulted in quantitative non-destructive release of amino acids. The method offers advantages over acid hydrolysis where destruction of serine, tyrosine, tryptophan, asparagine and glutamine occur.

To simplify the procedure we used a mixture of prolidase, aminopeptidase-M, trypsin and chymotrypsin bound to Sepharose (Hurwitz, Dietrich & Sela, 1970). Prolidase was prepared by using the first two steps of the method of Davis & Smith (1957). All the bound enzymes were found to be stable.

The bound peptidases (200 mg each of Sepharose-trypsin, Sepharose-chymotrypsin and Sepharose-prolidase and 400 mg of Sepharose-aminopeptidase-M) were stored as a mixture in 10 ml of tris buffer (50 mM, pH 7.4, 0.03% toluene, 0.5% thiodiglycol and 10 mM- Mn^{2+}). Incubations were performed with a 50–200 μl sample of the mixture (1 ml/mg of substrate) in a stoppered polystyrene tube. The mixtures were agitated gently for 24 h at 25°C . Blank amino acid contents when the slurry was incubated alone were equivalent to less than 3% of a single residue and were subtracted from the amino acid values obtained from substrates. Repeat digestions of synthetic β 1–24 adrenocorticotrophin (Synacthen; CIBA, Basle, Switzerland) revealed theoretical amino acid molar