which the contact between antigen and antibody molecules is much less likely to occur than when both are in solution.

The potential usefulness of the solid-phase radioimmunoassay for simplified determination of antigen in the micromicrogram range is shown by these experiments with human growth hormone as antigen. The technical aspect of this method involves no more than the addition of standard and unknown solutions to small quantities of the solid-phase-antibody, followed by a series of washing steps before and after the addition of tracer. The entire procedure can be performed in a vial suitable for counting in a well-type γ -radiation counter. The present method of assay has been further simplified by the preparation of the antibody-coated solid phase in the form of thin wafers of uniform size and surface area, one of which can simply be dropped into the assay vial with each portion of unknown or standard solution, and antigen-antibody systems other than human

growth hormone have also been investigated by this method (K. Catt, H. D. Niall & G. W. Tregear, unpublished work).

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The Isolation of *O*- β -D-Galactopyranosyl-(1 $\rightarrow$ 4)-D-galactose: a Product of the Partial Acid Hydrolysis of the Galactan of White-Lupin (*Lupinus albus*) Seeds

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Investigations in the last few years have shown that many mammalian tissues contain a range of neutral glycolipids as minor components of the total tissue lipid. They have been characterized as ceramide monohexosides, dihexosides, trihexosides and *N*-acetylhexosaminyltrihexosides with the carbohydrate moiety linked to the ceramide through the reducing group of either glucose or, more rarely, galactose. Some glycolipids were shown to be haptens (Rapport, Graf, Skipski & Alonzo, 1959; Joffe, Rapport & Graf, 1963) and the differences in immunological specificities were due to differences in the carbohydrate structures in the molecules. A ceramide dihexoside, cytolipin H, a lipid hapten found originally in human epidermoid carcinoma grown in conditioned rats

(Rapport *et al.* 1959) but now known to occur in a variety of normal and tumour tissues, has been completely identified by chemical synthesis (Shapiro & Rachaman, 1964) as 1-*O*- β -lactosyl-ceramide.

Recent evidence indicates that several ceramide dihexosides from natural sources contained not only ceramide lactoside but also a ceramide digalactoside, and methylation studies by Makita & Yamakawa (1965) show that the structure of a ceramide digalactoside in human kidney was galactosyl-(1 $\rightarrow$ 4)-galactosyl-(1 $\rightarrow$ 1)-ceramide. The pure ceramide dihexoside has not yet been isolated from natural sources.

Because of the close association of ceramide digalactoside with cytolipin H in mammalian

tissues its complete chemical identification by synthesis is of interest. The penultimate stage of the synthesis of ceramide digalactoside by the procedure of Shapiro & Rachaman (1964) involves the condensation of a ceramide derivative with the acetobromo derivative of *O*- β -D-galactopyranosyl-(1 $\rightarrow$ 4)-D-galactose. The synthesis of this dihexose is difficult, but Curtis & Jones (1965) have isolated β -(1 $\rightarrow$ 4)-galactobiose from a synthetic mixture of disaccharides consisting mainly of β -(1 $\rightarrow$ 6)-galactobiose. β -(1 $\rightarrow$ 4)-Galactobiose has, however, been isolated from the products of the partial hydrolysis of a naturally occurring galactan in spruce bark (Bouveng & Meier, 1959) and of an α -cellulose from white birch (Gillham, Perlin & Timell, 1958). Hirst, Jones & Walder (1947) found that white-lupin (*Lupinus albus*) seeds were a rich source of a galactan in which the galactose residues were joined through β -(1 $\rightarrow$ 4)-linkages. The present communication reports the isolation of β -(1 $\rightarrow$ 4)-galactobiose from the products of a partial hydrolysis of the galactan from *Lupinus albus*.

The method of preparation of the galactan described by Hirst *et al.* (1947) gave, in our hands, a product that was contaminated with xylose, arabinose and uronic acid. However, by a modification (W. J. Whelan, personal communication) of the method pure galactan was obtained. The lupin seeds (2 kg. of *Lupinus albus* from Carters Tested Seeds Ltd., Reading, Berks.) were allowed to swell in water overnight, the shells removed and the inner portions homogenized in 4 l. of 10% (w/v) NaCl solution. The homogenate was centrifuged at low speed (about 1200 rev./min. in the MSE Major centrifuge) and the residue was extracted by stirring with 4 l. of 10% (w/v) NaCl solution for 3 hr. at room temperature. The supernatant solution after centrifugation was removed and the residue extracted in the same way two more times. It was then extracted successively with four 4 l. portions of aq. 0.2% NaOH for 12 hr. at room temperature. The residue was at this stage free from protein, and the polysaccharide was obtained from it by boiling with 4 l. of aq. 0.2% NaOH for 3 hr. After centrifugation the supernatant solution was separated and the polysaccharide was precipitated from it by the addition of methanol (4 vol.). The precipitate was washed with ether and dried. The crude galactan was purified by extracting with 800 ml. of aq. 30% (w/v) formic acid for 15 min. at 100°. The extract was cooled to about 40° and centrifuged. The residue was extracted a further three times with 600 ml. of aq. 30% (w/v) formic acid for 15 min. at 100°. Methanol (5 vol.) was added to the supernatant from each of the four extractions, and the precipitates of polysaccharide were combined and washed with methanol and ether. At this stage the acid-hydrolysis products of a sample

of the polysaccharide consisted mainly of galactose, but appreciable quantities of xylose, arabinose and uronic acid were still present; amino acids were not detected. The galactan was extracted four more times, each with 500 ml. of aq. 30% (w/v) formic acid at 100° for 15 min. The product, recovered from the extracts by precipitation with methanol as before, was dissolved in 200 ml. of hot distilled water and poured, with stirring, into 1200 ml. of methanol. The precipitated galactan was recovered, washed with methanol and ether, and dried (yield, 42 g.). Galactose was the only product of acid hydrolysis of the galactan.

To obtain the disaccharide the galactan (40 g.) was hydrolysed by 3 l. of 0.066 N-H₂SO₄ at 100° for 8 hr., and the hydrolysate was cooled and neutralized (pH 7) with aq. NaOH. The disaccharide was separated from the other products of hydrolysis by chromatography on a column of charcoal-Celite (1:1, w/w) and eluted with aq. 0.01 N-formic acid, as described by Taylor & Whelan (1963). The eluate from the column (3 in. diam. $\times$ 48 in. long) was collected in 400 ml. fractions and their carbohydrate content determined with the phenol-H₂SO₄ reagent (Dubois, Gilles, Hamilton, Roben & Smith, 1956). Galactose was eluted in fractions 17–37. The eluting solvent was then adjusted to produce an ethanol gradient. An upper reservoir containing 21 l. of 15% (v/v) ethanol in aq. 0.01 N-formic acid and a lower reservoir of 21 l. of aq. 0.01 N-formic acid were connected to the column as described by Taylor & Whelan (1963). The disaccharide was recovered in fractions 72–102 (10–12% ethanol). The combined fractions were evaporated under reduced pressure to about 500 ml. and neutralized by adding D-Acidite E resin (CO₃²⁻ form). The residue was filtered off, the filtrate was freeze-dried and the residue obtained was decolorized by boiling with activated charcoal in 80% (v/v) methanol. The yield of disaccharide was 1.2 g.

The disaccharide was obtained crystalline by dissolving 50 mg. in 0.5 ml. of water, adding 5 ml. of acetone and keeping the solution at -10° for 2 days. The crystals had m.p. 202° (uncorrected) [reported: 200–202° (Curtis & Jones, 1965); 204° (Gillham *et al.* 1958)] and $[\alpha]_{25}^D + 66 \pm 1.5^\circ$ (c 2.47 in water). The infrared spectrum (KBr disk) showed bands at 11.07 and 11.4 μ . The characteristic bands reported for a methyl β -galactoside were 11.2 and 11.4 μ (Kuhn, 1950). Chromatography of the disaccharide on Whatman no. 1 paper in the solvent system ethyl acetate-pyridine-water (10:4:3, by vol.) gave a single spot $R_{\text{galactose}}$ 0.81 (cf. lactose, $R_{\text{galactose}}$ 0.78). Galactose was the only product of acid hydrolysis. The disaccharide (2 mg.) was methylated (Perila & Bishop, 1961) and then methanolysed with methanolic 2% HCl. The

methyl ethers were dissolved in hexane and analysed by gas-liquid chromatography.

Two columns were used: *A* (4mm. diam. $\times$ 120cm. long), 10% Carbowax 20M on Gas-Chrom CLH (80–100 mesh) used at 155°; *B* (4mm. diam. $\times$ 150cm. long), 15% EGSSX on Gas-Chrom CLH (80–100 mesh) used at 160°. Retention volumes on both columns were measured relative to that of authentic methyl 2,3,4,6-tetra-*O*-methyl- β -galactopyranoside. Peaks were obtained on *A* with relative retention volumes (*r*) 1.00, 1.9, 2.1, 2.5 and 2.65. The chromatographic characteristics of the last four peaks were similar to those obtained from an authentic methyl 2,3,6-tri-*O*-methylgalactoside. On column *B* six peaks were obtained with *r* values 1.00, 1.07, 1.93, 2.42, 2.63 and 2.89. The first two were similar to those of methyl 2,3,4,6-tetra-*O*-methyl- α -galactopyranoside and methyl 2,3,4,6-tetra-*O*-methyl- β -galactopyranoside and the last four to the different forms of authentic methyl 2,3,6-tri-*O*-methylgalactoside. The optical rotation and infrared spectrum showed that the disaccharide was a β -anomer, and thus it was concluded that its structure was *O*- β -D-galactopyranosyl-(1 $\rightarrow$ 4)-D-galactose.

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Heterogeneity of Glycopeptides from a Homogeneous Immunoglobulin

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Variations in the proportions of certain monosaccharide residues have been reported (Franklin & Dische, 1962; Cunningham, Clause & Ford, 1963; Beeley & Jevons, 1965; Clamp, Dawson & Putnam, 1965; Cunningham, Ford & Rainey, 1965) in a number of individual glycoproteins. However, these variations could have been due to the use of a pooled sample from several animals or, if the sample was from a single animal, the variations could have arisen from different cell types. If variations could be demonstrated in the oligosaccharide units of glycoproteins produced by a homogeneous cell population this would be of considerable value in investigations of the structure and the mechanism of biosynthesis of these units. Such heterogeneity has now been shown in a

myeloma globulin, which is not only the product of a single individual but which probably arises from a single clone of malignant cells.

An A myeloma globulin, type K, was prepared from human serum by a combination of $(\text{NH}_4)_2\text{SO}_4$ fractionation and chromatography on DEAE-cellulose (Bernier, Tominaga, Easley & Putnam, 1965), and after isolation appeared homogeneous by starch-gel and immuno-electrophoresis. The myeloma globulin was digested with Pronase, and glycopeptide material was isolated by exclusion chromatography on Sephadex G-25 followed by chromatography on paper (Clamp & Putnam, 1964).

The carbohydrate contents of the myeloma globulin and glycopeptide material were estimated