A Mannosyl-Carrier Lipid of Bovine Adrenal Medulla and Rat Parotid

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1. The transfer of mannose from GDP-[U-¹⁴C]mannose into endogenous acceptors of bovine adrenal medulla and rat parotid was studied. The rapidly labelled product, a glycolipid, was partially purified and characterized. 2. It was stable to mild alkaline hydrolysis but yielded [¹⁴C]mannose on mild acid hydrolysis. It co-chromatographed with mannosyl phosphoryl dolichol in four t.l.c. systems and on DEAE-cellulose acetate. Addition of dolichol phosphate or a dolichol phosphate-enriched fraction prepared from pig liver stimulated mannosyl synthesis. 3. The formation of mannosyl appeared reversible, since addition of GDP to a system synthesizing the mannosyl caused a rapid loss of label from the mannosyl. UDP-*N*-acetylglucosamine did not inhibit mannosyl synthesis except at high concentrations (2 mM), even though in the absence of GDP-mannose, *N*-acetylglucosamine was incorporated into a lipid having the properties of a glycosylated polyprenyl phosphate. 4. Mannose from GDP-mannose was also incorporated into two other acceptors, (i) being insoluble in chloroform-methanol (2:1, v/v) but soluble in chloroform-methanol-water (10:10:3, by vol.) and (ii) protein. These are formed much more slowly than the mannosyl. 5. Exogenous mannosyl served as a mannose donor for acceptors (i) and (ii), and it is suggested that transfer of mannose from GDP-mannose to mannosylated protein occurs via two intermediates, the mannosyl and acceptor (i).

Glycosyl-carrier lipids (polyisoprenol phosphates) have been shown to be involved in the biosynthesis of many bacterial glycans, catalysing the transfer of sugar from nucleotide sugars to growing glycan chains (Rothfield & Romeo, 1971). More recently a role for similar lipids has been demonstrated in many mammalian tissues and also yeast and plant systems (Lennarz & Scher, 1972). The microsomal fraction of these mammalian tissues catalyses the transfer of sugar from a nucleotide sugar to unidentified glycoproteins via an intermediate polyisoprenol phosphate sugar. The lipid intermediate involved in such a reaction in liver has been isolated and characterized as α -D-mannopyranosyldolichyl phosphate by Evans & Hemming (1973) and Baynes *et al.* (1973) and confirmed by synthesis of the compound by Warren & Jeanloz (1973).

Studies by the group of Leloir (Behrens *et al.*, 1971; Parodi *et al.*, 1972) on the transfer of glucose from UDP-glucose to endogenous acceptors in liver indicated the presence of a further intermediate. This acceptor, soluble in chloroform-methanol-water (10:10:3 by vol.), appeared to be a lipophilic oligosaccharide consisting of a chain of about 20 monosaccharide units linked through a pyrophosphate bond

to dolichol. A detailed kinetic study by Waechter *et al.* (1973) revealed that mannose from GDP-mannose was incorporated into three endogenous acceptors of hen oviduct, and from the relative rates of incorporation of mannose into each suggested the following scheme:

where B and C represent two unidentified endogenous acceptors (EA) differing in their solubility properties. Mannosyl s-EA is soluble in chloroform-methanol-water (10:10:3, by vol.); mannosyl r-EA is insoluble in aqueous and organic solvents and appears to be a glycoprotein.

The compound originally defined as hen mannosyl s-EA formed by hen oviduct membranes has been partially characterized as a mannose-containing oligosaccharide phospholipid and has been shown to serve as an oligosaccharide donor in glycoprotein assembly (Lucas *et al.*, 1975). Mannose-containing oligosaccharide phospholipids have also been shown to function in glycoprotein biosynthesis in rat liver (Behrens *et al.*, 1973) and mouse myeloma (Hsu *et al.*, 1974).

The present paper describes the incorporation of mannose from GDP-mannose into bovine adrenal medulla and rat parotid. Both tissues package

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secretory material into membrane-surrounded vesicles before export and are continually making glycoproteins, essential components of these membranes.

Materials and Methods

GDP, GMP and GDP-mannose were purchased from Boehringer Corp. (London) Ltd. (London W.5, U.K.). GDP-[U- 14 C]mannose was purchased from The Radiochemical Centre (Amersham, Bucks., U.K.). Tissue solubilizer TS1 was a gift from Koch-Light Ltd. (Colnbrook, Bucks., U.K.). All other chemicals and solvents were A.R. grade and obtained from commercial suppliers. Dolichol phosphate was a gift from Dr. F. W. Hemming (University of Liverpool). An endogenous acceptor lipid preparation was prepared from a total pig liver lipid extract as described by Behrens & Leloir (1970), followed by chromatography on silicic acid and DEAE-cellulose as described by Richards & Hemming (1972).

Protein was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as a standard, and lipid phosphorus by the method of Bartlett (1959).

Preparation of microsomal fractions

Bovine adrenal glands were obtained from Nottingham City Abattoir immediately after slaughter and stored in ice. A microsomal fraction was prepared as described by Smith & Winkler (1967) except that the final spin was at 100000g for 2h. The pellet was resuspended in 0.3M-sucrose, buffered with Tris-maleate to pH7.0, or in Tris-maleate buffer (100mM), pH7.0, to a final protein concentration of approx. 50mg of protein/ml. This suspension was stored in portions of about 500 μ l at -20°C until use.

Parotid glands were dissected from rats killed by cervical fracture and homogenized in 0.3M-sucrose buffered to pH7.0 with Tris-maleate (50mM) in a Potter-Elvehjem homogenizer (radial clearance 0.5mm). The fractionation scheme of Amsterdam *et al.* (1971) was followed to yield a microsomal preparation of approx. 30mg of protein/ml. This was stored in volumes of about 500 μ l at -20°C . Up to 30% of mannosyltransferase activity was lost on storage for 1 week, and the preparation lost activity rapidly on freezing and thawing.

Chromatographic methods

T.l.c. was performed on silica gel H in the following solvent systems: A, chloroform-methanol-formic acid-water (140:30:17:1, by vol.); B, chloroform-methanol-water (65:25:4, by vol.), C, di-isobutyl ketone-acetic acid-water (65:45:6, by vol.); D,

chloroform-methanol-aq. NH_3 (sp.gr. 0.880) (13:5:1, by vol.); and on Kieselgel G in solvent system E, ethyl acetate-propan-2-ol-water (17:6:3, by vol.). Phospholipids were detected by staining with iodine vapour or by spraying with the molybdate reagent of Dittmer & Lester (1964). Sugars were located by spraying with the anisaldehyde- H_2SO_4 reagent. Developed chromatograms were scanned for radioactivity with a Panax radiochromatogram scanner and confirmed by radioautography with Kodak X-ray-sensitive paper. For quantitative measurements, the radioactive areas were scraped from the plate and counted for radioactivity in the xylene-Triton-based scintillation medium described by White *et al.* (1971). Paper chromatography of sugars and sugar phosphates was carried out on Whatman 3MM paper in solvent F, ethyl acetate-pyridine-water (12:5:4, by vol.).

DEAE-cellulose chromatography as described by Baynes *et al.* (1973) was used to purify partially the mannosyl lipid from both adrenal medulla and rat parotid.

Hydrolysis of mannosyl lipid

(i) *Mild acid hydrolysis.* Partially purified mannosyl lipid (20000c.p.m.) was sonicated at 45kHz in a Kerry sonicator (Kerry Ultrasonics, Hitchin, Herts., U.K.) for 30s in 0.1M-HCl in aq. 50% (v/v) propan-1-ol (1ml) and incubated at 50°C . Samples (0.1ml) were removed at various time-intervals and added to chloroform-methanol (2:1, v/v) (4ml), and 0.01M-NaOH (1ml) was added to separate the phases. Both aqueous and organic phases were assayed for radioactivity.

(ii) *Strong acid hydrolysis.* Partially purified mannosyl lipid was sonicated as described above in 0.1M-HCl (1ml) and incubated for 10min in a boiling-water bath. The solution was cooled and neutralized with 0.4M-NaOH (250 μ l) and chloroform-methanol (1:1, v/v) (3ml) was added to give a two-phase system. Unlabelled mannose as carrier was added to the upper phase. A portion (0.1ml) of the upper phase was removed for radioactivity counting, and the rest was evaporated to dryness under reduced pressure and redissolved in water (100 μ l) before t.l.c. in solvent E or paper chromatography in solvent system F.

(iii) *Alkaline hydrolysis.* Purified mannosyl lipid (20000c.p.m.) was sonicated as described above in 0.5M-NaOH in 90% (v/v) ethanol (1ml) and incubated either at room temperature (25°C) for 15min or at 80°C for 2h. After neutralization with 0.05M-acetic acid aqueous and organic phases were obtained as described for the acid hydrolysis. Paper chromatography of the aqueous phase was performed in solvent F.

Assay of the incorporation of [U-¹⁴C]mannose from GDP-[U-¹⁴C]mannose into endogenous microsomal acceptors

The standard assay mixture contained 4 μ M-GDP-mannose (89000 d.p.m.), 5 mM-MnCl₂, approx. 2.5 mg of microsomal protein and 100 mM-Tris-maleate, pH 7.0, in a final incubation volume of 100 μ l. The reaction was started by addition of GDP-mannose and incubated at 37°C. Reactions were terminated by addition of chloroform-methanol (2:1, v/v; 2 ml) and three fractions were obtained as described by Waechter *et al.* (1973), i.e. (i) soluble in chloroform-methanol (2:1, v/v), (ii) soluble in chloroform-methanol-water (10:10:3, by vol.) and (iii) an insoluble fraction. Portions of fractions (i) and (ii) were taken for chromatography and the rest of each fraction was counted for radioactivity in the xylene-Triton phosphor described above.

Incorporation of [¹⁴C]mannose from mannolipid into endogenous acceptors

Approx. 80000 c.p.m. of partially purified mannolipid was sonicated in 1% Triton (700 μ l) to give a transparent solution and samples (50 μ l) were added to tubes containing microsomal protein (200 μ l) and 100 mM-Tris-maleate, pH 7.0 (25 μ l). After incubation at 37°C the reaction was terminated by addition of chloroform-methanol (2:1, v/v; 5 ml). The pellet was removed by centrifugation and re-extracted with chloroform-methanol (2:1, v/v; 2 ml). The extracts were combined and 0.9% NaCl (1.75 ml) was added to separate the phases. Each phase was assayed for radioactivity; the organic phase contained unchanged substrate and the aqueous phase any water-soluble activity derived from hydrolysis of the mannolipid.

The extracted pellet was washed three times by sonication at 45 kHz for 2 min in water and then extracted twice with chloroform-methanol-water (10:10:3, by vol.; 2 ml). This extract was also evaporated to dryness and assayed for radioactivity to give a measure of the formation of mannosyl s-EA. The residual pellet after the above extractions was air-dried, solubilized with TS1 and assayed for radioactivity.

Results

For all the experiments shown in the Materials and Methods section, results for bovine adrenal medulla and rat parotid were essentially the same, so only results from bovine adrenal medulla are given.

The optimal conditions for synthesis of mannolipid from GDP-mannose by using endogenous acceptor lipid of bovine adrenal-medulla microsomal fraction are shown in Fig. 1. There is a bivalent-cation

requirement, which is optimal at 5 mM-Mn²⁺, although other cations such as Ca²⁺, Mg²⁺, Co²⁺, Bi²⁺ and Pb²⁺ are also effective, but to a lesser extent. The activity in the absence of added bivalent cations can be eliminated by addition of EDTA. Incorporation of mannose into mannolipid is linear with protein to a concentration of approx. 20 mg of protein/ml and is optimal at pH 7.0. GDP-mannose is saturating at a concentration of approx. 2 μ M.

The time-course of incorporation of [¹⁴C]mannose from GDP-mannose into endogenous acceptors is shown in Fig. 2. There is rapid incorporation into a mannolipid, soluble in chloroform-methanol (2:1, v/v). This was linear for about 10 min and gradually reached a plateau, which was maintained even after 90 min. A second product, mannosyl s-EA, which is soluble in chloroform-methanol-water (10:10:3, by vol.), is formed at a much slower rate and reaches a maximum after 30 min. Incorporation of label into the insoluble pellet is very slow.

The effect of GMP and GDP on the formation of mannolipid is shown in Fig. 3. Mannolipid synthesis was allowed to proceed for 10 min and GDP or GMP (2 mM) was then added before further incubation. GDP caused an immediate rapid loss of activity from the mannolipid, whereas GMP had no immediate effect, although there was some loss after 20 min. This suggests that GDP and not GMP is the end product of mannolipid synthesis and that mannose is linked to the lipid via a phosphodiester rather than a pyrophosphate bond.

Properties of mannolipid

Stability to hydrolysis. Mannolipid, which had been partially purified on DEAE-cellulose, was unstable to mild acid hydrolysis in 0.1 M-HCl at 50°C. Approx. 50% of the starting material was hydrolysed in 14 min and mannose was identified as the only radioactive product, by co-chromatography with unlabelled mannose on Whatman 3 MM paper in solvent F and on t.l.c. in solvent E. Only one radioactive peak was seen on the radiochromatogram traces.

The mannolipid was stable to 0.5 M-NaOH in 90% (v/v) ethanol at room temperature; no water-soluble material was released. However, incubation at 80°C for 2 h caused release of a water-soluble product, which was chromatographically identical with mannose 1-phosphate in solvent F.

Chromatographic properties of mannolipid. The mannolipid preparations from both rat parotid and bovine adrenal medulla exhibited identical chromatographic properties. They remained adsorbed on silicic acid on elution with chloroform or acetone and were eluted with chloroform-methanol (1:2, v/v). When mixed with authentic [¹⁴C]mannosyl

Fig. 1. Properties of GDP-mannose-polyprenol phosphate mannosyltransferase of bovine adrenal medulla

The incubation medium (100 μ l) contained GDP-[^{14}C]mannose (4 μM , 89000 d.p.m.), MnCl_2 (5 mM), Tris-maleate buffer, pH 7.0 (100 mM), and approx. 2.5 mg of microsomal protein. Reactions were started by addition of the GDP-mannose and were incubated at 37°C. The curves were obtained by varying the concentration of individual components as follows: (a) Mn^{2+} ; (b) pH; (c) GDP-mannose; (d) protein.

phosphoryl dolichol and loaded on a DEAE-cellulose acetate column (1.2 cm $\times$ 11 cm) no radioactivity was detected in the material eluted with chloroform-methanol (7:3, v/v) or methanol. However, elution with 20 mM-ammonium acetate in 99% (v/v) methanol removed over 95% of the starting radioactivity in a single peak, suggesting that all three ^{14}C -labelled lipids were very similar if not identical. Further evidence that the mannolipids from rat parotid and bovine adrenal medulla were identical with authentic mannosyl phosphoryl dolichol is given in Table 1, where the R_F values for each in solvents A, B, C and D are given.

Stimulation of mannolipid synthesis by exogenous

dolichol phosphate. Although increasing concentrations of Triton X-100 had slight inhibitory effects on mannolipid synthesis the detergent was added as a routine to incubations with exogenous lipid to facilitate solubilization of the lipid. Addition of dolichol phosphate stimulated synthesis of mannolipid (Fig. 4), 6 nmol of dolichol phosphate causing an approximately twofold increase. A phospholipid fraction enriched in dolichol phosphate, prepared from pig liver, also stimulated mannolipid synthesis. In each case only one radioactive product, soluble in chloroform-methanol (2:1, v/v) was obtained and this was chromatographically identical with authentic mannosyl phosphoryl dolichol.

Fig. 2. Time-course of mannosid synthesis

The incubation medium was as described in Fig. 1 except that the final incubation volume was 200 μ l and contained approx. 4mg of microsomal protein and GDP-mannose (4 μ M, 168 000 d.p.m.). $\blacktriangle$, Mannolipid; $\blacksquare$, mannosyl s-EA; $\bullet$, mannosyl r-EA.

Fig. 3. Effect of GDP and GMP on mannosid synthesis

The incubation medium was as described in Fig. 1. Tubes were incubated for 10min in the presence of GDP-[U- 14 C]mannose (4 μ M), after which time GDP (2mM) or GMP (2mM) was added. $\bullet$, Control; $\blacktriangle$, +GMP; $\blacksquare$, +GDP.

Incorporation of [14 C]mannose from [14 C]mannolipid into other endogenous acceptors. Partially purified mannosid served as a mannose donor to two other endogenous acceptors (s-EA and r-EA) (Fig. 5). Incorporation into mannosyl s-EA was rapid and linear with time for about 20min, reaching a plateau after 30min. There was no loss of activity during a further 60min incubation. During the 90min incubation approx. 15% of the radioactivity in mannosyl phosphoryl dolichol was converted into mannosyl s-EA. The formation of mannosyl r-EA was very much slower, and after 90min only approx. 4% of

Table 1. *Chromatographic properties of authentic mannosyl phosphoryl dolichol and of the mannosids prepared from bovine adrenal medulla and rat parotid*

For details see the text.

Solvent	Mannosyl phosphoryl dolichol	Mannolipid of bovine adrenal medulla	Mannolipid of rat parotid
A	0.70	0.69	0.70
B	0.31	0.32	0.32
C	0.41	0.40	0.41
D	0.47	0.45	0.46

Fig. 4. Effects of exogenous dolichol phosphate on mannosid synthesis

The assay system was as described in Fig. 1 but included 0.1% Triton X-100. Dolichol phosphate was added at the concentrations indicated as a suspension in Triton X-100. The final Triton concentration in each case was 0.1%.

Fig. 5. Transfer of mannose from [14 C]mannolipid into endogenous acceptors of bovine adrenal medulla

The assay system contained partially purified mannosid (6000 c.p.m.) in Triton, as described in the Materials and Methods section, 8mg of microsomal protein and 0.1 M-Tris-maleate buffer, pH 7.0, in a final incubation volume of 275 μ l. $\bullet$, Radioactivity remaining in mannolipid; $\blacktriangle$, mannosyl s-EA; $\blacksquare$, mannosyl r-EA (protein).

the initial radioactivity appeared in this product. There was very little breakdown of the substrate to water-soluble products (less than 7% after 90 min incubation).

Effect of UDP-*N*-acetylglucosamine on mannosyl s-EA synthesis. UDP-*N*-acetylglucosamine had only a very slight inhibitory effect on the synthesis of mannosyl lipid from GDP-mannose. Even at a final concentration of 1 mM, i.e. 250-fold greater than GDP-mannose concentration, it caused an inhibition of less than 30%.

Chromatographic properties of mannosyl s-EA. Mannosyl s-EA was insoluble in chloroform-methanol (2:1, v/v) but soluble in chloroform-methanol-water (10:10:3, by vol.). It was adsorbed on silicic acid, being eluted with chloroform-methanol-water (10:10:3, by vol.). It did not move from the origin in solvents A, B, C and D, but had an R_F of 0.75–0.80 on paper chromatography on Whatman 3MM paper developed in isobutyric acid-aq. NH_3 (sp.gr. 0.880) water (59:43:9, by vol.).

Discussion

The formation of glycosyl polyprenyl phosphates by a variety of prokaryotic and eukaryotic systems has led to the suggestion that such lipids function as carriers of activated glycosyl units for macromolecular biosynthesis. Lennarz & Scher (1972) have reviewed the occurrence of such glycosyl carrier lipids in prokaryotic cells, fungi and higher plants and many animal tissues, and it is suggested that in animal tissues they serve as intermediates in glycoprotein synthesis. More recently the formation of mannosyl polyprenyl phosphates has been reported in pig liver (Richards & Hemming, 1972), bovine thyroid and hen oviduct (Waechter *et al.*, 1973), a mouse myeloma (Baynes *et al.*, 1973), bovine pancreas (Herscovics *et al.*, 1973), bovine and dog aorta (Chambers *et al.*, 1974), chick embryo retina (Kean *et al.*, 1974), the yeast *Hansenula holstii* (Bretthauer & Wu, 1974) and *Tetrahymena pyriformis* (Keenan *et al.*, 1974). In all these systems the mannosyl lipid appears to be a precursor of mannosyl-containing glycoproteins.

Indications that the lipid involved in rat liver was the polyprenyl phosphate, dolichol phosphate, were first published by Behrens & Leloir (1970), and final proof of the identity of dolichol phosphate was provided by chemical characterization and synthesis by Evans & Hemming (1973), Baynes *et al.* (1973) and Warren & Jeanloz (1973).

There appears to be some ambiguity about the nature of the final glycosyl acceptor. Behrens *et al.* (1971) suggested that the final acceptor was an oligosaccharide linked through a pyrophosphate bond to dolichol, which is tightly bound to protein. However, there is an increasing amount of evidence

that such a molecule serves as a second intermediate in the transfer of the sugar units to protein itself, and protein has been identified as the final acceptor in pig liver (Richards & Hemming, 1972), hen oviduct (Waechter *et al.*, 1973) and mouse myeloma (Baynes *et al.*, 1973).

The present paper describes the transfer of mannose from GDP-mannose into endogenous acceptors of bovine adrenal medulla and rat parotid. The results for production of mannosyl lipid are similar for both tissues.

The time-course of incorporation of mannose from GDP-mannose into endogenous acceptors of bovine adrenal-medulla microsomal fractions indicated that three endogenous acceptors were present, similar to those described by Waechter *et al.* (1973) for hen oviduct. Mannosyl phosphoryl dolichol was the most rapidly labelled product, but unlike the product from pig liver (Richards & Hemming, 1972) and hen oviduct (Waechter *et al.*, 1973) was not subject to endogenous mannosidase activity and lost radioactivity only very slowly. The low incorporation of radioactivity of mannosyl s-EA and mannosyl r-EA probably reflects the small amounts of endogenous acceptors available. Addition of UDP-*N*-acetylglucosamine did not compete for the dolichol phosphate associated with the mannosyltransferase except at very high concentrations, even though under normal assay conditions, in the absence of GDP-mannose, *N*-acetyl[U- ^{14}C]glucosamine from UDP-*N*-acetyl[U- ^{14}C]glucosamine ($4\mu\text{M}$) is incorporated into a chloroform-methanol (2:1, v/v)-soluble compound (D. A. White & E. T. Frommer, unpublished work). The formation of this glycolipid was stimulated by addition of dolichol phosphate to the incubation medium and exhibited chromatographic properties similar to the mannosyl lipid on silicic acid, DEAE-cellulose and t.l.c. in solvents A, B, C and D. In this latter respect it was similar to the *N*-acetylglucosamine-containing glycolipid of rabbit liver reported by Molnar *et al.* (1971).

If mannosyl phosphoryl dolichol is an intermediate in the transfer of mannose from GDP-mannose to protein then it should also serve as a mannose donor to the intermediates s-EA and r-EA. Experiments using partially purified mannosyl phosphoryl dolichol as exogenous mannosyl donor suggested that this was true. The incorporation of radioactivity into mannosyl s-EA was significant (approx. 15% of added mannosyl lipid radioactivity after 30 min) and was concomitant with the loss of radioactivity from the mannosyl lipid. However, incorporation of radioactivity into mannosyl r-EA was low and did not appear to follow any precursor-product relationship. No water-soluble radioactivity was obtained from these incubations, confirming the absence of mannosidase activity and making it unlikely that mannosylation of inter-

mediates s-EA and r-EA occurs via a water-soluble intermediate.

The true nature of the acceptors s-EA and r-EA is not known, since insufficient of each has as yet been obtained to characterize them. From its solubility and chromatographic properties it seems likely that acceptor s-EA is similar in structure to the lipophilic oligosaccharide described by Behrens *et al.* (1973), Hsu *et al.* (1974) and Lucas *et al.* (1975).

Thus these results are consistent with the hypothesis that transfer of mannose from GDP-mannose into glycoprotein in bovine adrenal medulla and rat parotid occurs, at least in part, by transfer first to dolichol phosphate, forming mannosyl phosphoryl dolichol. This glycolipid then serves as a mannose donor to an as yet uncharacterized intermediate having the solubility and chromatographic properties of a lipophilic oligosaccharide. The final acceptor is protein.

It has been suggested by Richards & Hemming (1972) that such a mechanism allows glycosyl transfer to hydrophobic proteins or proteins embedded in the hydrophobic environment of a membrane, and studies with whole cells show that mannose and N-acetylglucosamine, sugars which are generally located near the peptide backbone, become incorporated during peptide synthesis in the rough endoplasmic reticulum. Other sugars, glucose in rat liver (Behrens & Leloir, 1970), xylose in hen oviduct (Waechter *et al.*, 1974) and glucose and glucuronic acid in the livers of vitamin A-deficient rats (De Luca *et al.*, 1973), may also be incorporated into glycoproteins via a glycolipid having properties similar to mannosyl phosphoryl dolichol.

The function of glycoproteins synthesized via the glycolipid intermediate is not yet known. In the myeloma system of Baynes *et al.* (1973) the newly synthesized mannosyl glycoprotein is similar and may be related to the secreted light chain of immunoglobulin K-46. Waechter *et al.* (1973) showed that ovalbumin accounts for no more than 10% of the labelled glycoprotein formed in the hen oviduct system and suggested that membrane glycoprotein is a major product.

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