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THE ROLE OF DOLICHOL MONOPHOSPHATE IN SUGAR TRANSFER [1-2]

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The specificity of the transfer of monosaccharides from sugar nucleotides to dolichol monophosphate catalyzed by liver microsomes was studied. Besides uridine diphosphate glucose, uridine diphosphate-N-acetylglucosamine and guanosine diphosphate mannose were found to act as donors for the formation of the respective dolichol monophosphate sugars. Uridine diphosphate galacoste and uridine diphosphate-N-acetylgalactosamine gave negative results. The optimal conditions for transfer from dolichol monophosphate glucose to endogenous acceptor was determined. Studies were carried out on the glucosylation of ceramide by brain extracts and of collagen by skin enzymes in order to find out if dolichol monophosphate glucose is an intermediate in these reactions. The results, while not definite were not in favor of this assumption.

A polyprenol phosphate containing eleven isoprene residues has been found to be involved in the synthesis of various cell wall components in bacteria (1–3).

Work with animal tissues (4) has shown that a compound believed to be the monophosphate of dolichol has a role in sugar transfer. Dolichol (5) is a polyprenol containing about twenty isoprene residues, the first of them being saturated. The following reactions were found to be catalyzed by liver microsomes:

$$\text{UDPG} + \text{DMP} \rightarrow \text{DMPG} + \text{UDP}; \quad (1)$$

$$\text{DMPG} + \text{R} \rightarrow \text{DMP} + \text{G-R}; \quad (2)$$

$$\text{G-R} \rightarrow \text{G} + \text{R}^{4} \quad (3)$$

The identity of DMP was deduced by comparing samples purified from liver with preparations obtained by chemical phosphorylation of purified dolichol. The formation of UDP in Reaction (1) was not proved, but this compound was found to be an inhibitor of DMPG formation. The second step (Reaction 2) could be measured separately from the first and was found to have no specific ion requirements in contrast to the first step (Eq. 1) in which Mg^{2+} ions are necessary.

The nature of the compound represented by G-R in Eq. (2) has not been determined.

There are reports in the literature indicating that lipid intermediates may be involved in the formation of glycoproteins, but no direct transfer from glycolipid to glycoprotein has been reported. Thus Caccam *et al.* (6) and Zatz and Barondes (7) detected the formation of an acid labile mannolipid after incubation of GDP-mannose with various animal enzymes. Similarly Tetas *et al.* (8) described a liver microsome system which catalyzes the synthesis of acid labile lipids of mannose, *N*-acetylglucosamine, *N*-acetylgalactosamine, and galactose. As a continuation of our previous work in this field, several aspects of the problem have been studied further. Tests with several sugar nucleotides with DMP as acceptor and liver microsomes, showed that derivatives of *N*-acetylglucosamine and mannose were formed. Studies were also carried out in order to ascertain the optimal conditions of transfer from DMPG and also, to determine whether it is involved in the glucosylation of ceramide and collagen.

1 It is a pleasure to dedicate this paper to F. Lynen for his birthday. It is fortunate that DMP sugars have a lipophylic polyprene part which relates them to Dr. Lynen's main interest while it also has a carbohydrate part in keeping with our own field of research. L.F.L.

2 This investigation was supported in part by a research grant (No. GM 03442) from the National Institutes of Health, U. S. Public Health Service and by the Consejo Nacional de Investigaciones Científicas y Técnicas (R. Argentina).

3 Career investigator of the Consejo Nacional de Investigaciones Científicas y Técnicas.

4 Unusual abbreviations: DMP, dolichol monophosphate; DMPG, dolichol monophosphate glucose; DOC, sodium deoxycholate; UDPG, uridine diphosphate glucose.

MATERIALS AND METHODS

Substrates. Radioactive UDPG and UDP-galactose were prepared as described by Wright and Robbins (9). They were separated by paper chromatography in morpholinium borate (10). UDP-*N*-acetylglucosamine and UDP-*N*-acetylgalactosamine (both U-14C labelled in the sugar moiety) were purchased from New England Nuclear Corporation. GDP-mannose (U-14C-mannose) was a gift from Dr. Susana Passeron.

Natural DMP was prepared as described previously up to the DEAE-cellulose step (4). Synthetic samples were obtained by chemical phosphorylation of dolichol (4) as described by Popják *et. al.* (11). In order to decompose dolicholpyrophospate, the reaction product was head in n-butanol containing 1 N HCl for 7 min at 100° extracted with chloroform: methanol (2:1) and, washed according to Folch *et al.* (12).

Radioactive DMPG was prepared by incubation of liver microsomes with UDPG-14C and DMP as previously described (4), followed by extraction with chloroform: methanol (2:1). Ceramide-glucon was a gift from Dr. R. Caputto.

Analytical methods. Protein was determined as described by Lowry *et al.* (13) with bovine serum albumin as standard.

Chromatography. The following solvents were used for thin-layer chromatography on silica-gel plates: (A) chloroform: methanol: water (60:20:2; (B) the same as the preceding, but the silica gel was suspended in 1% sodium borate; (C) cloroform:methanol:water (84:15:1); (D) cloroform: methanol:water (65:25:4). For paper chromatography the solvent used was: (E) butanol: pyridine:water (6:4:3) (14).

Enzymes. Liver microsomes were prepared as described in a previous paper (4). The final protein concentration was 30-90 mg/ml. Newborn rat brain microsomes were prepared as described by Basu *et al.* (15) up to the 20.000 g centrifugation step. The pellet from 2 g of brain was resuspended in 0.5 ml of the solution used for homogenization (FIGS. 6 and 7) or in 0.5 ml of 0.1 M glycylglycine buffer, pH 7.8 (Fig. 8). Skin enzyme was prepared by homogenizing the skin from newborn rats in 0.25 M sucrose, 5 mM *EDTA*, and 10 mM 2-mercaptoethanol with a conical glass homogenizer. The homogenate was passed through chessecloth and centrifuged at 100 g for 10 min. The pellet was resuspended in water.

Assay procedures. In the incubation mixtures, DMP or DMPG plus Mg-EDTA, $MgCl_2$, or Na-EDTA were added first; the tubes were dried under reduced pressure, and then the remaining components were added.

RESULTS

Reaction of DMP with various sugar nucleotides. As shown in Table 1, incubation of labeled GDP-mannose or UDP-*N*-acetylglucosamine under the conditions previously used for DMPG formation (4), gave rise to the appearance of radioactive lipid. Natural and synthetic DMP were equally effective in increasing the formation of radioactive lipid.

TABLE I

THE FORMATION OF DIFFERENT DMP SUGARS [a]

Nucleotide	Amount (pmoles)	Additions: None	Natural DMP (pmoles of sugar transferred)	Synthetic DMP
UDPG	260	3.5	32.0	29.4
UDP-N-acetyl galactosamine	780	2.0	10.8	10.0
GDP-mannose	32.000	20.0	240	243
UDP-N-acetyl galactosamine	780	0.5	0.6	0.6
UDP-galactose	110	0	0	0

a The incubation mixtures contained: 90 mM 2-mercaptoethanol, 0.36 M glycylglycine buffer, pH 7.8, 0.55% Triton X-100, 9 mM Mg-EDTA, 9 mM $MgCl_2$, 20 μl of enzyme, natural or synthetic DMP (containing 13 and 3.4 nmoles of total phosphate, respectively) and radioactive sugar nucleotides in a total volume of 55 μl. After 20 min at 37°, the mixture was processed as previously described for DMPG (4). The specific radioactivity of the sugar nucleotides were (in Ci/mole): UDPG and UDP-galactose, 309; UDP-N-acetylglucosamine and UDP-N-acetylgalactosamine, 43; GDP-mannose, 1.

None was formed from UDP-*N*-acetylgalactosamine or UDP-galactose.

In other experiments UDP-glucuronic acid was tested and found to give some incorporation into lipid with natural DMP but none when synthetic DMP was used as acceptor. ADP-glucose also gave negative results. The fac that DMP stimulates incorporation of sugar into the lipid fraction, suggests that the compounds formed are the DMP derivatives. Furthermore the products obtained from the incubations with GDP-mannose and UDP-*N*-acetylglucosamine with natural DMP gave only one radioactive peak after thin-layer chromatography (solvent D) having the same R_F as DMPG (0.20-0.25).

Properties of the different DMP sugars. The course of decomposition of the different DMP sugars in 0.1 N acid in chloroform: methanol is shown in Fig. 1. The rate is slightly higher for the mannose derivative than for DMPG. The *N*-acetylglucosamine containing compound was considerably more stable. The reason for this difference is difficult to understand. Glucosaminides are known to be more acid-stable than other glycosides, and

this is attributed to the action of the positive $-NH^+$ group in the vicinity of the bond to be hydrolyzed (16). However, when the $-NH_2$ is substituted as in *N*-acetylglucosamines, the rate of hydrolysis is not very different from other glycosides. Comparison of the acid hydrolysis of UDPG and UDP-*N*-acetylglucosamine or glucose 1-phosphate and *N*-acetylglucosamine 1-phosphate showed that the *N*-acetylglucosamine derivatives were about two times more stable, but differences were not as great as those shown in Fig. 1. DMP-*N*-acetylglucosamine was completely decomposed by heating for 10 min at 100° in 0.01 N sulfuric acid in 50 % *n*-propanol. The products obtained were spotted on paper and developed with solvent E. The radioactive peak from

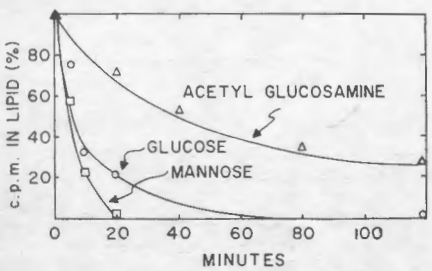

FIG. 1. — Acid treatment of DMP sugars. The DMP sugars were treated with 0.1 N HCl in chloroform: methanol (2:1). After different times at room temperature, the solutions were washed three times with Folch's theoretical upper phase (12) and counted.

hydrolyzed DMP-*N*-acetylglucosamine migrated 20.4 cm while acetylglucosamine migrated 20.3, glucosamine, 12.5, and glucose, 15.2 cm. The product of hydrolysis behaved, therefore, like *N*-acetylglucosamine.

It was reported previously (4) that DMPG is decomposed by alkali giving rise to the formation of 1,6-anhydroglucosan. The mannose and *N*-acetylglucosamine derivates are also decomposed by alkali but slightly more slowly than DMPG. Thus after heating at 100° for 10 min in 0.1 N NaOH, the amount decomposed (%) was as follows: DMPG, 100 per cent; DMP-*N*-acetylglucosamine, 74 per cent; and DMP-mannose, 45 per cent. The fact that the glucose-containing compound is more labile than the others may be understood by comparison with the data on the alkaline decomposition of β-aryl glycosides. The reaction is faster with the β-glucosides, which can form the 1,2-anhydride as intermediate. The formation of this compound requires an OH *trans* to the aglycone so that it is not formed with mannosyl or *N*-acetylglucosaminyl derivates (17). The identity of the products formed by alkaline treatment of the mannose and *N*-acetylglucosamine derivatives has not been investigated yet.

No transfer from DMP-*N*-acetylglucosamine or DMP-mannose could be detected when these compounds were incubated with microsomes under conditions in which DMPG reacted rapidly.

DMPG, DMP-mannose, and DMP-*N*-acetylglucosamine were also formed with the brain microsomal enzymes under the same conditions of Table I.

Conditions for the transfer from DMPG. It was reported previously (4) that transfer from DMPG takes place on incubation of liver microsomes at 30° and with a relatively high detergent concentration, but no detailed study of the optimal conditions had been carried out.

Measurements of the transfer to the insoluble fraction (insoluble in chloroform: methanol, and trichloroacetic acid) at different pH values are shown in Fig. 2. Maximal rate was obtained at pH 7.8 with 50 % values at about 6.3 and 8.3.

The optimal detergent concentration is somewhat variable with the conditions. Dif-

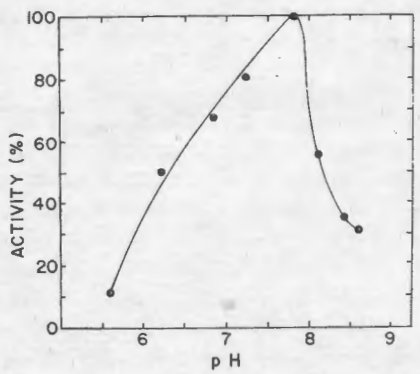

FIG. 2. — Optimal pH for the transfer froh DMPG. The tubes contained: 0.1 M Tris-maleate buffers of different pH, DMPG (4200 cpm, 207 Ci/mole), 8 mM EDTA, 40 mM 2-mercaptoethanol, 0.6% Triton X-100 and 0.47 mg of microsomal proteim in a total volume of 50 µl. After 10 min at 30°, 0.4 ml of methanol and 0.6 ml of chlroform were added. After centrifugation, the precipitate was dried in vacuum, then 1 ml of 5 % trichloroacetic acid was added and the precipitate was washed twice with 1 ml of *n*-butanol, resuspended in Bray solution (24) and counted.

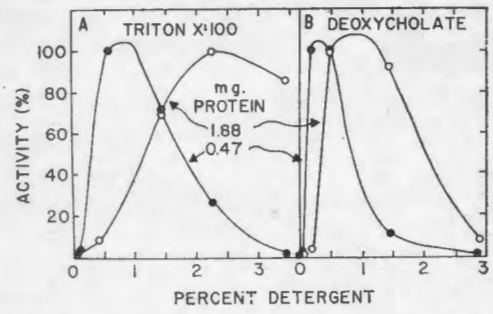

FIG. 3. — Optimal concentration of detergent. The incubation mixtures were the same as in Fig. 2 except that the pH 7.8 buffer and different detergent concentrations were used. Tubes with 0.47 mg of microsomal protein were incubated for 15 min (Fig. 3A) and 12 min (Fig. 3B) at 30° while those with 1.88 mg were incubated 4 and 5 min, respectively. The samples were processed as described in Fig. 2.

ferent optimal values were obtained when different amounts of enzyme were used. As shown in Fig. 3A, the optimal Triton X-100 concentration changes from 0.75 to 2.25 % with a fourfold increase in enzyme. The corresponding values for sodium deoxycholate were 0.3 and 0.90 % (Fig. 3B). Addition of a chloroform: methanol extract of the microsome fraction also produced a displacement of the optimal detergent concentration. The explanation of these changes may be that there are substances that fix detergent thus lowering its effective concentration. No activity was detectable if detergents were replaced by various amounts of organic solvents such as ethanol, *n*-propanol, isopropanol, *n*-butanol, or tert-butyl alcohol.

Time curves with Triton X-100 or DOC at different temperatures are shown in Fig. 4 A, B. At 35° the formation of product ceases after 5-10 min, and the amount decreases thereon. This decrease is due to the liberation of glucose (4). At 25° the formation of product continues for a longer time and reaches a higher maximum. These curves suggested that the enzyme became inactivated during the reaction. This possibility was tested directly by preincubation experiments, the results of which are shown in Fig. 5. In the presence of either DOC or Triton X-100 at 30°, the activity decreases rapidly with a half-time of approximately 9 and 6 min, respectively. No loss of activity occurred on preincubation for 20 min without detergent.

Table II shows the results obtained after incubation under different conditions. It may be observed that the activity obtained with DOC is nearly double that obtained with Triton X-100. These results are similar to those shown in Fig. 4 and 5. Manganese, which is required in many transfer reactions from sugar nucleotides to proteins (6, 18-23) acted as an inhibitor. Magnesium ions were slightly inhibitory, and addition of UDP or UDPG was without effect. No activity was detectable after heating the enzyme for 5 min at 100°.

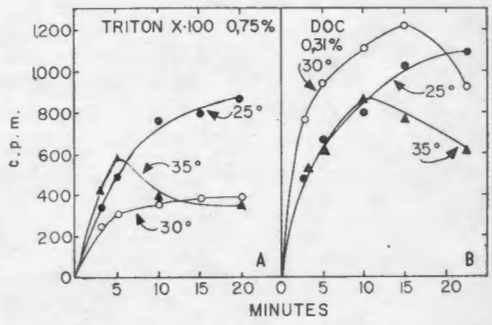

FIG. 4. — Temperature dependence of the glucose transfer to the cloroform:methanol insoluble material. The tubes contained 0.1 M Tris-maleate buffer, pH 7 8, 40 mM 2-mercaptoethanol, 8 mM EDTA, DMPG (33 600 cpm, 207 Ci/mole), 3.76 mg of enzyme protein and 0.75% Triton X-100 (Fig. 4A) or 0.30% DOC (Fig. 4B) in a total volume of 400 µl. Aliquots of 50 µl were taken after different times at the indicated temperatures and processed as described in Fig. 2.

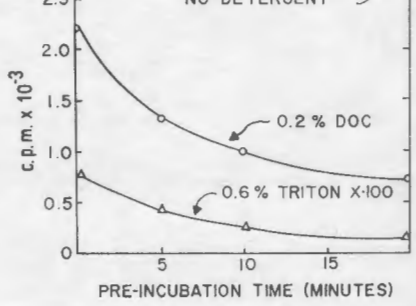

FIG. 5. — Inactivation of the transferring enzymes by detergents. Three different preincubation mixtures were used. The first contained 0.1 M 2-mercaptoethanol, 2.35 mg of microsomal protein and 0.1 M Tris-maleate buffer, pH 7.8, in a total volume of 250 µl. The second and third mixtures contained the same components plus 0.6 % Triton X-100 and 0.2% DOC, respecively. After different preincubation times at 30°, 50-µl aliqunts were taken from the mixtures and transferred to tubes to which DMPG (4200 cpm, 207 Ci/mole) and 0.4 µmole of EDTA had been previously added and dried under reduced pressure. The aliquots from the first incubation mixture were supplemented with DOC to give a final concentration of 0.2%. The tubes were then incubated for 20 min at 23° and processed as described in Fig. 2.

TABLE II

TRANSFER FROM DMPG TO THE CHLOROFORM: METHANOL INSOLUBLE FRACTIONS[a]

	Final concentration	cpm in precipitate
Complete	—	960
− EDTA	—	880
+ Mn^{2+}	10 mM	270
+ Mg^{2+}	10 mM	550
+ UDP	10 mM	900
+ UDPG	20 mM	925
− Triton X-100 + DOC	0.2%	1940
− Triton X-100 + DOC	0.5%	1580
− Triton X-100 + DOC	1%	470
Heated enzyme (5 min at 100°)		125
Nonincubated		105

[a] The complete system was the same as in Fig. 2 but with the pH 7.8 buffer. The indicated concentrations of Mg^{2+} and Mn^{2+} are the excess over the added EDTA. After 30 min at 30°, the tubes were processed as described in Fig. 2.

Transfer with the skin enzyme. One of the few glycoproteins which contain glucose is collagen. The transfer reaction from UDPG to collagen has been studied by Bosmann and Eylar (18) with a guinea pig fetus skin enzyme and by Spiro and Spiro (19) with kidney glomerular basal membrane. An experiment in which transfer from UDPG to protein and lipid was measured is shown in Table III. Gelatin was used as a protein acceptor. Under the conditions for transfer to collagen (18) that is with Mn^{2+}, radioactivity was incorporated to protein and about fourfold more in the presence of gelatin. DMP produced no change. Under the conditions favorable for DMPG formation no radioactivity was found in protein. DMP increased radioactivity in the chloroform-soluble fraction under both conditions.

Many tests were carried out under varied conditions in order to obtain transfer to protein from DMPG with the skin enzyme. The results were negative. The preliminary conclusion was that DMPG is not an intermediate in the glucosylation of collagen by the skin enzyme. In order to test if the acceptor protein of the liver system is different from collagen two rodioactive proteins were prepared (a) with skin enzyme, gelatin, and UDPG, and (b) with DMPG and liver enzymes. The products were hydrolyzed in 2 N KOH for 20 hr at 100°. Paper electrophoresis at pH 7.8 (0.1 M potassium phosphate) showed that the skin product had a positive charge while the product of the liver enzyme was negatively charged. They were, therefore, clearly different.

TABLE III

Transfer from UDPG with the Skin Enzyme[a]

Conditions	DMP	Gelatin	cpm in protein	cpm in lipid
A. Transfer to DMP	+	+	0	480
	−	+	0	70
	+	−	0	500
	−	−	0	80
B. Transfer to collagen	+	+	4200	480
	−	+	4500	250
	+	−	1200	240
	−	−	1000	140

[a] The incubation mixtures contained: (A) 5 mM Mg-EDTA, 5 mM $MgCl_2$, 0.2 M glycylglycine buffer, pH 7.8, 50 mM 2-mercaptoethanol, 0.3 % Triton X-100, UDPG (150 000 cpm, 309 Ci/mole), natural DRP, 2 mg of gelatin and 50 µl of skin enzyme in a total volume of 100 µl; (B) 20 mM $MnCl_2$, 0.01% Triton X-100, 0.1 M acetate buffer, pH 5.8, UDPG (150 000 cpm, 309 Ci/mole), natural DMP, 2 mg of gelatin and 50 µl of skin enzyme in a total volume of 100 µl. After 30 min at 37°, the reactions were stopped by the addition of 0.4 ml methanol plus 0.6 ml of chloroform. The soluble fractions were washed according to Folch *et al.* (12) and counted. The insoluble fractions were washed twice with 0.5 ml of 0.1 % phosphotungstic acid in 0.5 N HCl, twice with 0.5 ml of 66 % ethanol and counted.

Brain enzymes. The microsomal fraction from chick embryo brain catalyzes the transfer of glucose from UDPG to ceramide (15). Experiments were carried out with a similar enzyme preparation from newborn rats in order to find out if DMPG is an intermediate in this process.

Ceramide-glucose is relatively acid-stable while DMPG is completely hydrolyzed in 45 min at room temperature in choroform: methanol (2:1) containing 0.1 N HCl so that the two compounds can be easily distinguished. The result of incubating the brain enzyme with radioactive UDPG in the presence of DMP and Mg^{2+} is shown in Fig. 6A. A considerable amount of radioactivity appeared in the chloroform: methanol soluble fraction. Both acid-stable and labile compounds were

formed, and after 45 min there was about 50 % of each.

Magnesium ions are required for the transfer from UDPG to DMP with the liver enzyme (4). On the other hand, transfer to ceramide has been reported to have no specific cation requirements (15). As shown in Fig. 6B, in the presence of excess EDTA, the formation of the acid-labile compound was completely inhibited, while the synthesis of the acid-resistant one was hardly affected. Under the conditions of Fig. 6B, exogenously added DMPG was only slightly metabolized, but no chloroform: methanol soluble acid-stable material was formed.

Under the conditions of Fig. 6A, the presence of DMP greatly stimulated the formation of the acid-labile compound while that of the stable one was slightly diminished (Fig. 7). This inhibition of the acid-resistant incorporation is possibly due to some substance in the DMP solution and not to a competition between both substrates (DMP and ceramide) for UDPG, because the same inhibition occurred in the conditions of Fig. 6B, that is in the presence of excess EDTA where no incorporation to the acid-labile compound was detectable.

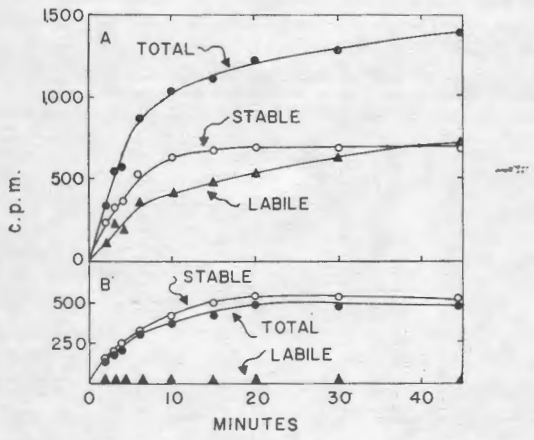

FIG. 6. — Glucose transfer to a chloroform: methanol soluble material by brain microsomes. The incubation mixtures contained: (A) Synthetic DMP, 8 mM $MgCl_2$, 8 mM Mg-EDTA, 0.5 % Triton X-100, 0.33 M glycylglycine buffer, pH 7.8, 250 µl of enzyme and 20 nmoles of UDPG (207 Ci/mole) in a total volume of 600 µl. (B) The same components except that instead of $MgCl_2$ and Mg-EDTA the mixture contained 33 mM Na-EDTA. Aliquots of 50 µl were taken after different times at 37° and placed in tubes containing 0.5 ml of chloroform: methanol (2:1) plus 120 µl of a 42 mM KCl, 21 mM EDTA solution. The lower phase was washed three times with 200 µl of chloroform: methanol: 0.1 M KCl (3:47:48) and carefully poured off to other tubes in order to separate the precipitates. The lower phase was then divided in two halves, *a* for total and *b* for acid-stable radioactivity. Sample *b* was made up to 1 ml with chloroform: methanol (2:1) and 20 µl of 5 N HCl were added. After 45 min at room temperature, the sample was washed twice as described above. The radioactivity *a-b* was taken as that of acid-labile compounds.

The identity of the compounds formed was established as follows. Thin-layer chromatography (solvent D) of the product obtained under the conditions described in Fig. 6A in which both acid-labile and stable compounds were formed by the brain enzyme, gave two peaks of radioactivity (R_F = 0.63 and 0.2). If the samples were treated with mild acid before chromatography, only the fast peak appeared. This substance seemed to be ceramide-glucose since its mobility in three different solvents (A, B and C) was the same as that of a ceramide-glucose standard and because it gave glucose by heating with 2 N H_2SO_4 for 4 hr at 100° (identified with solvent E). The acid-stable compound formed in the presence or absence of Mg^{2+} or of DMP seemed to be the same as judged by the above mentioned criteria.

As to the slow moving, acid-labile substance, its properties are those of DMPG. Its mobility during thin-layer chromatography was the same (solvent D, R_F: 0.2). As a further criterion of identity, the substance was treated with alkali, and it was found that 1,6-anhy-

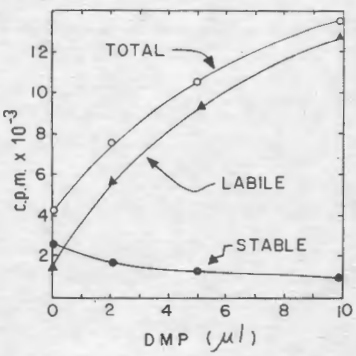

FIG. 7. — Stimulation by DMP of the incorporation to the chloroform: methanol soluble acid-labile fraction. The incubation mixtures contained: 8 mM $MgCl_2$, 8 mM Mg-EDTA, 25 µl of enzyme, 0.5 % Triton X-100, 0.33 M glycylglycine buffer, pH 7.8, 4 nmoles of UDPG (207 Ci/mole) and different amounts of synthetic DMP in a total volume of 60 µl. After 60 min at 37° the tubes were processed as described for the 50 µl aliquots in Fig. 6.

droglucosan was formed (identified with solvent E as previously described, Ref. 4). The acid-labile product formed in the presence or absence of DMP appeared to be the same as judged by its mobility during thin-layer chromatography and by the formation of 1,6-anhydroglucosan by alkaline treatment.

The results represented in Fig. 6 A, B show that the glucosylation of ceramide proceeds in the presence of EDTA while the formation of DMPG is inhibited so that it seems unlikely that the latter is a glucose donor for ceramide-glucose formation.

The brain extract was also examined for the presence of the enzyme which catalyzes the transfer from DMPG to acceptor. As shown in Fig. 8, the changes were the same as those reported before for the liver enzyme (4). The radioactivity which is initially all soluble in chloroform: methanol (2:1) was transferred to the protein fraction and afterwards appeared in the aqueous phase. The water soluble radioactivity was identified as glucose by paper chromatography with solvent E. No chloroform: methanol-soluble, acid-stable material was formed. In the same conditions ceramide-glucose was not metabolized. It seems therefore that the metabolism of DMPG is the same in brain and in liver.

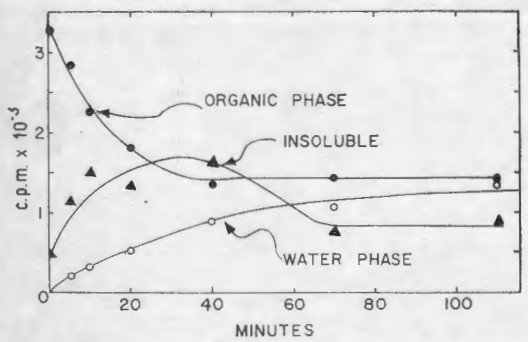

FIG. 8. — The metabolism of DMPG by brain microsomes. The incubation mixture contained: 7 mM EDTA, 1.6 % Triton X-100, 0.13 M glycylglycine buffer, pH 7.8 60 mM 2-mercaptoethanol, DMPG (38 000 cpm, 309 Ci/mole) and 600 µl of enzyme in a total volume of 835 µl. Aliquots of 100 µl were taken of different times at 30°. They were processed as described in Fig. 6 for the 50 µl aliquots except that the 0.5 ml of chloroform: methanol (2:1) contained 20 µl of a 0.25 M *KCl*-0125 M EDTA solution. The protein fraction was washed twice with 1 ml of *n*-butanol, resuspended in Bray solution (24) and counted.

DISCUSSION

According to the results reported in this paper, DMP sugars containing *N*-acetylglucosamine or mannose are formed from the respective nucleotides. These compounds are acid-labile like the glucose compound, but DMP-*N*-acetylglucosamine is more stable than the glucose derivative. This is a rather unexpected property since according to the data in the literature the rate of hydrolysis of β-methylglucoside is 9.3 times slower than that of β-methyl *N*-acetylglucosaminide. The relation is similar for the α glucosides (16). It is known that glucosaminides are more stable to acid presumably due to the effect of the positive charge on the amino group. However, acid hydrolysis of DNP-*N*-acetylglucosamine gave *N*-acetylglucosamine and not glucosamine.

As to the utilization of DMP-*N*-acetylglucosamine and mannose, nothing is known as yet. The reaction from DMPG to acceptor is catalyzed by the microsome fraction of liver and brain, but this does not occur with the other two compounds. Probably it is only a question of finding the appropriate conditions.

The other question which required investigation was whether DMPG is involved in other transfer reactions. For instance in the glucosylation of collagen, ceramide, and glycogen. In these cases the available evidence is against the participation of DMPG.

In the glucosylation of collagen catalyzed by skin enzymes with UDPG as donor, it was found that there was no relation between transfer to DMP and to the protein acceptor. The results were not in favor of DMPG as intermediate.

The transfer from UDPG to endogenous ceramide to give ceramide-glucose is catalyzed by a brain enzyme. Addition of DMP increased DMPG but not the rate of ceramide-glucose formation. Excess EDTA inhibited transfer to DMP but not to ceramide. This seems to be a fairly strong evidence in favor of the nonparticipation of DMPG in ceramide-glucose formation. Similarly, transfer from UDPG to glycogen can take place with EDTA concentrations which inhibit completely DMPG formation (25). Here again the evidence is against the intermediate formation of DMPG.

The role of DMP sugars as intermediates, therefore, appears to be restricted to some reactions, the significance of which is not known yet.

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