Effects of Turpentine-induced Inflammation on the Synthesis of Dolichol-linked Intermediates of N-Glycosylation and the Phosphorylation of Dolichol by CTP-dependent Dolichol Kinase

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Inflammation was induced in rats by the subcutaneous injection of turpentine. Microsomes were prepared from the livers between 2 and 72 h after injection. Mannose and glucose incorporation into mannosyl and glucosyl dolichyl monophosphate was increased 2-fold over saline-injected controls 24 h after induction of inflammation. Synthesis of glycosylated dolichyl pyrophosphoryl oligosaccharides was also increased compared to controls. Extraction and assay of dolichol monophosphate from inflamed and control rat liver microsomes indicated that the endogenous levels of the lipid were elevated in the inflamed state. CTP-dependent phosphorylation of endogenous dolichol was also found to increase in microsomes from inflamed rats 24 h after injection of turpentine. When exogenous dolichol was added to the microsomal system an increase in phosphorylation was observed as early as 6 h after turpentine injection. Furthermore, the increase appeared to be biphasic, there being two peaks of elevated activity at 12 and 36-48 h after induction of inflammation. The earlier peak was the greater of the two. The results suggest that the increase in glycosylation of dolichol derivatives was due to greater amounts of endogenous dolichol monophosphate. The increase in dolichol monophosphate was itself due to greater availability of dolichol and an increase in the levels of CTP-dependent dolichol kinase.

It is well known that certain serum glycoproteins, the acute phase reactants, increase in response to inflammatory agents such as turpentine (1, 2). Although the mechanism of action of the inflammatory response is not fully understood, it has been suggested that the initial response to inflammation is the release of an activator from the inflammatory site (1). The elevated levels of the acute phase reactants was shown to be the result of increased synthesis by the liver (3-6). Extensive work has been done on specific acute phase reactants, especially $\alpha_1$-acid glycoprotein and $\alpha_2$-macroglobulin (7-9), and the site of synthesis and pathway of secretion of the former has been determined (10, 11). Recent studies have shown increased activity of several NDP-glycosyl glycoprotein transferases in liver fractions from inflamed rats (12, 13). Most, if not all, serum glycoproteins are synthesized via dolichol-linked intermediates (14-16). Thus, inflammation might well be expected to increase the levels of these intermediates. This paper presents evidence that turpentine induced inflammation causes increased production of dolichyl monophosphoryl monosaccharides and dolichyl pyrophosphoryl gluco-oligosaccharides. We have also studied the effects of turpentine-induced inflammation on CTP-dependent dolichol kinase, the enzyme which has been shown to phosphorylate dolichol in brain matter (17) and other systems (18). This enzyme was found to increase in activity in the inflamed state due to increased availability of endogenous dolichol and elevated amounts of the enzyme itself.

MATERIALS AND METHODS

Chemicals
GDP [U-14C]mannose (193 mCi mmol$^{-1}$) and UDP [U-14C]glucose (281 mCi mmol$^{-1}$) were from New England Nuclear, Lachine, Quebec. Cytidine [y-32P]triphosphate (52 Ci mmol$^{-1}$) as triethylammonium salt, was from ICN Pharmaceuticals, Irvine, CA. Dolichol and dolichol monophosphate were from Sigma. Chloroform and methanol were redistilled before use.

Animals and Induction of Inflammation
Male Sprague-Dawley rats (200-250 g) were divided into two groups. Inflammation was induced in one group by the subcutaneous injection of turpentine (0.5 ml 100 g body weight$^{-1}$) in the dorso-lumbar region, equal volumes being injected either side of the midline. The second group were used as controls, being injected in an identical fashion with 0.9% NaCl. The rats had access to food and water ad libitum.

Preparation of Microsomes
Two methods were used to prepare microsomes, the method used being dependent upon the experiments to be performed.

Method 1, Based on That of Caccam et al. (19)—At times specified in the text rats were killed by exsanguination under ether anaesthesia and the livers removed. All subsequent procedures were performed at 4 °C. Weighed livers were homogenized using a Polytron homogenizer in 1.3 volumes of 0.05 M Tris-HCl, pH 7.4, containing 0.25 M sucrose. The homogenates were centrifuged at 20,000 × g for 30 min in a Sorvall RC5 using a type SS 34 rotor. The supernatants were decanted and recentrifuged at 145,000 × g using a type 40 rotor for 75 min in a Beckman L5-50. The subsequent microsomal pellets were washed and then resuspended in homogenizing buffer to a final protein concentration of 20 mg ml$^{-1}$.

Method 2, Based on Work of Moule et al. (20)—At times specified in the text, rats were killed by cranial fracture and the livers were removed after perfusion with ice-cold 0.9% NaCl. All subsequent procedures were performed at 4 °C. The livers were homogenized in 10 volumes of 0.88 M sucrose using a polytron homogenizer. The homogenates were centrifuged using a type GSA rotor in a Sorvall RC5 at 24,000 × g for 20 min. The supernatants were decanted and recentrifuged using a type SS 34 rotor in a Sorvall RC5 at 40,000 × g for 20 min. The resulting pellets were resuspended in 0.01 M glycylglycine, pH 7.4, containing 0.25 M sucrose to a final protein concentration of 10 mg ml$^{-1}$. This fraction constituted the rough micro-
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...omes. The supernatants from the 40,000 x g spin were further centrifuged using a type 60Ti rotor in a Beckman L5-50 centrifuge at 145,000 x g for 65 min. The pellets obtained were resuspended in an identical manner to rough microsomes and constituted the smooth microsomal fractions.

Extraction of Microsomal Dolichol Monophosphate

Dolichol monophosphate was extracted from microsomes prepared by method 1 using a modification of the procedure of Behrens and Leloir (21). Aliquots of microsomes were treated with 2 volumes of acetone and centrifuged. The dried pellets were extracted twice with 3 volumes of chloroform/methanol (2:1, v/v), the supernatants being removed by centrifugation and then combined. The supernatants were made 0.1 M with respect to NaOH and heated at 37 °C for 15 min. After adding sufficient HCl to make the extracts 0.1 M in free acid, they were then refluxed for 15 min. Following the addition of 1 ml of chloroform, the cooled extracts were washed three times with chloroform/methanol/water (4:48:47, v/v) (22). The washed extracts were applied to columns (0.6 x 6 cm) of DEAE-cellulose in the acetate form and eluted as described by Palamarczyk and Hemming (23). Successive elution was performed with chloroform/methanol (2:1, v/v), methanol, and 0.01 M ammonium acetate in chloroform/methanol (2:1, v/v). The ammonium acetate fractions were washed eight times with water and the residual chloroform layers containing purified dolichol monophosphate were taken to dryness under nitrogen and redissolved in a known volume of chloroform/methanol (2:1, v/v).

Assays for Glycosyltransferase Activities

Mannose to Dolichol Monophosphate—Microsomes (3.3 mg of protein) prepared using method 1 were incubated with GDP [U-14C]mannose at concentrations specified in the text and in a final volume of 225 μl containing 8.8 mM MgCl2, 2.2 mM EDTA, 38.4 mM Tris-HCl (pH 7.2), and 0.1% Triton X-100. Dolichol monophosphate, when included in incubations, was first taken to dryness and then solubilized in the required amount of Triton X-100. Incubations were performed at 37 °C for 5 min. Dolichyl monophosphoryl mannose was extracted as described previously (24), radioactivity in the extracts being determined by counting dried aliquots in 10 ml of toluene/Omnifluor (New England Nuclear). Glucose to Dolichol Monophosphate and Dolichyl Pyrophosphoryl Oligosaccharides—Microsomes (0.5 mg of protein) prepared using method 2 were incubated with 0.1 nmol of UDP [U-14C]glucose in a final volume of 115 μl containing 0.09% Triton X-100, 0.2 mM glycyglycine (pH 7.4), 0.087 M Mg-EDTA complex, and 0.087 mM 2-mercaptoethanol. Incubations were at 37 °C for times specified in the text. Dolichyl monophosphoryl glucose was extracted as described previously (24), radioactivity being determined by drying aliquots of the extracts in scintillation vials and counting in 10 ml of toluene/Omnifluor.

Galactosylated dolichyl pyrophosphoryl oligosaccharides were obtained by further extraction of the incubations with chloroform/methanol/water (65:25:4, v/v) (25, 26) followed by chromatography of the extracts on DEAE-cellulose plates as described below. The band migrating as dolichyl pyrophosphoryl oligosaccharide was scraped off and radioactivity assessed by counting in 10 ml of toluene/Omnifluor. Any radioactivity in bands migrating as dolichyl monophosphoryl glucose was determined similarly and taken into account when calculating the total amount of glucose transferred to dolichol monophosphate.

Characterization of Products from Glycosyltransferase Assays

Thin layer and DEAE-cellulose column chromatography was used to characterize the products of transferase assays as described previously (23, 24). Thin layer chromatography of dolichyl monophosphoryl monosaccharides was performed on silica plates (Eastman Kodak Co., Rochester, NY) using chloroform/methanol/water (65:25:4, v/v) as developing solvent. Dolichyl pyrophosphoryl oligosaccharides were chromatographed on cellulose plates (Eastman Kodak Co.) by the ammonium sulfate precipitation method of Burton et al. (17). In incubations not involving exogenous dolichol, 0.4 mg of microsomal protein was used with 2 nmol of [y-32P]CTP (initial activity 260 mCi/mmol) in a total volume of 50 μl incubating at 37 °C for 40 min. When exogenous dolichol was included, incubations contained 0.65 mg of microsomal protein and 4 nmol of [y-32P]CTP (initial activity 260 mCi/mmol) in a final volume of 105 μl incubating at 37 °C for 10 min. In both cases the other components of incubations were 20 mM UTP, 5 mM mercaptoethanol, 30 mM CaCl2, and 5 mM EDTA. Triton X-100 was included in incubations with exogenous dolichol at concentrations specified in the text.

Extraction of Dolichol [32P]Monophosphate from CTP-dependent Kinase Assays

Incubations were stopped with 2 ml of chloroform/methanol (2:1, v/v) and 2 ml of water. The tubes were vortex-mixed and centrifuged to separate phases. The lower phase was removed by aspiration and retained. The remaining upper phase and protein interface were re-extracted with a further 2 ml of chloroform/methanol (2:1, v/v), the resulting lower phase being combined with the first. This bulked extract was washed with sufficient water to remove all aqueous [32P], the remaining chloroform phase containing the dolichol [32P]monophosphate. Aliquots of the radioactive extracts were dried and counted in 10 ml of toluene/Omnifluor. Identification and purity of the product was ascertained by thin layer chromatography on silica plates using chloroform/methanol/water (65:25:4, v/v) as developing solvent, all the radioactivity migrating as a single spot with an Rf identical to that of authentic dolichol monophosphate.

RESULTS

Mannosyl and Glucosyl Lipid Intermediate Production—Using method 1, microsomes were prepared 24 and 48 h after injection of turpentine and incubated with 0–10 nmol of GDP [U-14C]mannose. The activities for production of dolichyl monophosphoryl mannose are shown as compared to controls in Fig. 1. At all concentrations of GDP mannose used, the activity in the inflamed microsomes was at least 2-fold higher than in the controls. Maximum production of dolichyl monophosphoryl mannose was observed in all cases using between 8–10 nmol of GDP mannose. At these saturating concentrations incorporation was 8 and 7 pmol/mg of protein1 in microsomes from 24- and 48-h inflamed rats, respectively, compared to about 3.5 pmol/mg of protein1 in both the corresponding controls.

Rough microsomes prepared using method 2 after 24 h of inflammation were incubated with 0.2 nmol of UDP [U-14C]glucose for 0–30 min. The incubations were extracted for dolichyl monophosphoryl glucose and dolichyl pyrophosphoryl oligosaccharides, the results being presented in Fig. 2.

![Fig. 1. Dependence upon GDP {U-14C}mannose concentration of incorporation of {14C}mannose into dolichyl monophosphoryl {14C}mannose. Control (•, □) and inflamed (○, △) liver microsomes from rat 24 (•, ○) and 48 (□, △) h after injection were incubated for 5 min with 0–10 nmol of GDP {U-14C}mannose. Preparation of microsomes (method 1), incubation conditions, extraction of products were as specified under "Materials and Methods." Means from 4 experiments as presented.](image-url)
inflamed rough microsomes were prepared from rat livers and dolichyl pyrophosphoryl glucose (\(0,0\)) and dolichyl pyrophosphoryl "C-glucosylated oligosaccharides.

Maximum dolichyl monophosphoryl glucose production in both inflamed and control rats was attained at about 30 min. Inflamed microsomes again showed 2-fold greater activity, giving 3.5 pmol .mg protein\(^{-1}\) as compared to less than 2 pmol.mg protein\(^{-1}\) for controls. Glucosylated oligosaccharide production was also higher in inflamed microsomes, giving a maximum of about 16.5 pmol.mg protein\(^{-1}\) at 20 min compared to 12 pmol.mg of protein\(^{-1}\) for controls.

Further incubations with microsomes prepared using method 1 from 24- and 48-h inflamed rats were performed in the presence of exogenous dolichol monophosphate (6.7 nmol) (Fig. 3). In both 24- and 48-h control microsomes activity was stimulated 6-fold over the endogenous control level to 18 pmol of \([^{14}C]\)mannose incorporated.mg of protein\(^{-1}\). Microsomes from 24 h inflamed rats gave stimulated activity 1.3-fold higher than that in the controls, incorporation of mannose being 24 pmol.mg of protein\(^{-1}\). However, activity in the microsomes prepared 48 h after turpentine injection was not significantly different from the controls.

Microsomes prepared from inflamed and control rats using method 1 were also incubated with 0-20 nmol of dolichol monophosphate and the resulting incorporation of \([^{14}C]\)mannose into dolichyl monophosphoryl mannose was calculated as ratio of activity in inflamed microsomes to that in controls (Fig. 4). With no addition of dolichol monophosphate the ratios obtained were 2.2 and 2.0 for 24- and 48-h microsomes, respectively. In incubations with 3.3 nmol of dolichol monophosphate, the ratios decreased to 1.7 and 1.3, respectively. As the amount of dolichol monophosphate used was further increased the ratios achieved unity, this being attained with a lower amount of dolichol monophosphate in the case of the 48-h microsomes.

Levels of Endogenous Dolichol Monophosphate in Microsomes from Control and Inflamed Rats—The effect of turpentine inflammation on dolichyl monophosphoryl mannose production (in microsomes prepared using method 1) over a 72-h period was established (Fig. 5). Increases in the levels of dolichyl monophosphoryl mannose synthesized were observed as early as 6 h after induction of inflammation. However, the maximum was attained 36 h after turpentine injection, after which time the activity in the microsomes from inflamed rats decreased towards the control values. A slight decrease in control activity was noted up to 12 h after injection of 0.9% NaCl. Aliquots of these microsomes prepared from control and inflamed rats (9, 6, 12, 18, 24, 36, 48, and 72 h after saline and turpentine injection) were extracted for dolichol monophosphate as described under “Materials and Methods.” These extracts were used to stimulate the transfer of mannose to dolichol monophosphate catalyzed by microsomes prepared from untreated rats (Fig. 6). The validity of this experiment was confirmed by extracting a number of 400-µl aliquots from a batch of control microsomes in the same manner and testing the stimulation of control microsomes with these extracts. Variation within this group was found to be less than 9%, with an average incorporation of 1950 dpm.mg of protein\(^{-1}\), corresponding to 4.59 pmol.mg of protein\(^{-1}\).

Extracts from control microsomes gave a basal level of 30% stimulation, as did extracts from microsomes prepared up to 18 h after turpentine injection. However, extracts from micro-
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In Fig. 5, the progression of the inflammatory effect on \[^{14}C\]mannose incorporation into dolichyl monophosphoryl \[^{14}C\]mannose over a 72-h period. Control (●) and inflamed (○) microsomes were prepared at the times indicated after injection and incubated with 6 nmol of GDP-[\(^{14}C\)]mannose for 5 min. Preparation of microsomes and other procedures were as specified under “Materials and Methods.” Means of 3 experiments are presented.

In Fig. 6, stimulation of incorporation of \[^{14}C\]mannose into dolichyl monophosphoryl \[^{14}C\]mannose by dolichol monophosphate extracted from microsomes prepared from control and inflamed rats. Liver microsomes from untreated rats were incubated with dolichol monophosphate extracted from control (●) and inflamed (○) microsomes. The dolichol monophosphate used in each assay was obtained from 400-μl aliquots of microsomes prepared at the times indicated after injection. Preparation of microsomes (method 1) and extraction procedures were as specified under “Materials and Methods.” Incubations were at 37 °C for 5 min and contained 6 nmol of GDP-[\(^{14}C\)]mannose. Other details of incubations were as described for Figs. 3 and 4 under “Materials and Methods.” Means of 4 experiments are presented for 18, 24, 36, and 48 h after injection; all other points represent 3 determinations.

Effect of Triton X-100 on the CTP-dependent phosphorylation of dolichol—In certain incubations investigating CTP-dependent phosphorylation of dolichol, exogenous dolichol was used. The dolichol was solubilized with Triton X-100, the optimum concentration of which was determined as shown in Fig. 7 using control rough and smooth microsomes. The dolichol monophosphate used in each assay was obtained from 400-μl aliquots of microsomes prepared at the times indicated after injection, were incubated for 40 min with 2 nmol of [\(^{32}P\)]CTP. Incubations were for 10 min. The results are expressed as incorporation of \(^{32}P\) into dolichol [\(^{32}P\)]monophosphate. Procedures were as specified under “Materials and Methods.”

In Fig. 8, effect of inflammation on the phosphorylation of dolichol by CTP-dependent dolichol kinase in rat liver microsomes. Control (●, □) and inflamed (○, □) rough (●, ○) and smooth (□, □) rat liver microsomes, prepared at the times indicated after injection, were incubated for 40 min with 2 nmol of [\(^{32}P\)]CTP. Incorporation of \(^{32}P\) into dolichol [\(^{32}P\)]monophosphate was measured (A). Inflamed activity is re-expressed in B as a percentage of control activity (taken as 100%) for rough (○) and smooth (□) microsomes. Procedures were as specified under “Materials and Methods.” Points are means of 3 determinations. Average activities for nontreated rats were 4.9 and 3.8 pmol of \(^{32}P\) incorporated·mg of protein\(^{-1}\) for rough and smooth microsomes, respectively.
using exogenous dolichol were performed using a final Triton X-100 concentration of 0.012%.

**CTP-dependent Phosphorylation of Dolichol**—Rough and smooth microsomes prepared using method 2 at various times after induction of inflammation were assayed for CTP-dependent phosphorylation of endogenous dolichol, the results being presented in Fig. 8A.

It was apparent that the control animals exhibited some reaction over the first 24 h after injection. When compared to activities in noninjected rats, both rough and smooth microsomes displayed reproducibly higher activities over the first 12 h, thereafter stabilizing to close to the noninjected control values.

Inflamed rough microsomes prepared after 24 h of inflammation showed increased capacity to phosphorylate endogenous dolichol. Between 24 and 72 h after turpentine injection this activity was at a maximum of 12-13 pmol·mg of protein⁻¹. Smooth microsomes gave generally lower activities than the corresponding rough microsomes. However, smooth microsomes from inflamed rats gave higher levels of activity compared to controls after the first 24-h period. These trends are emphasized in Fig. 8B in which the results for inflamed microsomes are expressed as a percentage of the corresponding NaCl-injected controls.

Exogenous dolichol was included in incubations of rough and smooth microsomes at a concentration of 80 nmol (Fig. 9A). Under these conditions smooth microsomes from control rats were nearly 2-fold more active in phosphorylating dolichol than were the corresponding rough microsomes. Actual activities were 110 and 60 pmol·mg of protein⁻¹ for smooth and rough microsomes, respectively. Inflamed microsomes were generally more active than the controls. In contrast to the activity towards endogenous dolichol, phosphorylation of exogenous dolichol in both rough and smooth microsomes from inflamed rats rapidly increased over the first few hours after induction, reaching a maximum at 12 h. These maximum activities were 105 and 280 pmol of ³²P incorporated·mg of protein⁻¹ in rough and smooth microsomes, respectively.

The maxima seen at 12 h for inflamed microsomes were followed by a drop in activity over the next 24 h in both rough and smooth microsomes and a further drop over the subsequent 12 h in rough microsomes. Between 24 and 48 h after injection in the case of smooth microsomes, and at 48 h after injection in the case of rough microsomes, there was a second increase in phosphorylation activity in the inflamed rats. This second peak of activity corresponded to 190 pmol·mg of protein⁻¹ in the smooth microsomes and 85 pmol·mg of protein⁻¹ in the rough microsomes. In rough and smooth microsomes prepared 72 h after injection, there was no significant difference between activities in inflamed and control rats. The trends are shown in Fig. 9B in which the activities of inflamed microsomes are expressed as a percentage of the corresponding controls.

**DISCUSSION**

This paper presents, in part, data showing that turpentine-induced inflammation in rats caused increased synthesis of glycosylated dolichol phosphate derivatives which are intermediates in the biosynthesis of asparagine-linked glycoproteins (10, 12). Production of mannosyl and glucosyl monophosphoryl dolichol was increased up to 2-fold. Glucosylated dolichyl pyrophosphoryl oligosaccharide synthesis was higher than that of dolichyl monophosphoryl monosaccharides in terms of picomoles of glucose transferred, perhaps reflecting the transfer of more than one glucose residue to the oligosaccharide. The identities of the products were confirmed using thin-layer and DEAE-cellulose column chromatography, but this did not allow quantitation of the number of glucose residues on the oligosaccharide chains.

Previous work has clearly established that the incorporation of leucine and glucosamine into specific acute phase reactants was increased in turpentine-induced inflammation (6, 7, 27). The present studies were designed to follow labeling into dolichol phosphate derivatives. Labeling of proteins in the incubations was found to be very low (not shown) and hence conclusions could not be drawn as to whether or not inflammation caused increased glycosylation of end product glycoproteins. However, it has been shown in this laboratory and by others (13) that sialic acid, galactose, and N-acetylglucosamine transfer to protein in rat liver homogenates and microsomes was elevated by turpentine-induced inflammation. The turpentine-induced increases in enzyme activities in Golgi were shown by Lombart et al. (13) to be accompanied by early proliferation of the Golgi complex. Consequently, the increases in specific activities of the enzymes were small (20%) compared to the 2-fold increase in total enzyme activities. Changes in rough and smooth endoplasmic reticulum (degranulation and vesiculation) were not seen until 96 h after induction of inflammation. The specific activities of the enzymes

1. T. Coolbear, I. H. Fraser, and S. Mookerjea, unpublished observations.
for CTP-dependent dolichol phosphorylation and dolichol phosphate-linked glycosylation in endoplasmic reticulum reported in this paper were increased 2-fold within 12 and 24 h, respectively after injection of turpentine. Thus, although increases in NDP sugar glycoprotein glycosyltransferase activity may be the result of increased Golgi complex protein relative to total cellular protein, there is no evidence to suggest that a parallel explanation applies to increases in the activities of enzymes of the dolichol phosphate pathway in the rough and smooth endoplasmic reticulum.

The observed increases in dolichyl phosphoryl glycoses may be interpreted on the basis of elevated levels of either endogenous dolichol monophosphate or the relevant enzymes in the inflamed state. Our investigations utilizing exogenous dolichol monophosphate would tend to support the former interpretation. In the presence of low amounts of dolichol monophosphate, activity in inflamed microsomes prepared 48 h after turpentine injection did not differ significantly from the controls. However, at 24 h after injection the activity towards exogenous dolichol monophosphate in the inflamed microsomes was higher than in the controls and also higher than at 48 h. This might indicate that more enzyme is present at 24 h in the inflamed microsomes but, when increasing amounts of exogenous dolichol monophosphate were used in the assay, it was apparent that the ratio of control and inflamed activity decreased to unity. Thus at 48 h after injection control and inflamed activity became equal with only 10-13 nmol dolichol monophosphate, while at 24 h after injection, 20 nmol were required. This indicates that enzyme levels in inflamed microsomes were not altered compared to controls, the increases seen being due to higher amounts of endogenous dolichol monophosphate. Furthermore, the data suggest that the amount of endogenous dolichol monophosphate in inflamed microsomes 24 h after turpentine injection was higher than at 48 h.

The progression of the inflammatory effect on dolichyl monophosphoryl mannose production over a 72-h period was also investigated. A steady increase in transferase activity in inflamed microsomes compared to controls was clear over the first 36 h after injection of turpentine, followed by a gradual decrease. Minor fluctuations in the controls over the first 18 h after NaCl injection may well be due to ether anaesthesia, the saline itself, or the handling of the animals.

Dolichol monophosphate extracted from inflamed microsomes caused stimulation of control microsomal mannosyl transferase activity. It was shown that extracts from inflamed microsomes prepared 24 and 36 h after turpentine injection caused the most stimulation, suggesting that these microsomes contained more dolichol monophosphate. Fluctuations in stimulation by control extracts over the first 18 h could again be a reflection of a reaction of the rats to trauma as mentioned earlier.

The probability of increased endogenous dolichol monophosphate levels prompted investigations into possible alterations of CTP-dependent phosphorylation of dolichol in inflammation. Initial experiments showed great sensitivity of the kinase to Triton X-100. The enzyme in rough microsomes was active within only a narrow concentration range of Triton X-100, whereas in smooth microsomes the enzyme remained viable over a greater range of the detergent.

This differential tolerance has been shown for other enzymes associated with the lipid-linked glycosylation pathway (24). Smooth and rough microsomal fractions have not been shown to differ in enzymic (28, 29) or lipid (30–32) composition except, perhaps, for a thickening of the smooth membrane bilayer using electron microscopy (33). Whether such a thickening results in altered physical properties of the membranes and could therefore account for the differences with respect to Triton X-100 interactions is unknown.

The obvious distinction between rough and smooth microsomes is the attachment of ribosomes in the former. There is evidence to suggest that glycosylation of proteins via the dolichol phosphate pathway occurs while the protein is being synthesized on the ribosomes with the acceptor part of the protein molecule penetrating into the lumen of the endoplasmic reticulum for glycosylation (34). Thus it is unreasonable to speculate that there is localized perturbation of the membrane in the immediate vicinity of the ribosomes, which is probably also the site of the glycosylating enzymes. If phosphorylation of dolichol is closely associated with other dolicholophosphate requiring glycosyltransferases, then the site of the kinase involved may be more exposed to Triton X-100 in the rough microsomes than in the smooth microsomes which do not have ribosomes. The juxtaposition of dolichol phosphate-dependent glycosyltransferases with CTP-dependent dolichol kinase may be plausible if phosphorylation of dolichol is a control point of the overall pathway. The fact that the Triton X-100 tolerance profiles for CTP-dependent dolichol kinase in rough and smooth microsomes are very similar to those obtained previously for NDP glucose dolichol monophosphoryl glucose transferases (24) can be construed as indicating a common Triton X-100-membrane interaction phenomenon.

The Triton X-100 profiles may also suggest that the smooth microsomes may be contaminated by rough membranes, the peak of activity at 0.012% being accredited to rough microsomes, and the broadness being due to the superimposition of the activity of smooth microsomes themselves with a Triton X-100 optimum of 0.042%. In our previous publication (24) the data from Triton X-100 dose profiles were interpreted, in conjunction with further data, as showing luminal and cytoplasmic membrane locations for the glycosyl transferases. Whether the data for the CTP-dependent dolichol kinase can also be interpreted in this way is uncertain without further supportive results. Investigations into the cross-contamination and Golgi content of the membrane fractions using RNA and sialyl- and galactosyltransferase assays indicated that contaminants alone could not account for the observed differences in the specific activity of the enzyme in the microsomal fractions.

Investigations into the effect of inflammation on CTP-dependent dolichol kinase showed that over a 72-h period endogenous enzyme activity was very significantly increased as a result of turpentine injection, especially in microsomes. It is of interest that activity towards endogenous dolichol is lower in smooth microsomes than in rough, compared to the higher activity in smooth microsomes when exogenous dolichol is used. Also, when exogenous dolichol is included in incubations, it is apparent that in the inflamed state there is a biphasic response of the enzyme, observable in both rough and smooth microsomes, wherein the activity increases sharply during the first 12 h after injection of turpentine, declines, and then rises again 36–48 h after injection.

These results can be interpreted as demonstrating two responses to inflammation. Since exogenous dolichol can be utilized early in the same sequence of events (i.e. within 12 h), it is logical that the amount of enzyme available for synthesis is rapidly increased. However, from the results on endogenous activity it would appear that the increase in phosphorylation is limited by the amount of endogenous dolichol present in the microsomes since the increases are gradual and only clearly observable after 24 h. Thus the demonstrated activity

\[ T. \text{ Coolbear and S. Mookerjea, unpublished observations.} \]
of CTP-dependent dolichol kinase towards endogenous dolichol may be interpreted as being indicative of an increase in the availability of CTP-dependent dolichol kinase towards endogenous dolichol, and activity towards exogenous dolichol may reflect the changes in the level of the CTP-dependent kinase itself.

The presence of large amounts of the enzyme in the smooth microsomal fraction is puzzling in view of the current models suggested that an "active substance" is released from the site of inflammation (1) which in turn causes increased glycoprotein synthesis. It would be of interest to determine if such an alleged factor has a controlling role in dolichol metabolism.

REFERENCES