The addition of 200 µg of (+)-catechin to the culture media of the various type of mutant fibroblasts produced invariably a considerable decrease in the amount of neutral salt-soluble collagen and an increase in the amount of the insoluble one.

In the case of the fibroblasts from the patient with cutis laxa, the solubility profile of the collagen was shifted toward a normal pattern and the excessive amount of collagen produced, as indicated by the level of intracellular hydroxyproline, was decreased to normal levels, to suggest that an adequate level of insoluble collagen might exercise some feed-back control on the process of collagen synthesis.

When the flavonoid was added to the medium of normal, control fibroblasts, changes in collagen solubility were minimal and, indeed, the amount of insoluble collagen decreased.

Although the exact mechanism of action of (+)-catechin in decreasing collagen solubility is not clearly defined, two hypotheses have been proposed: 1. that in complex with copper ions, it might increase the oxidative deamination of lysine ε-amino groups, especially in absence of lysyl oxidase[9]; 2. that it might establish hydrogen bonds between adjacent collagen chains[4] and, possibly, covalent bonds through formation of free radicals.

The effectiveness of the flavonoid in decreasing the solubility of collagen produced by skin fibroblasts derived from different genotypes would favor a non-specific mechanism of action rather than a specific one.

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Heavy Metal Hydrolysis of Polyisoprenoid-Phosphate Mono- and Oligosaccarides[1]

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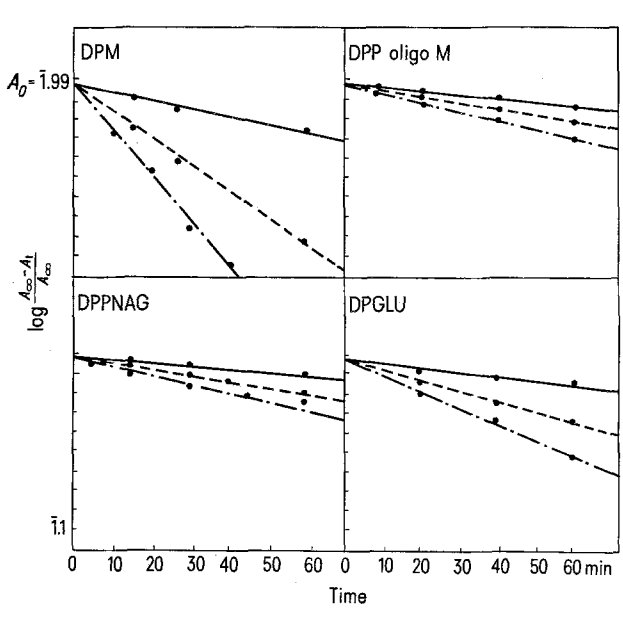
Summary. Mono- and oligosaccharide derivatives of dolichol phosphate can be hydrolyzed by heavy metal, preferably Zn^{++} at 100 °C or 65 °C. The reactions follow first order kinetics. The reaction proceeds through hydrolysis of the sugar phosphate bond.

Long-chain polyisoprenoid compounds function as glycosyl carriers in biosynthesis of complex polysaccharides of bacteria[2,3], yeasts[2], plants[4,5] and animals[6,7]. The first reaction in these pathways is the transfer of single sugar residue from sugar nucleotide to the phosphoryl derivative of dolichol[2,8], a C_{90}–C_{100} polyisoprenoid alcohol[9]. These sugar residues are believed to be transferred via a series of lipid-bound oligosaccharide intermediates[2]. Characterization of these pathways requires structural analysis of the oligosaccharide chains bound to dolichol. It would be facilitated by a technique for splitting of the intact oligosaccharide portion from the lipid-portion of the intermediate. A mild procedure for hydrolyzing polyisoprenoid-phosphate-sugars has been developed and is presented in this paper.



—, Kinetics of Zn^{++} hydrolysis at 100 °C; ——, Zn^{++} 0 M; – – –, Zn^{++} 10^{-3} *M*; —·—·, Zn^{++} 10^{-2} *M*; A_o = 1.99.

Materials and methods. Dolichol-phosphate-mannose-[^{14}C][10] (DPM), a dolichol-phosphate-glucose[^{14}C][7] (DPGlu) were prepared according to the literature in the following way. 50 µl GDP-mannose[^{14}C] (20 µCi/ml) or 50 µl UDP-glucose[^{14}C] (20 µCi/ml, both purchased from New England Nuclear, was incubated with 10 µl $MgCl_2$ 0.1 *M*. 15 µl AMP 20 m*M*, 60 µl *tris*-buffer pH 7.5 μ = 0.25, 100 µl microsomes (35 mg/ml of protein). After 15 min at 37 °C the reactions were stopped with 1 ml of chloroform, methanol, water (60:40:8). The organic phases were collected and washed several times with a solution of chloroform, methanol, water (1:16:16). Dolichol-P-P-N-acetyl-glucosamine [^{14}C] (DPPNAG) was prepared according to Ghalambor et al.[11] and separated from Dolichol-P-P-dichitobiose by thin layer chromato-

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graphy on Silicagel H with chloroform, methanol, water (60:25:4) as developing solvent, dolichol-pyrophosphate-oligosaccharide-mannose [^{14}C] (DPPoligoM) was prepared according to BEHRENS et al.[12,13]. DPM, DPGlu, DPPNAG, DPPoligoM were further purified on a sephadex LH-20 column (1.5 × 115 cm) eluted with chloroform, methanol, water (1:1:0.3) plus 50 m*M* ammonium acetate[14], followed by chromatography through a DEAE-cellulose-acetate column (1 × 20 cm) eluted successively with chloroform, methanol 2:1 (10 vol. column), methanol (10 vol. column), chloroform, methanol, water 1:1:0.3 (5 vol. column) then with a linear gradient of ammonium formate (0, 0.2 *M*) in chloroform, methanol, water (1:1:0.3). All those compounds were recovered as sharp peaks, washed exhaustively with water to eliminate all salt present then hydrolyzed as follows. The hydrolysis was carried out in a water bath at 65 °C and in boiling water. The samples were dryed in the bottom of a glass test tube and 1 ml of 25 m*M* NaCl, 25 m*M* ammonium acetate was added. Heavy metal was added to a final concentration of 10^{-3} or 10^{-2} *M*. Hydrolysis was stopped at desired time by addition of 1 ml of chloroform, methanol, water (60:4:8). The aqueous phase was removed for analysis. The organic phase was washed with chloroform, methanol, water (1:16:16) and the aqueous phase pooled with the first aqueous extraction. The various aqueous phases were counted in a LS-150 Beckman liquid scintillation spectrometer and a duplicate sample chromatographed in a sephadex G-15 or G-50 column (1.5 × 110 cm) eluted with buffer phosphate pH 7.0 μ = 0.1. All the reagents were of analytical grade.

Results and discussion. The incubation of DPM at 65 °C in ammonium acetate 25 m*M* and NaCl 25 m*M* at pH 7.0 was associated with an extremely slow release of a ^{14}C compound that was soluble in water. Addition of Zn^{++} to this mixture increased the rate of release of water soluble radioactivity from lipid. Over 90% of ^{14}C added as DPM [^{14}C] was recovered as water soluble compound after treatment with Zn^{++}, after 15 h at 65 °C or after 30 min at 100 °C. Also, Co^{++} enhanced the release of radioactivity into the water phase but was less efficient than Zn^{++}. Cu^{++}, Mn^{++}, Pb^{++}, Hg^{++}, Ni^{++} have no significant effect on the hydrolysis. The rate of Zn^{++} hydrolysis was not affected significantly by an anaerobic environment indicating the absence of intermediate on oxidative cleavage of ^{14}C from DPM [^{14}C]. The structure of the ^{14}C derivative released by Zn^{++}-catalyzed hydrolysis was examined by chromatography on sephadex G-15 and by paper chromatography (Whatman No.1) using *n*-butanol-pyridine-H_2O (6:4:3). In both experiments, the product had the same Rf as mannose [^{14}C]. The pH was checked before and after hydrolysis to be sure that no difference occurred during such hydrolysis. The reaction catalyzed by Zn^{++} therefore appears to be

$$\text{DPM} \xrightarrow{Zn^{++}} \text{DP} + \text{M}.$$

No presence of mannose-1-phosphate or other compounds was detected in any chromatographic test. The aqueous phase from the hydrolysis DP-Glu[^{14}C] and D-PP-NAG [^{14}C] on sephadex G-15 and paper chromatography give respectively Glu[^{14}C] and N-acetyl-glucosamine [^{14}C]. No other products were present. The aqueous phase from DPP-oligoM[^{14}C] on sephadex G-50 gives an homogeneous compound with 6–8 sugar residues[14]. The solvent was standardized with polysaccharides of different unit numbers between 3 and 7. DPGlu[^{14}C], DPPNAG[^{14}C], DPPoligoM[^{14}C] were submitted at the same Zn^{++} hydrolysis at 100 °C at different times. The kinetics of such hydrolysis are reported in the Figure. The kinetics of the hydrolysis catalyzed by Zn^{++} 100 °C was first order in all cases (Figure). However, the rate of hydrolysis varied with the substituent and followed the order DPM > DPGlu > DPPNAG and DPPoligoM.

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Activation of Particle Associated Rat Liver Guanine Deaminase by Lecithin and Interferences of Lecithin in Protein Precipitation

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Summary. Guanine deaminase solubilized from the 'light' mitochondrial fraction of rat liver was activated by lecithin. The activation was proportional to the concentration of lecithin taken in the system. A ratio of 1:1 between the two constituents (protein and lecithin) was at least necessary for complete precipitation.

Requirement of various lipids in membrane bound enzymes has been reviewed by COLEMAN[2]. Specific requirement of lecithin in the enzymes β-hydroxybutyrate dehydrogenase (from beef heart mitochondria) and acetyl CoA synthetase (from rat liver mitochondria) has been demonstrated[3–6]. Unspecific requirement for lecithin has been demonstrated in many cases[7–11].

In the present communication, we have demonstrated the activation of the solubilized particle associated rat liver guanine deaminase by lecithin and interference of lecithin in protein precipitation by perchloric acid.

Materials and methods. Albino rats were reared in the departmental animal house. All experiments were performed at 0–4 °C unless otherwise specified. Cell fractionation was according to KUMAR[12], excepting that the residue collected between 5,000 × *g* and 15,000 × *g*, designated as the 'light' mitochondrial fraction was washed twice with the sucrose medium instead of washing only once to get more purified fraction. Solubilization process includes freezing and thawing followed by Vir-Tis disruption and treatment with Triton X-100.

Guanine was procured from E. Merck (Federal Republic of Germany) and lecithin from V.P. Chest Institute (New Delhi). Other reagents used were of analytical grade.