qualitative results of the autoradiographs with tritiated MNDP for normal human tissues[11-13].

Conclusion. α-Particle track autoradiography provides a useful method for evaluating the detailed localization of the compound in cells and tissues. The uptake into critical tissues such as bone marrow, colon and lung was low.

These results are important from the point of view of the development of this endoradiotherapeutic drug.

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Transfer of oligosaccharide from oligosaccharide pyrophosphoryl dolichol to endogenous acceptor proteins in human breast malignant and normal tissues

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Summary. We have prepared dolichylpyrophosphoryl-[^{14}C]-oligosaccharide (Dol-PP-oligosaccharide) from calf thyroid. Microsomal fractions from human breast tissues catalyzed the transfer of labeled oligosaccharide to endogenous acceptor proteins. Malignant tumors showed higher activity of the oligosaccharide transferring enzyme than normal tissue. With kojibiose (Kj), and inhibitor of (Glc_3)-glucosidase, an increase in the radioactivity associated with glycoprotein was observed.

Key words. Oligosaccharide transfer; human breast cancer glycoproteins; kojibiose.

The fact that glycosylation of eucaryotic proteins involves lipid-linked saccharides as intermediates is well documented[1,2]. The final step in this metabolic pathway is the transfer 'en bloc' of the oligosaccharide from Dol-PP-$(GlcNAc)_2Man_9Glc_3$ to an asparagine residue of a protein acceptor.

We have already demonstrated the conversion of GDP-Man and UDP-Glc to Dol-P-Man and Dol-P-Glc respectively, catalyzed by the microsomal fraction from human breast tissues[3,4]. In addition, differences were detected between normal, benign and neoplastic samples[5].

Due to the present interest in glycoproteins as possible biological markers in human breast neoplasia[6-8] we extended our studies to the reaction involving the membrane-bound oligosaccharide transferring enzyme.

Materials and methods. Chemicals and substrates. All organic solvents were analytic grade. [^{14}C]-Glc (360 μCi/μmol) was from New England Nuclear. Kojibiose was supplied by Koch Light. All culture media were from GIBCO Laboratories. The hen oviduct Dol-PP-oligosaccharide, heterogeneous, consisting of four species with the composition $Glc_{0-2}Man_9GlcNAc_2$ and Glc_3- as a major component, used in the experiments described in tables 1 and 2, was kindly supplied by Dr Quesada.

Preparation of [^{14}C]-oligosaccharide-lipid. Calf thyroid slices (4 g) were incubated as described by Spiro et al.[9] with 250 μCi of [^{14}C]-Glc for 3 h at 37 °C. The tissue was washed 3 times with 0.25 M sucrose and homogenized in 0.2 M EDTA, pH 7.7. Chloroform and methanol were then added to achieve a mixture of chloroform/methanol/water (C/M/W) 3:2:1. To obtain the oligosaccharide-lipid, the interphase pellet was extracted as described by Spiro et al.[9].

Preparation of human breast microsomal fraction. Human breast tissues, obtained from mastectomy specimens or biopsies, were classified as benign or malignant according to their anatomopathological characteristics. The 'normal' sample was peritumoral tissue from the same patient. The tissues were homogenized as described[3]. Two types of membrane preparations were used. 'Unwashed' membranes were prepared as previously described[3], and 'washed' membranes according to Lucas et al.[10], omitting the incubation treatment.

Assay for transfer of labeled oligosaccharide from oligosaccharide-lipid to endogenous acceptor proteins. Standard reaction mixtures contained radiolabeled oligosaccharide-lipid ($6-10 \times 10^3$ cpm), 100 mM Tris-HCl pH 7.5, 8 mM $MgCl_2$, 8 mM $MnCl_2$, 2 mM EDTA, 40 mM 2-S-ethanol, 0.2% Triton X-100, with or without 1 mM Kj. The reactions were initiated by adding 350–500 μg of microsomal protein to the incubation mixture to a final volume of 50 μl, and incubated at 15 °C for 5–15 min. The reaction was stopped with 1 ml of C/M 3:2 and processed as described[3]. The 'lower phase' of the Folch's partition contains the lipid-bound monosaccharides and oligosaccharides up to 7–8 hexoses[11], while sugars are in the 'upper phase'. The extract C/M/W 10:10:3 contains polyprenyl-PP-oligosaccharides with more than seven hexoses[12] and the 'hot TCA resistant' material is considered as glycoprotein[13].

Other methods. Mild acid hydrolysis, radioactivity and protein determination, paper chromatography and microanalytic DEAE-cellulose chromatography used here were previously described[3].

Results and discussion. In our previous studies[3,4] the procedure used to assess protein glycosylation was based on the transfer of Man or Glc from GDP-[^{14}C]-Man or UDP-[^{14}C]-Glc respectively, to the endogenous protein fraction. In these studies the optimum temperature for glucoprotein formation was 15 °C[3].

Based on this observation we incubated microsomes from malignant and benign human breast tumors with labeled hen oviduct Dol-PP-oligosaccharide at 15 °C. Table 1 shows negligible radio-

activity associated with the hot TCA resistant fraction. At the same time the label present in the combined lower and upper phases suggests that the [^{14}C]-oligosaccharide underwent a remarkable degradative process involving glucosidases, copurified in the microsomal preparation.

Immediately after oligosaccharide transfer, at least two different membrane-bound glycosidases remove the Glc residues, permitting other processing reactions. One of these two, (Glc_3)-glucosidase, cleaves the external Glc(1–2)Glc linkage[14], the disaccharide Kj being its most potent inhibitor[15].

Table 2 illustrates the striking effect of Kj; a nearly 40-fold increment of the radioactivity associated with endogenous proteins of microsomes from malignant human breast tumors. This concords with the decrease in radioactivity found in the combined lower and upper phases derived from oligosaccharide breaking, probably *after* its transfer to proteins. This seems to be the sequence since the percentage of the compound present in C/M/W 10:10:3 was similar in both reactions. This hypothesis is reinforced by reports that Kj did not affect the disappearence of the Dol-PP-derivatives at detergent concentrations of 1–4%[15]. The detergent/protein ratio used here are similar although our assays were performed at 0.2% of Triton X-100[3].

For the experiment described in table 3, the Dol-PP-oligosaccharide prepared from calf thyroid was purified through a DEAE-cellulose column; its identity, tested by paper chromatography in butanol/pyridine/water 4:3:4, was similar to the hen oviduct oligosaccharide described above (data not shown). We used 'washed' microsomes from malignant tumor and peritumoral tissue from the same patient.

Lucas et al.[10] demonstrate that 'washed' and 'unwashed' membranes have similar characteristics except that in the former there is greater specific activity of glycosyl transferases and lower Dol-P-Man turnover.

Unexpectedly, Kj did not increase radioactivity in the hot TCA resistant fraction. One explanation for this finding is that a large quantity of (Glc_3)-glucosidase was lost during the preparation of 'washed' microsomes.

On the other hand, differences were observed between the two tissues. Thus, without Kj, malignant membranes show greater radioactivity in the hot TCA resistant fraction (nearly threefold) than the normal counterpart. Concomitantly, the label remaining in the C/M/W extract was lower. These findings, together with the similar amount of degradative products present in the combined lower and upper phases, allowed us to conclude that the transferring reaction is more efficient in malignant than in normal samples.

It should be pointed out that in no experiment did we achieve total transfer of the oligosaccharide to acceptor proteins, therefore we speculate that with these experimental conditions, the amount of endogenous acceptor and/or transferring enzyme was not sufficient.

We must consider that the transferring enzymatic system described here comes from microsomes prepared with whole tumor mass, consisting of subpopulations of cells with different properties[16]. The same rationale can be extended to the normal samples used. Therefore we cannot attribute any of the differences observed between tumor and normal samples to any particular class of cells. However, we believe that our results are significant because they represent the sum of the biochemical events that take place in these complex biological systems.

In summary, we have presented evidence that microsomes from human breast tissues are able to catalyze the transfer of the oligosaccharide to endogenous proteins. The differences observed between malignant and normal samples could be related to the atypical pattern of glycosylation observed in several antigens associated with malignancies[6–8].

Table 1. Labeled oligosaccharide transfer from Dol-PP-oligosaccharide to endogenous acceptor protein in benign and malignant human breast tumors

Fraction	Radioactivity distribution			
	Benign		Malignant	
	cpm/mg protein	%	cpm/mg protein	%
Lower + upper phases	5635	48.0	6083	52.7
C/M/W 10:10:3	5286	45.8	4885	42.2
Hot TCA soluble	485	4.2	375	3.2
Hot TCA resistant	133	1.2	215	1.9

520 µg of 'unwashed' microsomal protein from benign tumor (13.3 mg/ml) and from a pool of five malignant tumors (35.0 mg/ml) were incubated with 6000 cpm of hen oviduct Dol-PP-oligosaccharide for 15 min as described in 'Materials and methods'.

Table 2. Effect of kojibiose on the oligosaccharide transfer reaction

Fraction	Radioactivity distribution			
	Without kojibiose		With kojibiose	
	cpm/mg protein	%	cpm/mg protein	%
Lower + upper phases	5750	69.4	4152	45.9
C/M/W 10:10:3	2772	29.0	2660	29.5
Hot TCA soluble	88	0.9	110	1.2
Hot TCA resistant	62	0.6	2098	23.3

500 µg of 'unwashed' microsomal protein from a pool of three malignant human breast tumors (17.5 mg/ml) were incubated with 7000 cpm of hen oviduct Dol-PP-[^{14}C]-oligosaccharide for 5 min, with or without 1 mM Kj.

Table 3. Transference of the [^{14}C]-oligosaccharide in normal and malignant human breast tissues. Effect of kojibiose

Fraction	Radioactivity distribution					
	Malignant + kojibiose		Malignant		Normal	
	cpm/mg protein	%	cpm/mg protein	%	cpm/mg protein	%
Lower + upper phases	14600	53.4	14094	54.8	15577	50.5
C/M/W 10:10:3	7886	20.0	6897	26.8	13142	42.6
Hot TCA resistant	4648	17.0	4471	18.4	2122	6.9

350 µg of 'washed' microsomal protein from malignant tumor (25 mg/ml) and peritumoral tissue of the same patient (7 mg/ml) were incubated for 10 min with 10000 cpm of calf thyroid Dol-PP-[^{14}C]-oligosaccharide, with or without 1 mM Kj.

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