isoproteins of liver antitrypsin nearly or completely coincided in the focusing field with a corresponding isoprotein in an authentic sample of asialo M variant, that is, not containing sialic acid (compare sample 2 with sample 3, Fig. 3). The microheterogeneity of some glycoproteins is largely due to uneven sialylation of their individual components (*11*). From observations on the M variant, we concluded earlier that uneven sialylation was not the cause of the microheterogeneity of antitrypsin (*12*). The demonstration of microheterogeneity of sialic acid–free liver antitrypsin confirms the earlier conclusion. Our results indicate that the microheterogeneity of antitrypsin becomes established before it leaves the RER. Hence, microheterogeneity is probably caused by variations in the content of charged amino acids of the isoproteins.

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Decrease of Human Serum Fucosyltransferase as an Indicator of Successful Tumor Therapy

Abstract. *Surgical removal of colon carcinomas leads to a decrease in the rate of incorporation of [^{14}C]fucose into its endogenous acceptor in human serum; normal incorporation rates are attained within 14 days. A similar time course has been determined for α_2- and α_3-fucosyltransferase when either desialo- or desialodegalactofetuin are employed as exogenous acceptors. A correlation has also been seen between transferase activity and the therapeutic response of patients with breast cancer. These results indicate that the determination of fucosyltransferase activity can facilitate the diagnosis of neoplasia, and the success of surgery, chemotherapy, or radiation.*

The glycosyltransferases that add the terminal sugars L-fucose and *N*-acetylneuraminic acid to nascent glycoconjugates have been studied in different tissues (*1*), especially for their role in oncogenic processes (*2*). Elevations in the serum glycosyltransferases sialyltransferase (*3*), fucosyltransferase (*4*), and galactosyltransferase (*5*) have been found in patients with neoplastic disease. At least two fucosyltransferases have been detected in human serum: α_2-fucosyltransferase transfers L-fucose from guanosine diphosphate (GDP)-L-fucose to the terminal galactose residues of oligosaccharides, desialylated glycoproteins (*6*), or glycolipids (*7*) by forming (1 → 2) linkages; α_3-fucosyltransferase adds L-fucose at the C-3 atom of free or protein-bound *N*-acetyl-D-glucosamine (*6*). Fucosyltransferase activity is especially high in the serums of patients suffering from highly malignant or metastatic tumors (*4*). We now show a correlation between these enzyme levels and the patient's response to tumor therapy.

Randomly selected blood samples were collected in anticoagulant-free tubes, and the serum was separated for the assay. A characteristic decrease in the incorporation of [^{14}C]fucose into its endogenous acceptor was found 4 to 6 days after surgery for colon carcinoma (Fig. 1); this rate approached normal values within 14 days. However, a transient minor elevation of the incorporation rate may also be seen (Fig. 1). When desialofetuin, or fetuin from which both *N*-acetylneuraminic acid and galactose have been removed, were employed as exoge-

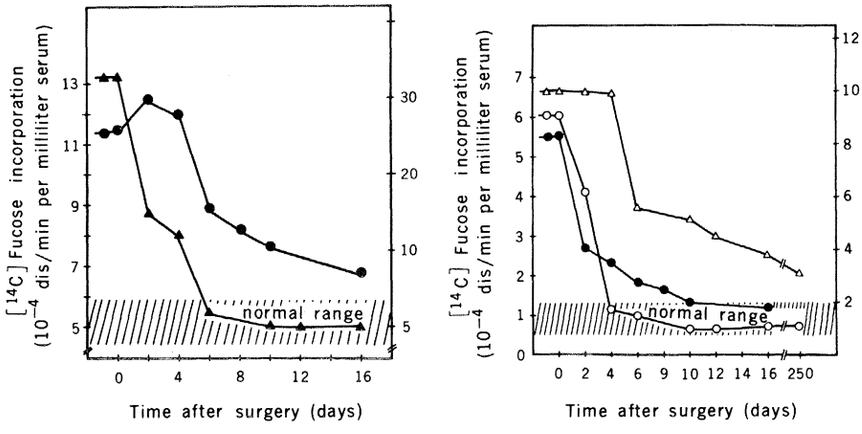

Fig. 1 (left). Incorporation of [^{14}C]fucose into endogenous acceptors of human serum before and after removal of colon carcinoma by T. W. (●) (72-year-old male, values indicated on left ordinate) and G.K. (▲) (59-year-old female, values indicated on right ordinate). The total volume of the incubation mixture (*p*H 5.5) was 120 μl, comprised of morpholinoethane sulfonic acid (4 μmole), $MgCl_2$ (2 μmole), sodium azide (0.4 μmole), GDP-L-[U-^{14}C]fucose (100,000 dis/min), and 30 μl of serum. The mixture was incubated for 21 hours at 37°C, and protein-bound radioactivity was determined (*4*). Each point represents the average value of duplicate determinations. The normal range is 40,000 to 60,000 dis/min per milliliter of serum. Fig. 2 (right). Pattern of fucosyltransferase activities in human serum at different times after surgery for colon carcinoma. T. W. (●) (values on left ordinate) and G. K. (○) (values on right ordinate) were assayed for α_2-fucosyltransferase. G.K. (△) (values on right ordinate) was assayed for α_3-fucosyltransferase. Incubation conditions were the same as described in the legend of Fig. 1, except that either 0.5 mg of desialofetuin or desialodegalactofetuin were added as exogenous acceptors. The normal range for α_2-fucosyltransferase was 4,000 to 20,000 dis/min per milliliter of serum; and for α_3-fucosyltransferase it was 10,000 to 30,000 dis/min per milliliter of serum.

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nous acceptors of [^{14}C]fucose, a similar time course for enzyme decrease was observed (Fig. 2). Immediately after surgery, the activity of α_2-fucosyltransferase decreased, and at day 10, only 20 percent of the original activity was still detectable. In comparison, α_3-fucosyltransferase sometimes exhibited a delay before its activity declined, and this decline was less pronounced. If anesthesia and surgery do not substantially alter the rate constants of protein degradation, a half-life for serum α_2-fucosyltransferase of about 1.8 days can be calculated from a plot of enzyme activity as a function of time after surgery.

Glycosyltransferases are thought to be either secreted by neoplastic cells (*8*) or released during cellular degradation (*9*). After colectomy, the main source of glycosyltransferases is removed, and the possibility that fucosyltransferase activity is due to "leakage" from surface membranes is negligible, because base line levels of the enzyme in healthy donors are quite low.

An unusual enzyme pattern was determined for several patients with malignancy. The incorporation of [^{14}C]fucose into the endogenous acceptor and the activities of α_2- and α_3-fucosyltransferase with the exogenous acceptor exhibited only a slight fall after surgery; this was followed by a steady increase in the enzyme a few days thereafter. Later analysis revealed that either only part of the tumor had been removed or that it had been impossible to excise all metastases. These findings indicate that serum fucosyltransferase activities can be of diagnostic value for evaluating treatment efficacy in patients with malignant disease. A good correlation between the transferase activity and the clinical condition of the patient was also found in women with breast cancer (Fig. 3); in general, a marked reduction of α_2-fucosyltransferase activity was noted, accompanied by a less dramatic decrease of [^{14}C]fucose incorporation into the endogenous acceptor. In addition, we tested patients who were responding favorably to a treatment regimen over several years that included therapy-free intervals; their enzyme activity with the endogenous acceptor was below the normal range.

An increase in glycoproteins during inflammatory or oncogenic processes has been described (*10*), and some of these proteins with free acceptor sites are responsible for the incorporation of [^{14}C]fucose into human serum. We attempted to characterize the endogenous acceptor by comparing the activities of the five major fractions of human serum: albumin, α_1-globulin, α_2-globulin, β-globulin, and γ-globulin. Serum was incubated in the presence of labeled GDP-fucose and fractions were separated on cellulose acetate strips. The strips were then fixed, stained, and analyzed on a densitometer to determine protein-bound radioactivity. When different types of malignancy were compared, the α_1-globulin fraction usually showed the highest [^{14}C]fucose incorporation, probably because of acid α_1-glycoprotein and α_1-antitrypsin, which are thought to increase in the presence of malignant growth, by a nonspecific response of the liver. However, no uniform pattern of fucosyltransferase activities with the endogenous acceptors was seen. In plasmocytoma cases, for example, substantial amounts of [^{14}C]fucose were also found in the α_2- and β-globulin fractions.

Molecular variants of serum glycoproteins have been reported (*11*), and the described electrophoretic and chromatographic heterogeneity probably reflects differences in amino acid sequence or carbohydrate composition. Alterations in the content of *N*-acetylneuraminic acid (*11*) are often responsible for these multiple forms, and it is conceivable that different types of tumors are responsible for different molecular variants of glycoproteins being synthesized and released into the blood stream as acceptors for [^{14}C]fucose. The malignant cells may also secrete still unknown carcinoembryogenic glycoproteins with free acceptor sites, and analysis of these proteins may aid in the diagnosis and differentiation of neoplastic diseases.

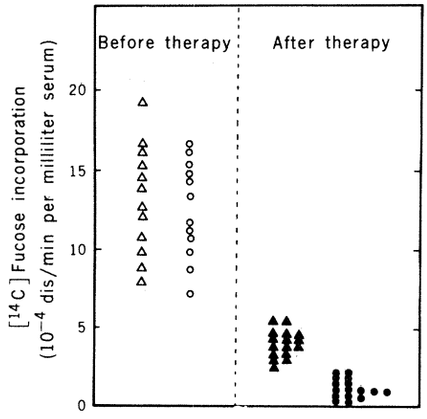

Fig. 3. Influence of therapy for mammary carcinoma on fucosyltransferase activities. Incorporation of [^{14}C]fucose into the endogenous acceptor in human serum (▲, △) was compared to the activity of α_2-fucosyltransferase toward the exogenous acceptor desialofetuin (●, ○). The size and location of the tumor were evaluated, taking into account the number of metastatic sites. Simple (or occasionally radical) mastectomy was performed, the patients were supplemented by radiotherapy if necessary. Especially in patients in whom metastases had spread—and if liver, lung, or bones were involved—the Cooper regimen of chemotherapy, with cyclophosphamide, methothrexate, 5-fluorouracil, vincristine, and prednisone, was also followed. Results from these patients show that both the incorporation rate of [^{14}C]fucose into the endogenous acceptor in human serum as well as the activity of α_2-fucosyltransferase decrease by 60 to 80 percent within 14 days after tumor removal. In these cases, a similar postoperative time course as was seen in colectomy patients was observed.

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