Upsala J Med Sci 86: 237–247, 1981

The Use of Con A Sepharose as an Affinity Adsorbent in a Simple Assay of Serum Sialyl and Fucosyltransferase and its Application in Tumour Diagnosis

Eric Cervén,[1] Gunnar Ronquist,[2] Åke Rimsten[3] and Gunnar Ågren[1]

From the Institute of Medical and Physiological Chemistry[1], Biomedical Center and Departments of Clinical Chemistry[2] and Surgery[3], University Hospital, University of Uppsala, Uppsala, Sweden

ABSTRACT

A considerably simplified assay for recording sialyl- and fucosyltransferase in human serum is presented. Serum samples incubated with labeled nucleotide-sugar and glycosylated endogenous acceptor molecules were adsorbed to Con A Sepharose and quantitated by scintillation counting. The results correlated with those of a much more timeconsuming acid precipition method, and displayed a higher diagnostic sensitivity due to the improved specificity of the method and the combined recording of the two activities.

A correlation between serum sialyl- and fucosyltransferase activities as well as quantitative agreement between the amount of incorporated sialic acid and fucose indicated that the endogenous acceptor molecules were rate-limiting for transfer and may themselves have diagnostic potential.

INTRODUCTION

It is known that the activity of certain serum glycosyltransferases can be correlated to malignant diseases (1,10,12,14,15). Particularly sialyl-, fucosyl- and galactosyltransferases are supposed to be of importance in the diagnosis and follow-up of some malignant diseases, although the laboratory procedures for the assay must be improved and simplified (1,14). In most previous studies of serum or plasma glycosyltransferases in connection with malignant diseases, overlapping activities of sera from other patients have been observed. The reason of this finding is unclear and several different factors are probably involved. In the future development in this promising field, however, an increased accuracy and precision in the analytical method as well as development of simpler laboratory assays seem to be necessary.

Evidence was obtained in our laboratory that sialyltransferase is located at the outer surface of Ehrlich tumor cells (5,6,7). Based on these observations we initiated a clinical study of serum sialyltransferases. A rationale for such studies is that glycosyltransferases and acceptor molecules located at the cell surface may be released to the extracellular fluid by membrane

shedding (9) or proteolytic cleavage (17). It is also assumed that shedding is more active on neoplastic cells when compared to resting normal cells. Using an acid precipitation assay, we measured the incorporation of sialic acid into endogenous serum glycoconjugates and also estimated the saturation of the endogenous acceptor molecules with sialic acid and breakdown (16). In a large clinical material comprising patients with several different tumours and benign surgical diseases, we observed a significantly elevated sialyltransferase activity in serum samples derived from patients suffering from colorectal cancer. Other types of neoplastic diseases displayed no changed transferase activity and in some cases decreased activity when compared to the control material (16).

Considering the possibility that cancer patient sera may contain low molecular weight acid-soluble glycopeptides derived from proteolytic cleavage at the tumour cell surface, and that these glycopeptides may be characterized by incomplete glycosidation (8,13,18), exposing mannose and glucose which bind to Con A, the assay was modified to include such glycopeptides. Thus, in the present work, the acid-precipitation step was substituted for a final short incubation in the presence of Con A Sepharose^{R} gel, followed by repeated washings. Labeled glycoconjugates exposing terminal glucose or mannose then bound to the gel and could easily be assayed for incorporated sugar. Serum samples derived from patients with colo-rectal cancers and with benign surgical diseases were analyzed for incorporation of sialic acid and fucose.

METHODS

Blood sampling. Blood was obtained by venipuncture from patients with colorectal cancers as well as from patients with benign surgical diseases. After clotting the serum was separated by centrifugation and stored at -20^{o}C for at the most 2 months until analyses.

Assessment of Con A-binding serum ^{3}H AcNeu-sialoconjugates and ^{14}C fucoconjugates.

Con A Sepharose (Pharmacia AB, Uppsala) was washed with 20 volumes of Tris-HCl (0.02 moles/l, pH 7.65) containing calcium chloride, magnesium chloride, manganese sulfate (1 mmole/l of each) and sodium chloride (0.05 moles/l), and kept overnight at 4^{o}C in two volumes of that buffer.
0.10 ml of NaEDTA (0.1 moles/l, pH 7.4), 0.20 ml of serum and 1.00 ml of sodium cachodylate-acetate buffer (0.05 moles/l with respect to cachodylate, pH 6.8) containing 10.8 · 10^{-6}moles/l of CMP-^{3}H AcNeu (18.9 Ci/mmole) were pipetted into the apical bottom of glass centrifuge tubes put on ice. (The final concentration of CMP- ^{3}H AcNeu was 8.31 · 10^{-6} moles/l). The tubes were then transferred to a water bath at 37^{o}C and incubated for 3 h. Thereafter, they were chilled fo · 10 min on ice. 0.75 ml of Con A Sepharose in a total volume

of 1.5 ml of buffer suspension were added to each tube, the tubes again transferred to the incubator and stirred manually in order to keep the gel suspended.

After incubation for 5 min at 37°C, the tubes were chilled on ice for 10 min followed by the addition of 5 ml of the ice-cold Tris-HCl buffer. The gel was allowed to sediment at unit gravity on ice or by centrifugation at 400 x g for 5 min followed by aspiration of the supernatant. The gel was subsequently washed 6 times with 10 ml of the ice-cold Tris-HCl buffer with the first 5 ml of buffer added to the upper margin of the tubes. Care was taken to suspend the gel completely during each washing. Finally, 0.5 ml of buffer was added, the gel suspended and transferred to a scintillation vial, and counted in the presence of 10 ml of Aquasol (NEN Chemicals, GmbH, Frankfurt on- Main, Germany).

The incubations with GDP-fucose (^{14}C fucose-labeled, 216 Ci/mole from NEN Chemicals) were performed identically with the aforementioned incubation except that the amount of the precursor nucleotide-sugar was 0.185×10^{-9} moles, added from the stock solution in 4 µl of ethanol-water.

The incubations with nucleotide sugar were performed on 8 different occasions with sera from patients with colorectal cancers and with benign surgical diseases. On each occasion, the mean value of incorporation of sugar into serum glycoconjugates from sera of patients with benign surgical diseases was indexed 1.00. Each serum probe was then related to this index in proportion to the amount of incorporated sugar. About 10 serum samples were analyzed consecutively on two occasions, constituting methodology controls. ^{3}H AcNeu-sialyzed glycoconjugates were also assessed by acid precipitation, as previously described (16). The results are thus given as indices and the reason is that the additive diagnostic value of serum sialyl and fucosyltransferase can be recorded in spite of different absolute amounts of incorporated sugar residues.

RESULTS

Serum samples of 0.2 ml from control patients with benign surgical diseases when incubated for 3 h with $8.31 \cdot 10^{-6}$ moles/l of CMP- ^{3}H AcNeu incorporated 66.6×10^{-12} moles $\pm$ 11.5×10^{-12}, n=48, of labeled AcNeu into glycopeptides having affinity for Con A-Sepharose. This corresponds to approximately 0.616% of the added CMP- ^{3}H AcNeu and 44% of the acid-precipitable ^{3}H AcNeu incorporated during an equilvalent incubation (Fig 1).

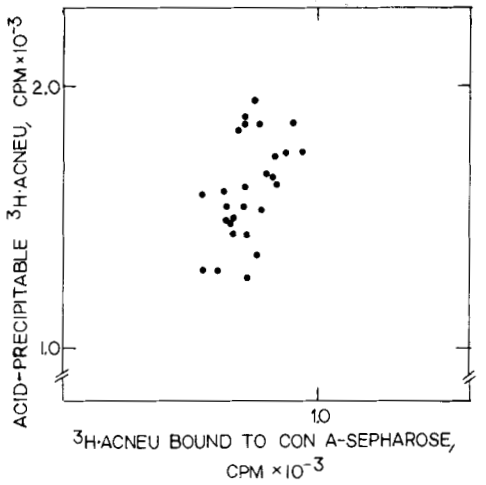


Fig. 1. Correlation of acid-precipitable ^{3}H AcNeu and Con A-bound ^{3}H AcNeu from identical serum samples of 0.2 ml incubated for 3 h at 37°C with CMP-^{3}H AcNeu.

In Fig. 2a the ^{3}H AcNeu bound to 0.75 ml of gel was indexed 100 % while, as shown in Fig 2b, this amount of gel is slightly suboptimal for binding the labeled glycopeptides. It can be calculated from the data presented in Fig. 2b that an infinite amount of gel would bind only 1.8 times more labeled glycopeptides than the actual amount of gel used routinely, 0.75 ml, which by this criterion is considered suitable for the assay.

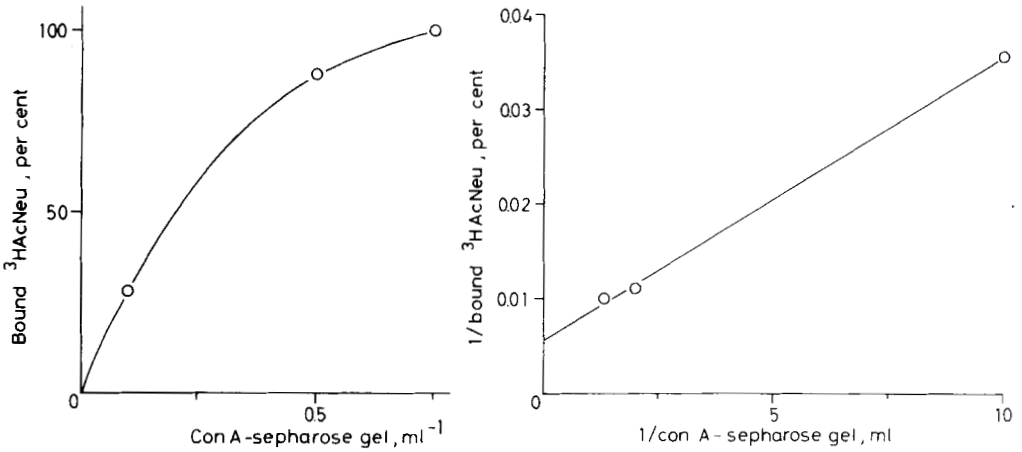


Fig 2A and B. Determination of the amount of Con A gel suitable for binding ^{3}H AcNeu-labeled glycoconjugates derived from incubating serum samples of 0.2 ml for 3h at 37°C. Samples derived from cancer patients and a normal correlate were incubated with CMP-AcNeu and the labeled glycoconjugates of each serum adsorbed to 0.1, 0.5 or 0.75 ml of gel. The mean of the cpm-value adsorbed to 0.75 ml of gel was set to 100 % and the other mean values of incorporation related to this percentage.
A. represents the actual percentage while B represents a graph with inverted values.

The incorporation of sialic acid into Con A -binding serum glycoconjugates from patients with benign surgical diseases was indexed 1.00 with standard deviations of 0.17 (n=48). This figure is derived from two separate incubations where 25 and 23 control serum samples were analyzed, giving a mean incorporation of 317 x 10^{-9} moles/l and 349 x 10^{-9} moles/l serum respectively. Thus the mean incorporation of ^{3}H AcNeu corresponding to the index 1.00 is 333 x 10^{-9} moles/l of serum/3h. The same serum samples were used in consecutive assays without significant loss of activity. The index for incorporation of ^{3}H AcNeu into Con A -binding glycopeptides in sera from patients suffering from colorectal cancer was 1.26 $\pm$ 0.22. This figure is derived from 72 different sera where the incorporation into colorectal cancer serum glycopeptides was correlated with control sera indexed 1.00 and corrected for slight variations of the absolute incorporation due to variations of methodology. Such variations which amounted to less than 15 % of the mean incorporation could be explained by the fact that the washing procedure following incubation with CMP- ^{3}H AcNeu was not completely standardized regarding temperature, time and sugar-binding capacity of the lectin-gel.

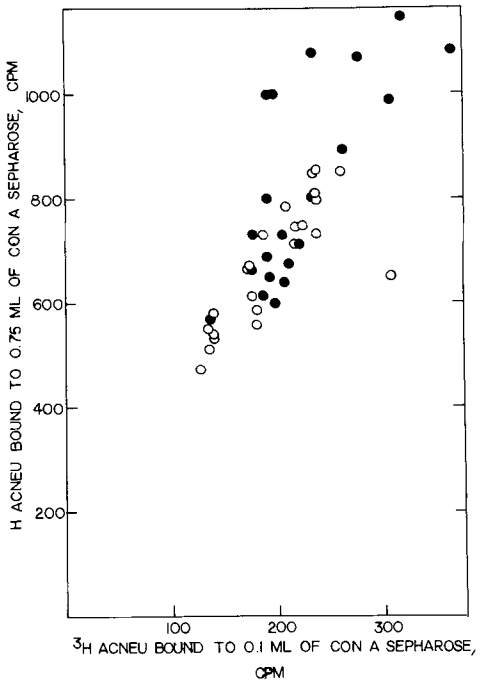


Fig. 3 A Representation of correlation between ^{3}HAcNeu-labeled material from serum samples incubated with CMP ^{3}HAcNeu and adsorbed to different amounts of gel. Filled circles represent serum samples derived from cancer patients while open circles represent samples derived from patients with benign surgical diseases. Serum samples are identical with those investigated in Fig 3 B. Abscissa: 0.1 ml of gel.

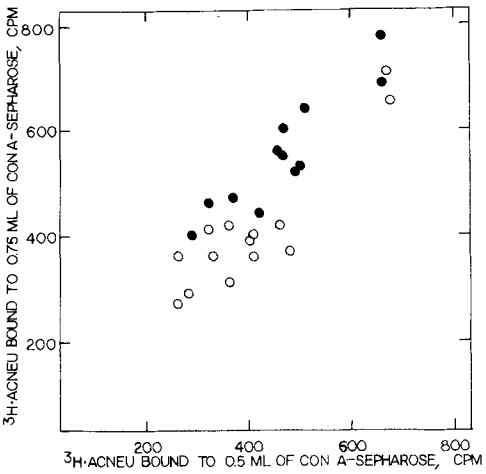


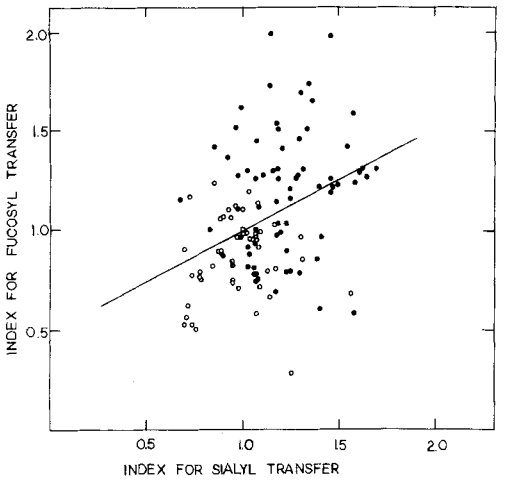
Fig.3 B. Representation of correlation between ^{3}H AcNeu-labeled material from identical serum samples incubated with CMP ^{3}H AcNeu and adsorbed to different amounts of gel. Filled circles represent serum samples derived from cancer patients while open circles represent samples derived from patients with benign surgical diseases. Abscissa: 0.5 ml of gel.

For comparison, the acid precipitation assay (16) yielded an index of 1.000 ± 0.288 from 152 patients with benign surgical diseases and an index of 1.189 ± 0.520 (n=21) for sera from patients with colo-rectal cancer after 3 h of incubation with CMP- ^{3}H AcNeu under equivalent conditions (16). Thus, the discriminative power of the present Con A -binding method seems to be somewhat better than the previously used acid precipitation of ^{3}H AcNeu -labeled glycopeptides. Furthermore, in some separate cases, it was possible to achieve a further slight improvement of the diagnostic usefulness of the Con A -binding assay by increasing the amount of gel (Fig. 3 A and B).

Aiming at an even better diagnostic value, serum fucosyltransferase was introduced in the assay. After 3 h of incubation of control sera with 0.142×10^{-6} moles/l of GDP- ^{14}C fucose, 2.21 % $\pm$ 0.44 of the added precursor had been incorporated into serum glycoconjugates that bound to Con A -Sepharose. This corresponds to 20.44×10^{-9} moles/l of serum or an index for normal sera of 1.00 ± 0.20 (n=48). The corresponding index for sera from patients suffering from colorectal cancer was 1.31 ± 0.32 (n=72). This finding is in agreement with previous reports (1,2) that serum fucosyltransferase may be useful in the diagnosis of colon cancer.

A correlation was observed between the incorporation of sialic acid and that of fucose (Fig. 4.). For 120 incubations of serum samples with CMP- ^{3}H AcNeu and GDP- ^{14}C fucose, the correlation coefficient was 0.37 in the sialyltransferase index range of 0.6-1.7. Contrary to sialyltransferase, serumfucosyltransferase was not stable to repeated freezing and thawing, which necessitated

that the change in activity of fucosyltransferase in consecutive incubations was compensated for.



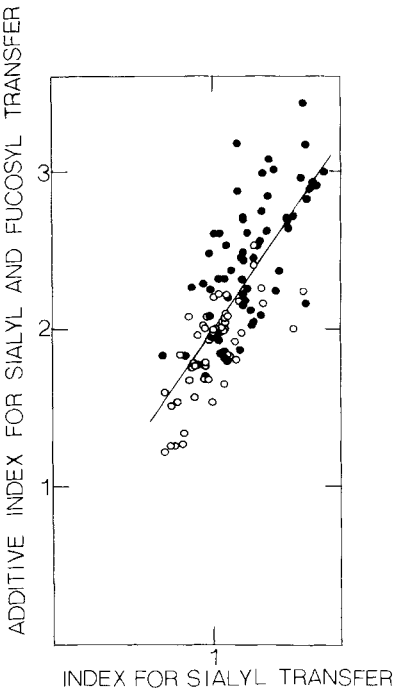


Fig. 4. A. Correlation between serum sialyltransferase activity and serum fucosyltransferase activity. (Upper figure)
B. Correlation between sialyltransfer and (sialyltransfer + fucosyltransfer). Serum samples from the same patients were incubated with either CMP ^{3}H AcNeu or GDP-^{14}C fucose for 3 h at 37°C and the labeled glycoconjugates bound to Con A Sepharose. The mean cpm-values for the incorporation into glycoconju-

gates in sera derived from patients suffering from benign surgical diseases was indexed 1.00 and the incorporation into each sample expressed in relation to this index. The representation with indices has been chosen in order to take advantage of the additive diagnostic sensitivity of sialyl- and fucosyltransferase activity in spite of the fact that they differed 16-fold in absolute amount of sugar that was incorporated into endogenous acceptor molecules.

The sum index of serum sialyltransferase and serum fucosyltransferase was 2.00 ± 0.29 for sera representing surgical diseases while that of colorectal cancers was 2.55 ± 0.41. This represents an improved diagnostic sensitivity when compared to either enzymatic activity only. Finally, the correlation between serum sialyl- and fucosyltransferase was explored by making a graph of sum index of sialyl- and fucosyl transfer versus the index for sialyl transfer (Fig. 4b). The resulting correlation coefficient was 0.77 which is better than that obtained when the two enzymatic activites were correlated and represents an improvement of discrimination between tumour and normal correlate sera.

DISCUSSION

The nucleotide metabolism of neoplastic and other rapidly dividing cells is characterized by a conversion of uridine triphosphate to thymidine triphosphate (4) for subsequent use in DNA replication. The drain of uridine nucleotides is *a priori* expected to cause diminished metabolism of uridine - linked sugars such as glucose, galactose, hexoseamines and glucuronic acid and a relative increase of the metabolism of glycoconjugate sugars that bind to other nucleotides like mannose, fucose and sialic acid. Therefore the heteropolysacharides of tumour and DNA-synthesizing cells are expected to contain a high proportion of these latter sugars in terminal position. For example, a glycopeptide rich in sialic acid was isolated from a number of tumour and rapidly dividing cells, while the corresponding glycopeptide class from their non-dividing counterparts lacked sialic acid (11).

When these peptides have been shed from the tumour cell surface into serum, they may be broken down by *ecto*- glycosidases (3), yielding new acceptor sites for mannose, fucose and sialic acid. These glycopeptides can act as substrates together with co-substrates such as CMP- AcNeu and GDP-fucose in reactions catalyzed by glycosyltransferases that are present in human serum. Provided glucose and mannose are not completely hydrolysed at the surface of the neoplastic cells, it should be possible to estimate the tumour-specific peptides by assessing the binding of such fucose and sialic acid- labeled glycopeptides to Sepharose-bound Con A, that binds mannose and glucose with high affinity. Thus, glycopeptides containing sialic acid and fucose and derived from the surface of neoplastic cells by shedding into serum have not been determined directly in the present investigation. Instead, their partially hydrolysed counterparts have been measured and this method has the advantage of compensa-

ting for the glycohydrolytic activity at the surface of the neoplastic cells.

Previous reports on serum glycosyltransferases have, with few exceptions, concentrated on the enzyme levels although the endogenous acceptor glycoproteins in serum may be important contributors to the elevated glycosyltransferase activity observed in serum of patients with certain malignant diseases. The present finding of a concomitant rise in sialyltransferase and fucosyltransferase activities in serum of many patients with colorectal cancers favours such a view. These glycosyltransferases share galactose as an acceptor molecule. The activities of sialyl- and fucosyltransfer seem to agree quantitatively, in that incubation with GDP ^{14}C fucose ($0.142 \cdot 10^{-6}$ moles/l) results in the incorporation of $20.44 \cdot 10^{-9}$ moles/l of serum while incubation with $8.31 \cdot 10^{-6}$ moles/l of CMP ^{3}H AcNeu gives an incorporation of $333 \cdot 10^{-9}$ moles/l of serum. Hence, it seems likely that with the same concentration of CMP AcNeu and GDP-fucose, the absolute incorporation of each sugar would correspond closely. This conclusion and the correlation between serum sialyl- and fucosyltransferase strongly suggest that the limiting concentration of serum acceptor molecules regulates the level of glycosyl transfer and its diagnostic potential. Terminal galactosyl residues, which can be used as acceptor molecules, for both sialyl- and fucosyltransferase are implicated.

ACKNOWLEDGEMENTS

Supported in part by grants from the Swedish Medical Research Council (project No:B81-03X-05916-01A).

REFERENCES

1. Bauer,C.H., Reutler, W.G., Erhart, K.P., Kottgen, E. and Gerok, W.: Decrease of human serum fucosyltransferase as an indicator of successful tumor therapy. Science 201:1231-33, 1978.
2. Bauer, C.H., Reutter, W.G., Kottgen, E. and Gerok, W.: Serum fucosyltransferases as an indicator of malignancy and a valuable marker for follow-up studies. 6:th Meeting of the International Research Group for Carcino-Embryonic Proteins held in Marburg/Lahn, W Germany, 17-21 sept 1978:1978.
3. Bosmann, H.B.: Elevated glycosidases and proteolytic enzymes in cells transformed by RNA tumor virus. Biochim Biophys Acta 264:339-343, 1972.
4. Buck C.A., Glick, M.C. and Warren, L.: Glycopeptides from the surface of control and virus-transformed cells. Science 172:169-171, 1971.
5. Cervén, E.: Demonstration of sialyl transfer from exogenous CMP-N-acetyl ^{14}C neuraminic acid to cell membrane-bound acceptor molecules at the surface of intact Ehrlich ascites tumour cells. Biochim Biophys Acta 467: 72-85, 1977.

6. Cervén, E.: Demonstration and Characterization of ecto-sialyl-transferase in surface membranes of intact Ehrlich ascites tumour cells. Acta Universitatis Upsaliensis 341, 1979.
7. Cervén, E., Ronquist, G. and Ågren, G.: Demonstration of endogenous sialyltransferase on the surface of Ehrlich tumour ascites cells. Uppsala J Med Sci 80:58-60, 1975.
8. Chatterjee S.K., Bhattacharya, M, Barlow, J.J.: Glycoprotein glycosyltransferase (GT) and glycosidase (GS) activities in the ovarian cancer patient. Proc Am Assoc Canc Res 19:165, 1978.
9. Chatterjee, S.K., Kim, U.: Galactosyltransferase activity in metastasizing and nonmetastasizing rat mammary carcinomas and its possible relationship with tumor cell surface antigen shedding. J Natl Cancer Inst 58: 273-280, 1977.
10. Douglas, A.P. and Chandler, C.: Galactosyltransferase isoenzymes in serum of patients with cancer and gluten-sensitive enteropathy. Gastroenterology 74: (5,part 2) 1120, 1978.
11. Fujioka, S.: Enzymatic regulation of DNA precursor synthesis in leukemic leucocytes. Nippon Ketsueki Gakki Zasshi 40: 1081:91, 1977.
12. Ganzinger, U., Dorner, F., Unger, F.M., Moser, K. and Jentzsch, K.: Elevated serum-sialyltransferase activity in human malignant diseases: A new diagnostic tool? Klin Wochenschr 55 (11) 553-555, 1977.
13. Kim, U.: Enzymatic basis for the alteration in membrane glycoproteins of colonic carcinoma. Pathophysiology of carcinogenesis in digestive organs. Proc 7:th Internat Symp of the Princess Takamatzu Cancer Res Fund held in 1976. The Princess Takamatzu Cancer Res Fund Tokyo, Japan 441 pp 1977.
14. Oncology Overview. Selected Abstracts on Changes in Glycosyltransferase and Glycosidase Activity in the Presence of Cancer. May 27, 1980. U.S. Dept Healt Education and Welfare.
15. Podolsky, D.K. Weiser, M.M., Isselbacher, K.J. and Cohen, A.M.: A cancer-associated galactosyltransferase isoenzyme. N Engl J Med 299:703-705, 1978.
16. Ronquist, G., Rimsten, Å., Westman, M. and Cervén, E.: Serum sialyltransferase activity in benign and malignant diseases. Acta Chir Scand 146:247-252, 1980.
17. Sinha, A.K. and Metnykovych, G.: Repression of sialo peptidase activity in Hela cells by prednisolone. J Biol Chem 21:1332-1335, 1973.
18. Speckart, S.F., Boldt, D.H., MacDermott, R.P.: Chronic lymphatic leucemia (CLL): Cell surface changes detected by lectin binding and their relation to altered glycosyltransferase activity. Blood 52:681-695, 1978.

Received June 10, 1981

Adress for reprints:

Docent Gunnar Ronquist
Department of Clinical Chemistry
University Hospital
S-750 14 Uppsala 14
SWEDEN