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CYTIDINE-5′-MONOPHOSPHATE-*N*-ACETYLNEURAMINIC ACID

ASIALOGLYCOPROTEIN SIALIC ACID TRANSFERASE ACTIVITY IN LIVER AND SERUM OF PATIENTS WITH JUVENILE HEPATIC CIRRHOSIS AND α-1-ANTITRYPSIN DEFICIENCY

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Summary

The molecular basis for the accumulation of a substance which displays the immunological reactivity of α-1-antitrypsin within vesicles of liver parenchymal cells of individuals with hepatic cirrhosis and serum α-1-antitrypsin deficiency remains unclear. We recently reported that serum from a patient with α-1-antitrypsin deficiency and hepatic cirrhosis was substantially deficient in sialyltransferease (EC 2.4.99.1) an enzyme which transfers sialic acid from cytidine-5′-monophosphate-*N*-acetylneuraminic acid to a variety of asialoglycoprotein acceptors. In the present report we have extended these studies to include serum from five additional patients with α-1-antitrypsin deficiency and juvenile hepatic cirrhosis as well as a liver specimen obtained at autopsy of one of these patients. We find the sialytransferase activity in serum from six patients with α-1-antitrypsin deficiency and hepatic cirrhosis to be 50% of healthy pediatric control values and 30% of pediatric patients with liver disease. However, serum from family members homozygous for α-1-antitrypsin deficiency but without hepatic cirrhosis, and serum from patients with a variety of other kinds of liver disease, failed to exhibit the marked sialyltransferase deficiency. Similar assays carried out on a homogenate of a liver sample from one patient with α-1-antitrypsin deficiency and hepatic cirrhosis indicated that the deficiency of sialyltransferase activity was not demonstrable in liver. Furthermore, a comparative kinetic analysis of serum and liver sialyltransferase in normal and afflicted in-

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dividuals failed to detect differences in substrate affinities which might account for a decrease in functional sialyltransferase capacity in individuals with α-1-antitrypsin deficiency and hepatic cirrhosis. These observations suggest that the serum sialyltransferase deficiency in such patients probably arises after chronic and extensive liver disease involving hepatic accumulation of α-1-antitrypsin rather than the enzyme deficiency being the primary cause of the hepatic cirrhosis and α-1-antitrypsin deficiency.

Introduction

Certain individuals who are homozygous (P_i^{ZZ} phenotype) for α-1-antitrypsin deficiency eventually develop a fatal form of hepatic cirrhosis. α-1-antitrypsin is the major sialic acid-containing glycoprotein of the α-1-globulin fraction of serum and is capable of neutralizing the activity of a variety of proteolytic enzymes of mammalian origin [1,2]. In the case of α-1-antitrypsin deficiency and hepatic cirrhosis, microscopic and immunologic studies on liver biopsies have shown that parenchymal cells accumulate a diastase-resistant, periodic acid-Schiff's base positive, amorphous material which reacts with antibody prepared against α-1-antitrypsin isolated from normal serum. This carbohydrate-containing material which accumulates within vesicles of the hepatocyte does not stain with reagents that react with sialic acid-containing macromolecules [3]. Prompted by this observation, we recently measured the sialyltransferase (EC 2.4.99.1) activity in serum from a patient with α-1-antitrypsin deficiency using a variety of asialoglycoprotein acceptors, including the asialo derivatives of fetuin, ceruloplasmin and α-1-antitrypsin. Although the galactosyl : glycoprotein glycosyltransferase activity of the patient's serum was significantly elevated along with other serum parameters indicative of liver disease, the serum sialyltransferase activity was markedly reduced. In an effort to evaluate the possible causal relationship between sialyltransferase activity and the disease state involving α-1-antitrypsin deficiency and hepatic cirrhosis, we conducted similar studies using serum from a larger group of similar patients and asymptomatic heterozygous and homozygous relatives. Also, we have determined the activity of these same glycoprotein-glycosyltransfereases in the liver of an α-1-antitrypsin-deficient patient who died with juvenile hepatic cirrhosis. The results which we present indicate that the decrease in serum sialyltransferase activity in these patients is probably a consequence of extensive liver damage involving hepatic accumulation of α-1-antitrypsin rather than the cause of such diasease.

Materials and Methods

Preparation of specimens. Serum was obtained by centrifugation (500 × *g*, 10 min) 1 h after drawing venous blood samples and was frozen immediately (-70° C). In the case of specimens requiring transportation between Minneapolis and Pittsburgh, serum samples were sent by air transport packed in solid CO_2; control sera drawn at the same time were also induced in such shipments. Liver from a patient with α-1-antitrypsin deficiency and hepatic cirrhosis was

obtained at autopsy 10 h post-mortem and was maintained for 1 year at -70° C. Control liver, obtained at autopsy from a 39-day-old female and a 6-month-old male, was stored under these same conditions for 9 months. Extracts of liver were prepared by homogenizing 1 g of liver tissue (by sampling tissue from several separate sites in the organ) with six volumes of deionized, distilled water in a Waring blender for 1 min at 4° C. The crude homogenate was passed through four layers of cheesecloth and centrifuged for 10 min at 800 × *g* to remove cellular debris. The supernatant fraction served as the source of enzymes.

Protein determination. Protein was determined by the method of Lowry et al. [4] using bovine serum albumin as a standard.

Preparation and assay of α-antitrypsin. α-1-Antitrypsin was prepared and assayed for trypsin inhibitory activity using methods described by Crawford [5]. α-1-antitrypsin (P_i) typing was carried out by the method of Fagerhol and Laurell [6].

Enzyme assays. Sialyltransferase activity was measured essentially as described elsewhere [3] using the asialo derivatives of α-1-antitrypsin, ceruloplasmin and fetuin, each of which was prepared by treatment of the native proteins with neuraminidase as described by Carlson et al. [7]. *Clostridium perfringens* neuraminidase (Worthington Biochemical Corp.) was used after further purification employing an affinity chromatographic procedure as described by Cuatrecasas and Illiano [8]. Neuraminidase was heat-inactivated (60° C, 1 h) as decribed by Carlson et al. [7] and potential *N*-acetylneuraminic acid acceptor sites were estimated using the thiobarbituric acid method of Warren [9] using *N*-acetylneuraminic acid (Sigma Biochemical Corp.) as a standard. Unless otherwise indicated, each incubation contained the following components in a final volume of 0.08 ml: 0.088 mg asialo-α-1-antitrypsin, 0.178 mg asialofetuin, or 0.075 mg asialoceruloplasmin, 8 mmol sodium acetate (pH 6.5), 0.8 mg Triton X-100, 0.205 μmol of cytidine-5′-monophosphate-*N*-acetylneuraminic acid, 111 000 cpm (4,5,6,7,8,9-^{14}C labeled) (New England Nuclear) and 2—8 μl of serum or 0.1—0.3 mg liver homogenate. Incubations were conducted at 37° C for 60 min and analyzed for extent of *N*-[^{14}C]acetylneuraminic acid incorporation into acid-insoluble product using trichloroacetic acid-phosphotungstic acid precipitation as described elsewhere [3]. Under these conditions, the incorporation of radioactivity into acid-insloble product was linear with time and amount of enzyme when using serum from both homozygous (P_i^{ZZ}) and normal individuals (Fig. 1).

The galactosyltransferase assay measured the transfer of [^{14}C]galactose from uridine diphosphate [^{14}C]galactose ([U-^{14}C]galactose) to ovalbumin and is essentially as described elsewhere [3] except that each incubation contained Triton X-100 at a final concentration of 1% (w/v).

Serum glutamate-pyruvate transaminase was assayed according to the procedure of Henry et al. [10]. Ceruloplasmin was quantitated using the phenylenediamine oxidase method of King [11]. Alkaline phosphatase was assayed as described by Glew and Heath [12]. γ-Glutamyltranspeptidase was assayed according to the procedure of Szasz [13]. Cytochrome oxidase [14], catalase [15], and β-glucosidase [16] were assayed according to the published procedures indicated, except that each incubation contained 0.5% Triton X-100 (w/v). All assays were linear with time and amount of protein under the condi-

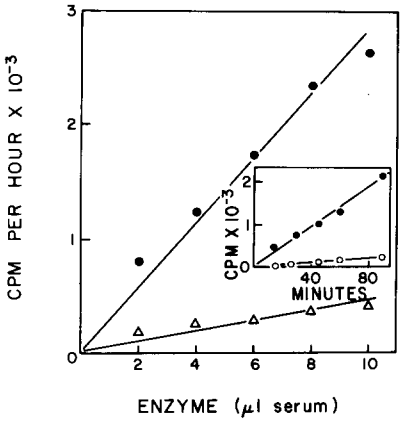

Fig. 1. The effect of increasing the amount of serum and time on sialyltransferase activity in a control and a patient with α-1-antitrypsin deficiency. Sialyltransferase activity was measured as described in Materials and Methods using 60 μl (2.94 mg/ml) of asialo-α-1-antitrypsin as acceptor prepared as described in Materials and Methods. The incubation was carried out for 60 min in the experiment where the amount of serum was varied. 5 μl of serum was used in the experiment where time of incubation was varied (insert). ●———●, control; ○———○, △———△, patient with α-1-antitrypsin deficiency.

tions employed and the values reported represent the average of duplicate determinations which did not differ from each other by more than 8%.

Results

Patient description

The core population of the present study involves six patients (Table I, patients I—VI) who share the following common properties: (1) homozygous with respect to serum α-1-antitrypsin deficiency (trypsin inhibitory capacity < 0.20), (2) α-1-antitrypsin, type P_i^{ZZ}, (3) presence of diastase-resistant, periodic acid-Schiff's base-positive amorphous deposits contained in cytoplasmic membranes of parenchymal cells confirmed by light and electron microscopy, and (4) extensive liver disease. Serum indices of current liver disease, including glutamate-pyruvate transaminase, alkaline phosphatase and γ-glutamyltranspeptidase, are substantially elevated in each of these patients (Table I), with the exception of the alkaline phosphatase level in patient II which is essentially normal. Circulating levels of ceruloplasmin in these patients with α-1-antitrypsin deficiency and hepatic cirrhosis are essentially normal with the exception of patient VI whose serum ceruloplasmin is slightly reduced (71% of the mean for controls). Also included in Table I are serum parameters for relatives of several of these patients. Two asymptomatic, homozygous siblings (IIIa and IVa) with residual α-1-antitrypsin of the P_i^{ZZ} type exhibit elevated levels of serum glutamate-pyruvate transaminase and alkaline phosphatase, whereas their serum levels of γ-glutamyltranspeptidase and ceruloplasmin were essentially normal. Serum from one other homozygous sibling (IIIb) was essentially normal in terms of the levels of glutamate-pyruvate transaminase, γ-glutamyltranspeptidase, alkaline phosphatase and ceruloplasmin present.

These same serum parameters in the heterozygous parents (IIIc and IIId) of patient III were not significantly different from control values.

TABLE I

SERUM PARAMETERS IN CONTROLS AND INDIVIDUALS WITH α-1-ANTITRYPSIN DEFICIENCY

All assays were performed as described in Materials and Methods and duplicated with less than 8% variation.

Serum source			Disease description	Trypsin inhibitory capacity	P_i type	Serum glutamate-pyruvate transaminase *	γ-Glutamyl-transpeptidase ** ($\times 10^3$)	Alkaline phosphatase ***	Ceruloplasmin (mg/100 ml)
Controls †				Range: 0.814—1.05	MM	5.0—9.0	3.8—10.0	10.8—16.2	19.8—29.0
				Mean: 0.926		6.7	6.3	14.1	24.0
Patients	(Sex)	(Age)							
I	M	8		0.169	ZZ	36	54	117	24
II	M	11	α-1-antitrypsin	0.124	ZZ	24	56	18	24
III	F	9	deficiency	0.093	ZZ	34	86	52	24
VI	M	13	and hepatic cirrhosis	0.158	ZZ	47	76	194	31
V	M	9		0.173	ZZ	37	61	131	29
VI	F	10		0.110	ZZ	39	36	140	17
Relatives									
IIIa (sister)				0.079	ZZ	43	11	70	21
IIIb (brother)			Asymptomatic	0.150	ZZ	9.0	11	14	34
IIIc (mother)			α-1-antitrypsin	0.380	MZ	9.0	12	26	21
IIId (father)			deficiency	0.550	MZ	9.0	12	4.0	25
IVa (sister)				0.273	ZZ	25	14	56	33

* Serum glutamate-pyruvate transaminase, measured in I.U., that amount of enzyme producing 1 μmol product per min per ml serum.

** γ-Glutamyltranspeptidase, measured in I.U.

*** Measured in I.U.

† Controls were average from five adults and two children.

Serum glycosyltransferase activities

Individuals I—VI are all pediatric patients. Therefore, control values for the various glycosyltransferase assays were established separately for serum from adults and children since the activities of children are consistently higher than those of adults. The sialyltransferase activity in serum from patients I—VI was consistently and significantly less than that from the control group; the ability of serum from these patients to catalyze the transfer of sialic acid from cytidine-5′-monophosphate-*N*-acetylneuraminic acid to various asialoglycoprotein acceptors including asialofetuin, asialoceruloplasmin and asialo-α-1-antitrypsin was 38, 56 and 48% respectively, of the mean values obtained for serum specimens from the pediatric control group (Table II). On the other hand, the activity of another serum glycosyltransferase, galactosyltransferase, in each of the

TABLE II

GLYCOSYLTRANSFERASE ACTIVITY IN SERUM FROM VARIOUS CONTROLS AND INDIVIDUALS WITH α-1-ANTITRYPSIN DEFICIENCY

		Glycosyltransferase activity (μmol/h per ml serum ($\times 10^{-1}$))		
		Sialyltransferase		Galactosyltransferase
Acceptor	Asialofetuin	Asialocerulo-plasmin	Asialo-α-1-anti-trypsin	Ovalbumin
Adult controls *:	Range: 3.92—4.53	1.50—1.72	0.78—1.17	0.249—0.322
	Mean: 4.17	1.61	1.15	0.296
Pediatric controls **:	Range: 4.04—6.64	1.68—2.09	1.24—1.66	0.259—0.588
	Mean: 6.06	1.88	1.38	0.400
Patients				
I	2.77 (46) ***	1.16 (62)	0.70 (51)	0.396 (99)
II	2.39 (39)	0.96 (51)	0.64 (46)	0.334 (84)
III	1.75 (29)	1.02 (54)	0.64 (46)	0.472 (118)
IV	2.14 (35)	1.08 (57)	0.74 (54)	0.404 (101)
V	2.12 (35)	1.05 (56)	0.69 (50)	0.414 (104)
VI	2.63 (43)	1.04 (56)	0.61 (44)	0.410 (102)
Mean (I—VI)	2.30 (38)	1.05 (56)	0.67 (48)	0.404 (101)
Relatives				
IIIa	3.54 (58) ***	1.80 (96) ***	1.51 (109) ***	0.294 (74) ***
IIIb	5.00 (120) †	1.31 (81) †	1.22 (106) †	0.242 (82) †
IIIc	3.89 (93) †	1.54 (96) †	1.21 (105) †	0.313 (106) †
IIId	5.86 (140) †	1.48 (92) †	1.26 (110) †	0.242 (82) †
IVa	4.53 (75) ***	2.00 (106) ***	1.37 (99) ***	0.300 (75) ***
Other				
VII ††	6.13 (147) †	2.78 (173) †	1.96 (170) †	0.352 (119) †

* The controls consisted of 10 adults, ages 24—47 years.

** The controls consisted of 5 children, ages 6—11 years.

*** Percentage of pediatric control group, mean value, All numbers in parentheses under Patients are percentages of pediatric control group mean value.

† Percentage of adult control group, mean value.

†† This patient is a 47-year-old male with advanced emphysema and homozygous for α-1-antitrypsin deficiency (P_i^{ZZ}) and a serum trypsin inhibitory capacity of 0.205.

TABLE III

SIALYLTRANSFERASE ACTIVITES IN SERUM FROM INDIVIDUALS WITH VARIOUS FORMS OF LIVER DISEASE

Patient description	Sialyltransferase activity (μmol sialic acid transferred/h per ml serum $\times 10^{-1}$)		
	Asialofetuin	Asialocerulo-plasmin	Asialo-α-1-antitrypsin
Controls *	Range: 3.98—4.75 Mean: 4.35	1.09—1.91 1.61	2.40—3.94 3.28 **
Wilson's disease-1	6.44	2.76	5.55
Wilson's disease-2	6.39	1.63	4.07
Wilson's disease-3	5.66	2.19	4.07
Cystic fibrosis-1	5.49	2.69	5.41
Cystic fibrosis-2	5.82	2.58	5.68
Cystic fibrosis, P_i^{MM}, with portacaval shunt	4.18	2.08	3.87
Cystic fibrosis, P_i^{MZ}, with portacaval shunt	4.75	1.92	4.30
Hepatitis, Australian antigen positive-1	3.62	2.28	3.20
Hepatitis, Australian antigen positive-2	7.72	3.70	7.06
Asymptomatic, Australian antigen positive-1	4.26	2.06	3.61
Asymptomatic, Australian antigen positive-2	6.05	2.27	3.74
Congenital polycystic liver disease	5.58	2.40	4.56
Hepatic cirrhosis P_i^{MS}	3.66	2.24	4.56
Mean for liver disease ***	5.39	2.41	4.76

* The controls consisted of three adults whose sialyltransferase activity is characteristic of our control population.

** The sialyltransferase activity using asialo-α-1-antitrypsin as acceptor is considerably higher here than in Table II because different preparations of asialo-α-1-antitrypsin were used in these two experiments.

*** These values are calculated having excluded the data obtained for the asymptomatic Australian antigen-positive individuals.

same six patients was within the normal range of values obtained for the pediatric control group. Thus, the serum sialyltransferase deficiency which we reported earlier [3] in one patient (III) with α-1-antitrypsin deficiency and hepatic cirrhosis is also observed in five additional patients with the same disease. Furthermore, of the serum parameters that we have measured, the sialyltransferase enzyme appears to be the only index that we have found to be consistently and significantly deficient in these patients. In addition, of the serum specimens that we have studied from patients exhibiting a variety of liver diseases, in no other instance have we observed a significant deficiency in sialyltransferase activity (Table III).

Included in Table III are the results of serum sialyltransferase assays in serum from individuals with Wilson's disease, cystic fibrosis, hepatitis, and congenital polycystic liver disease. In general, the sialyltransferase activity in the serum of these individuals with liver disease was at least 25—35% greater than that in controls. Therefore, if the serum specimens from individuals with liver disease are considered as reference standards, then patients I—VI, with α-1-antitrypsin defi-

ciency and hepatic cirrhosis, are characterized by serum that is approx. 70% deficient in sialyltransferase activity.

Also included in Table II are the results of sialyltransferase determinations on serum samples from homozygous and heterozygous relatives of patients III and IV. When compared with adult controls, both parents of patient III (IIIc and IIId) possess essentially normal sialyltransferase activities when assayed using all three asialoglycoprotein acceptors. This result is in contrast to results previously reported [3] where these two individuals showed a 52—72% deficiency of sialyltransferase when asialo-α-1-antitrypsin was used as the sialic acid acceptor. These conflicting results could be due to the fact that in the previous study asialoglycoprotein acceptors were prepared by acid hydrolysis, while asialoglycoprotein acceptors in the present study were prepared using neuraminedase.

In general, asymptomatic individuals, homozygous for α-1-antitrypsin deficiency (IIIa, IIIb, IVa) did not display the marked serum sialyltransferase deficiency characteristic of their siblings (Table II). However, in several instances, depending upon the sialic acid acceptor used in the assay, reduced levels of sialyltransferase activity were noted. For example, serum specimens IIIa and IVa contained only 58 and 75% as much sialyltransferase activity, respectively, as pediatric control serum when assayed using asialofetuin as the acceptor. Similarly, serum from IIIb contained moderately decreased sialyltransferase activity when assayed using asialoceruloplasmin as acceptor.

We have also evaluated the serum sialyltransferase activity in serum from an emphysematous adult who is homozygous (P_i^{ZZ}) for α-1-antitrypsin deficiency (Table II, patient VII). Light and electron microscopic examination of a liver biopsy from this patient revealed the presence of amorphous deposits characteristic of the periodic acid-Schiff's base-positive material observed in the pediatric patients (I—VI) with hepatic cirrhosis. The serum sialyltransferase activity in this individual was consistently elevated in comparison to healthy adult controls when assayed with all three asialoglycoprotein acceptors. Thus, the pronounced serum sialyltransferase deficiency is observed only in homozygous α-1-antitrypsin-deficient patients with extensive liver disease.

Kinetic properties of serum sialyltransferase in controls and patients with α-1-antitrypsin deficiency and hepatic cirrhosis

We measured the K_m for both cytidine-5'-monophosphate-*N*-acetylneuraminic acid and asialoglycoprotein acceptor using control serum and serum from patients with α-1-antitrypsin deficiency and hepatic cirrhosis in order to determine if the reduced sialyltransferase activity was due to altered affinity of the enzyme for its substrates. Asialoceruloplasmin was used as the glycoprotein acceptor (Figs. 2 and 3). Fig. 2 shows that the K_m values for cytidine-5'-monophosphate-*N*-acetylneuraminic acid for serum sialyltransferase from patient I and a pediatric control are not significantly different being 5.30 and 4.35 mM, respectively. Similar studies on the serum sialyltransferases of other patients with α-1-antitrypsin deficiency and hepatic cirrhosis did not reveal any specimen which exhibited a K_m for cytidine-5'-monophosphate-*N*-acetylneuraminic acid which significantly exceeded the value obtained for control serum (Table IV). The K_m value for asialoceruloplasmin was 77 μM (expressed on the basis

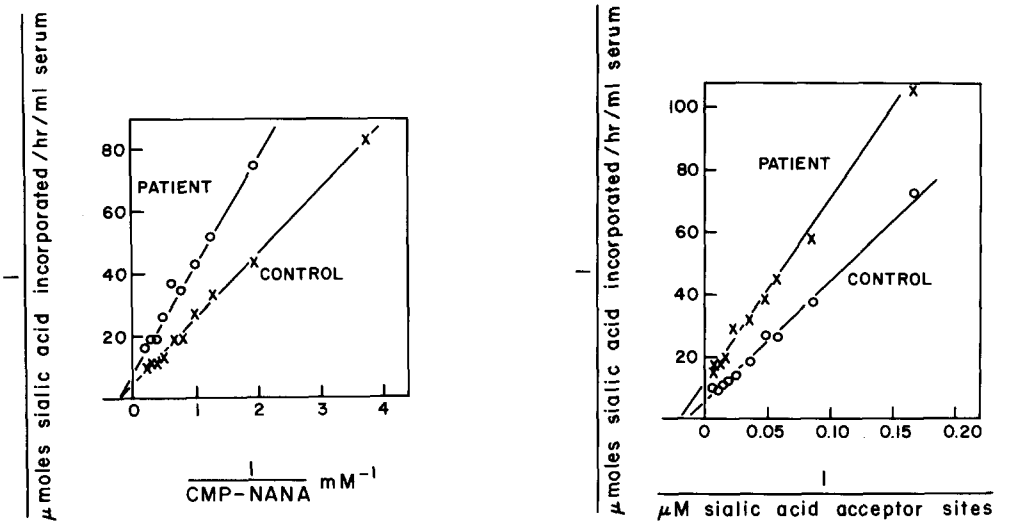

Fig. 2. The effect of increasing cytidine-5′-monophosphate-*N*-acetyl-neuraminic on acid sialyltransferase in serum from a control and a patient with α-1-antitrypsin deficiency. Sialyltransferase activity was measured as discussed in Materials and Methods using 0.1 mg (a concentration that is saturating with respect to the transferase) asialoceruloplasmin as acceptor. The incubation was carried out for 60 min. Cytidine-5′-monophosphate-*N*-acetyl[^{14}C]neuraminic acid (CMP-NANA) was diluted with non-radioactive cytidine-5′-monophosphate-*N*-acetylneuraminic acid (synthesized by the method of Roseman [18]) to give the final concentration listed on the abscissa. x———x, control; ○———○, patient with α-1-antitrypsin deficiency. The final volume of the incubation was 0.07 ml.

Fig. 3. The effect of increasing asialoceruloplasmin as acceptor on sialyltransferase in serum from a control and a patient with α-1-antitrypsin deficiency. Sialyltransferase activity was measured as described in Materials and Methods using 5 μl serum from a control (○———○) and a patient with α-1-antitrypsin deficiency (X———X). Asialoceruloplasmin was used as the acceptor and varied as indicated on the abscissa, in terms of μM sialic acceptor sites calculated from the amount of sialic acid released from α-1-antitrypsin as measured by the thiobarbituric acid method of Warren [9]. The cytidine-5′-monophosphate-*N*-acetylneuraminic acid concentration was 2.93 mM in a final volume of 0.07 ml.

TABLE IV

SUMMARY OF KINETIC PARAMETERS FOR THE SERUM SIALYLTRANSFERASE OF VARIOUS INDIVIDUALS

Serum source	K_m, cytidine-5′-monophosphate-N-acetylneuraminic acid * (mM)	K_m, asialoceruloplasmin ** (μM sialic acid acceptor sites)	V *** (μM sialic acid incorporated per h per ml serum)
Control	4.35	77.0	0.240 (100) †
Patient I	5.3	52.5	0.150 (63)
II	6.2	63.9	0.109 (45)
III	2.6	31.3	0.132 (55)
IV	3.8	136	0.130 (54)
VI	2.2	52.5	0.129 (54)

* Assayed in presence of 0.10 mg asialoceruloplasmin.

** Assayed at a cytidine-5′-monophosphate-*N*-acetylneuraminic acid concentration of 2.94 mM.

*** Assayed in presence of 0.1 mg asialoceruloplasmin, using 5 μl serum, 60-min incubation, cytidine-5′-monophosphate-*N*-acetylneuraminic acid increased to a concentration of 10.6 mM in a final volume of 0.07 ml.

† Expressed as percent of control

of sialic acid acceptor sites) for control serum and slightly less (52.5 μM) for serum from patient I (fig. 3). K_m values for asialoceruloplasmin in the sialyltranferase assay of several other patients with α-1-antitrypsin deficiency and hepatic cirrhosis are summarized in Table IV; in no case was the K_m value for asialoceruloplasmin more than 76% greater than the control value. Thus, the results of these kinetic studies indicate that serum sialyltransferase activities in the serum of the patients with α-1-antitrypsin deficiency and hepatic cirrhosis which we have studied possess essentially normal affinity for both substrates of the reaction, namely cytidine-5'-monophosphate-*N*-acetylneuraminic acid and asialoglycoprotein acceptor. The only apparent difference between the sialyltransferase of control serum and these patients is that the concentration of sialtransferase in the patients' serum is less than that in control serum; the *V* values of the patients' serum sialyltransferase were 45—63% of control (Table IV).

Glycosyltransferase activities in homogenates of liver from a patient with α-1-antitrypsin deficiency and hepatic cirrhosis

In order to determine if the level of sialyltransferase activity in serum might reflect the relative concentration of the same enzyme in tissues, we prepared homogenates of liver obtained at autopsy from patient VI (Tables I, II, and VI) who died with α-1-antitrypsin deficiency and hepatic cirrhosis and compared it with a pediatric control liver preparation. Since possible differences in the conditions (9—12 months) of tissue storage might have influenced the enzyme levels ultimately observed at the time of assay, we monitored the activity of several enzymes including catalase, alkaline phosphatase, β-glucosidase and cytochrome oxidase in these homogenates (Table V). In the case of catalase, β-glucosidase and cytochrome oxidase, the specific activity of these enzymes in the diseased liver were within the range of values obtained for the two control

TABLE V

VARIOUS ENZYME ACTIVITIES IN HOMOGENATES OF LIVER FROM CONTROLS AND PATIENT VI WITH α-1-ANTITRYPSIN DEFICIENCY AND JUVENILE HEPATIC CIRRHOSIS

Enzyme	Source of tissue		
	Control liver No. 1	Control liver No. 2	Liver from patient with α-1-antitrypsin deficiency and hepatic cirrhosis
Cytochrome oxidase *	181	33	57
β-Glucosidase *	0.73	0.10	0.22
Catalase *	$1.44 \cdot 10^6$	$2.66 \cdot 10^6$	$1.50 \cdot 10^6$
Alkaline phosphatase *	8.9	14.9	24
Glycosyltransferases **			
Galactosyltransferase	0.331	0.131	0.284
Sialyltransferase (acceptor)			
Asialo-α-1-antitrypsin	0.253	0.252	0.250
Asialoceruloplasmin	0.310	0.332	0.272
Asialofetuin	0.910	0.732	0.793

* Expressed as μmol of product produced per min per mg protein.

** Expressed as μmol of product produced per h per mg protein.

specimens. The specific activity of hepatic alkaline phosphatase was approx. 2-fold greater in the liver of the patient with α-1-antitrypsin and hepatic cirrhosis. These results indicate that the control liver tissues represent valid reference material.

The specific activity of the galactosyltransferase in the patient's liver was within the range of values displayed by the two control liver homogenates (Table V). In contrast to what we observed with serum (Table II), the sialyltransferase activity of the patient's liver was not markedly different from that of the homogenates of control liver when assayed using all three of the asialoglycoprotein acceptors. These observations demonstrate that the sialyltransferase deficiency in this particular patient was apparent in serum but not liver.

Kinetic properties of the liver sialytransferase in controls and a patient with α-1-antitrypsin deficiency and hepatic cirrhosis

Saturation curves were established for both cytidine-5′-monophosphate-*N*-acetylneuraminic acid and asialoceruloplasmin in order to compare kinetic properties of the sialyltransferase of normal liver and that from patient VI. With respect to cytidine-5′-monophosphate-*N*-acetylneuraminic acid, the K_m value (determined in the presence of a saturating concentration of asialoceruloplasmin) for the patient's liver sialyltransferase was 1.8-fold greater than the value obtained for the homogenate of control liver (Table VI); the V values (μmol sialic acid incorporated per mg of liver protein) for the two tissues were not significantly different (Fig. 4). In addition, the K_m values for asialoceruloplasmin for the sialyltransferase present in liver homogenates from both the control and patient VI were similar (Fig. 5). Also, the specific activity of the sialyltransferase in each preparation was essentially the same. These results add support to the concept that the predominant sialyltransferase activity which we have measured in this particular patient's liver is indistinguishable, both quantitatively (V) and qualitatively (K_m for cytidine-5′-monophosphate-*N*-acetylneuraminic acid and asialoceruloplasmin) from that of control liver.

TABLE VI

KINETIC PARAMETERS OF SERUM AND LIVER SIALYLTRANSFERASE IN PATIENT VI WITH α-1-ANTITRYPSIN DEFICIENCY AND HEPATIC CIRRHOSIS

Assay conditions for serum sialyltransferase activities are described in Figs. 2 and 3 varying the cytidine-5′-monophosphate-*N*-acetylneuraminic acid and asialoceruloplasmin concentrations, respectively. The liver sialyltransferase assay conditions for varying cytidine-5′-monophosphate-*N*-acetylneuraminic acid and asialoceruloplasmin are described in Fig. 4.

	K_m, asialoceruloplasmin (μM sialic acid acceptor sites)	K_m, cytidine-5′-monophosphate-*N*-acetylneuraminic acid (mM)	V
Control serum	77.0	4.35	0.240 *
Patient serum	52.5	2.20	0.135 * (56%)
Control liver	11.1	7.40	0.345 **
Patient liver	18.2	13.3	0.377 ** (109) ***

* Expressed as μmol product produced per h per ml serum.

** Expressed as μmol product produced per h per mg protein.

*** The numbers in parentheses indicate percent of control.

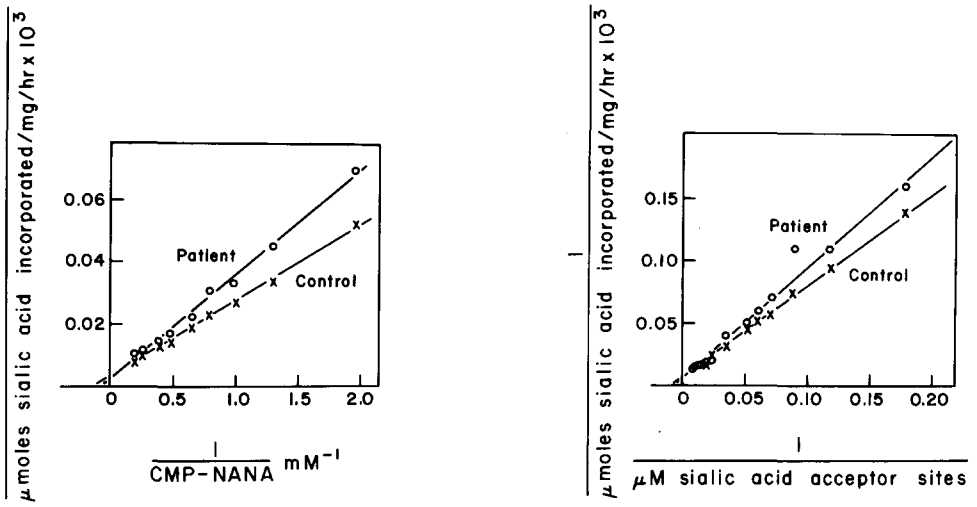

Fig. 4. The effect of varying the concentration of cytidine-5′-monophosphate-*N*-acetylneuraminic acid on sialyltransferase of liver homogenates from a control and a patient VI with α-1-antitrypsin deficiency. Sialyltransferase activity was measured as described in Materials and Methods using a 60-min incubation with 25 μl (2.5 mg/ml) of asialoceruloplasmin as acceptor, in a final volume of 0.075 ml. This concentration of acceptor was previously determined to be saturating for the transferase. The cytidine-5′-monophosphate-*N*-acetylneuraminic acid (CMP-NANA) was varied as described in Fig. 2. 7 μl of homogenate from the control (X———X) and the patient with α-1-antitrypsin deficiency (○———○) were used as the enzyme source.

Fig. 5. The effect of varying the concentration of asialoceruloplasmin as acceptor on sialyltransferase of liver homogenates from a control and patient VI with α-1-antitrypsin deficiency. The sialyltransferase activity was measured as described in Materials and Methods. The concentration of cytidine-5′-monophosphate-*N*-acetylneuraminic acid was 2.74 mM in a final incubation volume of 0.075 ml. The acceptor concentration varies as indicated on the abscissa and was calculated in terms of μM sialic acid acceptor sites as previously indicated in Fig. 3. X———X, control; ○———○, patient with α-1-antitrypsin deficiency.

Discussion

The most important observation of the present report is the consistent finding that all six pediatric patients with homozygous α-1-antitrypsin (P_i^{ZZ}) deficiency and hepatic cirrhosis exhibited significantly decreased serum sialyltransferase activity. This statement is supported by the results presented in Tables II and III in which the serum sialyltransferase activity of these individuals were approx. 50% of healthy pediatric controls and 30% of patients with liver disease unrelated to α-1-antitrypsin deficiency. This decreased level of sialyltransferase enzyme does not seem to be the result of a general inability of the serum of these patients to catalyze glycosyltransferase reactions since uridine diphosphate-galactose : ovalbumin galactosyltransferase activity, a closely related enzyme of the glycosyltransferase family [18], is either normal or elevated in these patients. The low level of sialyltransferase activity in the serum of these patients is unique in that all of the following serum parameters in these same cirrhotic patients were either elevated or within the control range: ceruloplasmin, alkaline phosphatase, serum glutamate-pyruvate transaminase, and γ-glutamyltranspeptidase (Table I), and β-glucosidase, β-galactosidase and acid phosphatase (Glew, R.H., unpublished results). The decreased sialyltransferase activ-

ity in α-1-antitrypsin-deficient individuals with hepatic cirrhosis is probably not due to their general state of liver dysfunction since serum from 11 patients with a variety of liver diseases unrelated to α-1-antitrypsin deficiency, did not exhibit a sialyltransferase deficiency (Table III). In fact, serum from several patients with cystic fibrosis, Wilson's disease, cirrhosis and hepatitis often displayed significantly elevated levels of sialyltransferase activity.

Another pertinent observation is that the sialyltransferase deficiency noted in the serum of α-1-antitrypsin-deficient, cirrhotic patient VI could not be demonstrated in a homogenate of homologous liver tissue (Table V). We do not know if the sialyltransferase of serum and liver are identical or if they represent different enzymes. Results of studies of Hudgin and Schachter [19] suggest that the serum enzyme arises from the liver and that the predominant sialyltransferase measured in crude homogenates of liver is similar to the serum enzyme. However, from our kinetic studies on liver and serum sailyltransferase we found the K_m value for cytidine-5′-monophosphate-*N*-acetylneuraminic acid for the liver enzyme to be 2—3-fold greater than for the serum enzyme (Figs. 2 and 4). Likewise, the K_m for the other substrate of the reaction, asialoceruloplasmin, was much lower for the liver enzyme (11—18 μM sailic acid acceptor sites) than for the serum enzyme (52—77 μM, see Figs. 3 and 5). These results indicate that, under our conditions of incubation, the sialyltransferase of serum and liver may be different. However, since crude enzyme preparations were used, the 2—3-fold difference may not be significant. A definitive statement concerning the possible common identity of the two enzymes must await their purification and a more extensive study of their kinetic, immunologic, acceptor specificity, and physical properties. At the present time, we cannot rule out the possibility that a sialyltransferase identical to the serum enzyme exists in normal liver as a minor fraction of the total sialyltransferase activity of liver and that this particular species of enzyme is in fact deficient in the liver of α-1-antitrypsin, cirrhotic individuals.

The results of glycosyltransferase measurements on serum from asymptomatic homozygous, P_i^{ZZ} siblings of cirrhotic patients indicate in general that a marked sialyltransferase deficiency does not exist in their serum. Nevertheless, serum from one healthy, homozygous α-1-antitrypsin deficient sibling (IIIa) was consistently reduced in sailyltransferase content when compared with the pediatric control group using asialofetuin as the sialic acid acceptor. It will be of interest to follow the level of serum sialyltransferase activity in this individual since the appearance of liver dysfunction may be paralleled by a decrease in serum sialyltransferase activity.

The heterozygous, P_i^{MZ}, father (IIId) and mother (IIIc) of patient III possessed normal serum sialyltransferase activity. In addition, an α-1-antitrypsin P_i^{ZZ} adult with emphysema, but no apparent liver dysfunction, displayed elevated serum sialyltransferase values. These results suggest that decreased serum sialyltransferase activity occurs as cirrhosis manifests itself clinically and is secondary to the extensive liver damage related to the α-1-antitrypsin deficiency and accumulation of periodic acid-Schiff's base-positive material in liver. It may be that the sialyltransferase of serum is a secreted protein and that the continued production and secretion of sialyltransferase requires a competent liver with respect to its secretory apparatus.

A recent report by Eriksson and Larsson [20] described the isolation and characterization of the inclusion bodies from cirrhotic livers of patients with homozygous α-1-antitrypsin deficiency. These investigators demonstrated that this material displays immunological properties and electrophoretic mobility in sodium dodecyl sulfate-polyacrylamide gels similar to native, P_i^{MM} α-1-antitrypsin, but that isolated globules lacked measurable sialic acid. These results confirm our findings [3] concerning the absence of sialic acid in these deposits and further suggest that this absence of sialic acid may be an important clue in the search for the pathogenesis of α-1-antitrypsin deficiency.

Theoretical considerations arising from the current study pertain to the application of the serum sailyltransferase assay to the management of patients with α-1-antitrypsin deficiency and hepatic cirrhosis. Invariably, these patients develop portal hypertension. Bleeding from esophageal varices is more frequently a terminal event when compared with other forms of childhood portal hypertension. In addition, these patients have had no evidence of hepatic failure prior to the terminal event (Sharp, H.L., unpublished observations). Clearly, it would be of value to have available non-invasive, objective criteria which would indicate the most opportune time for a surgical shunting procedure to decrease the portal hypertension and perhaps prolong life. It may be that the measurement of serum sailyltransferase activity in these patients would provide such a readily accessible, quantitative index. Also, this measurement may provide a suitable criteria in the future as to when to consider liver transplantation since preliminary evidence indicates that this procedure will correct the underlying metabolic defect [17].

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