Constancy of α_1 -Acid Glycoprotein Variants of Caucasian Patients under Conditions of Severe Stress*

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Recent studies on α_1 -acid glycoprotein (orosomucoid), a well-characterized globulin of normal human plasma (Schmid, 1953; Weimer, Mehl, and Winzler, 1950; Jeanloz, 1966), demonstrated that its sialyl-free form reveals on starch gel electrophoresis at pH 5 one or two bands depending on the individual serum from which this protein was isolated, and that these variants appear to be genetically transmitted (Schmid, Tokita, and Yoshizaki, 1965; Tokita, Burke, Yoshizaki, Fischer, and Schmid, 1966). In the present paper an investigation of the α_1 -acid glycoprotein variants of certain Caucasian diabetic patients who underwent hypophyseal stalk section is presented, affording additional evidence for the genetically determined transmittance of the variants of this plasma globulin.

Material and Methods

Blood from 21 Caucasian patients with long-standing diabetes mellitus and active haemorrhagic retinopathy was obtained by venepuncture. After clotting the blood at room temperature the sera were separated by centrifugation, shell-frozen, and stored at -40°C . until fractionation was initiated. The volume of the sera specimens varied between 7 and 28 ml. These patients subsequently underwent hypophyseal stalk section (HSS). Between the 3rd and 6th day after operation a second blood sample was taken. The haematocrits of these samples did not reveal any systematic changes either before or after the operation.

For the isolation of α_1 -acid glycoprotein, the sera were fractionated as described earlier (Schmid *et al.*, 1965). Chromatography on Amberlite IRC-50 of the resulting fraction VI was carried out as previously reported (Schmid, MacNair, and Bürgi, 1958). The weight of the resulting α_1 -acid glycoprotein preparations was corrected for losses occurring during the fractionation (fraction I-V was not extracted) and chromatography (Schmid *et al.*, 1958). Many of the preparations were analysed by the horizontal starch gel electrophoresis technique in pH 8.6 borate buffer (Smithies, 1959), by immunoelectrophoresis (Grabar, 1959) and double diffusion technique (Ouchterlony, 1962). For the latter two analyses a goat serum active against pooled normal α_1 -acid glycoprotein purchased from Behringwerke, Marburg/Lahn, Germany, was utilized.

For the removal of the sialyl residues 2 to 3 mg. of each α_1 -acid glycoprotein preparation were dissolved in 1.65 ml. of pH 4.5, $\Gamma/2$ 0.1 sodium acetate buffer, mixed with 0.35 ml. of neuraminidase solution (Schmid *et al.*, 1965; Tokita *et al.*, 1966) and incubated at 37°C . for 4 hours. In control experiments with pooled α_1 -acid glycoprotein it could be shown that 98 to 99% of the sialyl residues were cleaved off during this incubation period, while the individual specimens lost between 90 and 96% of this amino sugar. The neuraminidase used for these experiments was derived from a filtrate of *Clostridium perfringens*, and was shown to be free of proteolytic and other hydrolytic activities (Cassidy, Jourdan, and Roseman, 1965). It should be emphasized that all α_1 -acid glycoprotein preparations investigated in the present study were incubated simultaneously with this enzyme. The analysis for the variants of this protein was performed by starch gel electrophoresis at pH 5.0 (Schmid *et al.*, 1965).

The preparation of the seromucoid fraction and the determination of its polypeptide and hexose content were carried out with minor modifications according to the procedure of Winzler (1955). Since often only small volumes of blood were available, 3.0 ml. of each patient's serum were used for the preparation of this fraction. As controls for each set of analyses, normal human serum and normal α_1 -acid glycoprotein (Schmid, 1953) were included to check the entire procedure and the orcinol and biuret methods, respectively.

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TABLE

 α_1 -ACID GLYCOPROTEIN VARIANTS, BLOOD LEVELS OF α_1 -ACID GLYCOPROTEIN AND SEROMUCOID OF CERTAIN DIABETIC PATIENTS BEFORE AND AFTER HYPOPHYSIAL STALK SECTION OPERATION

Patient	Age (yr.)	Sex	α_1 -Acid Glycoprotein Variants (Type of Pattern)		α_1 -Acid Glycoprotein (mg./100 ml.)		Seromucoid (mg./100 ml. polypeptide)*	
			Pre-op.	Post-op.	Pre-op.	Post-op.	Pre-op.	Post-op.
M.J.	51	M	I	I	30	68	40	74
A.L.	35	M	I	I	38	92	35	119
W.A.	57	M	I	I	34	63	62	75
B.C.	39	M	I	I	44	109	73	127
B.C.	39	M	I	I	44	109	73	127
M.F.	41	M	II	II	35	57	79	101
B.R.	24	M	II	II	36	106	60	113
C.J.	30	M	II	II	39	79	57	136
B.L.	45	M	II	II	63	113	86	110
I.W.	38	M	II	II	44	89	73	109
M.A.	42	M	II	II	51	86	57	108
C.W.	34	M	II	II	23	52	49	77
S.L.	23	F	III	III	96	100	81	89
G.M.	31	M	III	III	68	122	108	157
A.C.	25	M	III	III	30	76	60	83
J.R.	33	M	III	III	68	122	68	153
Z.A.	42	F	III	III	24	63	45	79
C.A.	30	F	III	III	37	72	66	95
B.E.	32	F	III	III	41	122	62	144
O.F.	27	M	III	III	34	100	83	116
A.C.	20	F	III	III	33	41	46	103
T.S.	41	M	III	III	35	92	46	129
Average					44	87	62	109
SD					± 29	± 24	± 18	± 25
Range					23-96	41-122	35-108	74-157
Normal value					45	—	61	—

* Note that α_1 -acid glycoprotein is expressed in total weight and that the polypeptide moiety of this protein accounts for approximately 60% of its weight. Hence, to compare directly the seromucoid values, expressed as polypeptide, with those of α_1 -acid glycoprotein, the latter values must be reduced by 40%.

Results

Blood Levels of Seromucoid and α_1 -Acid Glycoprotein. The diabetic patients, before HSS operation, showed seromucoid levels (Table) within a relatively narrow range, with an average which was essentially identical with the normal value obtained in this study and that reported by Winzler (1955). The corresponding post-operative values were almost doubled. The α_1 -acid glycoprotein levels of these individuals were similarly increased. The varying, relative difference between the seromucoid fraction and the α_1 -acid glycoprotein content is due to the presence in the former of varying amounts of other plasma proteins, such as haptoglobin (Schultze, Heide, and Haupt, 1962), in these disease states. It should be added that the blood concentration of α_1 -acid glycoprotein, a value of major importance for this study, was measured by direct isolation of this protein and indirectly by determining the seromucoid fraction.

The seromucoid fraction was also measured in terms of neutral hexoses. The pre-operative values ranged from 8.5 to 20 mg./100 ml. and averaged 12 mg. The normal seromucoid fraction was found to be 12 mg., a value essentially identical with that reported earlier by Winzler (1955). The corresponding values of the post-operative analyses

ranged from 12 to 32 mg./100 ml. and averaged 20 mg. Both the pre- and post-operative analyses showed distributions that were very similar to those obtained by the polypeptide determinations.

Constancy of α_1 -Acid Glycoprotein Variants of Diabetic Patients with Hypophyseal Stalk Sections. The main finding of this investigation was the constancy of the type of the α_1 -acid glycoprotein variant(s) of each individual as observed over the period studied (Table). Representative starch gel electrophoresis patterns of the sialyl-free α_1 -acid glycoprotein preparations are given in Fig. 1.

Starch Gel Electrophoretic, Immuno-electrophoretic, and Immunochemical Analyses of α_1 -Acid Glycoprotein Preparations. Native α_1 -acid glycoprotein preparations derived from serum of these patients before and after hypophyseal stalk operation revealed, on starch gel electrophoresis at pH 8.6, homogeneity and the same apparent electrophoretic mobility (Fig. 2). On immunoelectrophoresis they each formed a single precipitin line and again showed the same apparent electrophoretic mobility. On double diffusion each preparation led to a single precipitin line. These lines fused complete with one another and

FIG. 1. Representative patterns of desialized α_1 -acid glycoprotein preparations obtained on starch gel electrophoresis at pH 5.1 in $\Gamma/2$ 0.02 sodium acetate buffer. These protein preparations were derived from sera of patients with diabetes mellitus. The Roman numerals indicate the type of α_1 -acid glycoprotein variant.

FIG. 2. Starch gel electrophoresis in pH 8.6, $\Gamma/2$ 0.02 borate buffer of native α_1 -acid glycoprotein preparations derived from sera of several patients (Nos. 1, 2, 3) with diabetes mellitus. This protein was isolated from blood taken before (a) and after (b) hypophyseal stalk section. Pooled normal α_1 -acid glycoprotein (A) was used as reference.

with that of pooled normal α_1 -acid glycoprotein used as reference. The sialyl-free α_1 -acid glycoprotein also appeared homogeneous on horizontal starch gel electrophoresis at pH 8.6.

Discussion

The present study on α_1 -acid glycoprotein preparations, isolated from sera of patients with diabetes mellitus, revealed the following two main findings: (a) The α_1 -acid glycoprotein variant(s) of each patient remained constant in spite of the greatly increased post-operative levels of this protein. This finding was interpreted to indicate that the α_1 -acid glycoprotein variants were probably genetically transmitted. (b) The α_1 -acid glycoprotein preparations isolated from the pre- and post-operative sera appeared identical with each other and with pooled normal α_1 -acid glycoprotein as judged by starch gel electrophoretic, immunoelectrophoretic, and double diffusion analysis. Hence, it was concluded that these patients probably produced normal α_1 -acid glycoprotein before and after operation. By contrast, in certain disease states α_1 -acid glycoprotein is synthesized on a sialic

acid deficient form (Schmid, Burke, Debray-Sachs, and Tokita, 1964; Caputo and Marcante, 1964).

Because of the stress of the HSS operation, the levels of the seromucoid fraction and α_1 -acid glycoprotein were increased approximately twofold as compared with the pre-operative values. A corresponding finding was recently reported for normal adults who had undergone certain major surgery and for pregnant women who were studied before and after delivery (Tokita *et al.*, 1966).

It should be emphasized again that the observed changes in α_1 -acid glycoprotein levels appeared to be unrelated to the antecedent and post-operative clinical courses of the patients and to whether or not their retinopathy was favourably influenced (Field, 1965). Thus, no evidence was obtained which would support a role for this plasma protein either in the pathogenesis or continuation of the retinopathy.

Summary

α_1 -acid glycoprotein (orosomucoid) was isolated from blood of 21 Caucasian patients with diabetes

mellitus and active haemorrhagic retinopathy, and was taken before and after hypophyseal stalk section. After enzymatic cleavage of their sialyl residues, these protein preparations were subjected to starch gel electrophoresis at pH 5 to identify the type of variants.

Constancy of the α_1 -acid glycoprotein variants was observed in each patient in spite of the considerable increase in the blood level of this protein due to the stress of the operation. The α_1 -acid glycoprotein produced by these patients before and after operation appears to be identical with normal α_1 -acid glycoprotein as judged by several criteria. It was concluded that this observation was consistent with the concept of the genetically determined transmission of the α_1 -acid glycoprotein variants.

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