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The estimation of two alpha$_1$ glycoproteins (orosomucoid and another alpha$_1$ acid glycoprotein) in health and disease

J. A. EASTON[1], J. HARDWICKE, AND P. H. WHITEHEAD

From the Department of Experimental Pathology, University of Birmingham

SYNOPSIS The normal values for orosomucoid and the α_1 acid glycoprotein of Schultze in young adults are reported, specifically estimated by an immunological technique. The use of purified orosomucoid as a standard allowed of absolute estimation of this protein. The α_1 acid glycoprotein is estimated relative to a standard normal serum.

These proteins were estimated in the serum of eight patients after surgery. Both proteins rise markedly in the serum, the α_1 acid glycoprotein reaching peak concentration in two to three days, and falling again equally rapidly, the orosomucoid rising more slowly to reach a peak after four to seven days, and returning to normal values in 10 to 14 days.

Estimations of serum levels in 24 patients with a variety of diseases show that the two proteins can vary independently; values as high as eight times normal were found for orosomucoid in Crohn's disease. In patients with proteinuria, orosomucoid is preferentially excreted in the urine. The losses of α_1 acid glycoprotein are, however, anomalous, being proportionately less than those of albumin, relative to the serum levels.

The significance of these preliminary findings is discussed.

Variations in the serum glycoproteins in disease have been appraised during recent years in a number of reports, most of which have been based on a chemical determination of the carbohydrate part of the glycoprotein molecules (anthrone reaction, determination of sialic acid, hexose, or fucose). The methods have been examined by Böttiger and Carlson (1960), and the clinical significance of bound polysaccharides has been reviewed by Schultze (1958); a raised serum level of bound polysaccharides has been recorded in many disease states (Winzler, 1960; Stary, 1957). These methods, however, estimate total bound polysaccharides and, since many of the plasma proteins contain carbohydrate, such estimations give no evidence about variations in individual protein fractions. Attempts to follow these variations have been made in a number of ways. Thus, estimation of the intensity of staining by the P.A.S. method following separation by paper electrophoresis has shown that the concentrations of the α_1 and α_2 globulins vary independently (Björnesjö, 1955); the value of this approach is reduced both by the limited accuracy of the method, and by the fact that both α_1 and α_2 globulin as separated electrophoretically include a number of protein fractions. Winzler, Devor, Mehl, and Smyth (1948) showed that the fraction of serum not precipitated by 0·6 M perchloric acid contained a glycoprotein which they termed 'seromucoid', and which could be estimated as protein by the biuret or the Folin method; such estimations have shown considerable variations of this fraction in disease. More recently, however, the 'seromucoid' fraction has been found to contain several proteins (Greenspan, 1954, 1955; de Vaux St. Cyr, 1960) and moreover it is probable that in this method a considerable amount of α_1 glycoprotein is non-specifically co-precipitated with the other serum proteins (Hardwicke, unpublished); this technique therefore is also non-specific, though clinically it has been found of value.

Darcy (1960) has used an immunological method to measure a specific α globulin in the serum of rats which is associated with tissue growth, although the

[1]Present address: Louis Jenner Laboratory, St. Thomas's Hospital, London, S.E.1.

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identity of this protein has not been definitely established. Specific estimations of orosomucoid (Schmid, 1953), a pure protein contained in the 'seromucoid' fraction, have however been obtained immunologically using chick antisera (Goodman, Ramsey, Simpson, and Brennan, 1957) and some changes have been recorded in malignant disease (Silberberg, Goodman, Kefalides, and Winzler, 1955).

Both orosomucoid and the α_1 acid glycoprotein of Schultze (Schultze, Göllner, Heide, Schönenberger, and Schwick, 1955) have recently been isolated from the urine of patients with proteinuria (Hardwicke and de Vaux St. Cyr, 1961). These proteins have been used to raise specific antisera in rabbits. This paper reports some immunological analyses of their varying concentrations in disease. In this context α_1 acid glycoprotein refers to the protein first described by Schultze *et al.* (1955).

METHODS

OROSOMUCOID A highly purified orosomucoid fraction was obtained from the urine of patients with proteinuria by a modification of the method of Hardwicke and de Vaux St. Cyr (1961). The step of precipitation with ammonium sulphate was omitted, urine being applied directly after dialysis against acetate buffer, *p*H 4·5 0·04 M, to a diethyl amino-ethyl cellulose column equilibrated with the same buffer. The fraction eluted at *p*H 4·0 0·1 M was then reapplied to a column of diethyl amino-ethyl cellulose and orosomucoid was eluted on a second run at *p*H 4·0 0·1 M. The preparation thus obtained appeared to be electrophoretically homogeneous on paper at *p*H 4·3 and 8·7, and a single symmetrical peak was observed in the ultracentrifuge over a *p*H range 4·0 to 8·7, giving an $S^{1\cdot0\%}_{20W} = 2\cdot72S$ in acetate buffer *p*H 4·5, 0·04 M.

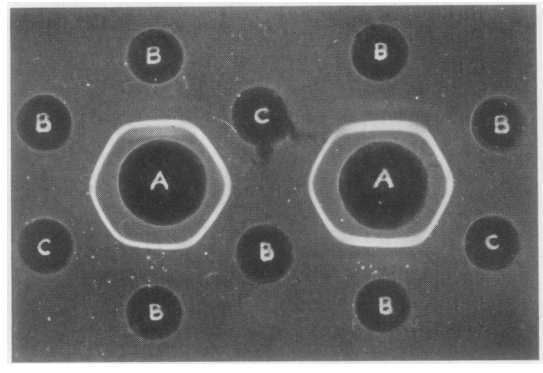

FIG. 1. *Reactions of identity between preparations of Schmid's orosomucoid and that prepared in this study.*
A = α_1 *antiserum*
B = *orosomucoid prepared during this study from urine*
C = *Schmid's acid* α_1 *globulin*

TABLE I

	Orosomucoid Prepared by Above Method	*Schmid's Orosomucoid Preparation (Schmid, 1953)*
Nitrogen content (%) (Pregl, 1945)	9·4	10·7
Polypeptide content (%) (Wolfson *et al.*, 1948)	50·0	66·0
Hexose (%) (Svennerholm, 1956)	16·7	17·2
Sialic acid (%) (Warren, 1959)	10·7	10·6
$E^{1\%}_{1\text{ cm.}}$ at 280 mμ	8·0	8·9

At concentrations of less than 1 g./100 ml. this preparation gave a single line on immunoelectrophoresis with a polyvalent horse antiserum (Pasteur H.S. 282) raised against whole normal human serum. At concentrations of greater than 1 g./100 ml., however, another faint line has been observed, possibly indicating the presence of another protein, estimated to be less than 1% of the total preparation. This line does not interfere with estimation as it readily dilutes out in the estimation method. Our orosomucoid preparation shows a reaction of identity with that prepared by Schmid (Fig. 1) and analysis gave the results shown in Table I.

Rabbits were immunized with approximately 100 mg. of this preparation in divided doses, the first injection containing Freund's adjuvant and being intraperitoneal, the remainder being intravenous (Soothill, 1962).

Estimates of orosomucoid were made by the method of Gell (1957). A preparation (optical density in 1 cm. cuvettes = 0·8 at 280 mμ) containing 100 mg. orosomucoid per 100 ml. by dry weight, and equivalent to a solution of 50 mg./100 ml. of human serum albumin when estimated by the Biuret method, was compared with standard serum (Fig. 2); five estimations showed the standard serum to contain 100 mg. orosomucoid/100 ml., or 50 mg./100 ml. by the Biuret method.

α_1 ACID GLYCOPROTEIN OF SCHULTZE This was obtained by the method of Hardwicke and de Vaux St. Cyr (1961). Antisera were raised as for orosomucoid and estimations performed by the same immunological technique.

SERUM ESTIMATION This method determines serum levels as a percentage of a standard serum; for orosomucoid this standard serum was found to contain 100 mg./100 ml. orosomucoid, so that concentrations can therefore be expressed in mg./100 ml. For the α_1 acid glycoprotein no sufficiently pure standard has yet been obtained; results are therefore expressed relative to the same standard serum (standard ≡ 100%).

URINE ESTIMATIONS The protein concentration in urine was not determined directly; instead the urine/serum ratio for the proteins was directly measured by the technique of Soothill (1962). Comparison of the urine/serum ratio with that for albumin, similarly determined, gives an estimate of the 'clearance' of the individual protein relative to that of albumin (Blainey, Brewer, Hardwicke, and Soothill, 1960).

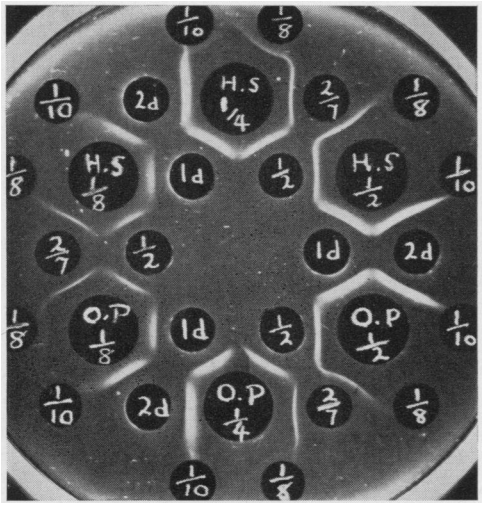

FIG. 2. *Gel diffusion plate showing concentration of orosomucoid in normal human serum (H.S.) at different dilutions, to be equivalent to the orosomucoid prepared (O.P.) at the same dilutions. Dilutions of antisera in saline in the small cups are shown (d = drops).*

Each large cup is surrounded by six dilutions of specific antisera. These are the same for each cup. The unknown dilutions are matched against the standards for best equivalence, i.e., H.S. ½ = O.P ½ (method of Gell, 1957).

RESULTS

NORMAL LEVELS Sera from 10 normal adult males and 10 normal females, between the ages of 17 and 47, were obtained.

Results are expressed as percentages of a standard serum from a healthy male subject, which was stored frozen in divided portions. Results vary from 75% of normal to 150% of normal for both proteins, with no significant sex differences. These values can be expressed as absolute values in the case of orosomucoid (Tables II and III).

AFTER SURGICAL OPERATIONS It has previously been suggested that variations in the glycoproteins are related to tissue breakdown and repair. The changes in these two proteins were therefore studied following acute surgical trauma, in parallel with estimations of the total serum α_1 by paper electrophoresis, and some estimations of the plasma fibrinogen; Table IV and Fig. 3 show the results obtained. It will be seen that both these α_1 glycoproteins rise very markedly in most cases. While there are variations between individual cases, the α_1 acid glycoprotein usually rises very rapidly to a peak between the second and fourth days, while the orosomucoid does not reach its maximum until about the sixth post-operative day; this rise in orosomucoid occurs approximately in concert with that of fibrinogen, but considerably exceeds it quantitatively, in one case rising to seven times the normal value. The α_1 acid glycoprotein not only rises with more rapidity, but also falls equally rapidly, suggesting that its turnover rate is even faster than that of fibrinogen. No close similarity between the post-operative rise and fall in the serum level of either of these α_1 glycoproteins estimated separately, and the level of the total α_1 protein estimated by paper electrophoresis, has been shown.

TABLE II

VALUES FOR OROSOMUCOID IN 20 NORMAL SUBJECTS

	Expressed as Dry Weight (mg./100 ml.)		*Expressed as Biuret (mg./100 ml.)*	
	Male	*Female*	*Male*	*Female*
1	87	100	43	50
2	125	125	62	62
3	100	100	50	50
4	100	125	50	62
5	125	125	62	62
6	150	125	75	62
7	75	87	37	43
8	75	75	37	37
9	125	75	62	37
10	87	75	43	37
Mean	105	101	52	50
Standard deviation	±25·9	±22·1	±12·7	±12·2

TABLE III

LEVELS OF α_1 ACID GLYCOPROTEIN IN 20 NORMAL SUBJECTS EXPRESSED AS A PERCENTAGE OF NORMAL SERUM

	Male	*Female*
1	125	125
2	125	150
3	100	150
4	150	125
5	87	125
6	125	150
7	150	75
8	125	150
9	150	150
10	100	75
Mean	125 ± 22·5	127 ± 29·9

MISCELLANEOUS CASES Results are given in Table V of estimations of these two proteins in the sera of 24 patients suffering from a wide variety of different conditions. Considerable and independent variations of the two proteins are found.

Very high values for orosomucoid were found in the cases of active Crohn's disease. Some rise in the orosomucoid level appears to accompany most forms of disease, and the α_1 acid glycoprotein level is significantly raised only in about half of these

TABLE IV

LEVELS OF α_1 ACID GLYCOPROTEIN AND OROSOMUCOID AND OF FIBRINOGEN AND TOTAL α_1 PROTEIN DURING FIRST TWO POST-OPERATIVE WEEKS

Patient No.		*Pre-operative Level*	*Post-operative Day* 2	4½	7½	10	13	*Diagnosis and Operation*
1	α_1 acid glycoprotein[1]	100	150	300	250	100	150	*Case 1* Duodenal ulcer, partial gastrectomy
	Orosomucoid	175	175	250	400	175	175	
	Fibrinogen[2]	360	440	—	590	600	420	
	Total α_1[2]	1110	590	530	460	640	700	
2	α_1 acid glycoprotein	125	300	200	200	250	200	*Case 2* Crohn's disease, resection of gut
	Orosomucoid	150	200	300	200	200	300	
	Fibrinogen	470	640	560	590	570	460	
	Total α_1	320	520	390	350	710	670	
3	α_1 acid glycoprotein	200	500	500	300	300	—	*Case 3* Carcinoma of bronchus, resection (pulmonary embolus—12 days)
	Orosomucoid	350	350	500	500	400	—	
	Fibrinogen	760	720	660	750	450	—	
	Total α_1	560	1070	670	540	510	—	
4	α_1 acid glycoprotein	150	150	175	100	—	150	*Case 4* Haemorrhoids, haemorrhoidectomy
	Orosomucoid	250	125	300	175	—	200	
	Fibrinogen	—	560	760	740	830	—	
	Total α_1	360	490	450	500	480	500	
5	α_1 acid glycoprotein	175	200	350	400	200	175	*Case 5* Carcinoma of bronchus, resection
	Orosomucoid	350	400	700	600	400	250	
	Fibrinogen	670	710	790	670	690	610	
	Total α_1	490	620	510	580	600	570	
6	α_1 acid glycoprotein	75	250	—	—	200	—	*Case 6* Intestinal obstruction (adhesions), laparotomy
	Orosomucoid	125	200	—	—	250	—	
7	α_1 acid glycoprotein	175	500	200	—	175	—	*Case 7* Carcinoma of stomach, gastrectomy
	Orosomucoid	200	350	125	—	350	—	
8	α_1 acid glycoprotein	125	250	250	250	—	—	*Case 8* Duodenal ulcer, partial gastrectomy
	Orosomucoid	150	175	250	350	350	—	
No. of patients reaching peak level on this day	α_1 Acid glycoprotein	0	5	2	1	0	0	
	Orosomucoid	0	1	4	2	1	0	

[1]Both expressed as percentage of normal. [2]Both given in mg./100 ml.

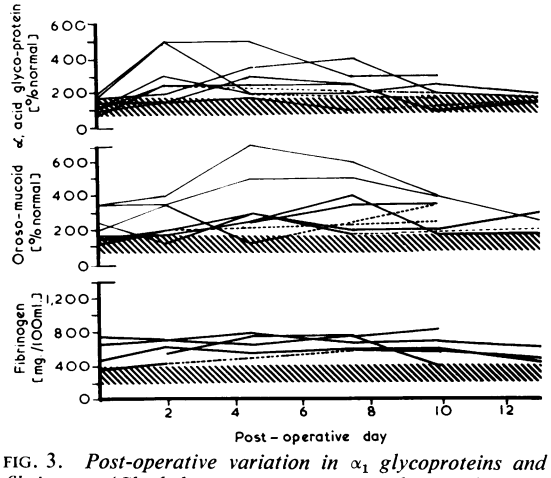

FIG. 3. *Post-operative variation in α_1 glycoproteins and fibrinogen. (Shaded areas represent normal ranges.)*

cases. Low levels of α_1 acid glycoprotein were found in two cases of gluten-induced enteropathy and in the untreated stage in a patient with diabetic ketosis.

PROTEINURIA AND THE NEPHROTIC SYNDROME In general the change in the level of any protein in the plasma of patients with the nephrotic syndrome varies in proportion to its molecular weight (Hardwicke, 1954) and hence its clearance in the urine. As the molecular weight of orosomucoid is 40,000 and that of the isolated α_1 acid glycoprotein 54,000 (Schultz *et al.*, 1955) both proteins should be excreted in the urine more readily than albumin (renal clearances should be greater than 100% of the albumin clearance); also, if production of the protein is normal then the serum level should be depressed relatively more than the serum albumin. Table VI shows the relevant figures on a number of cases of the nephrotic syndrome. Several points

TABLE V

SERUM LEVELS OF TWO α_1 GLYCOPROTEINS IN DISEASE

Patient	*Diagnosis*	*Orosomucoid (mg./100 ml.)*	*α_1 Acid Glycoprotein (% normal)*
	Carcinoma		
9	Stomach	100	150
10	Stomach	350	350
11	Stomach	200	175
12	Stomach	200	175
13	Bronchus	250	300
14	Bronchus	250	150
15	Bronchus	350	125
16	Bronchus	350	175
	Intestinal disease		
17	Steatorrhoea	175	150
18	Gluten-induced enteropathy	175	50
19	Gluten-induced enteropathy	100	37
20	Crohn's disease	800	300
21	Crohn's disease	700	150
22	Regional ileitis	200	250
23	Regional ileitis	500	400
24	Regional ileitis (in remission)	150	150
25	Diverticulitis	75	125
	Miscellaneous		
26	Cirrhosis of liver	400	150
27	Cirrhosis of liver	400	200
28	Cirrhosis of liver	150	125
29	Bronchopneumonia	300	500
30	Multiple myelomatosis	300	25
31	Disseminated lupus erythematosus	400	150
32a	Diabetic ketosis	100	37
32b	Diabetic ketosis (treated)	150	100

emerge from these data. First, the clearance of orosomucoid is usually higher than that of albumin, as expected, but in spite of the preferential loss of this protein in the urine, the serum level may be either raised or lowered; insufficient data are as yet available to interpret the significance of the high serum levels, which presumably reflect considerably increased production of this protein.

The loss of the α_1 acid glycoprotein in the urine is extremely variable. Although most patients excrete some of this protein in the urine, its 'clearance' is almost invariably less than that of albumin. Serum levels are usually low, consistent with the loss in the urine. One case (No. 35) is of particular interest. This patient had a nephrotic syndrome associated with minimal histological abnormality; unexpectedly he did not initially respond to cortisone treatment in the usual way, and in spite of a dose of cortisone of 150 mg. daily the serum steroid level remained low. When the drug was changed to prednisolone the proteinuria decreased, and a diuresis occurred. In this patient the α_1 acid glycoprotein level was consistently high or normal until effective therapy was started. It then fell to the expected subnormal values, this fall occurring rapidly, and before diminution in proteinuria could be confidently shown.

These cases are too few to provide more than preliminary data, but they do show a number of variations which are being further investigated.

TABLE VI

SERUM LEVELS OF α_1 GLYCOPROTEINS IN NEPHROTIC SYNDROME (10 PATIENTS)

Diagnosis	*Patient No.*	*Serum Level (% normal)*			*Clearance (% albumin)*	
		Orosomucoid	*Acid α_1 Glycoprotein*	*Albumin*	*Orosomucoid*	*Acid α_1 Glycoprotein*
Minimal change[1]	33a	25	75	12	300	100
	33b	75	75	37	200	125
	34	62	100	50	330	>125
	35a	37	200			
	35b	25	175			
	35c	25	100			
	35d	50	100			
	35e	75	50			
Proliferative glomerulonephritis[1]	36a	175	87	100	280	0
	36b	600	75	87	170	0
	37	87	125	75	0	0
Membranous glomerulonephritis[1]	38	100	125	25	200	30
	39	75	44		60	—
Nephrotic syndrome (no renal biopsy)	40a	100	25	22	250	50
	40b	20	37	25	130	50
	40c	50	<25	8	100	50
	41	37	25	31		
Renal vein thrombosis	42a	175	42	6	100	<12
	42b	62	75	10	600	75
	42c	125	42	50	200	8

[1]These diagnoses were based on renal biopsy histology (Blainey, Brewer, Hardwicke, and Soothill, 1960).

DISCUSSION

Estimations of these two α_1 glycoproteins in normal adult men and women show surprisingly little variation, the levels varying by only $\pm$ 25% while the coefficient of variation of the method itself is of the order of 16% (Soothill, 1962). This is surprising in view of the great lability of these two proteins following acute surgical trauma.

The site of synthesis of these two proteins is at present unknown. Considerable variations, however, occur in disease. In patients the values of orosomucoid of seven and eight times normal found in Crohn's disease represent a proportional increase greater than has been demonstrated in any other plasma protein; very high levels of 'seromucoid' have been reported in this condition (Cooke, Fowler, Cox, Gaddie, and Meynell, 1958). Both the α_1 glycoproteins estimated here are present in the perchloric filtrate 'seromucoid' and the demonstration that they can vary independently suggests that their separate estimation may prove of diagnostic value.

The sequential estimations following surgical trauma point to the great lability of these proteins. Studies on I^{131}-labelled orosomucoid have suggested a half-life of four to five days (Weisman, Goldsmith, Winzler, and Lepper, 1961). The results reported here have suggested that the α_1 acid glycoprotein is even more rapidly turned over, and that the half-life is likely to be of the order of one day. Previously, fibrinogen has been regarded as the most labile of plasma proteins, its rapid rise and fall in various infections and after surgical trauma being well studied (Ham and Curtis, 1938; Hodgson and Coon, 1954). The α_2 macroglobulin has also been estimated immunologically in parallel with the α_1 glycoproteins in the post-operative period, but no significant change in serum level has been found.

In the patients with proteinuria, orosomucoid is preferentially lost in the urine as would be expected (Hardwicke and Soothill, 1961) and the serum level is often diminished; those cases with high serum levels therefore presumably have an increased production considerably above normal. The other α_1 glycoprotein studied, in spite of its low molecular weight when isolated (Schultze *et al.*, 1955; Hardwicke and de Vaux St. Cyr, 1961), is consistently less readily excreted than albumin; this finding suggests that it is either preferentially reabsorbed from the glomerular filtrate, or that the effective molecular weight in the plasma is greater than that of albumin (69,000). This protein readily complexes with ceruloplasmin at acid *p*H (Hardwicke, unpublished) and it is possible that even at plasma *p*H and molarity it may circulate as a loose complex with other plasma proteins. The serum level, though usually low, is not readily predictable on the basis of urinary losses; and in one case with an initially raised serum level, the level only dropped to subnormal values when effective steroid therapy was started.

So far relatively large quantities of antiserum have been needed for each analysis, and this has limited the number of estimations which have been done. The development of a more specific chemical technique for estimation would be desirable, and may well be fairly simple for orosomucoid.

Although the data reported in this paper are insufficient to do more than indicate some of the variations found in disease, they show with certainty that these two proteins can vary independently. Since both proteins are present in the 'seromucoid' fraction, and since this 'seromucoid' has been shown to vary widely in a variety of diseases, it seems probable that their separate estimation may prove clinically even more valuable.

We would like to thank Professor J. R. Squire for his advice, Dr. J. F. Soothill, who kindly raised the antisera used in this work and has given much advice on technique, and Dr. K. Schmid for supplying us with a sample of his orosomucoid. We would also like to thank the physicians and surgeons of the United Birmingham Hospitals for permission to study their patients.

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