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Electrophoresis and Ultracentrifugation of Lipid-free Human Serum *

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In an earlier electrophoretic study of sera from which the lipids had been largely removed by precipitation and extraction with organic solvents at low temperature [1], it was *i. a.* found that the migration velocities of the protein components remained unchanged. In the experiments, Tiselius' electrophoretic apparatus was used, employing the *schlieren* method, hence no estimation of the quantitative relations of the different components could be made. It was observed, however, that in the extracted sera the *schlieren* band representing the α-globulin disappeared at pH values below 8. More recently, Zeldis, Alling, McCoord and Kulka [2], using the optical arrangement according to Longsworth, found certain alterations in total electrophoretic area and the relative areas of individual electrophoretic components in the extracted plasmas. Their findings confirmed the observations of Blix, Tiselius and Svensson [3] that the human β-globulin is particularly rich in lipid materials. The present study, which had already been completed when we became aware of the work just mentioned, was undertaken mainly to study the quantitative changes of the protein components brought about by delipidation. — The so called X-protein which is observed on ultracentrifugation of concentrated human sera owes its characteristic properties at least to a great extent to its high content of lipids [4]. Our work also aimed at giving some further elucidation of the question of the relation between the X-protein and other protein components of serum, especially the β-globulin.

* The ultracentrifugations were carried out at the Institute of Physical Chemistry by K. O. P., the rest of the work at the Institute of Medical Chemistry by G. B.

METHODS

Human serum samples were obtained from three normal men and from some patients from the Medical University Clinic in Upsala. Total serum protein was determined both by micro-Kjeldahl analysis and refractometrically with »Eintauch-refractometer». Phospholipid-P of the sera was determined on Bloor extracts using Teorell's method for the phosphorus analysis. Cholesterol was estimated according to the method of Schoenheimer and Sperry [5]. The lipid extraction was performed exactly in the same way as in the earlier work. The extracted sera were also analysed for lipid-P and cholesterol.

Electrophoresis was carried out at + 1° with a potential gradient about 5.5 volt/cm. The optical arrangement was that described by Svensson [6]. All samples were diluted with phosphate buffer of pH 7.68, ionic strength 0.10 plus 0.05 *M* NaCl making the total ionic strength 0.15. They were dialysed against 2 l of the same buffer for two days. In all experiments so much buffer was added to the sera as to make the total protein concentration 1.5 per cent. The electrophoreses were run for 140—145 minutes.

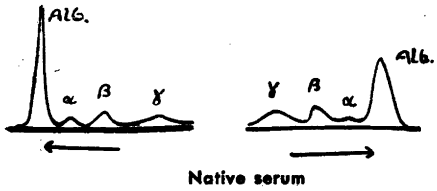
The ultracentrifuge runs were carried out in a Svedberg oilturbine ultracentrifuge at 59 000 R. P. M. and the observations were made with the Lamm scale method. Just before the runs, the sera were diluted with an equal volume of disodium phosphate in order to ensure a suitable separation between the »albumin» an the »X-protein» peak in the sedimentation diagram [4, 7].

EXPERIMENTAL OBSERVATIONS

Figs. 1—5 are reproductions of electrophoretic patterns obtained from some of the samples examined. The quantitative evaluation of these diagrams is beset with considerable errors and only the gross changes observed can be relied upon as real. A large error is encountered in the resolution of the individual peak areas when the separation is incomplete. This is especially the case in some of the extracted sera. The quantitative data obtained are given in Table 1 and 2.

From table 1 it is seen that the total protein, refractometrically determined, regularly decreased after extraction. As the proteins of the extracted sera dissolved completely on resuspension in buffer, this decrease is entirely or almost entirely due to the removal of the lipids. On the whole, both the greatest relative and the greatest absolute loss occurred in the β-globulin (Table 2). The same was found by Zeldis *et al.* in human sera. The reduction is in no case greater than reasonably can be expected from the removal of the lipid part of each fraction. The most marked change in the pattern is usually the

Fig. 1.—5. Electrophoretic patterns before and after extraction of lipids from human serum.



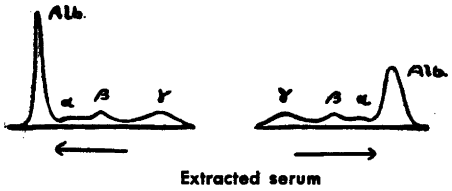
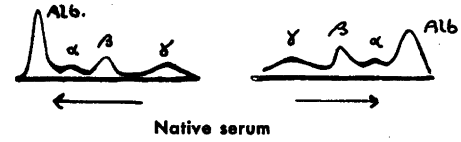


Fig. 1. Normal man (No. 1673/75).



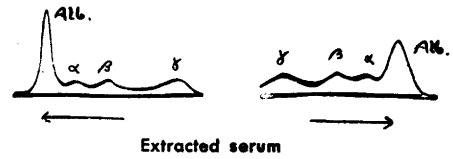


Fig. 2. Case of icterus (No. 1659/61).

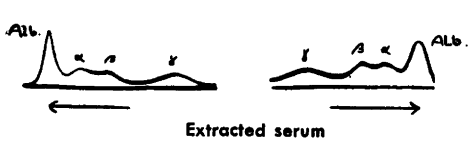
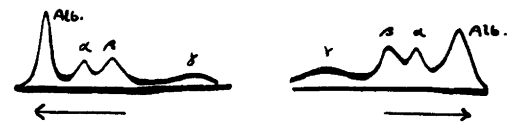


Fig. 3. Case of pregnancy toxemia (No. 1662/64).



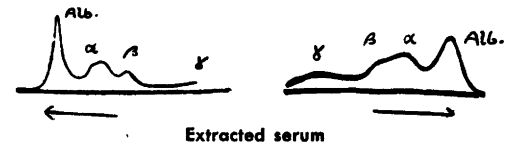
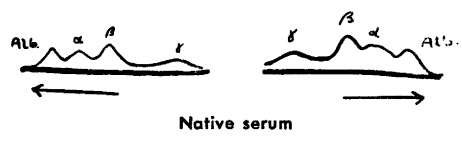


Fig. 4. Case of pregnancy toxemia (No. 1667/71).



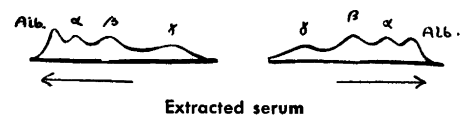


Fig. 5. Case of nephrosis (No. 1669/74).

Table 1. Total protein and lipid content of electrophoretically investigated sera.

		A Total protein Kjeldahl %	B Total protein refract. %	B—A %	Cholesterol + phospholipids * mg %
Normal	Native	7.68	7.75	+ 0.08	320
1653/55	Extracted	7.37	7.40	+ 0.03	42
	Difference	+ 0.31	+ 0.35		
Normal	Native	7.96	7.95	— 0.01	364
1651/54	Extracted	7.66	7.60	— 0.06	53
	Difference	+ 0.30	+ 0.35		
Nephrosis	Native	6.28	6.40	+ 0.12	389
1658/60	Extracted	6.02	6.00	— 0.02	49
	Difference	+ 0.26	+ 0.40		
Icterus	Native	5.79	6.05	+ 0.26	446
1659/61	Extracted	5.68	5.80	+ 0.12	46
	Difference	+ 0.11	+ 0.25		
Normal	Native	7.83	7.95	+ 0.12	450
1673/75	Extracted	7.58	7.35	— 0.23	67
	Difference	+ 0.25	+ 0.60		
Pregnancy	Native	6.63	7.40	+ 0.77	488
toxemia	Extracted	6.64	6.80	+ 0.16	152
1662/64	Difference	+ 0.01	+ 0.60		
Pregnancy	Native	8.11	8.50	+ 0.39	535
toxemia	Extracted	7.87	7.90	+ 0.03	81
1670/72	Difference	+ 0.24	+ 0.60		
Nephrosis	Native	4.55	5.70	+ 1.15	741
1669/74	Extracted	4.32	4.50	+ 0.18	116
	Difference	+ 0.23	+ 1.20		
Pregnancy	Native	5.33	6.05	+ 0.72	765
toxemia	Extracted	5.25	5.50	+ 0.25	127
1667/71	Difference	+ 0.08	+ 0.55		

* The small amounts of lipids remaining in the extracted sera contained no cholesterol.

Table 2. Electrophoretical distribution of α-, β- and γ-globulins in native and extracted sera.

No.	Diagnosis	Serum	Albumin %	α-globulin %	β-globulin %	γ-globulin %
1653/55	Normal	Native	3.79	0.81	1.10	2.05
		Extracted	3.39	0.87	1.05	2.09
		Difference	— 0.40	+ 0.06	— 0.05	+ 0.04
1651/54	Normal	Native	4.09	0.59	0.98	2.28
		Extracted	3.74	0.76	0.94	2.15
		Difference	— 0.35	+ 0.17	— 0.04	— 0.13
1658/60	Nephrosis	Native	1.84	0.65	3.91*	
		Extracted	1.77	0.55	3.69	
		Difference	— 0.07	— 0.10	— 0.22	
1659/61	Icterus	Native	2.46	0.77	1.24	1.59
		Extracted	2.52	0.76	1.08	1.44
		Difference	+ 0.06	— 0.01	— 0.16	— 0.15
1673/75	Normal	Native	4.14	0.60	1.22	1.99
		Extracted	4.05	0.67	1.01	1.62
		Difference	— 0.09	+ 0.07	— 0.21	— 0.37
1662/64	Pregnancy toxemia	Native	2.91	1.31	1.58	1.60
		Extracted	2.38	1.26	1.30	1.85
		Difference	— 0.53	— 0.05	— 0.28	+ 0.25
1670/72	Pregnancy toxemia	Native	— 3.86	0.95	1.58	2.11
		Extracted	— 3.80	0.91	1.29	1.90
		Difference	— 0.08	— 0.04	— 0.29	— 0.21
1669/74	Nephrosis	Native	1.01	1.46	1.68	1.55
		Extracted	0.89	0.95	1.48	1.18
		Difference	— 0.12	— 0.51	— 0.20	— 0.37
1667/71	Pregnancy toxemia	Native	2.27	1.06	1.38	1.34
		Extracted	2.02	1.69	0.70	1.09
		Difference	— 0.25	+ 0.63	— 0.68	— 0.25
Difference, mean			— 0.19	+ 0.03	— 0.24	— 0.15
Decrease in per cent of initial value, average			— 7.2 %	+ 5.3 %	— 15.9 %	— 8.6%

* Hardly any separation between β- and γ-globulin.

decrease in height of the peaks at the same time as the separation becomes incomplete. This means that each component after the extraction appears less uniform than before. Whether or not this is due to an incipient denaturation or to a breaking up of more uniform lipid-protein complexes cannot be decided (the same change is seen in the experiments of Zeldis *et al.* but is not so marked in them as in our cases). The apparent disappearance of the α-component in the earlier study did obviously not depend on an inclusion in some other fraction but on the flattening of the peak.

The figures given in Table 1 show some further facts of interest. The difference in total protein calculated on the Kjeldahl-values before and after extraction are due to removal of the main part of the non-protein nitrogen in the extraction. As could be expected this difference does not vary with the lipid content of the native serum. The Kjeldahl-values and the refractometric values for total protein do not differ considerably in unextracted sera with

Table 3. Relative amounts of components according to ultracentrifuge and electrophoresis experiments.

	Ultracentrifugal distribution				Electrophoretic distribution		
	Total protein concentration	Component	Per cent of total protein		Component	Per cent of total protein	
			Native	Extracted		Native	Extracted
Normal 1673/75	4.0	Albumin	42	88	Albumin	52	55
		Globulin	12	11	α-glob.	8	9
		X-protein	45	0	β-glob.	15	14
		»20-comp.»	1	1	γ-glob.	25	22
Pregnancy toxemia 1670/72	4.3	Albumin	40	86	Albumin	44	48
		Globulin	11	12	α-glob.	11	12
		X-protein	46	0	β-glob.	19	16
		»20-comp.»	3	2	γ-glob.	25	24
	2.8	Albumin	83				
		Globulin	15				
		X-protein	0				
		»20-comp.»	2				
Pregnancy toxemia 1667/71	3.0	Albumin	72	80	Albumin	38	37
		Globulin	11	14	α-glob.	18	31
		X-protein	11	0	β-glob.	23	13
		»20-comp.»	6	6	γ-glob.	22	20

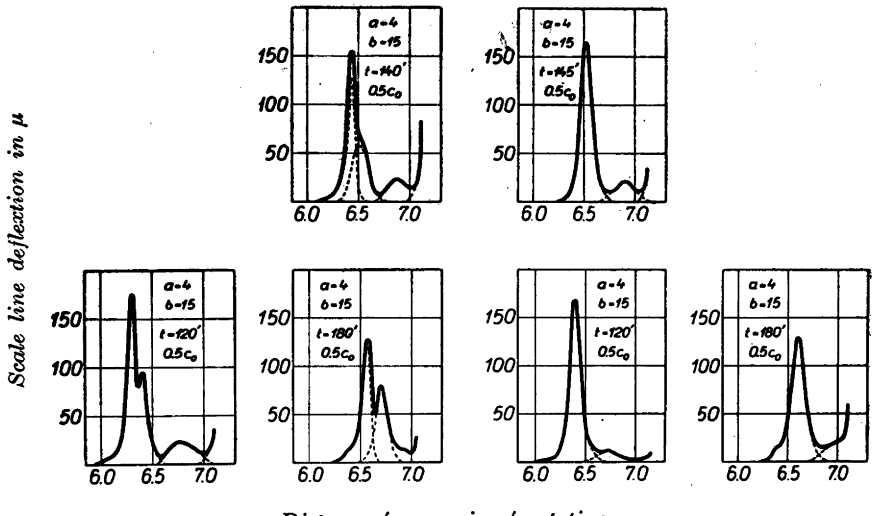


Fig 6. Sedimentation diagrams of human sera before (left hand diagrams) and after delipidation (right hand diagrams).
Upper row: Normal serum (1673/75).
Lower row: Pregnancy toxemia serum (1670/72). The slower sedimenting »X-component» is present in the native samples, but have disappeared from the extracted ones.

normal content of lipids. In hyperlipemic cases the difference increases. The decrease of the refractometric value for total protein on extraction bears also an obvious relation to the lipid content.

Fig. 6 shows the sedimentation diagrams from some of the sera before and after extraction of the lipids. It is quite evident from the diagrams that great changes take place in the sedimentation behaviour of the sera after delipidation. The values calculated for the various components from the diagrams are given in Table 3. The separation of the »X-component» and the »albumin» peak is not sufficiently good, however, to allow an accurate estimation of the amount of the two individual components, but only of their sum. The concentration of the slower sedimenting components will also come out somewhat too high, whereas that for the more rapid sedimenting ones comes out too low [8]. From Table 3 it appears that the »X-component» after extraction disappears into the »albumin» peak, whereas the γ-globulin and the »20-component» show insignificant changes only.

As one of us (K. O. P.) has earlier shown [4, 7] the X-protein, appearing in undiluted or slightly diluted sera, has an s_{20} which varies with the density of

the solution. On dilution the X-protein disappears in the albumin peak of the ultracentrifugal pattern. A mixture of electrophoretically isolated β- and γ-globulin showed in addition to a globulin peak also a component with the characteristics of the X-protein. As pure γ-globulin contained no X-protein, it was concluded that the X-protein was a β-globulin. In a recent work, Oncley, Scatchard and Brown [9] by low temperature ethanol fractionation isolated a β-globulin, designated as a β-lipo-protein which contained about 75 per cent lipids and had the properties of the X-protein. It is evident from their work that only a part of the β-globulin, *viz.* its lipo-protein part, in an isolated state behaves as X-protein. This lipo-protein seems to form only a few per cent of the total serum protein,* whereas the X-protein might amount to 50 per cent or more in concentrated sera. Obviously therefore the X-protein observed in the latter case must in the main be composed of other fractions than the β_1-lipo-protein. It has, indeed, been found earlier [4, 7] that on increasing the total protein concentration of serum solutions the appearance of the X-protein was accompanied by a decrease of the albumin and the globulin peaks. Furthermore, the X-protein, isolated by ultracentrifugation and purified by resuspension and respinning in the centrifuge, split at a suitable density of the solution into two peaks, the one of which sedimented as albumin and the other at a slower rate. Moreover, it was observed that when γ-globulin prepared by salt precipitation was added to a fresh human serum, the X-protein increased, whereas the globulin peak was augmented less than expected. From these observations it was concluded that the X-protein appearing in concentrated sera is a complex which in addition to β-globulin contains considerable amounts of albumin and globulin [4].

The results from the present work give no definite indication that part of the ordinary serum globulin with $s_{20} = 7$ S should enter into the ultracentrifugal X-component. It suggests, therefore, that the X-component of concentrated serum solutions might be a lipo-protein-albumin complex. On the other hand, Petermann [10] has found that when both isolated X-protein and whole serum containing X-component are treated with a lecithinase (from *Clostridium perfringens*), the X-component disappears from the sedimentation diagrams. At the same time the amounts of both the albumin and the glo-

* From the data published by the Harvard group until now, it appears that only 70—80 per cent of the plasma proteins may be accounted for by the products so far isolated by the ethanol technique. As, however, the lipid content of the isolated lipoproteins only amounts to one third of the total lipid content in normal human serum, or even less, it seems reasonable to assume that a considerable part of the remaining 20—30 per cent of the plasma proteins may consist of lipoproteins.

bulin showed a considerable increase. The electrophoretic pattern from isolated X-protein showed a very pronounced decrease for β_1-globulin after treatment with lecithinase, whereas those from whole serum showed only small changes.

It is of course possible that in addition to the β_1-lipo-protein, other serum lipo-proteins enter into the X-component also, *e. g.* the α_1-lipo-protein isolated by the Harvard group and other lipo-proteins not yet isolated.

As there has been some confusion regarding the relation between β-globulin and the X-protein, it must be emphasized that although a certain fraction of the β-globulin in the ultracentrifuge behaves like an X-protein, the total electrophoretic β-globulin should not be identified with the latter. Furthermore, it is evident from the present experiments that the main part of the electrophoretic β-globulin after delipidation retains its original electrophoretic mobility and does not become included in some of the other components. Finally, it must be concluded that the elctrophoretical β-globulin is not a complex of albumin and γ-globulin with lipids, although the X-protein isolated from *concentrated* sera besides the β-lipo-protein contains as well albumin as γ-globulin.

Many of the apparent contradictory results obtained from electrophoresis and sedimentation are evidently due to the fact that serum contains many more components than is generally recognized at present. Several of them have about the same sedimentation constants, but may have different electrophoretic velocities. On the other hand, some of the components show practically the same electrophoretic migration but give rise to several components in the ultracentrifuge.

SUMMARY

Extractions of most of the lipids of serum bring about certain alterations of the electrophoretic pattern, but no component is reduced more than could be reasonably expected from the removal of its lipid part. After delipidation the X-protein of serum entirely disappears; at the same time the albumin increases. The disappearance of the X-protein is accompanied by a corresponding increase of the albumin, whereas the globulin and the »20-component» remained practically unchanged.

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