ELECTROPHORESIS OF LIPID-FREE BLOOD SERUM

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The finding of lipid substances in all electrophoretically separable serum proteins, in the case of α- and β-globulins in remarkably large proportions (Blix, Tiselius, and Svensson, 1941), raised the question of the possible rôle of the lipids in the electrophoretic behavior of these proteins. Experiments with sera from which the lipids had been largely removed, according to the mild extraction principle introduced by Hardy and Gardiner (1910), seemed likely to give certain information in this respect. Such experiments were accordingly carried out. At the same time some questions pertaining to the transport function of the serum proteins were taken into consideration and experimentally investigated.

EXPERIMENTAL

The serum lipids were removed in the following way. 10 ml. of serum, cooled to $\pm 0°$, were precipitated with 60 ml. of acetone at $-14°$ and immediately centrifuged for 3 minutes at 3000 R.P.M. (the centrifuge was kept at a temperature not exceeding $+10°$). The supernatant fluid was poured off and the precipitate treated for $\frac{1}{2}$ hour with 60 ml. of fresh acetone of $-14°$. The mixture was placed in a refrigerator adjusted to this temperature. This treatment was repeated once. The precipitate was then well stirred up with 60 ml. of a mixture of acetone and dry ether, 2:1, allowed to stand for 5 minutes, and again centrifuged. The precipitate was at last treated with 60 ml. of dry ether for 15 minutes, this treatment being repeated once. The treatments with acetone-ether and ether were also carried out at $-14°$. The precipitate was then placed in a vacuum desiccator and freed from ether by

evacuation with an oil pump at ordinary room temperature. During the whole procedure great care was taken not to expose the precipitate for more than minimal periods of time to the moisture of the air. Subsequent extraction of the precipitate with dry ether in a Soxhlet apparatus was not carried out, as it did not appear that further lipids would be removed. In fact not more than traces of lipids were extracted after the two first acetone treatments, the subsequent treatments with ether serving mainly to remove the acetone.

With the extraction procedure thus employed the serum cholesterol was completely removed. On the other hand a certain amount of phospholipids (about 25 per cent of the total) was not extracted. An analysis according to the procedure of Brante (1940) showed the unextracted phospholipid to be mainly cephalin, whereas the phospholipid in the extract was chiefly lecithin. The proteins after the extraction dissolved easily and completely in the buffers used.

The electrophoresis apparatus of Tiselius (1937, *a*) was employed. All measurements were made at $\pm 0°$ with a potential gradient of about 5 volts per cm. The sera were diluted twice with and dialyzed against phosphate buffers of constant ionic strength, $\mu = 0.1$.

Table I shows the mobility values of the serum proteins found in native and lipid-free sera at different pH values. Sera I to IV were drawn from healthy men in the postabsorptive period. Serum V was a horse serum.

In the experiments with lipid-free sera the β-globulin, contrary to observations on native sera, always appears quite clear. This confirms the surmise that the opalescence of the β-globulin in native sera is due to the lipid substances present.

It may be gathered from Table I that no α-globulin appears in lipid-free sera at pH 6.1 and 7.4. In four further experiments performed at these pH values with sera to which certain foreign substances had for other purposes been added (see below) no α-albumin boundaries appeared either. The horse serum, in which the α-globulin is quantitatively more important than in human serum, agreed with the latter in this respect. On the contrary at pH 8 the α-globulin boundaries in most cases became visible also in the lipid-free sera, although—especially in horse serum—the

schlieren band was much less marked than in normal serum. In two experiments at pH 8, with foreign substances added to lipid-free sera, the α-globulin likewise appeared.

The changes affecting the α-globulin represent the only difference in electrophoresis found between the native and the lipid-free sera.[1]

Whether the effect on the α-globulin is due to the absence of the lipids or to an incipient denaturation of the protein cannot with certainty be decided. The easiest explanation is perhaps that the mobility of the α-globulin, due to an incipient denatura-

TABLE I

Electrophoretic Mobilities of Serum Proteins

The results are given in cm.^{2} sec.$^{-1}$ volt^{-1} $\times$ 10^5.

Serum No.	pH	Kind of serum	Albumin	α-Globulin	β-Globulin	γ-Globulin
III	6.1	Native	−3.8	−3.0	−1.9	+0.3
"	6.1	Lipid-free	−3.8		−1.9	+0.3
I	7.4	Native	−6.6	−5.2	−3.8	±0.0
"	7.4	Lipid-free	−6.5		−3.8	±0.0
II	7.4	Native	−6.5	−5.2	−3.7	±0.0
"	7.4	Lipid-free	−6.5		−3.8	±0.0
V	7.4	Native	−6.2	−5.0	−3.7	−0.4
"	7.4	Lipid-free	−6.2		−3.7	−0.3
III	8.0	Native	−7.5	−6.2	−4.7	−0.4
"	8.0	Lipid-free	−7.5	−6.2	−4.7	−0.4
IV	8.0	Native	−7.5	−6.5	−4.3	−0.5
"	8.0	Lipid-free	−7.7		−4.7	−0.3
V	8.0	"	−7.5	−6.2	−4.5	−0.9

tion, suffers such a change that its boundaries submerge in the broad schlieren band of the albumin or the β-globulin, the possible difference in mobility between one of these proteins and the changed α-globulin being so small that even in extended experiments no separate α-globulin boundaries become visible. The

[1] Tiselius (1937, *b*) mentions an experiment with serum from which the lipids had been removed in much the same way as in the present work. A large reduction of the mobility of the albumin was the sole change noted. Possibly the precautions taken in order to prevent protein denaturation were not in this case so strict as in the present experiments.

facts that the α-globulin appearing at pH 8 in lipid-free sera shows a quite normal mobility and that the mobility of the other protein fractions is, within the experimental error, unchanged nevertheless contradict such an explanation. That the α-globulin, deprived of its lipids, assumes as such a mobility coinciding with one of the other proteins or even that the native α-globulin has the nature of a compound with one of the other proteins with lipids seems also to be disproved by the appearance of α-globulin at pH 8. Lastly, it is perhaps conceivable that the lipid-free α-globulin particles become coated by one of the other lipid-free serum proteins, say the β-globulin, the resulting complexes having the mobility of the latter and not being stable on the alkaline side of about pH 7.5. The knowledge gained in the experiments given below might to some extent support this view.

A series of electrophoresis experiments was carried out with lipid-free (and native) sera to which had been added suspensions of either cholesterol, mastic, or the dye Sudan III.

The cholesterol and the mastic were dissolved in warm alcohol and poured into boiling water. The alcohol was driven off by concentration of the solution to one-third of the original volume. The cholesterol suspension was stabilized by addition of a trace of sodium oleate. The sols obtained contained about 1 per cent of the substance suspended. The Sudan III sol was prepared according to the method of Bennhold (1932). Of these suspensions usually 0.5 ml. was added to a mixture of 4 ml. of serum and 7.5 ml. of buffer solution.

In lipid-free sera the opalescence of the mastic and cholesterol respectively at pH 6.1 and 7.4 always coincided exactly with the β-globulin boundaries. At pH 8 the opalescence boundaries appeared between the α and β boundaries, save for one instance when they coincided with the α-globulin boundaries.

The results of the experiments with Sudan III are given in Table II.

From these results it is clear that the use of Sudan III as an indicator of the lipid substances of serum in electrophoresis experiments (Bennhold, 1932) is not justified. There is obviously reason to be cautious with the use of Sudan III as an indicator of lipid substances. This is further evidenced by the observation that in a native serum from a patient with marked alimentary

lipemia the opalescence boundaries migrated between the boundaries of the α- and β-globulins, whereas the Sudan III at the same time migrated exactly with the β-globulin. It should be added here that in two further sera from individuals with marked alimentary lipemia the opalescence boundaries likewise appeared between the α- and β-globulin boundaries.

In this connection another observation made in the above experiments should be pointed out. The albumin, which on electrophoresis of native sera always appears yellow, owing to the adhering bilirubin, in the lipid-free sera regularly appears colorless, or, in some instances, only faintly yellowish. As the bilirubin does not go into the extracts, it evidently shifts over to the only protein appearing colored in these sera; namely, the β-globulin.

TABLE II

Use of Sudan III As Indicator of Lipid Substances of Serum

Serum No.	pH	Kind of serum	Sudan III migration
II	7.4	Native	With β-globulin
"	7.4	Lipid-free	" "
IV	7.4	Native	Between α- and β-globulin
"	8.0	"	" " " "
V	8.0	Lipid-free	With β-globulin

From the observations thus made it appears that the β-globulin plays a special rôle in the transport of substances differing in chemical structure but similar in that they may occur in serum as hydrophobic colloidal particles.[2] As shown by Blix, Tiselius, and Svensson (1941), all the electrophoretically separable serum proteins contain lipids. However, whereas the lipids of albumin, α-globulin, and γ-globulin respectively probably should be regarded as relatively permanent elements in these proteins, it is suggested that the β-globulin lipids, which, to a great extent at

[2] The underlying observations may appear to be somewhat at variance with the results of Moyer and Moyer (1940) who worked on electrophoresis of quartz and collodion particles in serum and with those of Ludlum, Taft, and Nugent (1931), who investigated the electrophoresis of chylomicrons. The discrepancies are, however, not so great that they may not be in the main explained by the differences in the experimental conditions employed.

least, are present as comparatively large particles coated by a protein film, are lipids passing through the blood on their way from one part of the body to another. The β-globulin is thus supposed to be endowed with a special function in the fat transport of the body. In marked lipemic conditions the turbidity, as mentioned above, often appears between the α- and β-globulin boundaries. This probably indicates that, when larger amounts of fat have to be transported, the albumin or the α-globulin or both of them in addition to the β-globulin go into the surface film of the lipid particles and thus, in an auxiliary way, take part in the fat transport. The outcome of the experiments with mastic and cholesterol at pH 8 might indicate that a participation of proteins with a greater mobility than the β-globulin in the trans port of materials of the kind here in question takes place, especially at more alkaline pH values.

The substances which are transported with the different serum proteins may undoubtedly influence the transport conditions of each other. Competition for the transporting proteins and displacements certainly may occur. The distribution of the transported substances on the different protein components does not to all appearances represent a fixed state but rather a labile equilibrium which is often and easily changed in the one or the other direction. Such changes might have far reaching consequences, especially in pathological conditions.

SUMMARY

In sera from which the lipids had been largely removed by precipitation and extraction with organic solvents at low temperature the migration velocities of the different protein components were found to be unchanged, except that the α-globulin boundaries regularly disappeared at pH values below 8. The cause of this change is discussed.

Cholesterol, mastic, and the dye Sudan III, which were added to lipid-free sera in the form of suspensions in water, as a rule migrated with the β-globulin. In lipid-free sera the bilirubin is completely, or for the greater part, shifted over from the albumin to the β-globulin.

Certain aspects of the transport function of the serum proteins are discussed.

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