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Serum Protein Fractionation by Means of Ammonium Sulfate in the Presence of Phenolic Compounds

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Harms [1] described in 1946 a procedure, in which the fractional precipitation of horse serum proteins by means of ammonium sulfate was modified through the addition of phenolic compounds. The presence of 2 % phenol or 1 % tricresol caused a reversal of the order of precipitation of the different proteins, the albumin being precipitated first, thus making it possible to prepare a solution containing mainly β- and γ-globulins. The value of this procedure for the purification of antibodies was especially emphasized.

As this procedure might be more generally adaptable, it has now been studied by electrophoresis. Besides giving a more complete picture of the distribution of the different components, this method might disclose, whether the addition of the phenols caused an irreversible denaturation of the proteins, especially of the albumin, or whether it would be possible to recover the proteins unchanged.

Two kinds of horse serum were used. The first sample was prepared from fresh spontaneously coagulated blood and stored at −20° C, the other was a heat sterilized sample obtained from the State Serum Institute. The heat sterilization, which is generally applied to immune sera, is accompanied by profound changes in the electrophoretic pattern [2].

The experiments were performed as follows:

To a serum sample was added an equal volume of 4 per cent phenol or 2 % tricresol in water. This step as well as all the following were carried out in the cold room at 0—5° C. To the resulting only very slightly turbid solutions was added saturated ammonium sulfate and the amounts necessary to cause a suitable precipitation were estimated (Table 1). They may of cause be varied according to the special purpose of the fractionation. As already stated by Harms, much less ammonium sulfate is needed for this precipitation than in the absence of phenols. This is more marked in the case of the heat treated serum, indicating an increased lability caused by the heat sterilization. The samples were centrifuged, and the precipitates suspended in 0.9 % NaCl. These suspensions as well as the supernatants were dialyzed against phosphate-NaCl-buffer ($\mu_{\text{phosphate}}$ (ionic strength) = 0.075, μ_{NaCl} = 0.025, pH = 7.7)

Table 1.

Precipitant	Final $(NH_4)_2SO_4$ concentration in per cent saturation		Composition of sample in percentage		
Spontaneously coagulated serum:			Alb.	α-glob. (C-component)	$\beta+\gamma$-glob.
Control			53	8	39
2 % phenol	16	precipitate:	62	18	20
		supernatant:	10	16	74
1 % tricresol	20	precipitate:	69	8	23
		supernatant:	18	18	64
Heat treated serum:					
Control			24	39	37
2 % phenol	9	precipitate:	28	56	16
		supernatant:	9	17	74
1 % tricresol	14	precipitate:	25	61	14
		supernatant:	14	15	71

Distribution of the various proteins in the serum samples and in the fractions obtained by precipitation with ammonium sulfate in the presence of phenol and tricresol. Percentage calculated as the average from the ascending and descending pattern.

and studied by electrophoresis in our Tiselius apparatus [3]. Representative results are shown in Fig. 1 and Table 1.

It appears that the albumin is precipitated first, and that the β- and γ-globulins remain in solution in accordance with the findings of Harms. In the concentrations used, the α-globulin and the C-component of the heat treated serum appear in about equal amounts in both fractions. It is remarkable, that all components of the precipitates and supernatants showed completely unchanged mobilities as compared with the untreated samples. This indicates that the change in solubility of the albumin is not due to a simple denaturation. Probably the phenolic compounds form labile complexes with the proteins, the properties of which may be quite different from those of the free proteins. This behaviour is similar to that observed by Astrup and Birch-Andersen [4] in their experiments on protein fractionation by means of specific anions. The method may therefore probably find further applications than that suggested by Harms.

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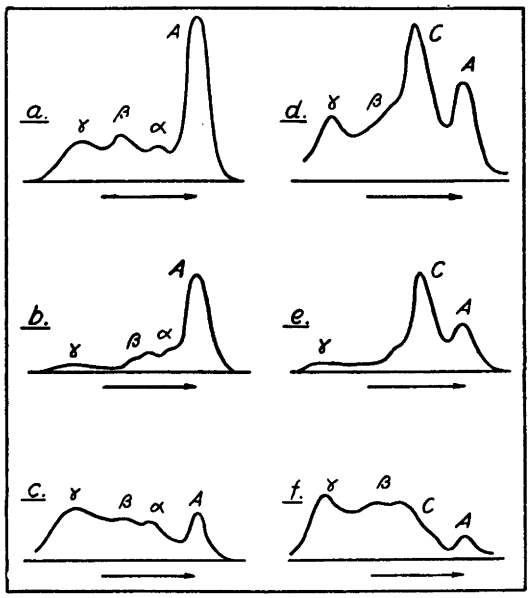

Fig. 1. Electrophoretic diagrams of the serum samples used and of some of the fractions (descending pattern).

a. Spontaneously coagulated serum.
b. Precipitate, and c. supernatant from precipitation of a. with 1 % tricresol and ammonium sulfate.
d. Heat treated serum.
e. Precipitate, and f. supernatant from precipitation of d. with 2 % phenol and ammonium sulfate.

SUMMARY

The precipitates obtained from horse serum by means of ammonium sulfate in the presence of phenol or tricresol were found by electrophoresis to contain mainly albumin and α-globulin. None of the components showed any sign of denaturation.

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