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Serum Protein Fractionation According to Henriques and Klausen's Method and with Methanol

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According to Sørensen's investigations [1], pure protein solutions may be considered systems of reversibly connected components in which the single dissociable factors are in a state of mutual equilibrium. In biological fluids, such as, for example, serum, several of these systems of components must be supposed to be active (Geill [2]) and to form *inter se* more or less firmly united complexes, the state of equilibrium of which may be displaced in case of changes of the composition of the solvent. Owing to this overlapping it is therefore possible, when a single protein is precipitated from such a solution, partly that only a small portion of the protein in question is precipitated, partly that the solution still contains small quantities of the substance united with the other components contained in the system and also that the precipitate contains small quantities of these components.

Under practical conditions this proves to be so. On comparison with electrophoresis Tiselius [3] thus found that after a 55 % saturation of serum with ammonium sulphate the filtrate still contained about 25 % of the globulin fraction. Similar observations have been made by Svensson [4] and by Cohn and collaborators [5] who, after precipitation in horse serum with a 2.05 molar solution of ammonium sulphate, found that the filtrate contained small quantities of globulin. After precipitation with 22.5 % sodium sulphate solution the filtrate contained about one-fourth of the beta-globulin and three-fourths of the alpha-globulin (Guttman and collaborators [6]). According to Taylor and Keys [7] the sodium sulphate precipitation gave 5.2 ± 3 % more nitrogen in the albumin fraction than stated by the electrophoresis values. On a direct comparison between the methods of precipitation of Howe [8] and of Henriques and Klausen [9] small but unquestionable differences were

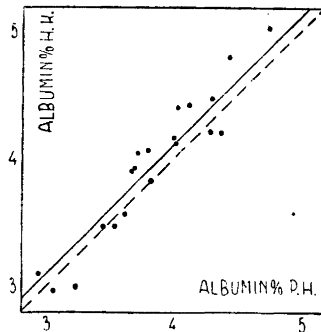

Fig. 1. Relations between the albumin per cents according to Henriques and Klausen and according to Pillemer and Hutchinson.

recently found by Bing, Næser, Rasch and Røjel[10], Howe's method giving slightly higher albumin values than that of Henriques and Klausen.

Whilst in case of precipitation the serum proteins occur as a more or less continuous system with mutual overlapping of the individual fractions, this does not seem to be the case in electrophoresis, in which the fractions can be defined with far greater accuracy. At present, therefore, electrophoresis is believed to afford the most correct picture of the proportion between the individual serum protein fractions. As mentioned above, several authors

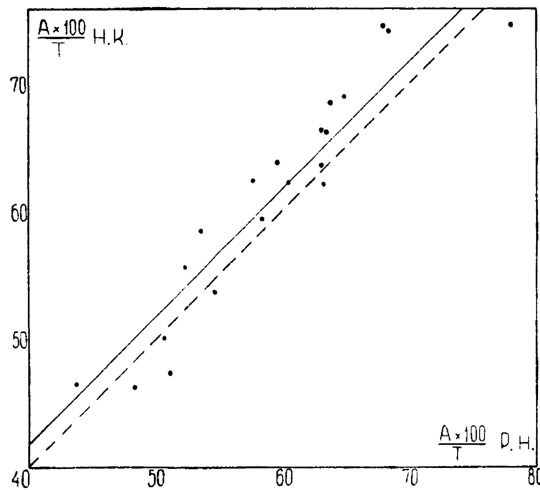

Fig. 2. Relation between the relative albumin per cents according to Henriques and Klausen and according to Pillemer and Hutchinson.

Table 1. Total serum protein, albumin and globulin in grammes per 100 ml. Relative albumin %.

No.	Total protein T	Pillemer and Hutchinson			Henriques and Klausen		
		Alb. A	Glob.	$\frac{A \times 100}{T}$	Alb. A	Glob.	$\frac{A \times 100}{T}$
1	5.45	3.44	2.01	63.1	3.46	1.99	63.5
2	6.30	3.99	2.31	63.4	4.16	2.14	66.1
3	6.33	3.05	3.28	48.2	2.96	3.37	46.8
4	6.75	4.29	2.46	63.5	4.48	2.27	66.4
5	6.46	3.53	2.93	54.7	3.47	2.99	53.7
6	7.05	3.68	3.37	52.2	3.92	4.13	55.6
7	6.92	3.70	3.22	53.5	4.04	2.88	58.5
8	7.14	3.61	3.53	50.6	3.57	3.57	50.0
9	6.36	3.79	2.57	59.6	4.06	2.30	63.8
10	6.78	4.28	2.50	63.3	4.21	2.57	62.1
11	6.45	4.11	2.34	63.8	4.42	2.03	68.5
12	6.70	2.93	3.77	43.7	3.10	3.60	46.3
13	5.61	4.37	1.24	78.0	4.20	1.41	74.8
14	6.52	3.81	2.71	58.4	3.87	2.65	59.4
15	6.63	4.01	2.62	60.5	4.12	2.51	62.2
16	5.90	4.02	1.88	68.2	4.40	1.50	74.6
17	6.47	4.42	2.05	68.3	4.80	1.67	74.2
18	6.33	3.65	2.68	57.6	3.93	2.40	62.2
19	6.32	3.22	3.10	51.0	2.99	3.33	47.3
20	7.29	4.73	2.56	65.0	5.03	2.26	68.9
		76.63			79.19		

$$\frac{79.19 - 76.63}{20} = 0.13\ \% \qquad s = \pm\sqrt{\frac{0.6998}{20 - 1}} = \pm\ 0.192$$

$$t_{0.01} = \frac{0.13}{0.0425} = 3.06 > 2.86 \quad \bar{x} - \bar{y} = 0.13 > 0.0425 \times 3$$

consequently use that method as their starting point in comparisons between the different methods of fractionation.

The separation between albumin and globulin may, however, also be effected in another manner, *inter alia* by precipitation with alcohol at —5° (Cohn [5]) or by precipitation with methanol at 0° (Pillemer and Hutchinson [11]). The two last-named authors found an extremely good correspondence between the albumin/globulin quotient determined by means of electrophoresis and determined by means of precipitation with methanol in normal individuals.

If, on the other hand, the albumin/globulin quotient was determined in the same sera according to Howe's method, considerable differences were found.

The present publication shows a comparison between Pillemer and Hutchinson's methanol method and Henriques and Klausen's method, which is the one most frequently used in this country, as both a possible difference and its magnitude will be of interest. The results will appear from Table 1 and from Figures 1 and 2.

The precipitation of globulin is effected according to Pillemer and Hutchinson's method at 0° in the course of 15 minutes with constant mechanical stirring with cooled reagents. According to Henriques and Klausen's method the globulin is precipitated under standard conditions by semisaturation with ammonium sulphate at constant pH and total protein concentration (Bing[12]). A duplicate analysis of the total protein and of the albumin was made in each serum according to the two methods and, to control the correctness of the nitrogen analyses, an analysis was made of the nitrogen in an aminoacetic acid solution of known concentration. All the patients are over 60 years, except no. 4, a 29-year-old woman.

It will be seen that Henriques and Klausen's method has a tendency to give slightly higher albumin values, on an average 0.13 % more albumin per analysis, than the methanol precipitation. (The deviation on the differences is 0.192 (19 degrees of freedom), the standard deviation on the mean error 0.0018, $t_{0.01} = 3.06 > 2.86, \bar{x} \frac{\varsigma}{\varsigma} \bar{y} = 0.13 > 0.0425 \times 3$). As the material only comprises 20 analyses, too great importance can hardly be attached to the statistical information. If the albumin values arrived at by means of Pillemer and Hutchinson's method can be considered a fairly reliable expression of the albumin values that would have been found by means of electrophoresis, it is still probable that with Henriques and Klausen's method the values will only show a slight discrepancy. This will hardly play any decisive rôle in the practical employment of the ammonium sulphate method of Henriques and Klausen.

SUMMARY

Using different methods of serum protein fractionation small differences always will be found. In this investigation the methanol fractionation of Pillemer and Hutchinson and the ammonium sulfate fractionation of Henriques and Klausen were performed on twenty sera in order to learn how great this difference might be. It was found that the ammonium sulfate method gave a little higher albumin values than the methanol method. As the difference between the albumin per cents of the two albumin series was 0.13 at a mean, and the mean error 0.0425, the difference between the two methods was

significant. The discrepancy is very small, however, and will be of no significance for the practical use of the method of Henriques and Klausen in clinical chemistry.

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