A CONSTANT ALBUMIN FACTOR FOR THE CALCULATION OF THE PERCENTAGE COMPOSITION OF THE SERUM-PROTEIN FRACTION OBTAINED BY ELUTION OF PAPER ELECTROPHORESIS STRIPS

THE AZOCARMINE STAINING OF STRIPS

by

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INTRODUCTION

The determination of the serum-protein fractions in paper electrophoresis can be carried out as follows:

1. After the electrophoresis strip has been made transparent it is run through a densimeter. The extinctions are then plotted on a graph and the resulting curve is measured planimetrically. This technique gives results that correspond with those of the "free" electrophoresis of Tiselius.

2. The electrophoresis strip is colored and then cut in strips, each containing one protein fraction. By means of sodium hydroxide the dye is extracted and the extinction of the extract measured. Since the albumin has a greater affinity for the dye than the globulins, it is necessary to apply a correction factor to the albumin extinction value in order to obtain results that are comparable to those obtained by the first method. This correction factor does not seem to be constant, and varies considerably.

CREMER AND TISELIUS[1] and ESSER *et al.*[2] used the multiplication factor 1.6 for the globulins in the bromophenol blue staining technique. KUNKEL AND TISELIUS[3] in a later paper, modified the correction factor to 1.3. Other investigators made use of factors lying between these two factors. VAN GOOL[4] found for his Azocarmine B staining technique the correction factor 1.5; DE JONG[5] for the Amidoschwarz 10 B staining, the factor 1.46. GRASSMANN AND HANNIG[6] found 1.4 the most suitable correction factor for the γ-globulins. A variation of from 1.1 to 2.9 for the globulin correction factor was given by KOÏW *et al.*[7]. They found far greater variations for the other globulin fractions. In their opinion, these variations are due to imperfections in the staining technique.

For the ratio of bromophenol blue bound by γ-globulin to the dye bound by albumin, JENKS *et al.*[8] found 0.75–1.2, according to the drying temperature of the paper strips. PEZOLD AND PEISER[9] encountered the same difficulties and found the following correction factors: for Azocarmine B: 1.8; for Amidoschwarz 10 B: 1.6; for bromophenol blue: 1.1.

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COMPARISON OF THE "SCANNING" METHOD ACCORDI

	A					E_A					E_T
	Scanning method					*Extinctions found with the elution method*					
Expt. No.	% *Alb*	% α_1	% α_2	% β	% γ	*Alb*	α_1	α_2	β	γ	*Total*
	1	2	3	4	5	6	7	8	9	10	11
1	42.3	9.6	12.3	11.2	24.6	1.351	0.177	0.247	0.215	0.537	2.527
2	35.7	10.0	16.0	12.6	25.7	0.835	0.181	0.313	0.212	0.507	2.048
3	33.6	10.2	12.2	16.0	27.9	1.185	0.237	0.292	0.365	0.722	2.801
4	54.4	4.4	12.4	12.0	16.8	1.735	0.105	0.253	0.233	0.409	2.735
5	50.3	4.7	11.5	13.8	19.6	1.795	0.136	0.257	0.258	0.435	2.881
6	52.8	5.1	11.6	13.0	17.6	1.757	0.100	0.224	0.202	0.364	2.646
7	54.3	6.5	13.3	17.0	8.7	1.877	0.152	0.315	0.391	0.146	2.881
8	57.3	6.2	11.5	16.9	8.0	1.679	0.135	0.225	0.318	0.165	2.522
9	40.8	6.8	10.0	12.2	30.2	1.355	0.157	0.203	0.286	0.747	2.748
10	61.3	4.4	7.7	11.4	15.0	2.617	0.090	0.136	0.211	0.381	3.435
11	54.3	3.81	11.3	13.2	17.4	2.468	0.132	0.258	0.333	0.432	3.623
12	58.8	4.7	9.3	12.2	15.0	2.637	0.109	0.187	0.267	0.332	3.532
13	35.7	7.2	11.9	12.8	32.4	0.852	0.146	0.220	0.251	0.732	2.201
14	60.9	4.4	9.3	11.3	14.1	1.886	0.077	0.135	0.166	0.232	2.496
15	50.8	5.1	5.9	8.8	29.5	1.366	0.079	0.083	0.125	0.357	2.010

An excellent survey of the correction factors in tabular form is given by HINSBERG AND LANG[10] in the latest edition of their well known book.

PROCEDURE

The following is an attempt to find a constant correction factor for albumin by means of a simple modification of the calculation. A constant correction factor would make it possible to compare the results obtained by both methods in different laboratories.

The calculation of the correction factor is generally done in the following way (Fig. 1). Let KL be the extinction of the albumin (E_A), LM of the total globulin fractions (E_G). If the extinction E_A is divided by the correction f_1 then we find the extinction NO which represents the corrected albumin extinction E_{AG}. The globulin extinction remains unchanged, OP = LM. The total extinction NP (after correction) is expressed in percentages (RT = 100%). The percent composition of albumin is RS% and that of the globulins ST%.

Fig. 1 makes it possible to calculate the correction factor. This has to be done before we are able to compare the albumin values of the "elution" method and the values of the scanning method.

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A_E

Uncorrected percentages calculated from extinctions found					*Calculated albumin correction factor*		*Albumin values calculated by the average factor found*		$A - \frac{E_A}{1.64}$		$A - \frac{A_E}{1.22}$	
Alb	α_1	α_2	β	γ	$f_1 = \frac{E_A(100-A)}{A(E_T-E_A)}$	$f_2 = \frac{A_E}{A}$	$\frac{E_A}{1.64}$	$\frac{A_E}{1.22}$	+	−	+	−
12	13	14	15	16	17	18	19	20	21		22	
53.4	7.0	9.8	8.5	21.2	1.58	1.26	41.2	43.8		1.1	1.5	
40.7	8.8	15.3	10.3	24.7	1.24	1.14	29.6	33.4		6.1		2.3
42.3	8.5	10.4	13.0	25.8	1.45	1.26	30.9	34.7		2.7	1.1	
63.4	3.8	9.2	8.5	14.9	1.45	1.16	51.4	52.0		3.0		2.4
62.3	4.7	8.9	9.0	15.1	1.63	1.24	50.3	51.1	--	—	0.8	
66.3	3.8	8.5	7.6	13.7	1.76	1.25	54.7	54.4	1.9		1.6	
65.2	5.3	10.9	13.6	5.1	1.57	1.20	53.3	53.5		1.1		0.9
66.6	5.4	8.9	12.6	6.6	1.48	1.16	54.8	54.6		3.6		2.8
49.4	5.7	7.4	10.4	27.2	1.41	1.21	37.2	40.5		3.6		0.3
76.1	2.6	4.0	6.1	11.1	2.01	1.24	66.2	62.4	4.8		1.0	
68.2	3.7	7.1	9.2	11.9	1.80	1.26	56.6	55.9	2.3		1.6	
74.7	3.1	5.3	7.6	9.4	2.06	1.27	64.3	61.3	5.5		2.5	
38.7	6.6	10.0	11.4	33.3	1.14	1.08	27.8	31.7		7.9		4.0
75.4	3.1	5.4	6.6	9.3	1.99	1.24	65.1	61.8	4.2		0.9	
68.0	3.9	4.1	6.2	17.8	2.05	1.34	56.3	55.8	5.5		5.0	
			Average		1.64	1.22						
			S.d.		0.29	0.06						

Since the triangle NPV and RTV are similar, it follows that:

$$RS : ST = NO : OP,$$

or

$$A : (100 - A) = E_{AG} : E_G.$$

The factor is now

$$f_1 = E_A/E_{AG},$$

therefore, if E_T represents the sum of the extinctions of albumin and globulins then

$$A : (100 - A) = E_A/f_1 : (E_T - E_A)$$

or

$$f_1 = \frac{E_A(100 - A)}{A(E_T - E_A)}$$

The correction can also be carried out in the following way.

Assume that AB and BC (Fig. 2) represent respectively the extinction values of albumin and the globulins, and DE and EF the uncorrected percentages of the protein fractions. The uncorrected percentage albumin is now corrected by using a factor f_2

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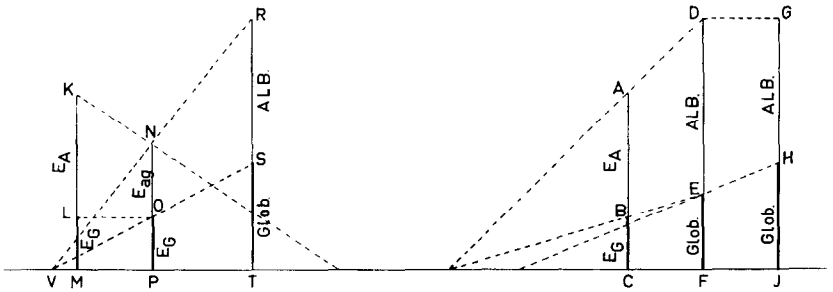

Fig. 1.

Fig. 2.

and is reduced to the percentage GH. The factor by which the globulins have to be corrected is

$$f_{glob} = \frac{100 - Alb/f_2}{100 - Alb}$$

The practical execution of the factor calculation took place as follows:

From 15 paper electrophoresis strips, that had previously been analysed by VAN KAMPEN AND ZONDAG[11] using the scanning technique, the protein fractions were cut out and eluted by means of 0.1 *N* NaOH, 10 ml for the albumin fraction and 5 ml for the remaining globulin fractions. The extinctions of the Azocarmine eluates were determined by use of a Beckman B spectrophotometer, using 520 mμ*.

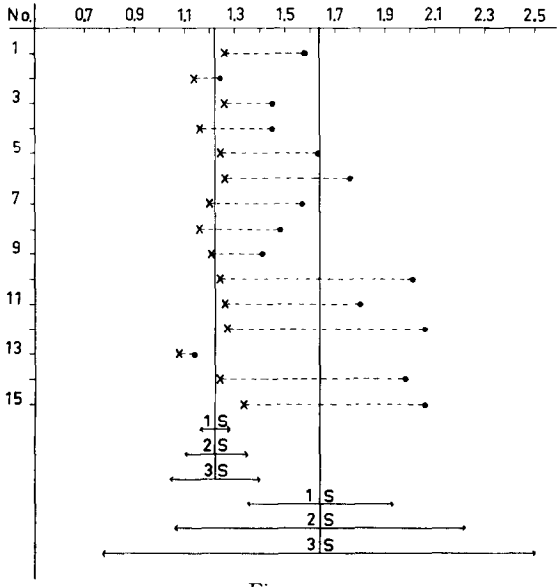

Fig. 3.

* It is evident that in this method of calculation, a correction is made for the Azocarmine dye that remains in the paper after the strip has been washed. This can be done by cutting a strip outside the protein fractions and weighing it. The remaining strip of the protein fractions is weighed and corrected in direct proportion to the weight.

As a result of this, all electrophoresis strips, even those which are not completely washed free from superfluous dye, can be used for the measurement.

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The results are given in Table I. The first five columns are the values found by VAN KAMPEN AND ZONDAG, using the scanning technique. In the next six columns (6–11) are noted the extinction values found after cutting and elution of the fractions. The uncorrected percentages of the extinction values appear in the next five columns (12–16).

The factor f_1 and f_2 (next two columns) were calculated as described above; they give the values by which the albumin extinction, or the uncorrected albumin percentage respectively, must be divided in order to arrive at the results obtained by VAN KAMPEN AND ZONDAG.

On comparing the values for f_1 and f_2 we see a wide distribution when the first method of calculation is used, while the factor f_2 exhibits a small distribution.

The average value for f_1 is 1.64 ± 0.29 and for f_2 1.22 ± 0.06.

The values of both factors and their average are shown graphically in Fig. 3. The accompanying standard deviation gives a clear picture of the value distribution.

Using average values to calculate the albumin amount, we get the results shown in the last columns (19–22). The difference between these values and those of VAN KAMPEN AND ZONDAG are noted in these columns.

CONCLUSION

From the above data we can conclude that the second method of calculation of the percentage of the protein fractions (f_2) is to be preferred to the first method (f_1). The values obtained using the second method correspond closely with results obtained by the scanning method. Also, the factor is not as variable as the albumin-extinction correction method of calculating.

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SUMMARY

A new method of calculating the percentages of serum protein is discussed. This method has a smaller distribution curve than the factor that is generally used for the correction of the extinction of the albumin fraction obtained with the elution method. The magnitude of the new factor is 1.22 ± 0.06, that of the old factor is 1.64 ± 0.29.

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