Electrophoretic Studies on Human Serum Albumin

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Prolonged electrophoresis of human serum has demonstrated the non-homogeneity of the albumin in phosphate buffer at pH 8 and ionic strength 0·1, the migration velocities of the boundaries of the two main components, as apparent from the patterns, differing by about 2% or less (Blix, Tiselius & Svensson, 1941; Hoch & Morris, 1945). Under the conditions of these experiments, i.e. similar mobilities and comparatively low ionic strength, the conventional methods of analysis of the patterns cannot be applied, since here the separation of the boundary peaks and the relative areas in the gradient curve are not even approximate measures of the difference in mobility and of the relative proportions of the two components in the original mixture. A detailed analysis of boundary anomalies of this kind was given by Svensson (1946). The calculation of the true proportions and relative mobilities by means of the theory available at present (Svensson, 1943; Dole, 1945; Svensson, 1946) can yield only approximate information on account of the necessity for assumptions concerning changes in mobility of the ions across the boundary. A simplified method of treating the data has been suggested for use under certain conditions (Hoch, 1948).

In the present paper, the ascending patterns of albumin obtained after prolonged electrophoresis of normal and pathological sera are studied by a procedure similar to that used previously.

THEORETICAL

Definition of symbols

V_1, V_2 = migration velocities of proteins 1 and 2 at infinite dilution in the buffer used as supernatant; V_{a1}, V_{a2} = actual migration velocities of proteins 1 and 2 below the ascending boundaries; C_1' = concentration of protein 1 in the region between the ascending boundaries of 1 and 2; $\mathbf{C}_1'$ = concentration of protein 1 in the region below the ascending boundary of protein 2; C_t' = total concentration of protein in the region below the ascending boundary of protein 2; C_1', $\mathbf{C}_1'$ and C_t' are expressed in g./100 ml.; K = coefficient as defined in the text; U_1 = mobility of a protein at infinitely low protein concentration in the buffer used as supernatant; U = mobility of this protein in the actual solution below the protein boundary; a = constant as defined in text; I = current/unit area of cross section; S = separation of two boundaries/unit time.

Notation for Svensson's (1946) *equation* (21). c_{i1} = ionic concentrations in electrochemical equiv./ml. above colloid boundary, with the signs of the charges; u_{i1} = ionic mobilities in cm.^{2} V.$^{-1}$ sec.$^{-1}$ above colloid boundary, with the signs of the charges; u_2/u_1 = mobility ratio across the colloid boundary; C = concentration of a leading ion in equiv./ml. with the sign of the charge; $\overline{U}$ = mobility of colloid ion above the boundary, defined by equation (22) of Svensson (1946); κ_2/κ_1 = ratio of conductivities of solutions above and below the colloid boundary.

The relation between migration velocity and concentration of serum albumin

The conditions obtaining in the region between the ascending boundary of a leading protein and the following ion depend only on the properties and concentration of the leading-protein ions in this layer, and on the buffer solution used as the supernatant fluid. This was the same in all experiments. Assuming (*a*) that in this region the relative change in migration velocity with protein concentration (expressed in g./100 ml.) is the same for the two main components of serum albumin at pH 8, and (*b*) that the relative rate of change of the migration velocity of one component, when at infinitely low concentration, with increasing concentration of the second component, is equal to the relative rate of change of the migration velocity of the second component with increasing concentration of this component, then

$$V_{a2} = V_2 (1 + K C_t') \quad \text{and} \quad V_{a1} = V_1 (1 + K C_1'), \qquad (1)$$

where the subscripts 1 and 2 refer to the faster and slower albumin components respectively; K was considered to vary little with the protein concentration, when this is low (Hoch, 1948). In the derivation of the expression for K on the basis of Svensson's (1946) theory, the mobility ratio for the protein, U/U_1, was taken to be equal to that of the buffer ions across the boundary.

An expression for K can also be derived, if the ratio U/U_1 for the protein ions is taken to differ from that of the buffer ions by amounts proportional to the protein concentration (expressed in equiv./ml.), i.e.

$$\frac{U_1}{U}(1 + aC) = \frac{u_1}{u_2} = \frac{\overline{U}}{U}. \qquad (2)$$

By combining (2) with Svensson's equation (21) (1946)

$$\left(\frac{\kappa_2}{\kappa_1} - \frac{u_2}{u_1}\right) \Sigma \frac{c_{i1} u_{i1}}{u_{i2} - U} + C = 0,$$

we have

$$\left(\frac{\kappa_2}{\kappa_1} - \frac{u_2}{u_1}\right) \frac{u_1}{u_2} \Sigma \frac{c_{i1} u_{i1}}{u_{i1} - U_1 (1 + aC)} + C = 0.$$

In the following, $\Sigma \dfrac{c_{i1} u_{i1}}{u_{i1} - U_1 (1 + aC)}$ will be written Σ.

$$\left.\begin{aligned} \frac{\kappa_2}{\kappa_1}\frac{u_1}{u_2} &= \frac{\kappa_2}{\kappa_1}\frac{U_1}{U}(1 + aC) = 1 - \frac{C}{\Sigma}, \\ \frac{\kappa_1}{\kappa_2}\frac{U}{U_1} &= (1 + aC)\frac{\Sigma}{\Sigma - C}, \\ \frac{\kappa_1}{\kappa_2}\frac{U}{U_1} - 1 &= \frac{aC\Sigma + C}{\Sigma - C} = \frac{1 + a\Sigma}{\Sigma - C} C. \end{aligned}\right\} \qquad (3)$$

K is defined as previously, or from (1),

$$K=\frac{1}{C_1'}\frac{V_{a1}-V_1}{V_1};$$

inserting

$$V_{a1}=\frac{UI}{\kappa_2} \quad \text{and} \quad V_1=\frac{U_1 I}{\kappa_1},$$

$$K=\frac{1}{C_1'}\left(\frac{\kappa_1}{\kappa_2}\frac{U}{U_1}-1\right),$$

and from (3)

$$K=\frac{(1+a\Sigma)\,Q_s\times 10^{-2}}{\Sigma - Q_s\times 10^{-2}\times C_1'}, \tag{4}$$

where the concentration of protein is expressed in g./100 ml. and Q_s is the net charge/g. protein. Solving (4) for Q_s we have

$$Q_s\times 10^{-2}=\frac{K\Sigma}{1+a\Sigma+KC_1'}.$$

If $a\Sigma$ is negative, the value of Q_s thus found is larger, if positive smaller, than that found from equation (4a) of the previous paper (Hoch, 1948):

$$K=\frac{Q_s\times 10^{-2}}{\Sigma\frac{c_{i1}u_{i1}}{u_{i1}-\mathbf{U}}-Q_s\times 10^{-2}\times C_1'},$$

derived by using the assumption that the mobility ratios of all ions are equal; i.e. the coefficient of C_1' in the denominator in (4) is larger (or smaller), so that variations in C_1' have more (or less) effect on K. The variation in Σ with C_1' is here comparatively small, as the numerical calculation showed. No data are available to estimate a, but for the present purpose it may be sufficient to evaluate the variation in K with C_1' for an arbitrary limiting case, in which this variation is greatest. For example, using the experimental value of $K=0{\cdot}06$ (Hoch, 1948), and taking the value of Q_s as being 25% higher than that of Q_s given in the literature (for references see Hoch, 1948), with the help of equation (4) $a=+0{\cdot}45\times 10^4$ and the change in K is calculated to be +11%, when C_1' increases from 0 to 1 g. albumin/100 ml.

In the present study the approximation is made that K is independent of the albumin concentration and the numerical value of 0·06 is used in all calculations. In consequence, all the values given below for the relative proportions and relative mobilities should be considered as approximations.

Calculation of the relative proportions and the relative difference in mobility of the two albumin components. The concentration of the faster albumin component ahead of the boundary of the slower component adjusts itself to such a value that the increase in concentration across this boundary is balanced by the decrease in migration velocity. The amount of the faster albumin ions/unit cross section which passes the boundary of the slower component must be the same whether calculated in terms of the migration velocity behind the slower boundary or that ahead of it. Using equation (1), this is expressed by

$$\begin{aligned}&(V_1-V_2)\,(1+KC_t')\,\mathbf{C}_1'\\ &\quad=V_1\,(1+KC_1')\,C_1'-V_2\,(1+KC_t')\,C_1'\\ &\quad=(V_1-V_2)\,(1+KC_t')\,C_1'-V_1K\,(C_t'-C_1')\,C_1'.\end{aligned}$$

Hence

$$\mathbf{C}_1'=C_1'-\frac{V_1K\,(C_t'-C_1')\,C_1'}{(V_1-V_2)\,(1+KC_t')}. \tag{5}$$

The separation per unit time, S, of the two boundaries, after they have migrated V_{a1} and V_{a2} respectively, is $\frac{\mathbf{C}_1'}{C_1'}(V_1-V_2)\,(1+KC_t')$ (Hoch, 1948) from which

$$V_1-V_2=S\frac{C_1'}{\mathbf{C}_1'}\frac{1}{(1+KC_t')}. \tag{6}$$

Inserting (6) into (5)

$$\left.\begin{aligned}&\mathbf{C}_1'=C_1'-\frac{V_1K\,(C_t'-C_1')\,\mathbf{C}_1'}{S},\\ \text{and}\quad&\mathbf{C}_1'=\frac{C_1'}{1+\frac{V_1}{S}K\,(C_t'-C_1')}.\end{aligned}\right\} \tag{7}$$

From equation (7) the proportion of the faster component in the total can be obtained. The relative difference in migration velocity at infinitely low albumin concentration (=relative difference in mobility) is best calculated from equation (6) after $C_1'/\mathbf{C}_1'$ has been found from equation (7).

EXPERIMENTAL

In most of the cases 4 ml. of fresh serum were diluted with 8 ml. phosphate buffer of pH 8 and ionic strength 0·1, and dialyzed against 2 l. of this buffer for 1–4 days at 0–4°. The serum protein concentration was determined by micro-Kjeldahl or by the specific gravity method of Linderstrøm-Lang (Hoch & Marrack, 1945). The 11 ml. Tiselius cell and the Philpot-Svensson optical system with a diagonal edge or wire (Svensson, 1939), or both, were used. The potential gradient was about 10 V. cm.$^{-1}$ and the bath temperature 0·1°. After 70–80 min. the serum patterns were photographed, and then a steady flow of buffer into the electrode vessel of the anode was started and kept up so that the albumin boundary did not migrate out of the top compartment of the U-tube. The patterns were evaluated by the method of Svedberg & Pedersen (1940) as used by Longsworth (1946), in which the areas of the peaks of the gradient curve are limited by lines similar to Gauss curves. This procedure involves much uncertainty in the analysis of patterns from the serum albumin, the components of which have not been completely separated (Pl. 10) and no more than a crude estimate of the apparent relative proportions of the albumin components was attempted. Moreover, the distance migrated per hour by the ascending albumin boundary was used in place of V_1 and this distance was not measured in every experiment, but was assumed to be 2·5 cm. at 20 mA. in all experiments. An accurate estimate was not considered necessary in view of the uncertainty involved in the measurements of the distances between the boundary peaks. The albumin concentrations on the ascending side were 0·8–1·4 g./100 ml. in all but four cases.

As an example for the calculation of the true proportions and relative mobilities, case 22 may be described: total albumin concentration, $C_t'=1{\cdot}2$; apparent proportion of faster component = 60%, $C_1'=0{\cdot}72$; distance between boundary peaks = 0·43 cm. $=S\times$ time; total distance migrated = 55 cm. $\cong V_1\times$ time; but from equation (7) the concentration of the faster component in the mixture,

$$\mathbf{C}_1'=\frac{0{\cdot}72}{1+55/0{\cdot}43\times 0{\cdot}06\times 0{\cdot}48}=0{\cdot}15 \quad \text{or} \quad 13\%\ \text{of}\ 1{\cdot}2;$$

$$\frac{C_1'}{\mathbf{C}_1'}=4{\cdot}8;\quad 1+KC_t'=1{\cdot}072;\quad \frac{S}{V_1}=\frac{0{\cdot}43}{55};$$

from equation (6)

$$\frac{V_1 - V_2}{V_1} = \frac{0{\cdot}43 \times 4{\cdot}8}{55 \times 1{\cdot}072} = 0{\cdot}035 \text{ or } 3{\cdot}5\%.$$

Only those patterns which showed two main peaks were analyzed in this way.

RESULTS

The results are summarized in Table 1 and Pl. 10. The proportion of the component represented by the faster main peak ranged from 0 to 42% of the total albumin. The albumin probably includes α_1-globulin, except in case 25 in which this had been removed. The mobilities of the two components differed by 2·5–6·5%. The findings in Table 1 were arranged into four groups in the order of increasing proportions of the faster component, irrespective of the clinical state of the subjects. Owing to the uncertainty in the estimations, little significance should be attached to the order within any one group.

The percentages of the faster component in the albumin from five healthy individuals were between 10 and 42. Cancer sera gave percentage values of the faster component of 0–5 in five, of 6–10 in two, of 11–20 in two, and of 21–30 in one out of ten cases. Five out of seven sera from patients suffering from non-malignant diseases fell into the range found with sera from clinically healthy subjects. The two low values were found in one case of cirrhosis of the liver and in one case of nephritis on two occasions.

Apart from the main components, a small component, migrating up to 12% faster than the albumins, was frequently observed. It was present in seven out of eight sera (including those which were electrolyzed undiluted) from clinically healthy subjects, in six out of eight sera from patients suffering from non-malignant diseases and in five out of ten cases of cancer.

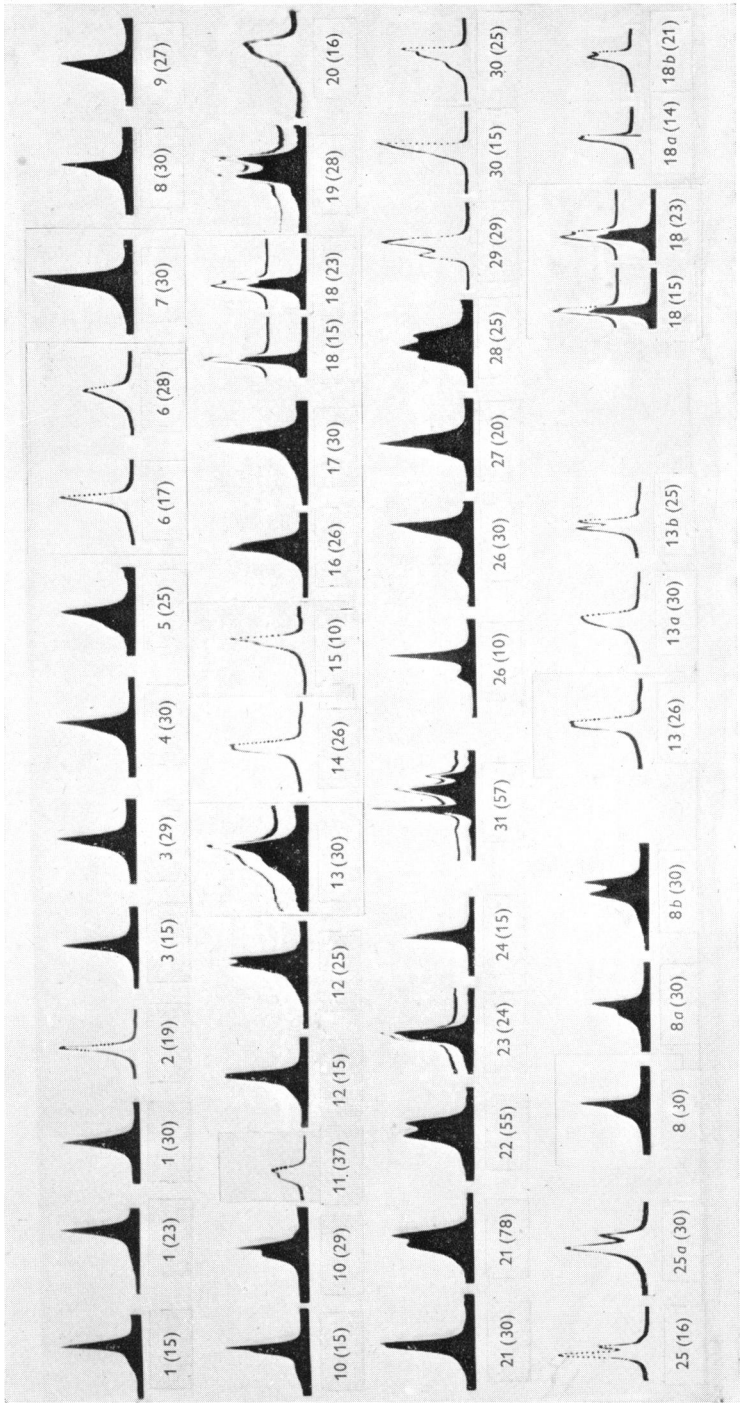
The fastest boundary peak in cases 26–30 (Pl. 10) was comparatively large and, except in case 26, its mobility differed little from that of the first main peak. On account of this large first component, the calculation of the relative proportions of the 'main' components was omitted in these cases, since equations (6) and (7) cannot be applied to more than two components. In four instances, portions of the same samples were tested before and after storage at 0–4° (Pl. 10, bottom row). The keeping of serum produced very little change in cases 13 and 18, while a marked change in the pattern was observed in case 8. When serum was first diluted, dialyzed and then kept at pH 8, there was little change in case 8, but in case 13 the separation of the two components and the proportion of the faster component was increased. No change on keeping at pH 8 was seen in a sample of serum albumin that had been freed from α_1-globulin by electrophoresis in veronal buffer (Longsworth, Curtis & Pembroke, 1945) and subsequently dialyzed against phosphate. The changes that occurred on keeping were in all instances in the direction of a greater separation of the main peaks. The following sera had been kept or dialyzed at 0–4° for more than 6 days before electrophoresis (the number of days is given in parentheses): 2 (9), 6 (8), 7 (12), 11 (18), 19 (8), 20 (21), 29 (11).

The albumin pattern no. 31 of Pl. 10 was obtained in an experiment with undiluted serum. A similar pattern was obtained with serum from the same subject taken 2 months later. In both cases there were three main and two small fast components. In the pattern shown here a slight disturbance can be seen in the region below the boundaries (i.e. to the right in the figure) which is probably due to convection. Undiluted sera from two other subjects gave albumin patterns with three and two main albumin peaks respectively. In addition one small fast and one small slow peak were present in either pattern. Although the small slow peaks behaved like genuine components, it is possible that they were due to convection, but it is unlikely that these convections arose from the heat generated by the current, since under identical conditions in many other experiments no small slow peaks were observed.

DISCUSSION

The assumption that two components are similar with regard to the change of mobility with protein or with salt concentration was used in the derivation of equations (6) and (7). The values for the relative proportions and the relative difference in mobility of two serum albumins calculated by means of these equations differ considerably from those apparent from the patterns. These calculated values are considered more reliable than those obtained by the conventional method (in which the relative areas are taken to represent the relative amounts in the mixture) even if the assumptions about the changes in mobility should prove to be not quite correct, provided the values for the K of the two components are similar. A formally similar treatment has been used by Johnston & Ogston (1946) to explain a boundary anomaly found in the ultracentrifugal sedimentation of mixtures.

In most of the patterns shown in Pl. 10, particularly patterns 6, 13 or 16, the extent of the separation of the two components might appear insufficient for a quantitative analysis. Greater separation could have been obtained by continuing the experiments for a longer time, but for technical reasons this was not done. The uncertainty in the extrapolation of the curves between the peaks can be assessed by considering the limits within which both curves could be obtained reasonably symmetrical. In case 6 of Pl. 10 these limits (ratio of the areas on both sides of the maximum ordinate not greater than about



Albumin patterns after prolonged electrophoresis of sera. The patterns 1–25 are numbered corresponding to Table 1.

Nos. 1–20, 26–30, albumin patterns from diluted pathological sera; 21–24, albumin patterns from diluted normal sera; 26, pleurisy; 27, menstrual oedema; 28, carcinoma of breast; 29, nephritis; 30, carcinoma of anus.

No. 31, albumin pattern from undiluted normal serum; 25, pattern of a normal serum albumin, free from α_1-globulin; 25*a*, dialyzed solution of 25 kept (at pH 8) for 15 days.

No. 8*a*, serum of 8 diluted and dialyzed 1 day (dialyzed solution kept (at pH 8) for 15 days); 8*b*, serum of 8 kept 18 days, then diluted and dialyzed 1 day.

No. 13*a*, serum of 13 kept 20 days, then diluted and dialyzed 2 days; 13*b*, serum of 13 diluted and dialyzed (at pH 8) for 27 days.

No. 18*a*, serum of 18 kept 18 days, then diluted and dialyzed 1 day.

The number in parentheses gives the distance in cm. migrated by the ascending albumin boundary. The direction of migration is from the right to the left.

Table 1. *Analyses of two-component patterns of serum albumin*

No.	Sex	Age	Diagnosis*	Additional fast component	Distance between boundaries A_1 and A_2 (cm.) ($S \times$ time)	Distance migrated (cm.) ($V_1 \times$ time)	Relative difference in mobility (%) †	Concn. of albumin (g./100 ml.) C_i'	Area of faster peak as % of total area of albumin peaks	Proportion of faster component as % of total albumin ‡	Number of cases: Ca.*	Other conditions	Healthy
1	♀	46	Ca. of antrum	+	—	—	—	1·2	—	—			
2	♂	18	Nephritis (13. iii. 46)	−	Faster component distinguishable			0·8	—	—			
3	♂	70	Ca. of soft palate	−	0·25	29	5·5	0·9	10	<2			
4	♀	34	Pregnancy, toxaemia	+	0·35 (±0·15)	30	6	1·0	10	2			
5	♂	63	Ca. of antrum ventriculi, liver metastases	−	0·45	25	6	0·8	10	3	5	4 (including 2 pregnancies)	0
6	♂	35	Cirrhosis of liver	+	0·35 (±0·15)	28	6·5	1·1	15 (?)	3			
7	♀	25	Pregnancy, chronic rheumatic carditis	±	0·25	30	5·5	1·1	25	4			
8	♂	60	Ca. of bladder	±	0·4	30	6·5	1·2	20	4			
9	♀	67	Ca. of rectum	−	0·3 (?)	27	—	0·8	—	Probably <5			
10	♀	49	Ca. of cervix	+	0·35	29	6	1·2	30	6			
11	♂	18	Same as no. 2 (22. v. 46)	±	0·3	37	5	1·5	50	8			
12	♂	70	Ca. of penis	+	0·25	25	5	1·4	50	10	2	2	1
13	♀	56	Cirrhosis of liver	+	0·6	30	6	0·7	30	10			
21	♀	—	Healthy	−	0·43	78	3	1·2	60	10			
14	♀	61	Ca. of breast	−	0·18 (±0·02)	26	3·5	1·3	60	11			
15	♂	22	Mitral stenosis, heart failure	+	0·3	10	6	0·7	25	12			
16	♀	30	Granulosa cell tumour	+	0·2	26	3	1·3	70	17			
17	♂	35	Adenoma of thyroid	+	0·2	30	2·5	1·2	75 (?)	20	2	2	2
22	♀	35	Healthy	+	0·43	55	3·5	1·2	60	13			
23	♂	—	Healthy, donor	+	0·25	24	4·5	1·2	50	11			
18	♀	—	Ca. of breast	Very small	0·2	23	2·5	1·4	75	22			
19	♂	19	Nephritis	+	0·48 0·63	20 28	4·5	0·8	50	25			
20	♂	38	Myelomatosis	+	0·45	16	5	0·8	50	27	1	2	1
24	♂	36	Healthy	Not looked for	0·13	19	2·5	1·2	75	21			
25	♂	62	Healthy, donor; electrophoretically purified albumin, free from α_1-globulin	−	0·3	16	3	1·0	75	42			

* Ca. = carcinoma.

† $\left(\frac{SC_1'}{V_1C_1'(1+KC_i')}\right)$.

‡ $\left(\frac{C_1'}{1+\frac{V_1}{S}K(C_i'-C_1')}\right)$.

2 or 2·5) were about 15 and 40 % of the total area. The value of 25 % was used in the calculation.

In the three cases which were tested, the separation into two albumin components could be reproduced after keeping either the serum or the dialyzed solution (at pH 8), but only in one case were the patterns of practically identical shape. The other patterns showed differences, all of which were in the direction of a greater separation of the peaks. It is therefore improbable that any sera which showed little or none of the faster component had altered in this respect on keeping or on dialysis.

The finding of a lower concentration of the faster main albumin component in a number of sera of patients with cancer warrants further chemical investigation of this component. Extensive work has been carried out with the purpose of establishing alterations in the protein composition of sera from persons suffering from malignant disease (for references see Stern & Willheim, 1943). Several changes have been found, but none of them was specific enough to be of definite diagnostic value. The present investigation might bear some relation to the findings of Kahn (1930). He found that the albumin fraction which is not precipitated by 37·15 % ammonium sulphate is diminished in sera of cancer patients. In these, as well as in the present experiments, sera from cases of pregnancy and liver and kidney diseases behaved similarly to those from cancer patients.

SUMMARY

1. The albumin patterns obtained after prolonged electrophoresis of diluted sera at pH 8 showed in general two main peaks, of which the faster covered an area of from 0 to 75 % of the total area of the albumin peaks. These proportions of the areas, however, do not represent the proportions in the amounts of the albumin components in the original serum.

2. A method for the calculation of the relative proportions and the relative mobilities of two albumin components is presented. Applying this method to the analysis of albumin patterns it was found that the amount of the faster 'main' component ranged from 0 to 42 % of the total albumin. In five sera from clinically healthy individuals the percentage was 10–42; in seven out of the ten cancer sera examined, the percentage was only between 0 and 10. Similarly low values were found in two samples of sera from a patient with nephritis, in one case of cirrhosis of the liver and in two pregnancies.

3. In two cancer and three non-cancer sera three main albumin peaks were found.

4. Electrophoresis of undiluted sera gave albumin patterns with three 'main' components in two out of three cases examined.

5. An additional small fast component was present in seven out of eight sera from clinically healthy subjects, in six out of eight sera from patients suffering from non-malignant diseases and in five out of ten cases of cancer.

6. No feature of the albumin pattern specific only for cancer sera could be established.

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REFERENCES

Blix, G., Tiselius, A. & Svensson, H. (1941). *J. biol. Chem.* **137**, 485.
Dole, V. P. (1945). *J. Amer. chem. Soc.* **67**, 1119.
Hoch, H. (1948). *Biochem. J.* **42**, 181.
Hoch, H. & Marrack, J. R. (1945). *Brit. med. J.* ii, 876.
Hoch, H. & Morris, C. J. O. R. (1945). *Nature, Lond.*, **156**, 234.
Johnston, J. P. & Ogston, A. G. (1946). *Trans. Faraday Soc.* **42**, 789.
Kahn, H. (1930). *Klin. Wschr.* **9**, 262.
Longsworth, L. G. (1946). *Industr. Engng Chem.* (Anal. ed.), **18**, 219.
Longsworth, L. G., Curtis, R. M. & Pembroke, R. H., jun. (1945). *J. clin. Invest.* **24**, 46.
Stern, K. & Willheim, R. (1943). *The Biochemistry of Malignant Tumors.* Brooklyn, N.Y.: Reference Press.
Svedberg, T. & Pedersen, K. O. (1940). *The Ultracentrifuge.* London: Oxford University Press.
Svensson, H. (1939). *Kolloidzschr.* **87**, 181.
Svensson, H. (1943). *Ark. Kemi Min. Geol.* **17**, no. 14.
Svensson, H. (1946). *Ark. Kemi Min. Geol.* **22**, no. 10.