Comparison of Electrophoretic Patterns of Human Sera obtained in Phosphate and in Diethylbarbiturate Buffer

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Diethylbarbiturate buffer of pH about 8.5 and ionic strength (I), 0.1 (Longworth, 1942), is considered to be best suited for the electrophoretic analysis of human sera on account of the good resolution of the α_1 -globulin from the albumin. With this buffer, however, which contains nitrogen, protein analyses cannot be made by the Kjeldahl method, the need for which may arise if fractions are to be studied further. Martin (1949) was the first to report analyses of pathological sera in which α_1 -globulin was estimated in phosphate of pH 8 and ionic strength 0.2; but the electrophoresis may take up to 4 hr. Since it had been observed that the α_1 -globulin separates from the albumin more readily in phosphate buffer at a pH around 8.8, the possibility of replacing diethylbarbiturate by this buffer was investigated.

EXPERIMENTAL

Serum or plasma was diluted to a protein concentration of about 1.4 g./100 ml. with a solution containing $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, 8.71, or 17.42 g./l. ($I=0.15$ or 0.3) or with diethylbarbiturate buffer as used by Longworth (1942) of pH 8.6 and $I=0.1$, and dialysed at $0-4^\circ$ for 2 days. The dilutions of serum equilibrated against the phosphate solution had a pH of 8.85 as measured with a glass electrode. The Tiselius apparatus with a cylindrical lens schlieren optical system was used. The patterns for the analysis were obtained with a diagonal wire (Svensson, 1939) throughout. The photographs of the ascending patterns were taken immediately after the current had been stopped, but those of the descending patterns after the β -anomaly had subsided sufficiently to show the outline of the curve clearly and within the measurable range. An example of the application of Pedersen's (1940) method for dividing the areas is given in Fig. 1. Each enlarged pattern was traced and analysed only once. The errors are therefore such as would be encountered in routine analysis. Care was taken in placing the base line so that the two ends of base line and pattern at which there was no elevation coincided exactly.

The values obtained from the ascending and descending patterns agreed within the error range of the mean values reported in the literature (Table 1*a*) (Dole, 1944; Seibert, Seibert, Atno & Campbell, 1947; and others). In Table 2, therefore, the mean of these values only is given in each case. Although, for theoretical reasons, exact agreement between the ascending and descending percentage values should not be expected, the uncertainty in dividing the areas does not seem to permit the measurement of these deviations, for

experiments at low protein concentration and high buffer ionic strength.

The wattage through the whole apparatus was kept near 6 or slightly under (Svensson, 1946). The potential gradients used were about 9.5, 6.8 and 4.9 V./cm. and the times of electrophoresis were about 1.5, 1.5-2.5 and 2.7 hr. for the diethylbarbiturate and the two phosphate buffers respectively.

Table 1. *Distribution of the differences between the percentage values for all components*

Difference ...	0.0-1.0	1.1-2.0	2.1-3.0	3.1-3.7	Total
Frequency (a)	41	32	9	6	88
(b)*	18	9	6	1	34

(a) Ascending and descending patterns, (b) patterns obtained in phosphate and diethylbarbiturate buffer.

* Albumin α_1 -globulin taken together.

RESULTS

In Fig. 2 the patterns of a normal serum and one of a patient who had cirrhosis of the liver are shown. The analyses of these and four other sera are given in Table 2. There was no evidence that the results for (albumin + α_1 -), α_2 -, β - and γ -globulin, obtained with phosphate of one ionic strength were in better agreement than the other with the results obtained using diethylbarbiturate; or that the two analyses using phosphate differed from each other. The distribution of the differences between the percentage values for (albumin + α_1 -), α_2 -, β - and γ -globulin in phosphate, including one case of phosphate at pH 8 and $I=0.1$, and in diethylbarbiturate, for total protein concentrations of 1.5 g./100 ml. or below are given in Table 1*b*. Theoretically the greatest discrepancies should be expected in the values for α_1 -globulin and albumin, since their relative difference in the mobility is the smallest. The percentages of α_1 -globulin did not differ significantly, but in phosphate at ionic strength 0.15 the percentages of albumin were, with one exception, always higher than in diethylbarbiturate (average difference +2.0). This was probably a consequence of 'boundary anomalies'. That boundary anomalies are noticeable in phosphate at $I=0.15$ may be seen in the experiments with serum 5 (Table 2), which was run at three different concen-

Fig. 1.

Fig. 2.

Fig. 1. Tracings of ascending and descending patterns of serum no. 6 at a protein concentration of 0.75 g./100 ml.
Fig. 2. Electrophoretic patterns of sera. *a* and *b*, pooled normal serum; no. 2 of Table 2; *c-e*, serum from patient with cirrhosis of liver; no. 4 of Table 2; (*a*) and (*c*) in diethylbarbiturate buffer, pH 8.6, *I*, 0.1; (*a*) after 99 min. at 9.5 V./cm.; (*c*) after 92 min. at 9.5 V./cm.; (*b*) and (*e*) in Na₂HPO₄, *I*, 0.15; (*b*) after 135 min. at 7 V./cm.; (*e*) after 105 min. at 6.8 V./cm.; (*d*) in Na₂HPO₄, *I*, 0.3, after 160 min. at 4.9 V./cm.

Table 2. Apparent composition of serum or plasma in diethylbarbiturate and phosphate buffers

(The figures for diethylbarbiturate are the mean percentages of the ascending and descending sides, those for phosphate are the differences in the percentage values from these means.)

Designation: serum proteins (g./100 ml.), sample diluted to protein concentration of g./100 ml.		Globulins					Albumin + α ₁ -globulin	α ₁ + α ₂ + β
Buffer	Albumin	α ₁ -	α ₂ -	β- (+φ)	γ-			
1. Normal plasma, 7.2; 1.4	Diethylbarbiturate Na ₂ HPO ₄ <i>I</i> =0.3 <i>I</i> =0.15	57.3 -3.0 -0.7*	5.2 +1.5 +2.1*	9.5 -0.9 -1.1	19.6 +0.1 0.0	8.4 +2.3 +0.5	62.5 -1.5 +0.6	— — —
2. Normal serum pool, 7.3; 1.4	Diethylbarbiturate Na ₂ HPO ₄ <i>I</i> =0.15	51.7 +2.4	7.9 -2.2	11.1 +0.5	14.3 -0.5	15.0 -0.2	59.6 +0.2	— —
3. Multiple myeloma serum, 7.6; 1.4	Diethylbarbiturate Na ₂ HPO ₄ <i>I</i> =0.3 <i>I</i> =0.15	42.8 -0.2 +2.1	3.7 (+0.5 +0.5	9.7 -2.7 -1.3	10.9 +4.7) -0.3	32.9 -2.3 -1.1	— — +2.6	24.3 +2.5 bad separation of α ₁ -, α ₂ - and β- globulin -1.1
4. Cirrhosis of liver serum, 11.3; 1.4	Diethylbarbiturate Na ₂ HPO ₄ <i>I</i> =0.3 <i>I</i> =0.15	18.8 -0.4 +0.4 +1.7	3.8 +1.1 +1.4 +0.4	5.5 +0.4 +1.1 +0.1	71.9 -1.9 -2.9 -2.2	— +1.5 +1.8 +2.1	22.6 +1.5 +1.8 +2.1	— Tilt 15° Tilt 25° —
5. Pregnancy serum, 7.7	1.5 Diethylbarbiturate 0.75 Na ₂ HPO ₄ 1.5 <i>I</i> =0.15 2.25	45.2 +1.6 +2.8 +4.6	7.9 -1.9 -1.2 -1.9	15.7 -2.8 -3.5 -4.2	16.7 +2.0 +1.7 +1.2	14.5 +1.1 +0.2 -0.3	53.1 -0.3 +1.6 +2.7	— — — —
6. Normal serum, 7.3; 1.2	Diethylbarbiturate Phosphate pH 8, <i>I</i> =0.1	53.1 —	5.4 —	8.9 -0.4	13.7 +0.1	18.9 0.0	58.5 +0.3	— —

* From ascending pattern only.

trations of protein. The rate of increase in the apparent proportion of albumin with protein concentration was 2.0 %/g./100 ml. Perlmann & Kaufman (1945) have measured the same relationship in diethylbarbiturate buffer. From their Fig. 3 the rate of increase worked out at about 2.1. Although comparison is not strictly possible, since the ratio of albumin to α_1 -globulin was larger than in serum 5, it may be said that the variation of the apparent proportions with total protein concentration in phosphate at ionic strength 0.15 is probably not greater than that in diethylbarbiturate at ionic strength 0.1. The larger the ratio albumin/ α_1 -globulin, the smaller should be the variation in the apparent proportion of the albumin.

The analyses of serum 6 confirmed the agreement observed earlier (Hoch, 1948) between the results obtained with diethylbarbiturate of pH 8.6 and $I = 0.1$ and phosphate buffer pH 8 and $I = 0.1$.

DISCUSSION

Diethylbarbiturate and phosphate buffers were compared with each other on the assumption that the fractions separated are the same in both, though this need not be the case. In experiments with sera 2 and 5 (Table 2) it might be suggested that in phosphate part of the α_1 -globulin has been included in the albumin, or conversely with serum 1, that this has happened in diethylbarbiturate. It does not seem easy to test this because of the uncertainty in extrapolating the curve of the albumin, which was assumed to be symmetrical.

With one serum the separation of α_1 -globulin from albumin was better in phosphate at $I = 0.3$ than at 0.15, while with another α_1 , α_2 and β -globulin separated better at $I = 0.15$. The use of phosphate at $I = 0.3$ is not practicable for routine examinations because the time of electrophoresis is unduly long, and because the spreading of the globulin boundaries causes too much overlapping. The discrepancies between diethylbarbiturate and phosphate, at a total protein concentration of 1.4 g./100 ml., were within the experimental error of dividing the areas, except for albumin. The magnitude of these discrepancies is clinically of little significance. One may suggest,

therefore, that for routine clinical work disodium phosphate can replace diethylbarbiturate if the concentration of total protein is between 1.0 and 1.4 g./100 ml. While the resolution of the components in diethylbarbiturate buffer is superior without doubt, phosphate may be preferred when protein estimations have to be made by the Kjeldahl method. The greater cost of diethylbarbiturate may also be taken into consideration.

Phosphate at pH 8.8 has very little buffering capacity. This, however, is of no serious concern to the experiments described here, since the protein itself exerts sufficient buffering action in all sections of the U-tube except where the concentration is small, such as in the region of the γ -globulin of normal sera on the descending side. The effect of a higher pH would be to increase the migration velocity of the γ -globulin in this region, resulting in a higher apparent relative proportion of the γ -globulin. The error introduced by this is opposite to that due to the boundary anomalies. No significant differences in the percentage values for the γ -globulin were found.

In some of the runs in phosphate slight convectional disturbances were noticed ahead of the descending albumin boundary, such as can be seen in Fig. 2*d*. These may have been caused by the separation of a faster component in conjunction with the heat generated by the electric current.

SUMMARY

1. The α_1 -globulin of human serum can be resolved in phosphate at a pH around 8.8.

2. The differences in the apparent relative proportions of the components of serum in this buffer and in diethylbarbiturate buffer of pH 8.6 were less than 3 % of the total area in 33, and less than 2.1 % in 27 out of 34 cases.

3. These differences are not of clinical significance and phosphate at pH 8.8 and $I = 0.15$ may therefore be used in place of diethylbarbiturate buffer at pH 8.6 and $I = 0.1$.

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