LI. THE PROTEIN ERROR IN ESTIMATING p_H WITH NEUTRAL RED AND PHENOL RED.

BY ELIZABETH HERDMAN LEPPER
AND CHARLES JAMES MARTIN.

(From the Department of Experimental Pathology, Lister Institute, London.)

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SØRENSEN [1910, 1912] first systematically studied the protein error of the indicators then available, including neutral red. The solutions of protein contained sodium chloride and a phosphate buffer, and the p_H was estimated electrically and colorimetrically. He found that 2 % gelatin had no colorimetric error, 2 % peptone gave readings about 0.13 p_H too low and 2 % crude egg-white 0.6 to 0.7 too low.

Homer [1917], using the undiluted sera of horses immunised against diphtheria containing 4 to 8 % of proteins, found that the colorimetric p_H with neutral red was in the majority of cases 0.02 to 0.11 too low, but that occasionally it was as much as 0.2 too high. Phenol red under the same conditions gave colorimetric results which were always 0.84 to 0.92 too low, the error being greater with the more alkaline sera. Atkin (private communication), using horse-serum diluted five times with 0.9 % saline, found that the colorimetric error with phenol red was 0.17 at p_H 6.74 and 0.41 at p_H 8.33. Cullen [1922] found that human plasma had to be diluted twenty times before the colorimetric p_H , as estimated with phenol red, agreed with the electrometric determination. A one-in-three dilution of the plasma determined colorimetrically gave 0.4 p_H lower than the electrical measurement.

In the course of some experiments connected with the concentration of horse-sera containing antitoxin, we had occasion to examine the error of the colorimetric determination of the p_H of a strong solution of horse-pseudoglobulin when phenol red and neutral red were used as indicators. The results obtained were so different from those which we had previously observed when using these indicators for the colorimetric determination of the p_H of whole serum that they suggested that pseudoglobulin and albumin behaved differently. We accordingly carried out a parallel series of observations with serum-albumin of the horse which had been six times recrystallised. We found that with both these indicators the error caused in the colorimetric determination of p_H by pseudoglobulin was in the opposite direction to that caused by serum-albumin. Serum-albumin diminished the colour of the indicators; pseudoglobulin increased it. The divergence between colorimetric

and electrometric is, as might be anticipated, in all cases opposite with neutral red to that with phenol red.

These results led us to examine also the effect of egg-white and recrystallised egg-albumin.

OBSERVATIONS ON NEUTRAL RED.

The p_H of a solution of pseudoglobulin, twice fractionated with $(NH_4)_2SO_4$, containing 16.5 % protein, was determined electrically. The solution was not entirely free from euglobulin. It was titrated electrometrically to the isoelectric point. From the $N/10$ acid required the amount of base in the globulin solution was calculated. Some $M/45$ phosphate having the same p_H electrically

Fig. 1. Globulin error of neutral red.

as the globulin solution was prepared. This contained the same concentration of Na as the globulin solution. Standard phosphate tubes at intervals of 0.1 p_H containing neutral red as indicator were used for the colorimetric determinations.

Various dilutions of the globulin solution were made by adding the phosphate solution. The results are shown in Table I and graphically in Fig. 1.

Table I. *The pseudoglobulin error of neutral red.*

Globulin %	p_H colorimetrically	Globulin %	p_H colorimetrically
16.35	5.75	2.7	6.35
10.8	5.77	1.35	6.45
8.17	5.87	0.67	6.55
5.4	6.0	0.33	6.60
4.09	6.15	0.17	6.60
		0.0	6.60

A similar experiment was carried out with recrystallised serum-albumin. In this case the strongest solution contained 3 % albumin, $M/60$ phosphate and 0.85 % sodium chloride. It was diluted with $M/60$ phosphate which contained the same concentration of sodium and had the same p_H as that found electrically for the albumin solution. The results are shown in Table II and Fig. 2. The effect is in the opposite direction to that with globulin. The serum error of neutral red will therefore depend on the proportion of albumin to globulin in any particular sample.

Table II. *The serum-albumin error of neutral red.*

Albumin %	p_H colorimetrically	Albumin %	p_H colorimetrically
3.0	6.93	0.095	6.45
1.5	6.86	0.047	6.35
0.75	6.75	0.23	6.35
0.38	6.65	0.0	6.35
0.19	6.55		

Ledingham [1907] found that in horses immunised against diphtheria the globulin content of the serum increased. The serum of one horse at the beginning of treatment contained 5 % globulin and 2.7 % albumin. This serum would, from our curves, have a colorimetric error of 0.58 less 0.60 so that the p_H would be 0.02 too low. Six weeks later this horse's serum contained 8 % globulin and 2 % albumin and the colorimetric error would now have been 0.51 less 0.74 or 0.23 too low.

We next tested the effect of purified egg-albumin and found to our surprise that a 7 % solution of six times recrystallised egg-albumin showed no colorimetric error. Crude egg-white, however, 20 % by volume, gave a p_H 0.52 too low. Sørensen [1910] with 2 % egg-white found the p_H colorimetrically 0.6 to 0.7 too low.

OBSERVATIONS WITH PHENOL RED.

In these experiments the protein was always made up with $M/60$ phosphate and enough sodium chloride added to bring the molecular concentration of sodium to approximately $M/7$, for we have shown [Lepper and Martin, 1926] that the dissociation of phenol red is sensitive to cations and in order to get identical results electrically and colorimetrically when using $M/15$ phosphate

solutions as standards it is necessary to have the molecular concentration of the sodium in the solution to be tested approximately the same as in the standards. The dilutions were made with $M/60$ phosphate containing sodium chloride adjusted colorimetrically to the p_H required.

Fig. 2. The albumin error of neutral red.

The results with globulin are shown in Table III. Pseudoglobulin causes a slight rise in the apparent p_H which is more easily detected at the alkaline end of the indicator range. 2 % has no effect.

Table III. The pseudoglobulin error of phenol red.

Globulin %	p_H colorimetrically	Globulin %	p_H colorimetrically
11.0	8.05	12	7.42
7.0	8.02	8	7.41
3.5	7.95	4	7.40
1.75	7.93	2	7.38
0.0	7.93	0	7.38

Table IV. *The serum-albumin error of phenol red.*

Albumin %	p_H colorimetrically	Albumin %	p_H colorimetrically
3	7.69	0.125	7.99
2	7.73	0.0625	8.0
1	7.80	0.031	8.02
0.5	7.86	0.0	8.02
0.25	7.94		

Albumin, however, produces a marked decrease in the apparent p_H as shown in Table IV and Fig. 3; this was slightly greater the higher the p_H . With serum the albumin error will, therefore, always predominate. Cullen [1922] with human serum and a bicarbonate buffer found a higher colorimetric error than we have done with serum-albumin of the horse.

Fig. 3. The albumin error of phenol red.

The presence of pure egg-albumin again caused no error but crude egg-white, 20 %, decreased the p_H by 0.1.

DISCUSSION.

The results obtained show that the protein error of an indicator must be determined for each protein. The effect of serum, for instance, is a summation of the opposed effects of the albumin and globulins. These may almost balance each other, as happens in the case of neutral red, or one error may predominate as in the case of phenol red. Proteins belonging to the group of albumins but derived from different sources do not react in the same way with a particular indicator; with neutral red the error is considerable with

horse-albumin but negligible with egg-albumin. The experiments do not throw any light on the way in which proteins interfere with the colorimetric determination of p_H . With the indicators used, there was no precipitation of the dye or alteration in colour such as occurs, for instance, with bromo-o-cresolsulphonephthalein which gives a blue colour in the presence of proteins impossible to match with the standards. The diminution of colour of both types of indicator in the presence of albumins might be due to diminished dissociation or adsorption on the colloid particles, but an increase in the presence of globulins requires the assumption of complexes between the dye and protein possessing a higher dissociation coefficient.

SUMMARY.

The protein errors with neutral red and phenol red for pseudoglobulin and serum-albumin of the horse and for egg-albumin and egg-white in varying concentrations, have been determined.

With both these indicators the colorimetric error due to the presence of this pseudoglobulin is in the opposite direction to that caused by serum-albumin. In either case the errors with neutral red are in the opposite sense to those with phenol red.

Pure egg-albumin up to 7 % does not cause any colorimetric error with either neutral red or phenol red.

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