CCLVIII. MICRO-POTENTIOMETRIC TITRATIONS OF NORMAL HORSE SERUM GLOBULINS.

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THE measurement of titration curves affords a method of investigating protein solutions which is simple to manipulate and in principle easy to interpret; the interpretation in detail in terms of the dissociation constants of individual groups has not yet been carried out with success, although a semi-quantitative explanation has been given in some cases; it appears likely (for reasons to be published elsewhere) that a detailed interpretation is a matter of great complexity.

For the present purpose interpretation was not the first object; it was required rather to be able by this means to compare the behaviour of different globulins. Identity or otherwise of the titration curves would be a strong argument for the identity or difference of two specimens of protein. It was intended to use this as a companion method to that of spectrographic analysis described by Holiday [1936].

The first object was to establish the titration curves of normal horse pseudoglobulin and euglobulin. As well as measuring their titration curves, titrations were performed in the presence of formaldehyde to the amount of about 1.2 *M* in order to estimate the amounts of free primary amino-groups, by the method described by Kekwick & Cannan [1936].

Experimental. The method of titration was designed to give curves of moderate accuracy using as small an amount of solution as possible; with hydrogen electrodes it is necessary to use the rocking type of half-cell described by Clark to avoid frothing of protein solutions; cells were made on this principle [described by Ogston & Peters, 1936] with a maximum capacity of 0.2 ml. which can be used with rather less than 0.1 ml. of solution. The electrodes are small platinum plates lying parallel to the length of the cell; hydrogen flows continuously over the surface of the liquid, escaping through a trap; titrant solutions *N*/10 are added from capillary microburettes after removal of the hydrogen exit trap. Contact is made with the 3.5 *N* calomel electrode through a flexible connexion of rubber pressure tubing; closed unlubricated taps prevent any flow of liquid to or from the titration half-cell.

In order to obtain accurate and reproducible curves the following precautions were found necessary.

(1) The very small volume and titre of the solution make it especially sensitive to acid or basic impurities; trouble was experienced from the former. The hydrogen from a cylinder was washed first with ammoniacal cuprous chloride to remove oxygen, then very efficiently with 20% sulphuric acid to remove ammonia; it was then passed through two U-tubes containing "protosorb" soda-lime to remove acid spray and carbon dioxide; finally it was saturated with

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water vapour at the temperature of the thermostat. As much as possible of the hydrogen tubing should be of glass, since it appears that carbon dioxide can diffuse through rubber at a rate sufficient to cause marked drifting of the potential in alkaline solutions. A further source of acid impurity was the soft-glass of the first cell: a cell was therefore constructed of pyrex glass and used only for alkaline titrations since it evolved acid for two days after exposure to acid solutions.

(2) Large errors (up to 15%) were found in volume delivery of protein solutions into the cell; it was found necessary to deliver these solutions by weight by means of a pipette made from 2 mm. capillary tubing having a long fine jet at one end and a rubber nipple at the other; this was weighed before and after discharge of solution, the error being estimated at not more than 0.2%.

(3) It was advisable to platinize the electrode afresh before every run.

Each branch of the curve was determined from two or three concordant runs with different electrodes and different portions of solution; thus each complete titration curve requires 0.4–0.6 ml. of solution which may be as dilute as 0.5%. Runs with formaldehyde were usually not repeated unless there was reason to suspect their reliability; misbehaviour of these was easy to detect. Discordant runs were very uncommon.

All measurements were made in an air thermostat at $25 \pm 0.2^\circ$; potentials were measured with a Tinsley valve electrometer and slide wire potentiometer.

Materials. Horse serum proteins were used for this work since they were easily available. They were prepared by ammonium sulphate precipitations as described by Holiday [1936], except for NEG $\alpha\gamma$ vii which was made by dialysing whole serum; details in which the preparations differed are given in a column of the table.

The concentrations of the protein solutions were estimated by total nitrogen determination by the micro-Kjeldahl method of Parnas & Wagner [1921], using a factor of 6.45 to convert into weight of protein. These measurements were done in parallel pairs with a control, and agreed to within 0.3%.

A strong stock solution of formaldehyde was prepared and estimated according to the directions of Levy [1933]. Formaldehyde solutions of the proteins were made up by weight in small vessels and used immediately.

Results. The results are given in the form of corrected titration curves in Fig. 1. Fig. 2 shows the actual points obtained in the titration of NPG β vi to show the degree of reproducibility of the method. Fig. 3 shows a specimen formaldehyde curve and the subtraction curve [*vide* Kekwick & Cannan, 1936] giving the amino-titre.

Titration have been performed only between pH 3 and 11; outside these limits the correction for the water blank leads to large uncertainties (*a*) because of the increasing importance of reproducibility of the liquid junction potential at high or low pH; 1 mv. is the best that can be claimed with this small cell; (*b*) because of the increasing importance at high or low pH of the uncertainty about the values of activity coefficients in protein solutions; for lack of better information values of the activity coefficients have been assumed to be those of solutions in the absence of protein. Correction for the base-binding of formaldehyde has been applied using the formula of Levy [1934].

In Table I are given the amino-titres estimated by formaldehyde titration and the tyrosine and tryptophan contents estimated spectrographically [quoted from Holiday, 1936], expressed in milliequivalents per g. protein.

Fig. 1.

Fig. 2.

Fig. 3.

Table I.

Protein	Milliequivalents/g.				Remarks
	% form- aldehyde	Amino	Tyrosine	Trypto- phan	
	Pseudoglobulins.				
NPG v	3.5	0.44	0.28	0.13	Three times precipitated
NPG α vi	3.9	0.47	0.29	0.13	Twice precipitated
NPG vii	3.7	0.41	0.35*	0.15*	Twice precipitated
NPG iii	3.6	0.64	0.31	0.14	Three times precipitated: dried P ₂ O ₅
Euglobulins.					
NEG v	3.7	0.41	0.33	0.14	Dialysis of whole globulin
NEG vi	3.8	0.44	0.37*	0.15*	
NEG β vii	3.7	0.42	0.38*	0.15*	
NEG α γ vii	3.8	0.60	0.39*	0.18*	Dialysis of serum
NEG iv	3.1	0.61	0.41	0.11	Dialysis of whole globulin; dried P ₂ O ₅

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DISCUSSION.

Apart from the two dried proteins NPG iii and NEG iv, which show an abnormally large titre at all values of pH and abnormal amino-contents, differences in the titration curves of both pseudoglobulins and euglobulins are evident; these differences are well outside the experimental error, as is shown by the identical curves given by NPG α vi and β vi. Whilst the pseudoglobulins give the same shape of curve, differences in shape are found between the curves of the euglobulins; these latter have in general a greater titre than the pseudoglobulins. It is of interest that the two differently prepared vii euglobulins have different titration curves. The smallness of variation in the amino-figures is remarkable. They are of the same order as the highest figure obtained for globulin by Dulière [1936].

McFarlane [1935] showed that a protein cannot be dried without becoming polydisperse, and that euglobulins prepared by ammonium sulphate precipitation are more polydisperse than pseudoglobulins so prepared. If polydispersion is accompanied by any breaking up of protein molecules, new groups should become available for titrations; this might account for the large titre of the dried proteins and for the greater titre of euglobulins as compared with pseudoglobulins. But it appears that new groups appearing in euglobulins cannot be primary amino-groups; nor is there any obvious correlation between the titre in alkaline solutions and the tyrosine contents of the proteins.

The differences, at any rate of the pseudoglobulins, suggest that there may be differences in the ampholyte properties of globulins prepared from different individuals; it is interesting to note that preliminary experiments on pseudoglobulins prepared from four different specimens of the same human blood show no significant differences. These results will be published in full in due course.

SUMMARY.

1. A micro-method is described for determining the titration curves of small amounts of protein solution.

2. The titration curves of several specimens of globulins from different horse sera have been determined; further evidence is obtained of the polydispersion in solution of dried proteins and of euglobulins prepared by ammonium sulphate precipitation.

3. The results suggest that there may be differences in the properties of globulins prepared from bloods of different individuals.

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REFERENCES.

- Clark (1920). Hydrogen ions. (Williams and Wilkins Co., Baltimore.)
Dulière (1936). *Biochem. J.* **30**, 770.
Holiday (1936). *Biochem. J.* **30**, 1795.
Kekwick & Cannan (1936). *Biochem. J.* **30**, 235.
Levy (1933). *J. biol. Chem.* **99**, 767.
— (1934). *J. biol. Chem.* **105**, 157.
McFarlane (1935). *Biochem. J.* **29**, 407, 660, 1209.
Ogston & Peters (1936). *Biochem. J.* **30**, 736.
Parnas & Wagner (1921). *Biochem. Z.* **125**, 253.