CLIII. THE ESTIMATION OF PHOSPHORUS IN BLOOD.

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The results obtained in the colorimetric estimation of phosphorus are known to be affected by a number of factors [Fiske and Subbarrow, 1925] and various workers have applied more direct methods to the small quantities of phosphorus present in blood. Using Neumann's method [1903] Iversen [1920] was successful in estimating 0·015 mg. of phosphorus with an error of ± 0·003 mg. Stewart and Archibald in a later paper [1925] estimated 0·05 mg. with an error of ± 0·0015 mg.

It has been found that the introduction of various modifications simplifies the method and allows 0·013 mg. to be estimated with an error of ± 0·0004 mg. The precipitate is washed with the ammonium nitrate solution described below and the washing is carried out in small centrifuge tubes similar to those described by Trevan and Bainbridge [1926]. The micrometer syringe described by Trevan [1925] has been used for the titration.

Titration readings.

After 1 washing 0·591 cc. of $N/10$ HCl
„ 2 washings 0·544 „ „
„ 3 washings 0·541 „ „
„ 4 washings 0·541 „ „
„ 5 washings 0·542 „ „

To remove the ammonia that is formed when ammonium phosphomolybdate is dissolved in soda it is necessary to boil the solution until its bulk is considerably reduced [Iversen, 1920] but more consistent results have been obtained by titrating in the presence of the ammonia.

To determine the optimum p_H for the end-point, the titration curves of these solutions have been plotted with indicators. They have also been plotted by Dr Trevan with a glass electrode and similar results have been obtained. It will be seen that the best end-point is about 7·4 (see Fig. 1). This is an unexpected result because the phosphate solutions themselves buffer well in this region.

The titration in a CO_2-free atmosphere involves a slight modification of the usual technique for the micrometer syringe. A long glass needle drawn out

fine is made to dip below the surface of the liquid which is kept stirred by a constant stream of bubbles of CO_2-free air. It has been found convenient to pump the air with a 2-foot Kekulé pump [Barr, 1924]. The air then passes through a jar of soda lime (which is renewed each morning) to the titration tube. The bubbles cause a certain amount of splashing on the sides of the tube. The error so introduced is less than 1 % of the total titration and the liquid soon runs down again. When the titration is nearly completed the tube may be taken out and tilted to ensure that no unneutralised acid remains on the sides of the tube. Paradoxically, splashing may often be abolished by increasing the rate of bubbling.

Preparation of the blood.

"Inorganic phosphate" is conveniently measured in whole blood. A quantity of blood is measured straight into trichloroacetic acid in accordance with the advice of Fiske and Subbarrow [1925]. It was found that a concentration of 3·3 % trichloroacetic acid in the final solution was sufficient for the precipitation of proteins if it was left for 2 hours before centrifuging. 0·5 cc. of blood is measured into 2·5 cc. of 4 % trichloroacetic acid and 2 cc. of the supernatant fluid are taken for phosphorus determination.

Solutions containing "total acid-soluble phosphate" have been prepared by the method of Fiske and Subbarrow [1925] and solutions for "ether-soluble phosphate" by the method of Randles and Knudson [1922].

ESTIMATION OF PHOSPHORUS.

Solutions required.

(1) Sulphate-molybdate reagent.

50 mg. of ammonium sulphate are dissolved in 500 cc. of nitric acid of specific gravity 1·36 in a litre flask. 150 g. of powdered ammonium molybdate are treated with 400 cc. of boiling water in a porcelain dish and stirred until solution is complete. The solution is rinsed into a flask with a little water, cooled to the temperature of the room, and poured in a thin stream with stirring into the nitric acid solution of ammonium sulphate. The resultant liquid is diluted to 1 litre, allowed to stand for 2 days, filtered and kept in a well stoppered bottle in a dark cool place [Pregl, 1924].

(2) 30 cc. of sulphuric acid (S.G. 1·84) are poured into 1 litre of nitric acid (S.G. 1·19–1·21). The latter acid is obtained by mixing 357 cc. of nitric acid of S.G. 1·4 with 500 cc. of water.

(3) 2 % solution of ammonium nitrate in distilled water containing CO_2 with the addition of one drop of 2 % nitric acid per 100 cc. so that the p_H is about 4.

(4) Normal HCl and NaOH (CO_2-free).

2 cc. of the unknown phosphate solution are measured into a small hard glass test-tube which is drawn out to a point at one end. 0·25 cc. of sulphuric-nitric acid is added with a syringe and the test-tube is heated to boiling point in a water-bath. It is then removed and 2 cc. of the molybdate reagent are

added. This must be measured as accurately as the phosphate solution, since it was found that a 12 % error in this measurement might make a 10 % error in the titration.

It is allowed to stand for a minute or two because it was found not to pack well when centrifuged at once though the titration values were correct within 1 %. The tube is centrifuged at about 3000 revolutions per minute for 15 minutes at a radius of 10 cm. If it has previously been cleaned by boiling in potassium dichromate and sulphuric acid the precipitate packs neatly in the bottom of the tube. The liquid is poured off carefully and the tube inverted and its mouth dried with blotting paper. 5 cc. of the ammonium nitrate solution is then added from a pipette, and two tubes may be centrifuged for 5 minutes without rebalancing. Two washings with this solution are sufficient. The delivery tube of the CO_2-free air is placed in the test-tube and the air is allowed to run for a minute to drive out the CO_2. A standard quantity (about 0·5 cc.) of CO_2-free $N/10$ soda is measured with a micrometer syringe and added through a long tube and the precipitate rapidly dissolves in this. A drop of phenol red (0·02 %) is added, and the soda titrated without undue delay to p_H 7·4. The difference between this reading and a standard reading taken without any precipitate gives the volume of HCl equivalent to the phosphorus. By titrating a known solution of KH_2PO_4, it was found that 253 cc. of $N/10$ HCl corresponded to 31 mg. of P.

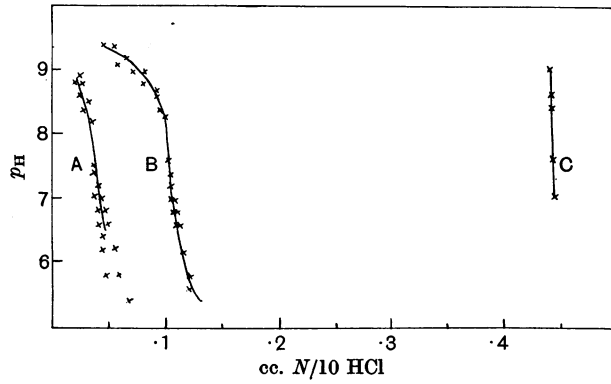

Fig. 1. Curves obtained with indicators by dissolving the phosphomolybdate from 0·0415 mg. of P in the same quantity (about 0·44 cc.) of $N/10$ soda and titrating with $N/10$ HCl. Curve *A*, after boiling off the ammonia. Curve *B*, without boiling off the ammonia. The points on each of these curves were obtained in two separate titrations. Curve *C*, standard titration without any phosphomolybdate. The best end-point is at p_H 7·4.

Results.

cc. of N acid calculated to be equivalent to 31 mg. of P.

At p_H 7·4 Neumann's formula would give	28·5
Pregl's formula would give	*ca.* 29·5
Stewart and Archibald [1925]	22·35
Titrated after boiling off the ammonia	*ca.* 30·4
Titrated without boiling off the ammonia	25·3

0·5 cc. of a batch of normal horse serum gave readings:

0·0124 mg.
0·0126 mg.
0·0131 mg.
0·0132 mg.

Average 0·0128 mg. or 2·56 mg. per 100 cc.
Maximum deviation 0·0004 mg.

2 cc. gave readings:

0·0518 mg.
0·0522 mg.

Average 0·0520 mg. or 2·6 mg. per 100 cc.
Maximum deviation 0·0002 mg.

SUMMARY.

The "inorganic phosphate" in 0·5 cc. of blood may be estimated by dissolving the precipitate of phosphomolybdate in $N/10$ soda and titrating the excess soda with $N/10$ HCl by means of a micrometer syringe.

I wish to thank Dr Trevan for his encouragement and advice.

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