THE ESTIMATION OF PHOSPHORUS IN URINE

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As a preliminary to some investigations on the metabolism of Phosphorus, an examination of some of the methods that have been employed for the estimation of P_2O_5 in urine was carried out. In view of the probable presence of organic phosphorus compounds, Neumann's method, as modified by Plimmer and Bayliss (1), was also employed.

First Neumann's method, precipitation with magnesia mixture and precipitation with magnesium citrate mixture,¹ were compared on a solution of K_2HPO_4 , and were found to give results concordant within one per cent. For Neumann's method 10 c.c. of urine are combusted with 10 c.c. of sulphuric acid, repeated small amounts of nitric acid being added towards the end of the combustion. The rest of the procedure is that described by Plimmer and Bayliss.

For inorganic phosphates, about 4 c.c. of the magnesium citrate mixture are added to 10 c.c. of urine, and enough ammonia to make the mixture smell distinctly of ammonia. The mixture is well stirred and let stand over night, and is then filtered through an ash free paper. The precipitate is dried and ashed; P_2O_5 is calculated from the ash, $Mg_2P_2O_7$.

Since the magnesia mixture method was found unreliable, it is not described in detail.

When the three methods mentioned were applied to urine, the highest values were given by Neumann's method, while magnesia mixture gave higher values than magnesium citrate. Neumann's method or magnesium

1. This reagent is prepared as follows :—Dissolve 40 grams of citric acid in 500 c.c. of water, add to the hot solution 20 grams of light magnesium oxide. Cool; add 400 c.c. of 0.880 ammonia and water to 1500 c.c. Let stand twelve to twenty-four hours, and then filter.

citrate gave practically constant results, but those given by magnesia mixture showed considerable variations. The P_2O_5 in the filtrates after precipitation with magnesia mixture or magnesium citrate was determined by Neumann's method.

TABLE I—COMPARISON OF NEUMANN, MAGNESIUM CITRATE AND MAGNESIA MIXTURE METHODS

Grams P_2O_5 in 100 c.c. Urine						
		Neumann	Magnesium Citrate		Magnesia Mixture	
			Ppt.	Filt.	Ppt.	Filt.
I	...	{ 0.153	{ 0.144	—	{ 0.151	{ 0.005
		{ 0.156	{ 0.143	0.011	{ 0.156	{ 0.004
II	...	{ 0.242	{ 0.223	—	0.238	0.004
		{ 0.238	{ 0.223	0.013	—	—
III	...	{ 0.224	{ 0.204	{ 0.017	{ 0.228	{ 0.006
		{ 0.230	{ 0.207	{ 0.019	{ 0.242	{ 0.012
IV	...	{ 0.160	{ 0.153	0.016	{ 0.160	{ 0.004
		{ 0.166	{ 0.151	—	{ 0.164	{ 0.007

The sum of the magnesium citrate precipitate and filtrate values corresponds fairly with the Neumann value; but the sum of the magnesia mixture precipitate and filtrate values is considerably higher. This discrepancy will later be shown to be due to errors inherent in the magnesia mixture method.

The magnesium citrate results are very constant, even when quantities varying from 2 c.c. to 10 c.c. of the solution are used to 10 c.c. of urine. The precipitate is insoluble in 1:3 ammonia, for the results are not affected by prolonged and frequent washing.

To obtain evidence of complete precipitation of phosphates, Scott's reagent (2), which is capable of detecting 0.005 milligram inorganic P_2O_5 , was applied to the filtrate after separation of the citrate precipitate. No reaction was obtained. It was found, however, that in the presence of citrates, Scott's reagent was far from delicate, so that the desired evidence had to be obtained in a less direct way, which will be described later.

The non-agreement between magnesium citrate and magnesia mixture values required some explanation. Neumann's method, applied to the ash from citrate precipitate, showed the theoretical amount of P_2O_5 to be present; applied to the ash from magnesia mixture precipitate, it showed less P_2O_5 than the theoretical, the amount being variable.

TABLE II—ESTIMATION GRAVIMETRICALLY AND BY NEUMANN'S METHOD OF P_2O_5 PRESENT IN ASH FROM (i) CITRATE, (ii) MAGNESIA MIXTURE PRECIPITATES

Grams P ₂ O ₅ in 100 c.c. Urine					
		Magnesium Citrate		Magnesia Mixture	
		Gravimetric	Neumann	Gravimetric	Neumann
I	...	0.151	0.149	0.160	0.154
II	...	0.144	0.143	0.153	0.148
III	...	0.207	—	0.228	0.217
		0.204	0.204	0.224	0.211
IV	...	0.194	—	0.270	0.211
V	...	0.226	0.222	0.235	0.227

If the magnesia mixture ash is dissolved in acetic acid, the addition of potassium oxalate shows the presence of calcium, which accounts for part of the error.

By adding magnesia mixture to the filtrate after precipitation with magnesium citrate, a further precipitate is obtained. This contains a little calcium, and also some phosphorus. The P_2O_5 was estimated in the ash gravimetrically and by Neumann's method.

TABLE III—TOTAL P_2O_5 , INORGANIC P_2O_5 AND P_2O_5 PRESENT IN THE 'ADDITIONAL' MAGNESIA MIXTURE PRECIPITATE

Grams P ₂ O ₅ in 100 c.c. Urine						
		‘ Additional ’ Ppt. with Mag. Mixture			Residual P ₂ O ₅ by difference	
		Total	Inorganic	Gravimetric		Neumann
I	...	0.224	0.204	0.025	0.016	0.003
		0.230	0.207	0.020	0.012	0.011
II	...	0.220	0.194	0.025	0.018	0.008
		0.218	0.194	—	—	—
III	...	0.244	0.226	0.028	0.010	0.008
		0.241	0.222	0.024	0.011	0.009
IV	...	0.222	0.203	0.014	0.006	0.013

This additional precipitation of P-containing substances suggested that magnesium citrate had failed to precipitate all the inorganic phosphates. Scott's reagent was applied to about 50 milligrams of the additional precipitate, and failed to give any reaction. Thus evidence was afforded of the complete precipitation of inorganic phosphates by magnesium citrate. The inaccuracy of the magnesia mixture method is thus due to two factors, first, that some calcium is precipitated; second, that some of the organic phosphorus is precipitated.

Ammoniacal solutions of barium chloride and calcium chloride have been used to precipitate phosphates. Since these reagents also precipitate sulphates, they are not convenient for quantitative estimations in urine. But in the filtrate from these the organic phosphorus of urine can readily be determined by Neumann's method. Indeed, this direct determination is much more readily performed in the barium chloride filtrate than in that from magnesium citrate, since the combustion of the latter is a matter of considerable difficulty.

TABLE IV—COMPARISON OF ORGANIC P_2O_5 VALUES BY DIFFERENT METHODS
Grams P_2O_5 in 100 c.c. Urine

		Total (Neumann)	Inorganic (Citrate)	Organic		
				Total, minus Inorganic	Citrate Filtrate	BaCl ₂ Filtrate
I	...	0.242	0.222	0.020	0.018	0.016
II	...	0.156	0.143	0.013	0.011	0.011
III	...	0.142	0.127	0.015	0.012	0.013
IV	...	0.180	0.170	0.009	0.009	0.011
V	...	0.196	0.183	0.010	0.015	0.011

The substantial agreement of these methods affords further evidence of the accuracy of the citrate method for inorganic phosphates. The difference between Neumann and magnesium citrate values represents the amount of phosphorus present in organic combination.

The uranium acetate method was found useless for accurate work.

Répiton (3) has shown that uranium acetate solutions must be standardized for the particular phosphate which is being estimated. Thus the method is inaccurate in a solution of mixed phosphates.

TABLE V—COMPARISON OF NEUMANN, MAGNESIUM CITRATE AND URANIUM ACETATE¹ VALUES

Tincture of Cochineal as a warning and Potassium Ferrocyanide as final indicator.
Grams P_2O_5 in 100 c.c. Urine

		Neumann	Mag. Cit.	Uran. Acet.
I	...	{ 0.229 0.233	— 0.205	{ 0.213 0.217
II	...	0.201 —	0.187 —	{ 0.198 0.191
III	...	0.166	0.156	0.166
IV	...	0.204 —	0.194 —	{ 0.208 0.198
V	...	{ 0.196 0.198	0.183 —	{ 0.192 0.186
VI	...	0.154	0.142	0.160

1. This uranium acetate was standardized against the K_2HPO_4 solution originally employed to test the different methods.

The uranium acetate values are usually somewhere between the total and the inorganic values, but may be above the total. Any attempts to determine organic P_2O_5 by methods involving the use of uranium acetate must give incorrect results.

THE DISAPPEARANCE OF ORGANIC PHOSPHORUS

In the course of the investigation it was several times noticed that duplicate samples left some days before being analysed, gave lower values for organic phosphorus than samples analysed immediately; in some cases no organic phosphorus was found. It was thought that this might be due to the destruction of organic phosphorus compounds, perhaps by the enzymes present in the urine.

Samples of urine were kept in aseptic flasks, in an incubator, at $37^\circ C.$, a little toluol being added. Small quantities were removed at intervals of a few days, care being taken to cool the flask so as to obtain the correct volume, and estimations of inorganic P_2O_5 made. In other cases ammonia was added to the urine, which was similarly incubated. Owing to the precipitation of phosphates it was impossible to obtain uniform samples, so the organic P_2O_5 was estimated in the filtrate from 50 c.c. of urine after treatment with magnesium citrate.

The following are a few of the results:—

I	Jan. 12.	250 c.c. Urine + toluol at $37^\circ C.$	Total P_2O_5 = 0.196 grams in 100 c.c. Urine
			Inorganic P_2O_5 = 0.183 " "
	" 15.	Inorganic P_2O_5 = 0.187 grams in 100 c.c. Urine	
	" 22.	" " = 0.194 " "	
	" 29.	" " = 0.194. " "	No phosphorus present in filtrate
II	Feb. 13.	250 c.c. Urine + toluol, at $37^\circ C.$	Total P_2O_5 = 0.247 grams in 100 c.c. Urine
			Inorganic P_2O_5 = 0.230 " "
	Feb. 27.	Organic P_2O_5 = 0.009 grams in 100 c.c. Urine	
	Mar. 25.	" " = 0.007 " "	
	III Feb. 13.	250 c.c. Urine + 10 c.c. Ammonia at $37^\circ C.$	
	"	Total P_2O_5 = 0.282 grams	
	"	Inorganic " = 0.255 "	
	Feb. 20.	Organic P_2O_5 = 0.007 "	
	Feb. 27.	No trace of Organic P_2O_5 present	

These results show that the organic phosphorus compounds are partially or completely broken down when urine is kept at body temperature. This decomposition is accelerated by ammonia. It is, therefore, important not to leave urine standing too long after adding the magnesium citrate mixture, and also to make estimations of organic P_2O_5 in fresh urine.

As it has been suggested, though without adequate proof, that glycerophosphoric acid is the form in which organic phosphorus is present in urine (4), 10 c.c., containing 1.2 grams organic P_2O_5 were neutralized and added to urine containing a known amount of organic P_2O_5 . The urine was left in the incubator for a month. The increase of inorganic phosphates was no greater than could be accounted for by the breaking down of the pre-existing organic phosphorus compounds; the glycerophosphoric acid escaped destruction.

THE RESULTS OF DIALYSIS

It was thought that by dialysis it might be possible to remove the inorganic phosphates and leave organic phosphorus behind. Urine in quantities of 200 c.c. was dialysed for some days, the dialysing fluid being changed three times a day. In some cases continuous dialysation was employed during the last twenty-four hours. Nearly all the phosphorus dialysed out in the first twenty-four hours; at the end of four days no phosphorus could be detected in the residual fluid. By the addition of Folin's ammonium sulphate and uranium acetate reagent to this fluid a nitrogen-containing substance was precipitated, but it contained no phosphorus. Filtrates obtained after precipitating urine with magnesium citrate were similarly dialysed, but no phosphorus-containing substances remained in the tube. The addition of Hedin's tannic acid reagent to urine occasionally produced a slight precipitate, but no phosphorus could be found in this. The organic phosphorus compound of urine is thus not identical with the protein-like material sometimes present.

SUMMARY

1. The estimation of total phosphorus in urine is most conveniently carried out by Neumann's method as modified by Plimmer and Bayliss.
2. Inorganic P_2O_5 is best estimated by precipitation with magnesium citrate mixture, incineration and calculation from the weight of the ash, $Mg_2P_2O_7$. This method is shown to precipitate inorganic phosphates completely.
3. Magnesia mixture gives incorrect and variable results, partly owing to the precipitation of calcium, partly to precipitation of a portion of the organic phosphorus.

4. Organic P_2O_5 can be determined either by subtracting the inorganic from the total P_2O_5 , or directly by applying Neumann's method to the filtrate after precipitation of inorganic phosphates by magnesium citrate or barium chloride.

5. The uranium acetate method is unsuitable for accurate work. Statements as to the existence or non-existence of organic phosphorus in the urine based on uranium acetate estimations are valueless.

7. It is important to make the determinations of organic P_2O_5 in fresh samples of urine, as the organic compound is partially or completely decomposed in the course of a few weeks, or, if ammonia be present, in a few days.

8. The organic phosphorus compound is readily dialysable, and is not precipitated by reagents that precipitate traces of protein.

It is with great pleasure that I acknowledge my indebtedness to Dr. Aders Plimmer for his freely given help and suggestions as to methods in this investigation.

REFERENCES

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