CCIV. THE ORGANIC PHOSPHATES OF URINE

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IN view of the fact that phosphoric esters have been shown to play important roles in nerve, muscle and bone physiology it is surprising that so little accurate information is available concerning the organically bound phosphate of urine. While the absolute amounts have been determined, the nature of the organic phosphates in urine is still unknown.

Many years ago Ronalds [1846] reported the excretion by the kidney of phosphorus compounds in an incompletely oxidized state. Sotnitschewsky [1880] investigated the organic phosphate fraction of urine and attempted to identify it. Although he isolated no pure compound he did obtain evidence for the presence of glycerophosphoric acid. Immediately following this work Zuelzer [1881] and Lepine *et al.* [1882; 1884] made quantitative studies on the urinary excretion of organic phosphate in various diseases. Since then numerous workers have published somewhat conflicting data as to the quantities of organic phosphate occurring in normal and pathological urines. Mathison [1910] made a comparative study of the methods of removing inorganic phosphate and believed the magnesium citrate reagent to be the best precipitant. Later, Forbes & Keith [1914] published a comprehensive review of the literature dealing with the metabolism of phosphorus in animals.

The estimation of urinary organic phosphate by determining the difference between the total and inorganic phosphate, as usually has been done in the past, is obviously an unsuitable procedure because of the comparatively small amount of phosphoric esters present. The experimental error involved in phosphate determinations, whether made gravimetrically or colorimetrically, is of sufficient magnitude seriously to affect the reliability of the calculation of organic phosphate by difference. This point was mentioned by Taylor & Miller [1914] but many investigators continued to use different methods. Youngburg & Pucher [1924] determined the organic phosphate after removal of the inorganic phosphate and report that in man 0.131 mg. of organic phosphate per kg. of body weight every 24 hr. is the average excretion.

The removal of inorganic phosphate by means of uranyl acetate [Pincus, 1859] has several advantages (inorganic phosphate is precipitated rapidly, in the cold and at a mildly acid reaction, conditions favourable for avoiding hydrolysis of esters), but the precipitate is amorphous and adsorbs 60–80 % of the organic phosphates. The classical basic ferric acetate method for removal of inorganic phosphate was considered but dismissed for two reasons: the esters might be hydrolysed during the necessary heating of the mixture, and the bulky, flocculent precipitate would probably adsorb large amounts of the esters. A study of the use of magnesia mixture, already recommended by Youngburg & Pucher [1924] for the removal of inorganic phosphate from urine, led to a

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modification of this standard procedure which proved suitable for the study in hand.

There are two obvious possible sources of serious error in such a method: (1) the phosphoric ester fraction may be hydrolysed by long standing with the strongly alkaline magnesia mixture which is usually added in considerable excess; (2) the precipitate may carry down some of the organic phosphate. The first source of error was minimized by adding to each urine sample (after preliminary analysis for inorganic phosphate) a slight, calculated excess of magnesia mixture (made without ammonium hydroxide), followed by just enough N NH_4OH to bring the $p\text{H}$ to 9.0. Precipitation of inorganic phosphate is complete in 10 min. While these conditions may not give a precipitate of constant composition suitable for gravimetric determinations, they do ensure removal of practically all inorganic phosphate and are therefore quite satisfactory for an investigation of this kind. The fear of hydrolysis was probably exaggerated in the preliminary stages of the investigation since subsequent work confirmed the report [Youngburg & Pucher, 1924] that there is no appreciable hydrolysis of the organic phosphates in urine during precipitation with magnesia mixture.

Loss of phosphoric esters by adsorption on the crystalline magnesium ammonium phosphate precipitate was investigated by adding various organic phosphates to urine and determining the amounts which could be recovered. At the concentrations usually found in normal urines no appreciable loss was observed. Data of a typical experiment are shown in Table I.

Table I. *Loss of phosphoric acid esters during removal of inorganic phosphate by magnesia mixture*

Organic P present in urine mg./100 ml.	mg. organic P added per 100 ml. of urine			Organic P found mg./100 ml.	Recovery %
	Sodium glycero- phosphate	Disodium phenyl- phosphate	Aminoethyl- phosphate		
0.84	0.95	—	—	1.69	95
0.84	—	0.95	—	1.66	93
0.84	—	—	0.95	1.65	92

The above figures are average recoveries found in four similar experiments. When double the above quantities of each ester were added to urine, the recoveries were again over 90%; with ten times the quantities added, the recoveries were 88–92%. Possibly these losses are due to slight hydrolysis rather than to adsorption on the magnesium ammonium phosphate precipitate.

EXPERIMENTAL

Details of the method

(a) *Removal of inorganic phosphate.* The amount of inorganic phosphate in the urine is determined colorimetrically on a suitable aliquot by King's [1932] method. To 10 ml. of the urine (in a 15 ml. volumetric flask) are added 20% excess of magnesia mixture (55 g. hydrated MgCl_2 and 70 g. NH_4Cl per l. prepared without added ammonia) and a volume of N NH_4OH equal to that of the magnesia mixture required. This should produce a $p\text{H}$ between 8.8 and 9.0. The mixture is thoroughly shaken, kept 10 min., made to volume and filtered through a Whatman No. 32 paper.

(b) *Digestion.* To a 3.0 ml. aliquot of the filtrate in a pyrex digestion tube 1.2 ml. of HClO_4 (60%) are added and the mixture is heated to destroy organic matter. The process is accelerated by the addition of a drop or two of 30%

H₂O₂ towards the end of the digestion. (For details see King [1932].) When the mixture becomes water-clear the solution is cooled and rinsed into a 15 ml. volumetric flask.

(c) *Estimation of organic phosphate.* The organic compounds of phosphorus originally present in the urine filtrate will now be converted into inorganic phosphate which is determined, colorimetrically, in the usual way. An aliquot of the filtrate is analysed in the same way for inorganic phosphate in view of the possibility of its incomplete precipitation by the magnesia mixture. If any inorganic phosphate is found in the filtrate, it must be subtracted from the "total phosphate" value found after digestion.

Four urines can be analysed in duplicate by this procedure in about 1½ hr.

RESULTS

In Tables II and III are presented the results obtained on analysing a few normal and pathological urines by the method described above. The "normal" urines were obtained from supposedly healthy, young, male adults working in the laboratory. The 24 hr. volumes are in some cases suspiciously small, but it might be worth noting that these urines were collected during a period of

Table II. *Excretion of organic phosphate in normal human urine per 24 hr.*

Subject no.	Volume ml.	Sp. gr.	Inorganic P mg.	Organic P mg.	Organic P % of total P
1	650	1.028	664	7.2	1.1
2	858	1.022	883	7.0	0.8
3	440	1.032	344	6.5	1.9
4	1100	1.027	1100	10.9	1.0
5	800	1.026	785	6.4	0.8
6	590	1.035	565	8.6	1.5
7	850	1.027	815	10.7	1.3
8	490	1.033	728	7.6	1.1
9	1862	1.027	1660	19.4	1.2
10	800	1.029	935	11.6	1.2
Average values			848	9.6	1.2

Table III. *Excretion of organic phosphate in urines from pathological subjects per 24 hr.*

Subject no. and sex	Volume ml.	Sp. gr.	Inorganic P mg.	Organic P mg.	Organic P % of total P	Pathology
1 M.	1130	1.020	460	4.3	0.7	Diabetes
2 M.	530	1.033	440	9.6	2.2	Diabetes
3 M.	700	1.030	742	8.8	1.2	Diabetes
4 M.	373	1.015	179	5.1	2.8	Diabetes
5 F.	320	1.022	297	8.5	2.9	Myxedema, diabetes
6 F.	1200	1.010	441	12.7	2.9	Diabetes
7 M.	1010	1.014	510	11.1	2.2	Pancreatitis
8 M.	1674	1.018	775	12.2	1.6	Catarrhal jaundice
9 M.	680	1.019	441	6.3	1.4	Diabetes
10 M.	1220	1.020	1220	10.0	0.8	Obstructive jaundice
11 F.	2900	1.011	1342	13.8	0.9	Hyperthyroid
12 M.	692	1.022	554	5.8	1.0	Hyperthyroid
13 M.*	750	1.016	367	4.4	1.2	Malnutrition
14 F.*	320	1.008	192	1.8	0.9	Malnutrition
15 M.	275	1.025	393	13.7	3.4	Cirrhosis of liver
16 M.	515	1.029	515	10.6	2.0	Lymphatic leukemia
17 M.	2020	1.020	1334	21.2	1.5	Lymphatic leukemia

* 22 hr. specimens on children.

extremely hot weather (105° F.) which was experienced in Ontario during July 1936. The data in Table II require very little comment. The average value (9.6 mg. organic phosphate, as P, per 24 hr.) is slightly higher than that found by Youngburg & Pucher, but is considerably lower than some of the values found in the earlier literature.

In the pathological group (Table III) the values are less regular but the variations are not sufficiently marked to justify drawing any conclusions as to the clinical significance (if any) of the figures until a larger number of patients have been examined.

In order to determine to what extent the urinary excretion of organic phosphates depends upon the nature of the food consumed, four normal subjects were given a diet rich in organic phosphorus: each person ate 11 fresh eggs and drank 1 quart of milk and $\frac{1}{3}$ pint of cream, except no. 4, who consumed 8 eggs and 1 pint of cream. The data in Table IV show a definite effect of the diet on the excretion of organic phosphates.

Table IV. *Effect of diet on urinary excretion of organic phosphate*

Subject no.	mg. organic P in urine per 24 hr.		
	Normal mixed diet	Diet rich in organic P	% increase over normal
1	7.2	12.6	75
2	7.0	12.7	81
3	19.4	27.0	39
4	7.45	14.83	99

Since the data presented in Table IV were based on a 24 hr. experiment, it was considered advisable to continue this diet for a longer period. Subject 4 consumed 8 eggs and 1 pint of cream, in addition to his ordinary diet, for 7 days. In Table V are presented the results of this experiment.

Table V. *Effect of continued feeding of a diet rich in organic phosphate*

Day	mg. organic P in urine per 24 hr.	% increase over normal
Control day (normal diet)	7.45	—
1	14.83	99
2	13.28	78
3	15.16	103
4	13.99	88
5	14.40	93
6	15.54	109
7	11.50	54

These results definitely indicate that diet does affect the excretion of organic phosphates. While these results are what one might expect they are not in accord with the findings of earlier workers, who believed that diet had no appreciable influence on the excretion of organic phosphate [Keller, 1900; Mandel & Oertel, 1902; Plimmer *et al.* 1909].

SUMMARY

A method for the estimation of organic phosphate in urine is described. The improvements over the older methods are: (1) it is more rapid; (2) the precipitation of the inorganic phosphate takes place in a less alkaline medium; (3) an

improved method for the colorimetric determination of phosphate is used; (4) an inorganic phosphate determination on the filtrate is made to ensure that complete precipitation has been effected.

Results obtained, using this method, on normal and pathological human urines are presented.

A diet rich in organic phosphate definitely raises the excretion of organic phosphate in the urine.

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