Urinary inorganic phosphorus determinations

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SYNOPSIS The Parekh-Jung method for determination of inorganic phosphorus in serum was applied to its determination in urine. Accuracy is good. The mean percentage recovery of added phosphorus was 100.2%. Forty analyses of a sample gave a mean value of 46.71 mg/100 ml $\pm$ 0.76 standard deviation (between-batch precision). The relative usefulness of the Parekh-Jung method compared with those other methods is discussed.

In an earlier publication Parekh and Jung (1970a, 1970b) introduced the use of p-phenylenediamine dihydrochloride as a reducing agent for the determination of inorganic phosphorus in serum. This new method possessed advantages over the existing methods in terms of simplicity, speed, reproducibility, reagent stability, etc. Recently, the authors applied this method to the determination of inorganic phosphorus in urine.

We now describe our experience with various interfering substances which may be present in urine in considerable concentrations. The results are compared with those obtained by employing the methods of Dryer, Tammes, and Routh (1957), Fiske and Subbarow (1925), and Gomori (1941-42).

Materials and Methods

REAGENTS

The reagents used are the same as those used for the determination of inorganic phosphorus in serum.

Trichloroacetic acid

Ten per cent solution in water.

Sulphuric acid solution

Concentrated sulphuric acid to water in a ratio of 3:7 (v/v).

Molybdic acid solution

Five g ammonium molybdate is dissolved in 100 ml of the sulphuric acid solution.

Molybdic-trichloroacetic acid reagent

One volume of molybdic acid solution is mixed with 2 volumes of 10% trichloroacetic acid solution.

Reducing agent

p-Phenylenediamine dihydrochloride (Eastman Kodak No. 207) 0.5 g is dissolved in 100 ml of a 5% sodium meta-bisulphite solution in water.

Phosphorus standard

For the stock solution, 0.3514 g of anhydrous monopotassium phosphate is dissolved in 10 ml of 10 N sulphuric acid and diluted to 1 litre with water to give 8 mg inorganic phosphorus/100 ml. For the standard solution, an aliquot of the stock solution is diluted with an equal volume of water to give 4 mg inorganic phosphorus/100 ml.

Hyland urine control

A commercial reference preparation is available from Hyland, Division of Travenol Laboratories, Inc, Costa Mesa, California, USA.

PROCEDURE

(1) Dilute an aliquot of a 24-hour urine specimen 1:10 (v/v) with water. (2) Deliver 0.1 ml of diluted urine to a 3 ml centrifuge tube, and add 0.9 ml of molybdic-trichloroacetic acid reagent. (3) Substitute standard solution and water for urine in the standard and blank tubes respectively in step 2 above. (4) Mix well, allow the tubes to stand for five minutes and centrifuge for five minutes. (5) Transfer 0.5 ml of the supernatant to a 12 $\times$ 75 mm cuvette and add 2 ml of the reducing reagent. Mix by inversion and allow to stand for 20 minutes for colour development. (6) Read against blank in a spectrophotometer at 680-700 m μ .

No precipitation occurs with urine in step 4, except in cases of proteinuria.

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CALCULATION

$$\frac{\text{OD Unknown}}{\text{OD Standard}} \times \text{concentration of standard} \times 10 \times \frac{\text{ml of 24-hour specimen}}{1\,000} = \text{g P/24-hr urine.}$$

Results

Known quantities of inorganic phosphorus in the form of monobasic potassium phosphate were added to aliquots of a urine pool. Analyses were performed in triplicate for each set of the recovery study outlined in Table I. The percentage recoveries ranged from 98.8 to 102.0 with a mean percentage recovery of 100.2.

Urine	Added P	Total Found	Recovered	Percentage Recovered
3-62	4	7.60	3.98	99.5
3-62	6	9.68	6.06	101.0
3-62	8	11.66	8.04	100.5
3-62	10	13.50	9.88	98.8
3-62	12	15.85	12.23	102.0
3-62	16	19.50	15.88	99.5

Table I Recovery of phosphorus (mg/100 ml) added to urine

In order to compare the performance of the four different methods of analysis, a Hyland urine control for inorganic phosphorus (given value of 46 mg/100 ml with a range of 42-50 mg/100 ml) was employed. Over a period of two months 40 analyses were carried out by each of the methods using the above control. The results obtained were statistically treated and are shown in Table II.

Eight substances were investigated for possible interfering activity in the measurement of inorganic phosphorus in urine. Analyses were performed in triplicate on each set by the four different methods. In Table III the percentage difference from the true value is shown in each case.

Method	Range (mg/100 ml) P	Mean	SD	Coefficient of Variation
Parekh-Jung	45.6-48.2	46.71	±0.76	1.62
Fiske-Subbarow	45.5-48.6	46.96	±0.86	1.85
Gomori	45.4-48.5	46.92	±0.87	1.85
Dryer	45.7-48.4	46.94	±0.78	1.67

Table II Comparison of the four methods showing between-batch precision

Discussion

In the literature, a separate study of urinary inorganic phosphorus determinations has not usually been described. However, interference by a number of substances in serum or plasma has been reported by Denis and von Meysenbug (1922), Negrin (1964), and Baginski, Foa, and Zak (1967). Such problems encountered in the measurement of inorganic phosphorus in normal urine could be considerably magnified owing to the very variable composition of urine.

Very little or no interference was observed on applying the proposed method to urinary inorganic phosphorus analyses in the presence of 20 mg/100 ml of creatinine, L-histidine, inositol, D-glucose, salicylate, or 50 mg/100 ml of oxalate or 100 mg/100 ml of citrate. Maximum interference was caused by the addition of 20 mg/100 ml of L-ascorbic acid and this amounted to less than 1% of difference from the true value. It is noteworthy that seven of the eight compounds investigated are normally present in urine. The results in Table III are therefore indicative of the accuracy of the method.

The results shown in Tables II and III indicate that the Fiske-Subbarow and Gomori methods are closely comparable. Similarly, it can be concluded that the Dryer method compares favourably with the Parekh-Jung method. However, the Dryer method has certain serious shortcomings. Not only does it lack the simplicity of the Parekh-Jung method, but the insolubility as well as the instability

Substance Added	Concentration of Added Substance (mg/100 ml)	Method			
		Dryer (Percentage difference from true value)	Fiske-Subbarow	Gomori	Parekh-Jung
L-Ascorbic acid	20	+0.85	+1.27	+1.06	+0.85
Creatinine	20	0.0	0.0	0.0	0.0
Histidine	20	0.0	0.0	0.0	0.0
Inositol	20	0.0	+0.4	+0.5	0.0
D-Glucose	20	0.0	+0.2	+0.4	0.0
Salicylate	20	0.0	-0.2	0.0	0.0
Oxalate	50	-0.6	-0.4	-0.4	-0.4
Citrate	100	-0.4	-0.6	-0.6	-0.4

Table III Effects of interfering substances with different methods when added to urine containing 362 mg phosphorus per 100 ml

Urinary inorganic phosphorus determinations

of its reducing agent (Henry, 1964) is such that, for all practical purposes, it would have a limited application. The reagents of the Parekh-Jung method are stable for up to at least six months on the shelf. Also the reducing agent, *p*-phenylenediamine dihydrochloride, is a stable chemical and is freely soluble in water. The final reaction yields a blue colour, free of turbidity, which is very stable and obeys Beer's law.

The Parekh-Jung method is therefore at least as accurate and precise as the other methods. The procedure is simple and the reagents are stable. After the preliminary dilution, the urine sample is treated exactly as the serum sample thus facilitating simultaneous analyses. Urinary proteins, when present, are removed and hence cause no interference in this method. The Parekh-Jung method is thus ideal for routine use.

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