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A METHOD FOR ESTIMATING Tm FOR PHOSPHATE IN MAN

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The methods used by Ollayos & Winkler (1943), Lambert, van Kessel & le Plat (1947) and others for estimating the Tm for phosphate depended on measuring clearances on rapidly falling blood levels following the injection of a large amount of phosphate solution. Pitts & Alexander (1944), on the other hand, gave dogs varying infusions of phosphate to maintain a series of high constant blood levels and during these periods determined phosphate clearances. In all these methods the glomerular filtration rate (GFR) was determined either by inulin or creatinine clearances.

The method described here for determining the Tm for phosphate, expressed throughout as Tm_P, in man needs only a 3 hr phosphate infusion. It was originally devised in collaboration with Drs C. E. Dent and B. Senior who propose, with the present author, to publish later the results of its applications to patients with disorders of renal phosphate excretion. The theoretical basis of the Tm calculations was described by Govaerts (1950), when he discussed the determination of the Tm for glucose. This is shown in Fig. 1. A substance like inulin, which is entirely filtered at the glomerulus and is neither reabsorbed nor excreted by the renal tubule, will give results which lie along a straight line (*OC*) which passes through the origin. The slope of this line is directly related to the glomerular filtration rate. Substances like glucose which have a renal threshold are excreted according to a curve *OB*. At low concentrations in the blood there is minimal excretion. At the point *H*, the minimum threshold, the excretion begins to increase rapidly. After the point *I*, the maximum threshold, has been reached the substance is excreted at a rate determined only by concentration in the plasma and glomerular filtration, as the renal tubules are now reabsorbing at their constant maximum capacity. The mean threshold (*A*) is determined by extrapolating the line *BF* to cut the abscissa. If phosphate is also excreted in this way, then it should be possible to determine its mean threshold as well as the glomerular filtration rate from a single phosphate

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infusion, during which determinations of plasma and urinary phosphate are carried out. The Tm_P can then be calculated as it is the product of the mean threshold and the glomerular filtration rate.

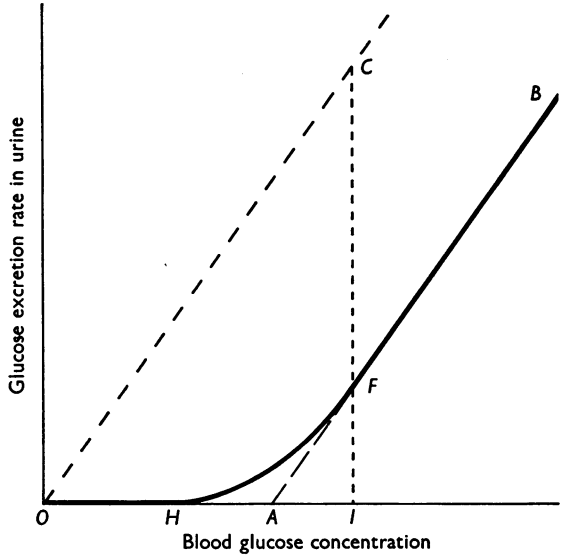

Fig. 1. Glucose excretion in man. Curve *OB* represents the rate of glucose excretion in the urine at varying blood glucose concentrations (modified from Govaerts, 1950). *H* = minimum threshold; *A* = mean threshold; *I* = maximum threshold. *OC* represents glomerular filtration of glucose and excretion of inulin at increasing plasma concentrations.

METHODS

In this work an attempt has been made to avoid sources of error present in other techniques. After a suitable control period the infusion procedure was aimed to achieve a slow linear rise in plasma phosphate concentration in 3 hr. Frequent urine collections were made during the infusion with mid-point blood collections. As it is eventually intended to use the method for studying disorders of renal phosphate excretion, conditions have been standardized to avoid effects due to diurnal variations and so forth. Simultaneous inulin clearances were also determined as a check on the determination of GFR from the phosphate data.

Solutions. Sodium phosphate solution (pH 7·4). This contains 1% of phosphorus and is prepared from $NaH_2PO_4 2H_2O$ (11·49 g), and $Na_2HPO_4 2H_2O$ (44·31 g) made up to 1 l. with pyrogen-free distilled water. 2% (w/v) inulin prepared by diluting the 10% inulin solution supplied by Kerfoot and Co. with 0·45% (w/v) sodium chloride solution.

Calculation of phosphate infusion rate. In the experiments on healthy subjects it was assumed, for the purpose of calculating the infusion rate, that the GFR was 120 ml./min/1·73 m^2 of body surface area, that the phosphate 'space' was 20% of body weight and that the Tm_P was 3·5 mg/min. A linear rise of plasma phosphorus concentration of 10 mg/100 ml. was aimed at in each subject during the course of the 3 hr infusion. It was also assumed that during the course of the infusion the phosphate 'space' was constant, that the GFR was constant, and that the kidney was working above Tm_P during the infusion so that the rate of kidney phosphate loss was proportional to plasma level.

The phosphate infusion consists of two components, one being to counteract the loss of phosphate by the kidney, the other a constant rate component to increase the phosphorus concentration of the phosphate 'space'. The required rate of the first component will increase with time as the plasma concentration rises.

The method of calculation is shown for subject 1, F.C., whose height was 178 cm, weight 84·5 kg and surface area 2·1 m^2. His GFR was estimated to be 145 ml./min. The factors in the graphic calculation of the infusion rate are as follows:

(1) If the plasma phosphorus concentration rises linearly from 0·03 to 0·13 mg/ml. over the 3 hr of the experiment, then the phosphorus filtered at the glomerulus will be 0·03 × 145 mg/min initially, rising to 0·13 × 145 mg/min at the end of the infusion (line *A*, Fig. 2).

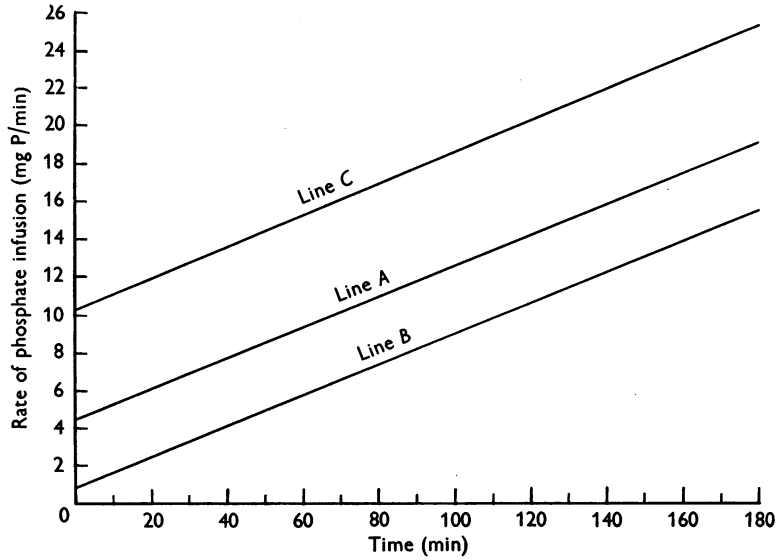

Fig. 2. Graphic calculation of phosphate infusion in subject F.C. Line *A*, change in rate of infusion to raise plasma phosphorus concentration from 3·0 to 13·0 mg/100 ml., when GFR is 145 ml./min, and no other factors are considered. Line *B*, change in rate of infusion when Tm_P of 3·5 mg/min is considered. Line *C*, change in rate of infusion when phosphorus 'space' of 17 l. requires to be filled at increasing plasma phosphorus concentrations in addition to Tm_P and GFR changes.

(2) If the Tm_P is 3·5 mg/min the rate of phosphate excretion by the kidney will be decreased by this amount (line *B*, Fig. 2).

(3) The infusion is also required to raise the phosphate concentration of the phosphate 'space' as well as to compensate for the kidney loss. The total increase in concentration required is 0·1 mg/ml. over 180 min, i.e. 0·556 mg/l. per min. With an estimated phosphate space of 17 l. the required infusion rate is 17 × 0·556, i.e. 9·5 mg/min. Addition of this constant infusion rate to line *B*, Fig. 2, gives line *C*, the total phosphate infusion rate required.

The equation for this line is

$$R = FKt + Fc + SK - \mathrm{Tm},$$

where R = rate of phosphate infusion (mg/min); F = GFR (ml./min); S = phosphate 'space' (ml.); K = rate of rise of plasma phosphorus concentration (mg/ml. min); t = time (min); c = plasma phosphorus concentration at zero time (mg/ml.) and $\mathrm{Tm} = Tm_P$ (mg/min).

It is convenient to convert the rates obtained from the graph (Fig. 2) into amounts of phosphate solution to be given from the burette. This may be done by determining the mean rate, i.e. the rate at the mid-point of each 10 min period and calculating the amount of phosphorus in mg/min to be given in each 10 min period. The total amount of phosphate solution to be given in any period in mg/min is converted to the amount of 1% phosphorus solution to be given in ml. as in Table 1. To obtain the burette reading for each minute these 10 min values are added together and the cumulative total plotted against time in the form of a graph. From this graph the burette reading for each minute can be determined if the scale is large. These minute readings were recorded on a table showing the burette reading at each minute interval. This enabled an accurate flow to be maintained.

TABLE 1. Calculation of burette values from rate graph

The mean rate—i.e. that at the mid-point of each 10 min period—is determined from the rate graph (Fig. 2) and from it the cumulative amount of 1% phosphorus solution to be given in each 10 min period can be determined and the total amount of phosphorus solution required.

Time (min)	Mean rate of P infusion (mg/min)	Amount of P infused by 10 min (mg)	Total amount P given (mg)	Total amount 1% P solution infused (ml.)
0–10	10·7	107	107	10·7
10–20	11·5	115	222	22·2
20–30	12·3	123	345	34·5
		and so on		
170–180	24·6	246	3157	315·7

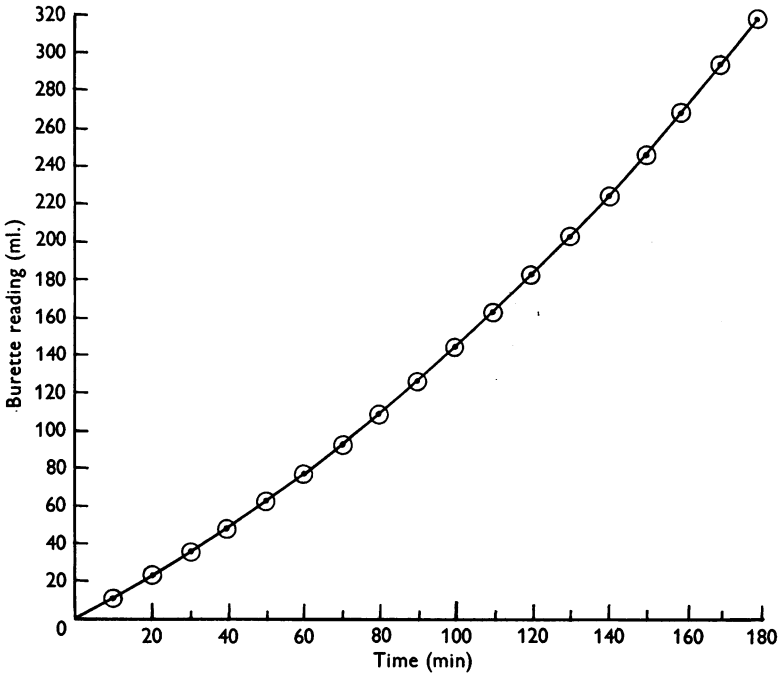

Fig. 3. Graph of burette reading against time. The readings for the delivery of the 1% phosphorus solution can be read off the graph, if the scale is large, for each minute of the infusion. Graph plotted from the values given in Table 1.

The total amount of phosphate solution to be given as calculated from the graphic procedure was 315·7 ml. of 1% phosphorus solution.

Alternatively, the equation of line C may be integrated as follows:

$$\int_0^t R.\,dt = \tfrac{1}{2}FKt^2 + Fct + SKt - \mathrm{Tm}t = P,$$

where P is the total amount of phosphate (in mg) to be infused by time t. The value obtained for the total amount to be infused by substituting the above values in this equation for subject F.C. was 316·4 ml. In practice the result of the graphic procedure was accepted and the other value was used as a check on calculation.

Calculation of inulin infusion rate. A constant plasma inulin concentration of about 40 mg/100 ml. was aimed at. The priming dose given was that calculated to raise the concentration in the extracellular fluid, assumed to be 20% of body weight, to this value. The maintenance dose was, of course, constant, and was calculated from the expected GFR (above).

Procedure. The apparatus used for the infusion consisted of two 100 ml. burettes, two standard transfusion sets, Luer lock syringes, needles and one 3-way tap. All apparatus was sterilized by autoclaving at 120° C for 20 min.

All subjects were healthy male students. They were fasted overnight, had no breakfast and were kept in bed. All infusions were given at the same times and in the morning. An hour before the infusions began the subject drank a priming dose of 13 ml./kg body weight of water and maintained a diuresis by drinking about 150–200 ml. of water every 20 min during the infusion. At urine flow rates of 8–12 ml./min it was found that bladder emptying with suprapubic pressure was good and catheterization was not necessary.

The two 100 ml. burettes, one for the phosphate solution and one for the inulin solution, were clamped to a tall stand and connected by means of the tubing from the transfusion sets to the same 3-way Luer lock tap which carried the intravenous needle. The dropping chambers were left connected to the tubing and rates of flow were controlled with large screw clamps. It was important to keep the fluid levels of the two burettes equal to prevent one flowing into the other.

The hour before the beginning of the inulin infusion was used for determining basal phosphate clearances for comparison with the clearances also determined during the inulin infusion. The priming dose of inulin was given 2 hr before the phosphate infusion. This was at once followed by infusion of a 2% inulin solution at the required constant rate. The first hour of this period served as an equilibrium period for the inulin. During the next hour two 30 min inulin clearances were determined for comparison with the later clearances during the phosphate infusion. The phosphate infusion from the second burette was then started by altering the 3-way tap and was continued at the required steadily increasing rate for the next 3 hr, the constant rate of inulin infusion being also maintained. Infusion rates were checked continually throughout, accuracy of delivery being thus obtained.

The working details are summarized in Table 2, where the data of the infusion on subject 1, F.C., are given. The blood was collected in heparinized tubes and the plasma separated without delay. Plasma and urine were analysed for inorganic phosphate using the method of Fiske & SubbaRow (1925), and for inulin using the method of Hagashi & Peters (1950). Plasma calcium was determined by the method of Clark & Collip (1925). All samples were analysed for phosphate and inulin, those before 7 a.m. serving as blanks for the determination.

RESULTS

Figures for the analyses of individual samples are not shown, except in Table 2, which gives the data obtained on subject 1, F.C. No significant differences were found for the basal phosphate clearances before and during the inulin infusion, although they vary widely, nor for the inulin clearances before and after the phosphate infusion.

TABLE 2. Results of inulin and phosphate infusion in subject F.C.

(Height 178 cm, weight 84·5 kg, surface area 2·1 m².) This table shows the procedure adopted about blood and urine collections, as well as the results of estimations carried out on these samples.

Time		Plasma P (mg/ 100 ml.)	Plasma inulin (mg/ 100 ml.)	Urine vol. (ml.)	Urine P (mg/ 100 ml.)	Urine inulin (mg/ 100 ml.)	Plasma calcium (mg/ 100 ml.)
06.00	Empty bladder, reject urine. Start drinking water	—	—	—	—	—	—
06.30	Blood taken	3·0	—	—	—	—	10·6
07.00	Empty bladder. Priming dose of inulin and inulin infusion started	—	—	430	4·1	—	—
08.00	Empty bladder	—	—	800	1·9	525	—
08.15	Blood taken	2·95	45·0	—	—	—	10·6
08.30	Empty bladder	—	—	420	1·0	425	—
08.45	Blood taken	2·90	44·0	—	—	—	—
09.00	Empty bladder. Phosphate infusion started	—	—	270	1·08	661	—
09.15	Blood taken	4·9	41·25	—	—	—	10·6
09.30	Empty bladder	—	—	415	1·72	362	—
09.45	Blood taken	6·4	41·0	—	—	—	10·4
10.00	Empty bladder	—	—	475	17·2	345	—
10.15	Blood taken	7·6	41·0	—	—	—	10·4
10.30	Empty bladder	—	—	310	45·5	461	—
10.45	Blood taken	9·4	41·0	—	—	—	10·2
11.00	Empty bladder	—	—	230	87·5	630	—
11.15	Blood taken	11·0	41·0	—	—	—	10·3
11.30	Empty bladder	—	—	305	90·0	525	—
11.45	Blood taken	13·4	40·0	—	—	—	10·0
12.00	Empty bladder and discontinue infusion	—	—	440	86·0	318	—

The plasma phosphate rise in the six healthy subjects is shown in Fig. 4. It will be noted that a rise which was approximately linear with time was achieved. It may be significant that subject 3, whose plasma P concentrations were less satisfactory, was later discovered to be still feeling the effects of a reunion dinner the night before. The plasma and urine data are plotted in Fig. 5. It was necessary to displace individual results to the right to avoid overlapping. In all cases once the plasma P concentration was above the maximum threshold it was possible to draw a straight line through the points. The slope of this line should measure the glomerular filtration rate if phosphate excretion mechanisms are similar to those postulated for glucose (Govaerts, 1950). Extrapolation of the line should also cut the abscissa at the mean threshold for phosphorus. The mean threshold and the glomerular filtration rate calculated from both phosphate and inulin methods are shown in Table 3. Tm data are also included.

Plasma calcium determinations were done on all samples in subject 1, and on the first and last samples in all the other subjects. The concentration in the same subject did not vary by more than 0·6 mg/100 ml., which is within the

range of normal diurnal variation (Dent & Philpot, 1954—unpublished observations).

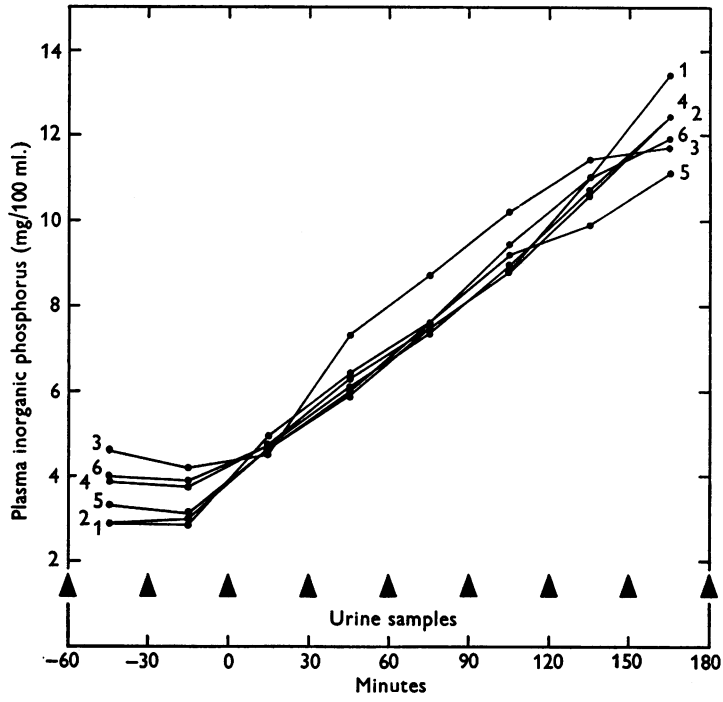

Fig. 4. Plasma phosphorus rise during infusions in normal subjects. Approximately linear rise of plasma phosphorus concentration during the course of the infusion in six normal subjects.

DISCUSSION

It will be seen that it has been possible here to raise the plasma phosphorus concentration in a linear manner by this technique of infusion. Thus it can be assumed that the mid-point plasma sample will represent a true average concentration during the clearance period. When determining clearance periods on rapidly falling concentrations as some previous workers have done, it is not possible to assume that their mid-point plasma represented the average plasma concentration during the period, for the plasma phosphorus then falls in an approximately exponential rather than in a linear manner. Moreover, with a more rapid fall, errors arise on account of the dead space in the kidney and renal tract.

The present work on man supports that of Pitts & Alexander (1944) on dogs, which suggested that there is a Tm for phosphate which can be measured. This is a rather unusual situation with an inorganic ion but may be easier to understand when it is recalled that Pitts & Alexander (1945) have evidence in favour

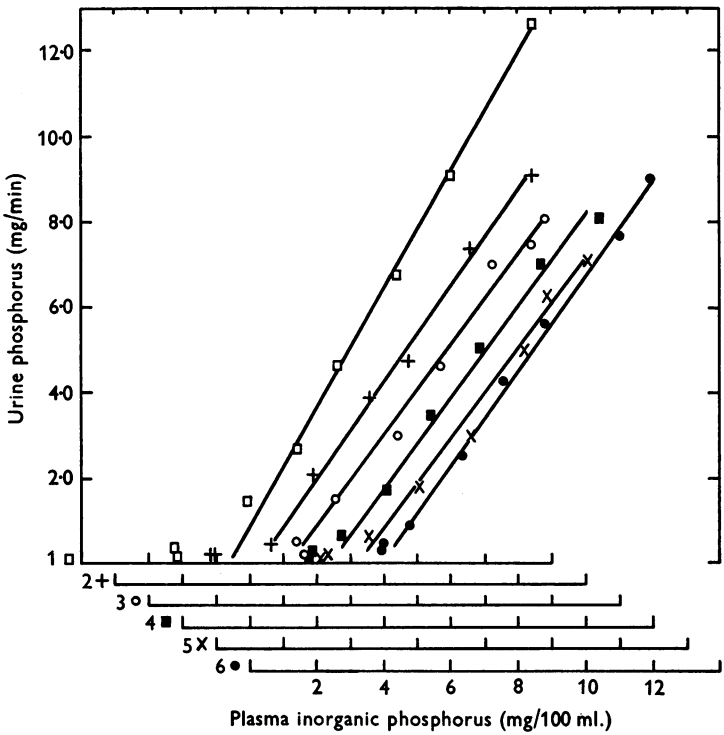

Fig. 5. Infusion results in six normal subjects. Individual results displaced to the right because of overlapping. It is possible to draw a straight line through the points determined by the phosphate clearances and determine the GFR from the slope of this line. The mean phosphate threshold is the point at which this line cuts the abscissa.

TABLE 3. Results of phosphate infusions in six normal subjects

This shows the mean phosphorus threshold, the GFR as determined by inulin and phosphate clearances, and the Tm_P.

Subject	Mean phosphorus threshold (mg/100 ml.)	GFR (ml./min) Inulin	S.D.	PO_4	S.D.	Tm_P (mg/min)	$Tm_P/1{\cdot}73m^2$
1. F.C.	4·2	124	3·0	132	2·9	5·5	4·1
2. B.B.	4·3	123	3·8	117	3·0	5·0	4·6
3. R.A.	4·7	118	4·5	117	3·4	5·5	5·1
4. A.P.	4·3	112	3·2	108	2·6	4·4	4·3
5. K.L.	4·3	111	2·8	106	2·1	4·5	4·0
6. D.L.	4·0	107	3·4	112	2·3	4·5	4·1
Average	4·3	117		114		4·9	4·5

of a common pathway of reabsorption for glucose and phosphate in the renal tubule. The results of Ollayos & Winkler (1943), who hold a contrary view, have been reconsidered. If one ignores their rather improbable filtration rates (from creatinine clearances) and calculates them by the phosphate method used here, the results for the mean threshold for phosphorus in three of the four normal subjects are similar to those reported in this paper, being 4·7, 4·0 and 4·1 mg/100 ml. The data for the fourth subject are insufficiently complete to allow of such calculation.

The basal clearances determined before the phosphate infusion began have shown that the clearance was very variable when plasma P concentrations were near the threshold. This has also been observed by other workers (Dean & McCance, 1945; Richmond, Kravitz, Segar & Waisman, 1951) and is the reason for attempting here to measure Tm_P as being better for eventual comparison with disorders of phosphate excretion. The results at normal plasma P concentrations suggest that there is a variability in the capacity of individual tubules to absorb phosphate and that measurement of basal clearances at these concentrations tells little about the real capacity of the kidney tubule to handle phosphate. Measurements of the minimum and maximum thresholds for phosphate would, however, shed light on the range of the tubule reabsorptive capacity in normal and diseased organs.

The constancy of our Tm_P data at various plasma P concentrations does not suggest any formation of colloidal calcium phosphate complex. Lambert *et al.* (1947) have found that at concentrations of 12–16 mg/100 ml. the Tm for phosphate appeared to increase. They suggested that this was due to the formation of colloidal calcium phosphate which was not filterable at the glomerulus. Such high concentrations were therefore avoided in this work.

It might be expected that the infusion of large amounts of phosphate over a short period of time could act as an assault on the homœostatic mechanism of the body. The results do not suggest that in the 3 hr infusion period there is any marked degree of alteration of the renal threshold or of Tm such as one might expect if, for instance, there were a marked change in the activity of the parathyroid. The insignificant changes in plasma calcium concentration during the infusion also support this.

The glomerular filtration rate as determined by phosphate infusion agreed well with that determined from the inulin clearance. This agreement must be partly due to the fact that the same samples were used for phosphorus and inulin determinations, so that some of the errors of collection would cancel out. Each phosphate Tm and clearance determination represented the mean of six readings, the inulin clearances the mean of eight. It may be significant that the standard deviation was less for the GFR determination by phosphate than by the inulin method, presumably because phosphate is more easily and accurately determined. Thus it is not essential when measuring the Tm for

phosphorus to determine the glomerular filtration rate by other means. This will greatly simplify the procedure. It is probably necessary, although troublesome, to deliver the infusion fluids from large burettes, with the rate of flow continually checked.

SUMMARY

1. A phosphate infusion method is described which enables phosphate clearances to be determined in man over a wide range of plasma P concentration.

2. The kidney excretes phosphate as if a definite Tm existed.

3. From the phosphate determinations both phosphate Tm and GFR could be calculated. The GFR thus obtained agreed well with the simultaneously determined inulin clearance.

I wish to thank Dr C. E. Dent for his advice and encouragement and the six University students who volunteered. Sister Buck, the other nurses of the Metabolic Ward, Mr C. D. Cusworth and Miss J. McAllister also helped. Dr M. D. Milne gave advice on inulin clearance.

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