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HANDLING OF PHOSPHATE BY THE HUMAN KIDNEY AT HIGH PLASMA CONCENTRATIONS

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The diurnal rhythm in phosphate excretion in man (Fiske, 1921) is associated with a parallel rhythm in plasma phosphate concentration (Stanbury & Thomson, 1951; Mills & Stanbury, 1955). The renal response to a varying plasma phosphate concentration is said to depend upon a simple mechanism of maximum tubular reabsorption (Tm) in man (Schiess, Ayer, Lotspeich & Pitts, 1948) and dog (Pitts & Alexander, 1944), but not in the cat (Eggleton & Shuster, 1954) nor the rat (Crawford, Gribetz & Talbot, 1955). It is possible that this response may be different at different times of day, since there is said to be a diurnal rhythm in adrenocortical function (Elmadjian, Bayliss & Thorn, 1955), and cortisone is said to depress the renal absorption of phosphate in the dog (Roberts & Pitts, 1953). The renal response to increased loads of phosphate at different times of day has therefore been studied. The results suggest that a simple Tm mechanism provides an inadequate description of the renal 'handling' of phosphate in man under certain conditions.

METHODS

Details of the five subjects used are given in Table 1. They were not in a basal state, and ate a light meal before experiments, but took no caffeine nor related substances. During the experiment the subject remained seated apart from rising to void urine; no food was taken, but water was drunk freely in order to maintain a large urine flow. An initial blood sample was collected to determine plasma phosphate concentration and inulin blank, the bladder was emptied, and intravenous infusion of phosphate and inulin began. This was given in earlier experiments from a burette. Complete asepsis was difficult, and occasional febrile reactions and rigors suggested that pyrogens may have been accidentally introduced. In later experiments a constant infusion device similar to that described by Clutton-Brock (1954) was used, whereby a syringe delivering a constant volume of about 0·4 ml. is operated by a solenoid activated by a monostable multivibrator which is controlled by a master oscillator of variable frequency. This permits automatic infusion of 0–4 ml./min, whilst faster rates can be obtained by hand operation. No further rigors were experienced after the introduction of this apparatus, though subjects often suffered malaise after infusion of the larger amounts of phosphate.

The usual procedure was as follows: priming phosphate, a buffered mixture of sodium dihydrogen

phosphate and disodium hydrogen phosphate, pH 6·9–7·1 at 37° C, phosphate concentration 480–630 mM, 45–135 ml. given in 10–45 min. Priming inulin (Kerfoot, Vale of Bardsley, tested by the manufacturers and found to be pyrogen-free), 25 ml. 10% inulin given in 2–4 min. Sustaining infusion, a buffered mixture of the two phosphates, pH 7·25–7·30, phosphate concentration 48–63 mM, inulin concentration 26–30 mg/ml., 180–260 ml. given in $1\frac{3}{4}$–$3\frac{1}{4}$ hr. This procedure usually resulted in a fairly constant plasma inulin concentration of around 0·3–0·4 mg/ml., and a plasma phosphate concentration falling slowly from a peak of 3–5 mM to about 2 mM.

In a few experiments, M2, M3, T3 and Y7, a high plasma phosphate concentration was maintained for up to 3 hr by using a stronger sustaining infusion, phosphate 165–200 mM, inulin 25–30 mg/ml. This always led to painful thrombosis, and permanent loss, of a vein. In one experiment, M1, a rising plasma phosphate concentration was obtained by giving a second priming dose of phosphate.

Blood and urine samples were collected at intervals of 10–25 min for up to 3 hr, beginning usually some 10 min after the start of the sustaining infusion. Blood was obtained from an indwelling cannula in a vein in the opposite arm to that receiving the infusion; it was heparinized and centrifuged immediately.

Plasma phosphate concentration was plotted against time, to permit interpolation. It was assumed that urine samples corresponded to the plasma composition $2\frac{1}{2}$ min earlier (Smith, 1951, p. 42). Phosphate filtered was calculated in the conventional way, as the product of plasma phosphate concentration and inulin clearance, and reabsorbed phosphate as the difference between that filtered and excreted.

Analytical methods

Phosphate—Fiske & SubbaRow (1925); inulin—Dick & Davies (1949); creatinine (urine)—Bonsnes & Taussky (1945); creatinine (plasma)—Brod & Sirota (1948); potassium—flame photometer, EEL (Collins & Polkinhorne, 1952); pH—glass electrode at 37° C.

Creatinine outputs and clearances are not here reported. At first, clearances were determined and were found, as claimed by Brod & Sirota (1948), to be close to inulin clearance. We later confirmed the observations of Roscoe (1953), indicating that this agreement is purely fortuitous and dependent upon slow development of colour from the acid plasma filtrate. Henceforward creatinine *output* was compared with inulin clearance, for a rough proportionality and similar directional changes. In one experiment only, these quantities changed in opposite directions: the results of this experiment were therefore regarded as erroneous, and ignored, as insufficient material was available for repeat analyses.

The standard error of the mean of duplicate plasma or urine inulin determinations, calculated from 171 pairs of analyses, was 0·025 mg/100 ml., and the diluted samples analysed contained usually 2–2·5 mg/100 ml. The standard error was thus about 1% of the actual value. Protein precipitation was performed upon duplicate aliquots of sixteen plasma samples, and the variance between analyses upon these separate filtrates of the same plasma was little more than that between separate aliquots of the same filtrate. Inulin clearance is calculated from the ratio of urine to plasma concentration, each subject to a standard error of about 1%, so the clearance has a standard error of about $1\frac{1}{2}$% (1% × $\sqrt{2}$). Only one determination in twenty should therefore be in error by more than 3% (twice the standard error) through inaccuracy in inulin estimation.

RESULTS

Twenty-three experiments were performed upon five healthy male subjects (Table 1). The relationship between phosphate excretion and plasma phosphate concentration will first be considered, without reference to the inulin data.

Fig. 1 shows a form of relationship that was observed in ten experiments, referred to as group I. The regression of phosphate output upon plasma phosphate concentration ($[P]_p$) is linear. Each increment of plasma phosphate

concentration resulted in a proportional increment of phosphate excretion. If plasma phosphate concentration was proportional to phosphate filtered at the glomerulus, that is, if G.F.R. was constant, this represents the conventional Tm hypothesis (Smith, Goldring, Chasis, Ranges & Bradley, 1943). Even the lowest plasma concentration appears to exceed the 'saturation threshold', above which the tubules can reabsorb no more phosphate. On this interpretation G.F.R. is equal to the slope of the regression line, and Tm is the vertical

TABLE 1. Subjects examined

Subject	Age (yr)	Surface area (m^2)	No. of expts.
D	30	1·94	4
L	23	1·73	1
M	40	1·83	6
T	29	1·67	4
Y	23	1·71	8

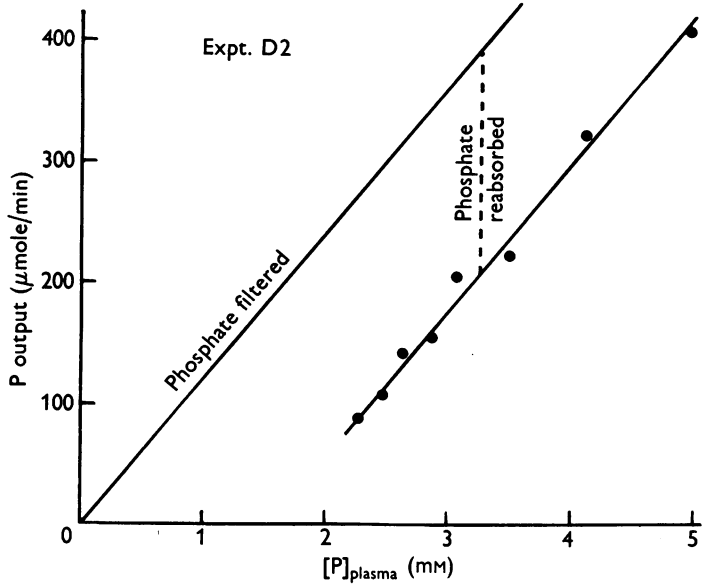

Fig. 1. Expt. D2. Phosphate output at different plasma concentrations, with linear regression inserted. The parallel line through the origin represents phosphate filtered, and the vertical discontinuous line phosphate reabsorbed.

distance between the lines representing phosphate filtered and phosphate excreted, determined geometrically as the negative intercept of the regression line upon the ordinate. The G.F.R. thus calculated in this experiment, from phosphate data alone, was 121 ml./min, and the inulin clearance was constant and almost identical, 118 ml./min. The phosphate Tm calculated from the regression line was 148 μmole/min, and that determined from inulin clearance was 139 μmole/min.

In Table 2 the calculated G.F.R. and Tm are shown for comparison with those determined with inulin in these ten experiments, and it will be seen that the agreement is good, though better for the G.F.R. than for Tm. The mean excess of the determined over the calculated value is 1 ml./min for G.F.R. and 4 μmole/min for phosphate Tm (Tm_P).

Also included are five further experiments compatible with this form of relationship, but in which regression coefficients could not be accurately calculated, owing to the small number of clearance periods, or in one (D4) to suspected errors in bladder emptying. Plasma concentrations and filtered loads are included in the table, to show the levels at which 'saturation threshold' had been reached.

TABLE 2. Group I experiments, including comparison between glomerular filtration rate and phosphate Tm, calculated from relationship between phosphate excretion and plasma phosphate concentration, or determined with the aid of inulin clearance

Expt.	Time of day	P infused (m-mole)	No. of clearance periods	G.F.R. (ml./min) Calc.	G.F.R. (ml./min) Det.	Tm_P (μmole/min) Calc.	Tm_P (μmole/min) Det.	Range of $[P]_p$ (m-mole)	Range of P filtered (μmole/min)
D1	10.10–13.00	?	5	120	117	148	139	1·8–3·1	237–390
D2	14.50–17.50	88	5	121	118	185	174	2·3–5·0	257–593
D3	10.50–12.40	91	8	110	113	153	147	2·2–4·4	290–500
D4*a*	14.40–16.40	?	6	—	102	—	96	4·0–5·2	376–507
D4*b*			3	—	113	—	121	3·7–4·1	406–454
L1	14.00–17.00	52	9	107	104	142	137	1·8–3·0	170–312
M5	13.30–16.50	75	8	106	109	129	137	2·0–3·5	215–365
M6	13.20–15.50	66	7	104	116	102	134	2·0–3·1	235–367
T1	14.15–17.50	76	8	83	82	120	141	2·5–3·4	193–383
T2	10.20–13.00	76	8	98	94	124	110	2·1–4·1	185–357
T3	10.20–11.30	?	4	—	97	—	142	4·2–4·5	382–468
T4	13.40–15.40	65	6	79	85	77	93	2·2–3·5	191–267
Y1	10.00–12.00	33	4	—	143	—	186	1·8–2·1	244–294
Y2*	10.00–12.10	50	4	—	143	—	250	2·3–3·7	351–517
Y3	15.00–17.10	52	6	150	154	197	202	1·8–2·8	285–447
Y8	13.20–15.40	62	4	—	103	—	123	2·0–2·9	211–293

In Expt. D4, $a = 6$ clearance periods before K ingestion and $b = 3$ periods following.

* Omitting 2 points below saturation threshold.

The figures in Table 2 give no support to the possibility of a regular diurnal variation in phosphate Tm. If such exists, it is swamped by the quite large variations from one day to another.

In eight other experiments by contrast, referred to as group II, there was little or no relationship between phosphate excretion and plasma phosphate concentration. Examples are shown in Fig. 2 of an experiment (Y7) in which phosphate excretion rose steadily despite falling plasma phosphate concentration, and another (M3) in which excretion varied widely despite a nearly constant plasma phosphate concentration. In all such experiments the inulin clearance varied considerably, and phosphate excretion was related to filtered load rather than to plasma concentration, as shown for three experiments in Fig. 3. These have been chosen to include one with a progressive rise in

inulin clearance, Y7, one with random variations, M3, and a third with a progressive fall, Y5. It appears that phosphate excretion is related to plasma phosphate concentration only if G.F.R. is fairly constant, when filtered load is directly proportional to plasma concentration.

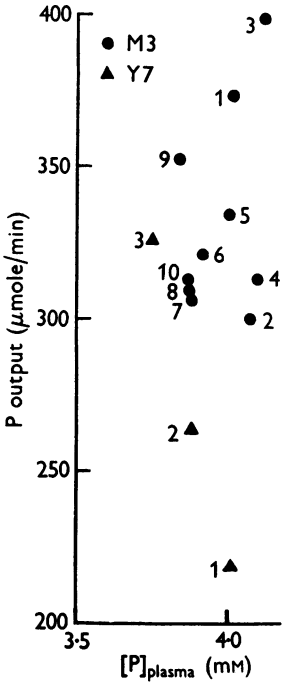

Fig. 2.

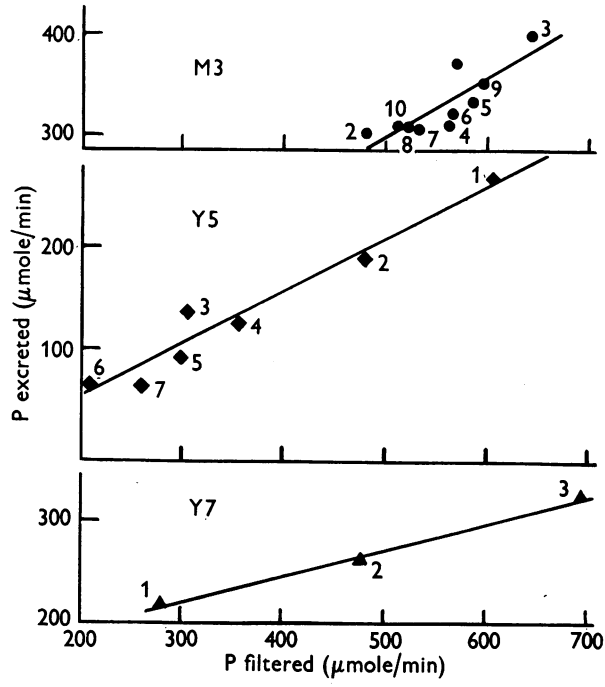

Fig. 3.

Fig. 2. Expts. M3 and Y7, showing phosphate output apparently unrelated to concentration in plasma. Clearance periods are numbered in sequence in each experiment.

Fig. 3. Phosphate output and phosphate filtered in three experiments, with linear regressions inserted. Clearance periods are numbered in sequence.

In two experiments, shown in Fig. 4, this plot of phosphate excretion against phosphate filtered showed that at the lowest filtered loads the tubular maximum had not been attained. The two points in each of these experiments which appear to be below 'saturation threshold' have been omitted from further calculations. With these omissions one of these experiments falls into group I, and the other into group II. For this subject, Y, the 'saturation threshold' can thus be fixed at between 310 and 350 μmole P/min in one experiment, and between 280 and 320 in another; in experiment Y3, Table 2, it appears that this threshold had been attained at a filtered load of 285 μmole/min, whilst other subjects had much lower 'saturation thresholds'. There

appears to be considerable variation between subjects, and even from day to day in one subject.

On the conventional Tm hypothesis the regression coefficient of phosphate excretion upon filtered load should be unity, provided that the filtered load is large enough; when Tm has been attained, any increment in the filtered load,

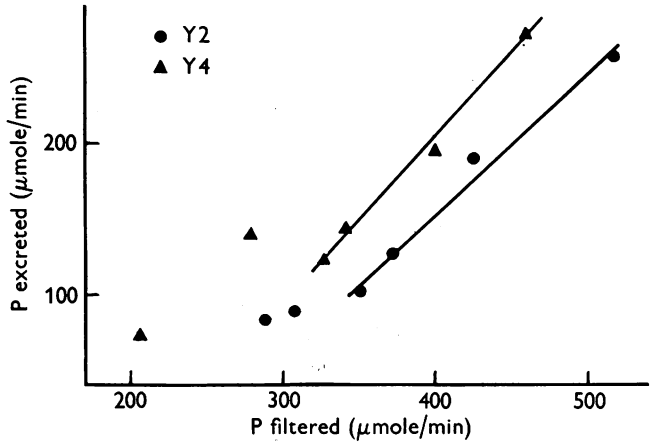

Fig. 4. Phosphate output and phosphate filtered in two experiments in each of which two clearance periods appear to be below saturation threshold. Regression lines are fitted to the other four points only of each experiment.

TABLE 3. Group II experiments, with variable G.F.R.

Expt.	P infused (m-mole)	No. of observations	Range of G.F.R. (ml./min)	Range of $[P]_p$ (m-mole)	Range of filtered loads (μmole/min)	Range of P output (μmole/min)	Range of P reabsorbed (μmole/min)	b	S.E. of b	P*
Y4†	52	4	115–145	2·4–4·0	330–460	120–270	190–210	1·099	0·074	0·4–0·3
Y5	51	7	94–158	2·1–3·9	210–600	60–270	150–330	0·525	0·055	<0·01
Y6	60	5	99–151	2·1–3·4	210–460	80–340	130–220	0·967	0·204	0·9–0·8
Y7	103	3	95–185	3·8–4·0	380–700	220–330	160–370	0·349	0·036	0·05–0·02
M1	100	6	114–146	2·3–3·6	270–410	110–225	160–230	0·705	0·053	<0·01
M2	100	4	112–141	3·9–4·4	440–630	280–370	160–260	0·462	0·062	0·02–0·01
M3	160	10	118–155	3·8–4·2	480–650	300–400	180–260	0·591	0·134	0·02–0·01
M4	83	3	130–165	2·3–3·2	380–420	90–190	230–290	2·196	0·274	0·2–0·1

b = regression coefficient of P output upon P filtered.

* Probability that $b = 1$.

† Omitting two points below 'saturation threshold'.

whether due to increment of G.F.R., plasma phosphate concentration, or both, should lead to an equal increment in phosphate excreted. It is, however, apparent from Fig. 3 that the regression coefficients of the three experiments here represented were well below unity; that is, as filtered load increased, phosphate excretion did not increase equally, so that more phosphate was being reabsorbed. In Table 3 are collected the regression coefficients from all eight experiments, with their standard errors and the probability that each

coefficient differs from unity. It will be seen that only two of the regression coefficients approach unity, five are considerably less, and one is over two. In many of these experiments the lowest filtered loads were far in excess of those at which phosphate Tm was usually attained; whilst in others, such as those shown in Fig. 3, the graph shows no appearance of being compounded of an upper part, with a slope of unity, and a lower part with a more gentle slope, as might occur if some points were below saturation threshold. The possible interpretation of these regression coefficients will be considered in the Discussion.

Comparison of figures for G.F.R. and phosphate reabsorbed in experiments on subject M in Tables 2 and 3 shows that in the group II experiments these quantities were variable, but even so, their lower limit was above that of group I experiments. A large and variable G.F.R. was apparently associated with a large and variable phosphate reabsorption. For subject Y in the only experiment with a low G.F.R., Y8, the phosphate Tm was also low. This experiment was the only one performed on this subject after adequate precautions against pyrogens had been introduced.

TABLE 4. Effect on G.F.R. and phosphate Tm of oral administration of 30 m-equiv $KHCO_3$, after six clearance periods

Expt.	... ...	M3	D4
K deficit (m-equiv)	During first 6 clearance periods	19	3
Mean G.F.R. (ml./min)	First 6 periods	140	102
	Last 3 or 4 periods	141	113
Mean Tm_P (μequiv/min)	First 6 periods	229	96
	Last 3 or 4 periods	220	121

No regularly progressive change in phosphate Tm was observed during an experiment. A variable potassium loss always occurred, however, as sodium phosphate was infused and a part of the excreted phosphate was 'covered' by potassium. In two long experiments 30 m-equiv $KHCO_3$ was taken by mouth after the first six clearance periods, and the effect observed over a further three or four periods. Adequate absorption was indicated by a progressive rise in urinary pH and potassium excretion. Table 4 shows that in one experiment, in which a considerable potassium deficit had developed, Tm_P and G.F.R. were unaffected by potassium administration, whilst in another, in which negligible potassium deficit had developed, potassium administration was followed by increased G.F.R. and Tm.

DISCUSSION

Renal delay times

Smith (1951, p. 42) suggested that in calculating renal clearances, excretion should be related to the plasma composition $2\frac{1}{2}$ min earlier. Brun, Hilden & Raaschou (1949) suggested a mean delay of 5 min at flows of 2–5 ml./min, decreasing at higher flows. Bradley, Nickel & Leifer (1952) calculated that

75% of human nephrons have delay times of under 10 min, but that 5% of nephrons have delay times of up to 30 min. Their figures are probably an overestimate, for Emery, Holmes, Davies & Black (1955) have advanced an alternative explanation for the findings; and more recently Childs, Wheeler, Cominsky, Leifer, Wade & Bradley (1955) have calculated that 62% of glomerular filtrate reaches the bladder in 4–6 min. It appears that 10 min is an outside limit for the mean delay time. If plasma concentration changes are small and regular, correction can be made by using a single figure for mean delay. All clearances and phosphate Tm's here reported have been calculated on the assumption both of a $2\frac{1}{2}$ min and of a 10 min delay time. At the fairly constant plasma and inulin concentrations used the figures derived from the two calculations have rarely differed by more than 10%.

Experiments at normal, constant G.F.R.

The opinion that phosphate shows, in man, a stable renal tubular maximum appears to rest upon the experiments of Schiess *et al.* (1948), performed upon a single subject and published without data for G.F.R. The present experiments with a constant G.F.R., shown in Table 2, are in agreement, and it is shown that such an excretory mechanism may plausibly be deduced from a consideration of the relationship between plasma phosphate concentration and phosphate excretion. No assumptions need therefore be made concerning inulin clearance, upon whose identity with G.F.R. some workers have cast doubt (Wolf, 1950; Ladd & Gagnon, 1954). However, the G.F.R. calculated from these experiments was in fact very close to the inulin clearance; and in those experiments in which phosphate excretion bore no simple relationship to plasma phosphate concentration, the excretion appeared related to filtered load as calculated from inulin clearance. There is therefore internal evidence that the inulin clearances determined in these experiments were fairly close to the G.F.R., and hence that the inulin did not suffer from the defects found in some samples by Ladd & Gagnon.

Few figures are available to fix the 'saturation threshold', that is, the filtered load at which tubular reabsorptive capacity is first saturated, since we attempted in all clearance periods to exceed this level. Subject L had reached his threshold at a filtered load of 170 μmole/min, and T at 185; Y had attained his threshold on some occasions at 210, 240 and 285 μmole/min, whilst on two occasions he did not reach threshold until his glomeruli were filtering over 300 μmole/min. Since this subject usually showed a high Tm, it is not surprising that his saturation threshold was also high. The subject of Schiess *et al.* (1948) had a saturation threshold around 200 μmole/min.

No evidence has been found of regular diurnal changes in phosphate Tm. Experiments (unpublished) at normal or slightly elevated plasma phosphate concentrations support the findings of Schiess *et al.*, that below saturation

threshold the tubules reabsorb less than their maximal capacity, leaving some non-absorbed phosphate in the lumen; even at subnormal plasma phosphate concentrations some phosphate is still excreted. Since the filtered load is normally below saturation threshold, it is possible that an exploration of the renal 'handling' of phosphate at such moderate plasma concentrations might throw light upon diurnal rhythmicity. Schiess *et al.* claim, for instance, that increased phosphate excretion in acidosis is due to a depression of reabsorption at moderate plasma concentrations, despite an unaltered Tm.

It would be premature to speculate upon the cause of the day-to-day variations in phosphate Tm. Various factors, such as cortisone (Roberts & Pitts, 1953) and vitamin D (Harrison & Harrison, 1941), are said to depress phosphate reabsorption, but there is no evidence whether these or other factors are responsible for the variations here reported.

Hogben & Bollman (1951) found in the dog that phosphate Tm declined progressively to 25–50% of its initial value during a series of clearance periods extending over 3–4 hr, and that this decline was usually prevented by adding potassium to the infusate. Correction of the negative potassium balance in experiment M3 had no effect on G.F.R. or Tm_P. In experiment D4, potassium administration was followed by an increase in Tm_P, but only in association with an increase in G.F.R.

Experiments at raised, variable G.F.R.

In most of the group II experiments shown in Table 3—those with regression coefficient below unity—phosphate reabsorption increased with filtered load, even at values obviously above saturation threshold. An apparent rise in Tm with high filtered loads has been interpreted by Lambert, van Kessel & Leplat (1947) as due to the presence in plasma of non-filterable phosphate, when the total concentration exceeds about 3 mM. This cannot account for our results; when a high filtered load was due to a high plasma phosphate concentration, the reabsorbed phosphate remained unaltered, and the reabsorption only increased when a high filtered load was due to a high G.F.R. Govaerts (1952) also argues that a certain fraction of plasma phosphate is non-filterable at the glomerulus, since after infusion of isotopic phosphate the specific activity of the urine exceeds that of the plasma. This is a common observation after the administration of a variety of isotopes, and has been interpreted by Bradley *et al.* (1952) as due to renal delay. Another explanation is suggested by the work of Emery *et al.* (1955), so that the interpretation put upon his findings by Govaerts has little foundation. Harrison & Harrison (1941) claimed that some non-filterable phosphate appeared in the plasma soon after an infusion, but disappeared within an hour. We usually allowed nearly an hour between the start of infusion and the first clearance period, for stabilization of plasma phosphate and inulin concentrations; an occasional very high phosphate re-

absorption in the first period has been disregarded, since it could be due either to the non-filterable phosphate described by Harrison & Harrison, or to faulty allowance for delay times through ignorance of plasma concentration immediately before the clearance period. Hogben & Bollman (1951) found no non-filterable phosphate in the plasma of dogs even when the concentration had been raised to about three times the highest level we have ever achieved. It seems therefore unlikely that error has been introduced into our experiments through the presence of non-filterable plasma phosphate.

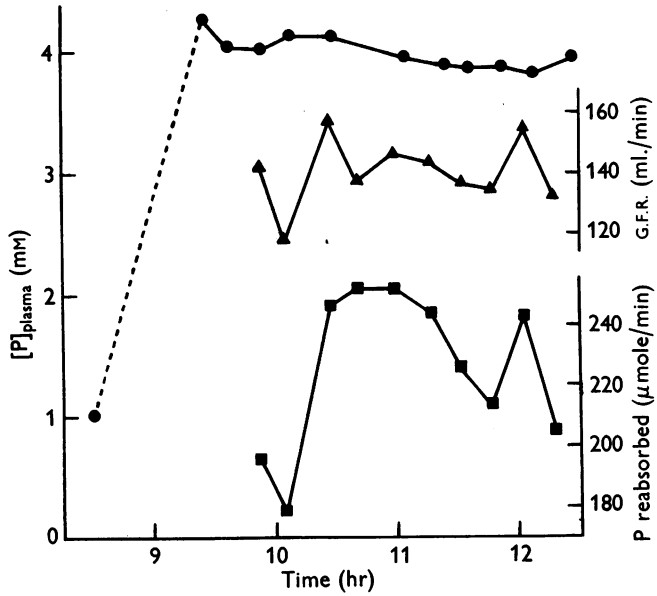

Fig. 5. Expt. M3. Successive determinations of plasma phosphate concentration, ●, G.F.R., ▲, and phosphate reabsorbed, ■. Urinary data are plotted at the mid-point of the collection period.

High phosphate reabsorption was however more closely related to high G.F.R. than to high filtered load or plasma phosphate concentration. When variations in filtered load were due largely to varying plasma phosphate concentration, with constant G.F.R. (experiments of Fig. 1 and Table 2), phosphate reabsorption was independent of filtered load above the 'saturation threshold'. When variations in filtered load were due largely to varying G.F.R., with nearly constant plasma phosphate concentration, phosphate reabsorption rose and fell with every rise and fall of filtered load. This is strikingly shown in Expt. M3 (Fig. 5; see also Figs. 2, 3 and 6). Here a high steady plasma phosphate concentration was maintained, and the random changes in G.F.R. were accompanied by more or less proportional changes in phosphate reabsorption.

This apparent dependence of phosphate Tm upon G.F.R. needs careful consideration, since it leads to certain unorthodox conclusions. A direct regression of phosphate reabsorption upon G.F.R. would be suspect for two reasons: first, although the two quantities have no necessary physiological connexion, and, upon orthodox theory, they should be independent when saturation threshold has been exceeded, they cannot be determined independently; and secondly, they were measured within a time series. Phosphate reabsorbed is calculated as the difference between filtered and excreted phosphate, and filtered phosphate as the product of plasma phosphate concentration and G.F.R. Hence any errors in determination of G.F.R. will lead to a spurious correlation. This was appreciated by Harrison & Harrison (1941), who observed a high phosphate Tm at high G.F.R. in dogs, but were reluctant to draw any conclusions. We have attempted to circumvent the difficulty by exploring the relationship between filtered and excreted phosphate.

Suppose the hypothesis to be correct, that phosphate reabsorption increases with G.F.R. Then if an increase in filtered load be due largely to increase in G.F.R., the phosphate excretion will not increase equally with filtered load, and the regression coefficient of excreted on filtered phosphate will be below unity. This can be observed in most experiments of Table 3. In one experiment, however, M4, the regression coefficient exceeds 2, suggesting that at high filtered loads much *less* phosphate was reabsorbed. In fact in this experiment the highest filtered load, 420 μmole/min, corresponded to the lowest G.F.R., 130 ml./min, so again dependence of phosphate reabsorption upon G.F.R. is suggested.

The correlation coefficients of Table 3 and Fig. 3 are incompatible with the simple Tm concept. They are immediately explicable if Tm varies with G.F.R. It therefore seems justifiable to plot phosphate reabsorption against G.F.R. This has been done in Fig. 6 for Expts. M 1–4, and a single regression line has been fitted to all the data. Similar plots for Expts. Y5 and Y7 are shown in Fig. 7. It might seem more logical to have plotted the data in this way in the first place, were it not for the possibilities of spurious correlation discussed above.

We claim no high reliability for the inserted regression lines, since determination of plasma and urine inulin, as well as urine flow, enter into the calculation of both variables. Even so, the random error of determination of G.F.R., assessed from our duplicate inulin analyses and duplicate extractions from plasma, can seldom have exceeded 3%, so that the apparent relationship is probably valid. As for the possibility of false association if both variables were changing progressively through unknown factors acting through the lapse of time, this is negatived by the fact that the variations were random. In Fig. 7 the successive periods are numbered, and it will be seen that there was no regular change of either variable with time.

Gross errors of bladder emptying could lead to spurious correlation between phosphate reabsorption and G.F.R., or between phosphate excretion and filtered load. The four subjects chiefly used are those whose ability to empty their bladders was investigated by Mills, Thomas & Yates (1955). Subject M seemed always to empty his bladder consistently; and the small residual volumes occasionally shown by T and Y are of little importance with the usual urine samples of over 100 ml. Subject D, however, sometimes had a

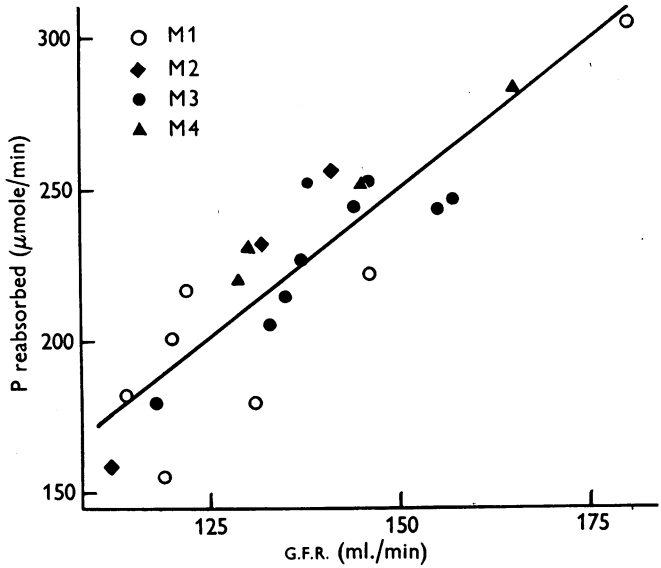

Fig. 6. Phosphate reabsorbed plotted against G.F.R. for four experiments upon subject M, with linear regression fitted to all the points.

large residual volume. Random irregularities in flow and output for this subject have therefore been disregarded and no experiment upon him has been included in group II. The linear relationship between phosphate output and plasma phosphate concentration found in Expts. 1–3 on this subject (Table 2) suggests, however, that bladder emptying was in these experiments adequate.

Apart altogether from the correlation between phosphate reabsorption and G.F.R. in a consecutive series of clearance periods, a similar association can be seen on comparing experiments on different days on the same subject, particularly on comparing the low G.F.R. experiments, Y8 and M5 and 6, with the high G.F.R. experiments, Y1–7 and M1–4. Neither bladder emptying errors nor spurious correlations within a time series could explain this association.

A fairly constant G.F.R. can be achieved under suitably constant conditions (Davies & Shock, 1950), but our subjects were not strictly basal. Quite wide variations in G.F.R. can occur: Robinson (1954) mentions an increase to

285 ml./min (more than twice the initial value) on infusion of 10% NaCl. Variations between subjects may be quite large, as may those in one subject on different diets (Pullman, Alving, Dern & Landowne, 1954). In most of the experiments here reported, G.F.R. was similar to values reported by others, though in a few experiments in group II (Table 3) and some on Y in Table 2, some values were rather high. Two factors which may have been responsible were accidentally introduced pyrogens in early experiments, such as Y 1–7,

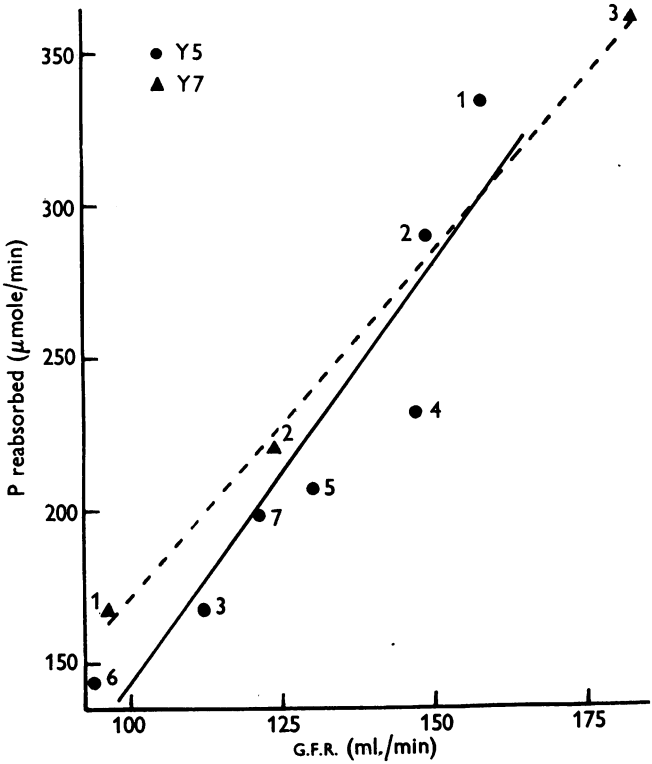

Fig. 7. Phosphate reabsorbed plotted against G.F.R. for two experiments upon subject Y. Continuous line is linear regression for experiment Y 5, discontinuous line for Expt. Y 7. Clearance periods in each experiment are numbered in sequence.

or the infusion of large amounts of phosphate. Smith (1951, pp. 446–451) found that pyrogens increased the renal circulation, and though he states that G.F.R. was unaltered, his fig. 83 shows an increase from about 100 to 150 ml./min. We have found no reports in the literature of the effect upon G.F.R. of large phosphate infusions, either direct or secondary to depression of ionized calcium. In Expts. M 1–4 however, very large amounts of phosphate, 80–160 m-equiv, were infused, and this may perhaps account for the unusually high and variable G.F.R. in these experiments. Experiments (unpublished) in-

volving infusion of inulin without phosphate confirm that the inulin was not pyrogenic.

Such variations in G.F.R. in these experiments could be due to the operation of more or fewer glomeruli or to the filtration of greater or smaller volumes at the same glomeruli. The classical 'glucose titration' of the kidney (Smith *et al.* 1943) merely indicates that among the functioning nephrons there is no wide dispersal of glomerular activity. It in no way excludes the possibility of a certain proportion of totally inactive glomeruli, as was pointed out by Kruhoffer (1950). The filtration of a greater volume of fluid through the same glomeruli should not affect Tm, although it would reduce the plasma concentration at which Tm was achieved. The opening up of reserve nephrons would, however, be expected to increase proportionally the maximal reabsorptive capacity of the kidney. Our data thus suggest the operation of a varying proportion of glomeruli at different G.F.R. This is contrary to the conclusions of Ayer, Schiess & Pitts (1947), who found that G.F.R. in dogs could be varied over a range of 100% by altering the protein content of the diet, without altering phosphate Tm. In contrast, Handley & Moyer (1955) found that adrenaline, noradrenaline, morphine and haemorrhage in dogs, and walking and tilting in man, caused parallel reductions in G.F.R. and glucose or PAH Tm; and they interpreted their results, as we interpret ours, as indicating that a certain fraction of nephrons may be inactive. It is possible that in different circumstances G.F.R. may vary by changes in the number of active glomeruli, or in the volume filtered by each, or both. Our observations and those of Ayer *et al.* (1947) are thus not incompatible. Other observations upon phosphate infusion in man with measurement of G.F.R. are somewhat sparse. Those of Barclay, Cooke & Kenney (1949) are difficult to interpret since they did not publish the actual results, but only indices of renal function devised by themselves; and they appear to have used a single-injection technique for inulin clearance determination, which probably leads to a fall in plasma concentration too steep to permit accurate allowance for renal delay time.

It is probable that high and variable G.F.R.'s were here observed under abnormal conditions, i.e. infusion of very large amounts of phosphate in M, and perhaps pyrexia in Y. In a more recent experiment upon subject Y (Y 8) after pyrogens were effectively excluded, and two experiments on M (M 5 and 6) with infusion of smaller amounts of phosphate, G.F.R.'s were at the lower limit of, or below, those of group II (Table 3), and around the values reported by others under basal conditions. It thus appears that under the somewhat abnormal conditions of the group II experiments a number of additional glomeruli were open, producing a Tm considerably above those recorded by Schiess *et al.* (1948). The precise cause of these abnormal conditions is irrelevant to the argument.

Handley & Moyer (1955) suggested that in conditions of reduced renal blood

flow some glomeruli might become inactive; the present work suggests that in conditions of abnormally increased renal blood flow more glomeruli may become active. This implies that, under normal resting conditions, in which most figures for renal clearances are obtained, a fair proportion of glomeruli are inactive, only to be opened up by vasodilator agents such as pyrogens. Miller's (1953) observations on aminophyllin vasodilatation are most simply interpreted in the same way, as are also the observations of Selkurt (1954) upon the dependence of sodium reabsorption upon G.F.R. More work, with other tubular maxima and other vasodilator agents is, however, required before such a conclusion can be safely established.

SUMMARY

1. Measurement of phosphate excretion in healthy subjects during intravenous phosphate infusion has revealed two forms of relationship: group I, in which phosphate output was linearly related to plasma phosphate concentration, and inulin clearance was fairly constant; and group II, in which phosphate output was not obviously related to plasma phosphate concentration, and inulin clearance varied over a wide range.

2. Group I experiments are entirely compatible with the classical Tm hypothesis, and from them can be inferred a G.F.R. and a phosphate Tm close to those calculated from inulin clearance.

3. Considerable variations were observed between phosphate Tm from one day to another, in the same subject and at similar G.F.R. These were not regularly related to time of day.

4. In group II experiments G.F.R. was considerably and variably increased, as a result perhaps of pyrexia or infusion of large amounts of phosphate, and phosphate reabsorption increased with it, even at high plasma phosphate concentration.

5. The dependence of phosphate reabsorption upon G.F.R. suggests the operation of varying proportions of nephrons, even at G.F.R.'s considerably above normal. It thus appears that under normal conditions a proportion of nephrons may be inactive.

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Note added in proof. Since this paper went to press, Anderson (1955) has reported experiments with results very similar to those of our Group I. The differences between G.F.R. calculated from phosphate data and from inulin clearance are very similar to ours. Anderson fails however to indicate that small errors in calculation of G.F.R. lead to proportionately much larger errors in Tm_P. We have calculated the Tm_P from his inulin figures and the data represented in his Fig. 5, and find that they differ from his values calculated from phosphate data in much the same way as do our own. Calculation of Tm_P from phosphate data alone would lead to gross unsuspected errors should there be any progressive change in G.F.R. during the period of phosphate infusion.

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