THE EXCRETION OF CALCIUM AND PHOSPHORUS BY THE HUMAN KIDNEY

BY

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TO

J. F. BROCK M.D., F.R.C.P.

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INDEX

INTRODUCTION.

In this thesis I have attempted to assemble in some coherent form the fruits of two years' research into the renal handling of calcium and phosphorus.

The experimental techniques that I have used are based upon clearance analysis and presume throughout that the clearance of inulin is an acceptable measurement of the glomerular filtration rate. For the sake of brevity and because I did not think it important, I have not defended this presumption against the attacks of Welf[277]. The measurement of the inulin clearance is well established as a research tool, and even if it does not measure what it purports to, it is a good standard of reference.

The thesis is divided into five main sections. The first deals with the renal handling of phosphorus, the second with calcium, the third with the metabolic effects of calcium infusion, the fourth with the action of parathyroid hormone and the fifth with the renal abnormality in two cases of Sarcoidosis.

There are many important topics that fall under the heading of this thesis that I have not discussed for the reasons that I have not investigated them myself and that I have not considered them relevant to my argument. Had I reviewed the literature that has accumulated around these topics it would have been little short of plagiarism. I have, however, included many references in the bibliography that I thought would help to fill the gaps. These references do not appear in the text.

For the convenience of the reader I have included in the main text only such experimental data as I considered necessary. Detailed accounts of the conduct of the experiments appear in Appendix A at the end of the text.

In Appendix B can be found a brief account of the various analytical and statistical procedures that have been employed.

There are many problems that have arisen out of my own experimental results that are, for the moment, unsolved. I am pleased that it is so, because this thesis records the beginning and not the end of my interest in renal research.

NOTE ON ABBREVIATIONS AND TERMINOLOGY

The term "renal titration experiments" has been used to describe experiments where the relationship between plasma concentration of a solute and the rate of excretion in the urine is studied under conditions of progressively increasing plasma concentration.

P	denotes plasma concentration of the solute indicated by the subscript. e.g. P_p denotes plasma phosphorus concentration.
U	denotes urine concentration in mg./ml.
V	denotes minute volume of urine in mls./min.
UV	denotes the rate of excretion in the urine in mg./min. of the solute indicated by the subscript.
C	denotes plasma clearance in mls./min. of the solute indicated by the subscript.
T	denotes the rate of tubular reabsorption of the solute indicated by the subscript.
G.F.R.	denotes glomerular filtration rate in mls./min.
F.	denotes the filtered load of the solute indicated by the subscript.

S E C T I O N I.

The Renal Handling of Inorganic Phosphorus.

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THE RENAL HANDLING OF INORGANIC PHOSPHATE

The inorganic phosphorus in the plasma is generally accepted to be a threshold substance with regard to its urinary excretion. By "inorganic phosphorus" is meant that fraction of total plasma phosphorus that is acid soluble and will react directly with molybdate to produce, on reduction, a blue colour. By "threshold substance" is meant something less easy to define.

The term "threshold" in its broadest, and to me most acceptable, sense implies the presence of some teleological or, to use the modern euphemism, homeostatic, mechanism that operates to conserve phosphorus during periods of deficit and to excrete phosphorus during periods of excess. By so doing this mechanism helps to maintain the plasma phosphate concentration within limits optimal for the organism. One could not require more acceptable proof of this function than the observation that phosphate deprivation as a result of low phosphorus feeding will result in a lowering of urinary phosphorus(38) and that intravenous injection of a solution of phosphate will result in an outpouring of phosphorus in the urine(101,231)

To translate this rather nebulous concept of a threshold into the more attractive though probably less valid terms of modern theory, it can be said that the kidneys conserve phosphate by a process of tubular reabsorption of phosphate filtered by the glomeruli. The ability of these tubules to reabsorb phosphate

is said to be limited by a maximal value or "Tm"[210] similar to that described by Shannon and his co-workers for glucose[235,236]. When the filtered phosphorus exceeds this Tm, the difference will be excreted in the urine. It can therefore be said that the kidneys excrete phosphate by not conserving it. To shift the emphasis slightly, the rate of excretion of phosphate is largely the responsibility of the glomeruli, whereas its conservation is that of the tubules. As the threshold is the net result of these two processes acting simultaneously it will be affected by either of them in such a manner that its level will vary inversely with the glomerular filtration rate and directly with the maximal ability of the tubules to reabsorb phosphate. This "modern theory" to judge by its inclusion in modern textbooks relating to the subject, is generally accepted as that best explaining observations made during health and disease. On dissecting this theory, it is apparent that it makes four assumptions which I propose to discuss.

Assumption (i) <u>The kidney is an important organ in the excretion and conservation of phosphorus</u>. That this assumption is valid is indicated by the fact that nephrectomy in dogs is followed by a very rapid and marked rise in plasma inorganic phosphate concentration[184,192,136,97] and by the fact that cyaniding of the kidney produces a rapid depletion in body phosphate[68], and by the fact that artificially induced hyperphosphataemia is rapidly corrected largely by urinary excretion of the excess.[101,231]

Assumption (ii) That the phosphorus is dealt with by the kidney by a process of filtration and reabsorption in accordance with the concept of renal function elaborated by Ludwig in 1844[168]. That this is so was convincingly demonstrated in micro-puncture studies in amphibian kidneys by Walker[258,259,261], Wearn and Richards[262] and White[268]. This has never been seriously disputed, although the work of Taugener et al.[252] does indicate that the renal tubules may, under normal circumstances, secrete small amounts of inorganic phosphate into the tubules as a result of hydrolysis of organic phosphoric ester compounds by kidney phosphatases. An unhappy abstract records a case where the phosphate clearance exceeded the inulin clearance in man.[137]

Assumption (iii) That the inorganic phosphorus in the plasma exists entirely in a form available for glomerular filtration so that, allowing for minor and unimportant Donnan effects, the concentration in plasma and glomerular filtrate will be identical. Evidence in support of this assumption has come from several sources using a variety of experimental procedures. The most convincing evidence has been that obtained by direct estimation of the concentration of phosphate in the glomerular fluid obtained by micropuncture of the glomeruli of Necturi and frogs[258,259,261, 262,268]

The results of renal titration experiments on dogs have indicated that very high concentrations of plasma phosphate can be achieved without the formation of non-filterable

complexes[143,210]. In vitro ultrafiltration through collodion or cellophane membranes has similarly lent support to this assumption [240,96,24,28,95,194,126,256], as has in vivo compensation dialysis[89,97].

That this assumption is not necessarily valid, however, is indicated by the fact that the concentration of inorganic phosphate in the Cerebrospinal fluid is only approximately half of that in the blood plasma[197]; by the observations of Lambert et al.[157] who interpreted their results from renal titration experiments as indicating the presence of non-filterable compounds at high plasma levels and confirmed this by in vitro ultrafiltration, and by the observations of Jacobs et al.[142] who compared the relative permeabilities of endogenous phosphate and infused P^{32} and came to the conclusion that the latter was slightly (7.7%) more filterable. These observations of Jacobs et al. may however have been apparent rather than real and due to renal delay time effect. I shall discuss this assumption in greater detail when discussing my own experimental results.

Assumption (iv) <u>That a true Tm for phosphate does exist in man</u> that is independent of glomerular filtration rate or filtered load once the Tm value has been exceeded. This assumption has aroused more controversy than any other and opinions, substantiated by experimental results, have been expressed favouring one of three schools of thought.

a) <u>That the assumption is unconditionally valid</u>. Representative

of this school of opinion are Pitts[210], Ayer et al.,[13] Letspeich et al.[167], Smith[239] and Anderson[12]. Apart from these workers who have expressed their beliefs, this assumption is implicit in the work of many others.

b) That the assumption is invalid in that the absolute amount reabsorbed at maximal levels varies directly with the glomerular filtration rate, but is independent of the plasma concentration. Adherents to this belief are Barclay and Cooke[15,16], Longson et al.[166] and Harrison and Harrison[114]. If the renal handling of other ions can be said to be representative of the manner in which the phosphate anion is dealt with by the kidney, analogous support is lent to this belief by the fact that a directly proportional relationship between glomerular filtration rate and rate of tubular transport exists for chloride[167], bicarbonate[212] and sodium[126]. Ayer et al.[13], in an experiment designed to test this criticism of the Tm hypothesis, found that elevation of the creatinine clearance in dogs by high protein feeding produced no change in absolute amount of phosphate reabsorbed. This criticism, if valid, would mean that the glomerular filtration rate would have no effect upon the threshold level.

c) The third body of opinion, represented by Crawford et al.[50] and Smith, Ollayos and Winkler[240] claims that Assumption (iv) is invalid in that the amount reabsorbed is directly proportional to the filtered load, or that the renal tubules will reabsorb a constant fraction of the filtered phosphorus at all loads. In this respect, the human kidney would behave in a manner similar to that

of the cat where there is no true Tm, but where an increased amount of phosphorus is reabsorbed as the plasma level is elevated. This was well shown by Eggleston and Shuster[65,66], and confirmed by Taugener et al.[252]

To return to the term "threshold", it should mean in its strictest sense, that there exists for inorganic phosphorus a critical plasma concentration below which it will disappear from the urine, at which it will suddenly appear in the urine and above which increases in the plasma concentration should be accompanied by proportional increments in the rate of urinary elimination. That there is no such sharply defined threshold level, however, was shown as early as 1928 by Brain et al.[28] who demonstrated that even with very low plasma levels produced by insulin injection or overbreathing, it is virtually impossible to obtain a phosphate free urine. There is thus no known "Minimal threshold level" for phosphate in man or threshold of appearance for phosphate. When the plasma level of phosphate is raised artificially by intravenous infusion of phosphate in the performance of renal titration experiments, and the rate of urinary elimination is plotted on the ordinate of a graph against the plasma concentration as the abscissa, the linear relationship that exists between the two at very high plasma concentrations is preceded by a curvilinear relationship at moderately raised concentrations. This curved line is spoken of as the "heel of the titration curve" and is illustrated in Fig. 1.

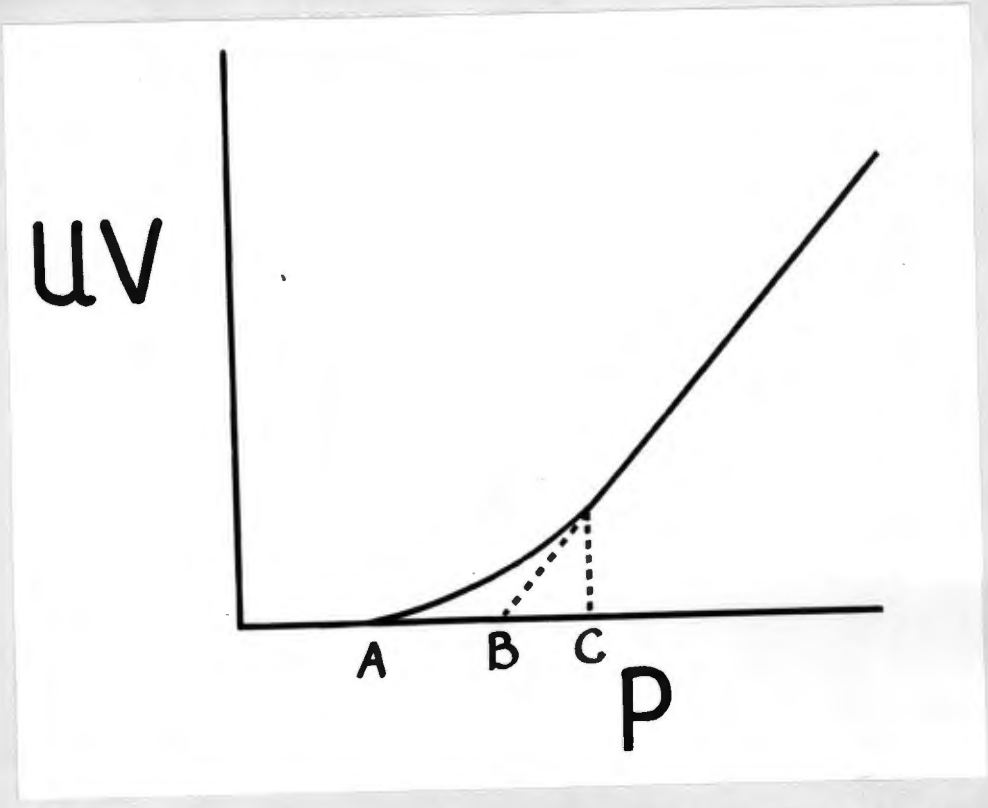

Fig.1. The curvilinear relationship between UV and P between P = A and P = C is shown in this diagram and is referred to as the "heel of the titration curve".
A, the threshold of appearance, is known as the "minimal threshold level"
B, the point at which the extrapolated straight line intercepts the abscissa is known as the "mean threshold level"
C, the plasma concentration at which the curvilinear relationship becomes linear, is referred to as the "maximum threshold level" and represents that plasma concentration at which, presumably, the entire nephron population is "saturated".

The plasma concentration at which the curvilinear relationship becomes linear is referred to as the "Maximal threshold level", and the intercept of the extrapolated line upon the abscissa is referred to as the "mean threshold level". Were it not for the fact that such a maximal threshold does exist, the use of the term "threshold" would scarcely be justified with regard to phosphorus. Fortunately for this thesis, there is such a limiting maximal threshold and it has been repeatedly demonstrated for

phosphate and most other threshold substances[1,143,207,208,217].

The phosphate threshold can therefore be defined as a range of plasma phosphate concentrations with no known minimal limiting value but with a fairly well defined maximal limiting value at which phosphorus will appear frankly in the urine.

This "heeling" of the titration curve is interpreted as indicating heterogeneity of the tubule population, so that shorter, or less "active" tubules will become saturated at lower filtered loads than will others with consequent spillover into the urine of filtered phosphate at low levels. The maximal threshold level indicates that plasma concentration at which the reabsorptive capacity of the entire nephron population is exceeded. Had it not been for this heel of the curve in glucose titrations, Shannon and Fisher need only have spoken about a glucose "T". The "m" would have been redundant.

An alternative, and to me more acceptable, explanation for the presence of phosphate in the urine at sub-maximal threshold levels follows from the suggestion of Eichels and Starling[67,68] recently supported by Taugener [252] that the tubule cells may contribute to the urine phosphorus by hydrolysis of organic phosphorus compounds. The kidney is known to contain large amounts of phosphatases, and it is clear that this minor contribution of endogenous phosphate would assume decreasing significance as the rate of excretion of phosphorus in the urine is accelerated by

progressive elevation of the plasma concentration, until, at the maximal threshold level, it would cease to assume significance and the relationship thereafter between UV_p and P_p would be linear.

Nevertheless, although the reason for this "heel of the curve" may be obscure, it does exist and its only immediate importance lies in the fact that if its existence is ignored, it may give rise to error in the interpretation of results. This has been emphasised by Pitts[210] and formed the basis of his criticism of the work of Harrison and Harrison[115] whose conclusion regarding the tubular handling of phosphate during periods of acidosis were founded on experiments performed at submaximal plasma levels.

To summarise at this stage :-

i) A threshold exists for phosphate that is not represented by a critical plasma level but covers a range of plasma concentrations limited maximally by a maximal threshold level at which phosphate will appear "frankly" in the urine.

(ii) The level at which this threshold is fixed is determined largely by renal mechanisms or mechanisms mediated through the kidney about the nature of which controversy still exists.

It was in an attempt to enter this controversy and to add to it the meagre weight of my opinion that I embarked upon the series of experiments to be described shortly. As all of these experiments took the form of renal titrations I shall first discuss the theory underlying the conduct and interpretation of these experiments.

An Analysis of Renal Titration Experiments.

In conducting these experiments one is concerned with the relationship between two variables - the plasma concentration of inorganic phosphate(P_p) and the rate of excretion of phosphate in the urine (UV_p).

That there is a relationship between the two was observed in man in 1927 by Schultz and Keith[231], and has since been repeatedly confirmed. Haldane et al.[101] demonstrated that this parallel relationship was unaffected by rate of flow of the urine and Brain et al. in 1928 [28] derived a mathematical formula to express this relationship. They found that the rate of excretion of phosphorus in the urine could be expressed as a function of "r" (an excretory constant), the plasma phosphate concentration and the threshold value.

In the experimental procedure, the plasma phosphate concentration is raised by the intravenous infusion of a sterile buffered phosphate solution of known phosphorus content. If this is administered accurately and at progressively increasing speeds of infusion over the experimental period (as for instance was done by Anderson[12]) a predictable and linear rise in plasma phosphate concentration can be achieved. If, during the infusion, accurately timed urine collections are made, and the rise in plasma phosphate is linear with relation to time, the concentration of phosphate in samples of plasma taken at the midpoint of each period of urine collection may (ignoring for the moment the complication of renal delay time) be justifiably

assumed to be representative of the average plasma concentration during each urine collection period. The effects of such calculated elevation of the plasma phosphate concentration upon the rate of urinary excretion of phosphate may then be studied and plotted graphically.

Since the plasma phosphate is the controlled variable in such an experiment it will, by convention, be plotted on the abscissa and the rate of urinary excretion of phosphate, as it is the dependent variable will be plotted on the ordinate. If there is no connection between the two variables the points on the graph will be scattered and without obvious trend. If there is a relationship, there will be a regularity in the arrangement of the points with a degree of correlation dependent upon the relationship that exists and upon the accuracy with which the values of the two variables can be estimated.

As the relationship between these two variables is normally linear, I propose to digress briefly to discuss the mathematical expression for a straight line.

<u>The Mathematical Expression For a Straight Line</u>.

In co-ordinate geometry, any equation relating y to x of the form $y = ax + b$ where a and b are constants will yield, when values for y obtained for any value of x are plotted against these values of x, a straight line. This fact is illustrated in Fig. 2.

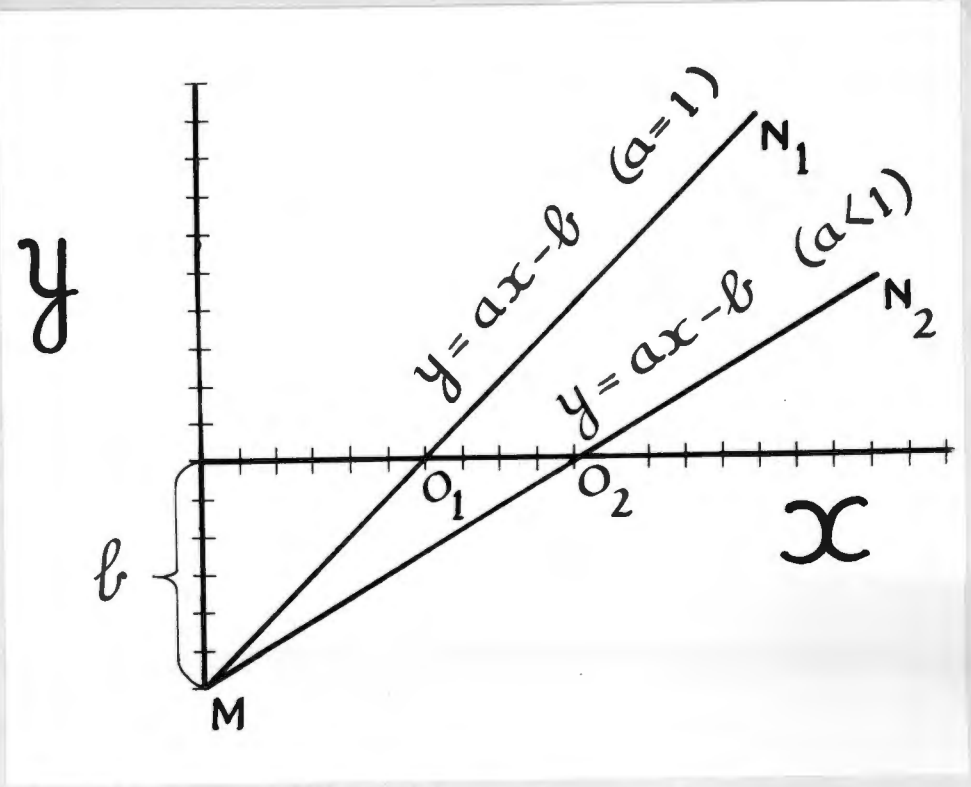

Fig. 2.

Here y has been plotted on the ordinate and x on the abscissa (This convention will be adhered to in all subsequent graphs.) The following points are illustrated.

(i) When the same scales are chosen to represent the numerical values of the co-ordinates, the line y = ax-b where a = 1 (line M,O_1,N_1) lies at 45° to both axes and intercepts the ordinate at M where y = -b.

(ii) When, under similar circumstances the line y = ax-b is drawn with a = less than 1 (line M,O_2,N_2) the line will still intercept the ordinate at y = -b, but now lies to form a more acute angle with the abscissa, and the tangent of this angle will be equal to a.

The constant "a" is referred to as the regression coefficient of x and determines the slope of the line. The constant b determines the value of the ordinate at which the line will intercept.

(iii) The line will intercept the abscissa at $x = \frac{b}{a}$.

<u>The application of co-ordinate geometry to the interpretation of renal titration experiments</u>.

In determining the amount of phosphate reabsorbed by the renal tubules every minute, it is customary to calculate the filtered load of phosphate as the product of the glomerular filtration rate and plasma phosphate concentration. The rate of reabsorption is then determined as the difference between the filtered load and the amount excreted each minute.

Expressed in symbols :-

Let P_p = plasma phosphate concentration in mg. of phosphorus per ml.

Let GFR = glomerular filtration rate in mls./min.

Let F_p = filtered load of phosphorus in mg./min.

$\therefore F_p = GFR \times P_p$ mg./min.

Let T_p = Rate of tubular reabsorption of phosphorus in mg./min.

Let U_p = Urinary concentration of phosphorus in mg./ml.

Let V = rate of secretion of urine in ml./min.

$\therefore$ rate of excretion of Phosphorus = $U_p \times V = UV_p$.

Then $UV_p = GFR \times P_p - T_p$ .....(1)

this equation can also be written

$UV_p = P_p - T_p$ .....(2)

or

$\frac{UV_p}{GFR} = P_p - \frac{T_p}{GFR}$ .....(3)

In equation (1), if UV_p and P_p are chosen as the co-ordinates, the slope of the line will give an estimate of the glomerular filtration rate and the negative intercept on the ordinate an estimate of the rate of tubular transport of phosphate. The intercept on the abscissa will give the mean threshold value for phosphate or that plasma concentration of phosphate numerically equal to the rate of reabsorption divided by the glomerular filtration rate.

In equation (2), where UV_p and P_p are chosen as the co-ordinates, the regression coefficient will be unity and the negative intercept will be equal to the rate of phosphate reabsorption.

In equation (3), where $\frac{UV_p}{GFR}$ and P_p are the co-ordinates the regression coefficient will, once more, be unity and the negative intercept will be equal to the rate of reabsorption of phosphate divided by the glomerular filtration rate or the threshold level.

We have then, from these three equations, a choice of three pairs of co-ordinates that can be used in the graphic depiction of renal titration results.

Let us take, as theoretical models, three renal titration experiments conducted under ideal circumstances where the parameters are subject to exact measurement and where such complications as renal delay time, 'heeling' of the titration curve and the formation of non-filterable phosphate complexes do not exist.

Let each experiment consist of six urine collection periods of equal duration and let there be linear increase in Plasma inorganic phosphorus concentration by 1 mg.% over each urine collection period.

EXPERIMENT A.

On this experiment let the glomerular filtration rate remain constant at 120 ml./min. and let the rate of tubular reabsorption remain constant at 3 mg./min.

The data for plotting of the graphs are given in Table A.

TABLE A.

PERIOD	GFR (ml./min)	P_p (mg.%)	F_p (mg./min)	T_p (mg./min)	UV_p (mg./min)	$T_p/GFR \times 100$*	$UV_p/GFR \times 100$*
I	120	3	3.6	3.0	0.6	2.5	0.5
II	120	4	4.8	3.0	1.8	2.5	1.5
III	120	5	6.0	3.0	3.0	2.5	2.5
IV	120	6	7.2	3.0	4.2	2.5	3.5
V	120	7	8.4	3.0	5.4	2.5	4.5
VI	120	8	9.6	3.0	6.6	2.5	5.5

* The multiplicand of 100 is introduced because P_p is expressed in mg.% instead of mg./ml.

In Fig. 3(a) the graph is drawn with co-ordinates UV_p and P_p
In Fig. 3(b) " " " " " " UV_p and P_p
In Fig. 3(c) " " " " " " $\frac{UV \times 100}{GFR}$ and P_p

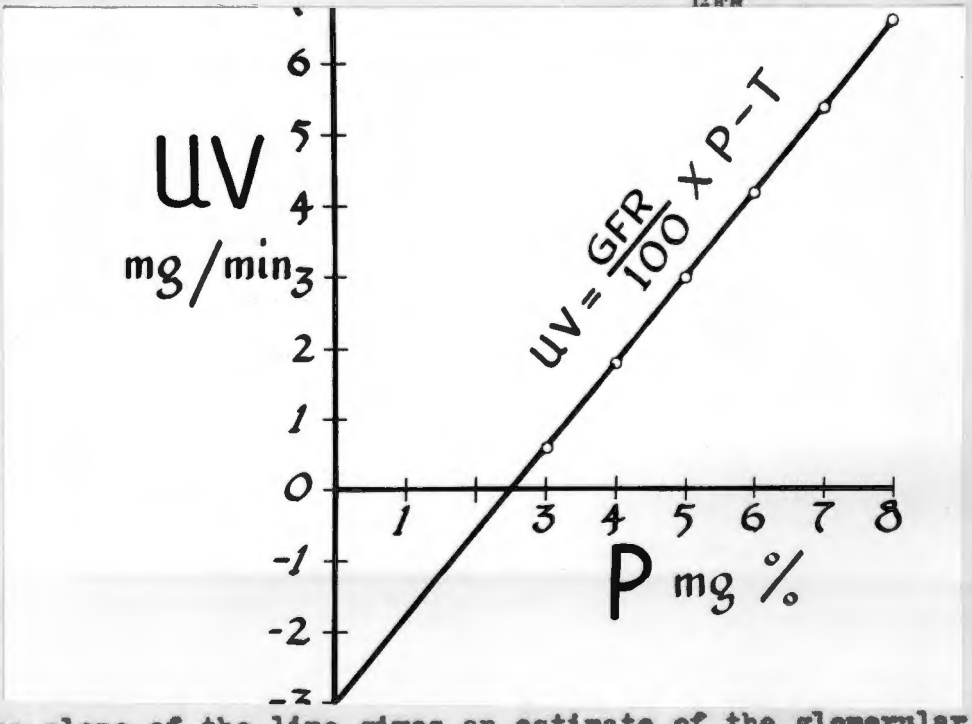

Fig.3(a):- The slope of the line gives an estimate of the glomerular filtration rate. The negative intercept on the ordinate gives the rate of tubular reabsorption of phosphorus. The line intercepts the abscissa at the threshold value of 2.5
The points lie on a straight line with good correlation.

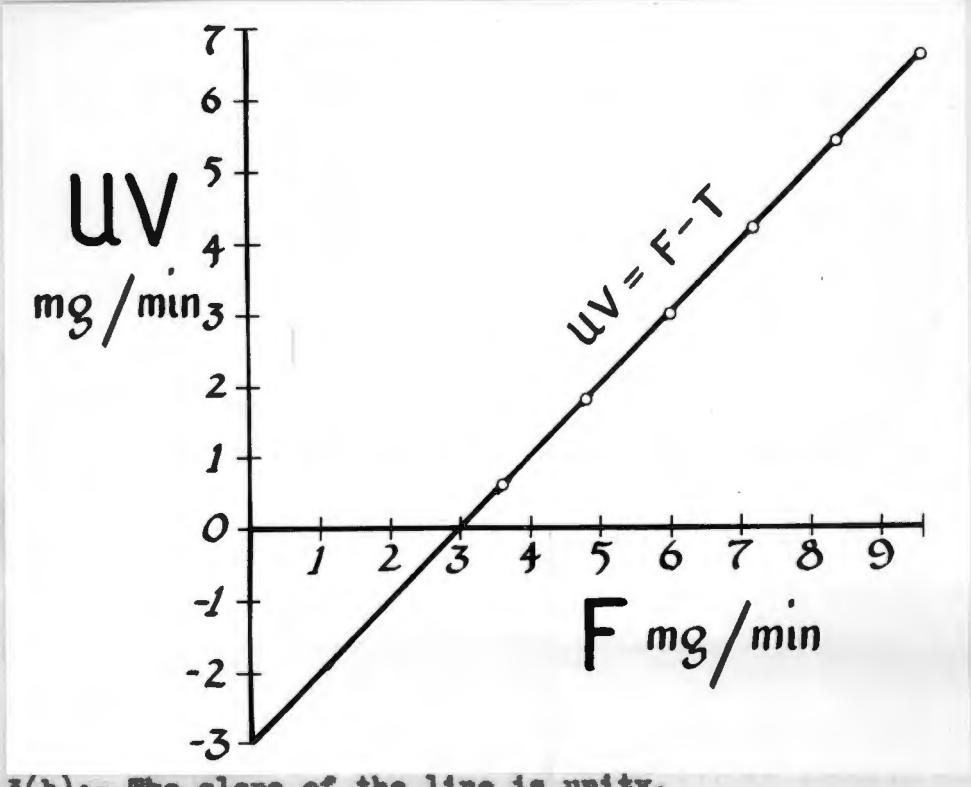

Fig. 3(b):- The slope of the line is unity.
The line intercepts the ordinate and the abscissa at the same value corresponding to the rate of reabsorption of phosphorus.
The points lie on a straight line with good correlation.

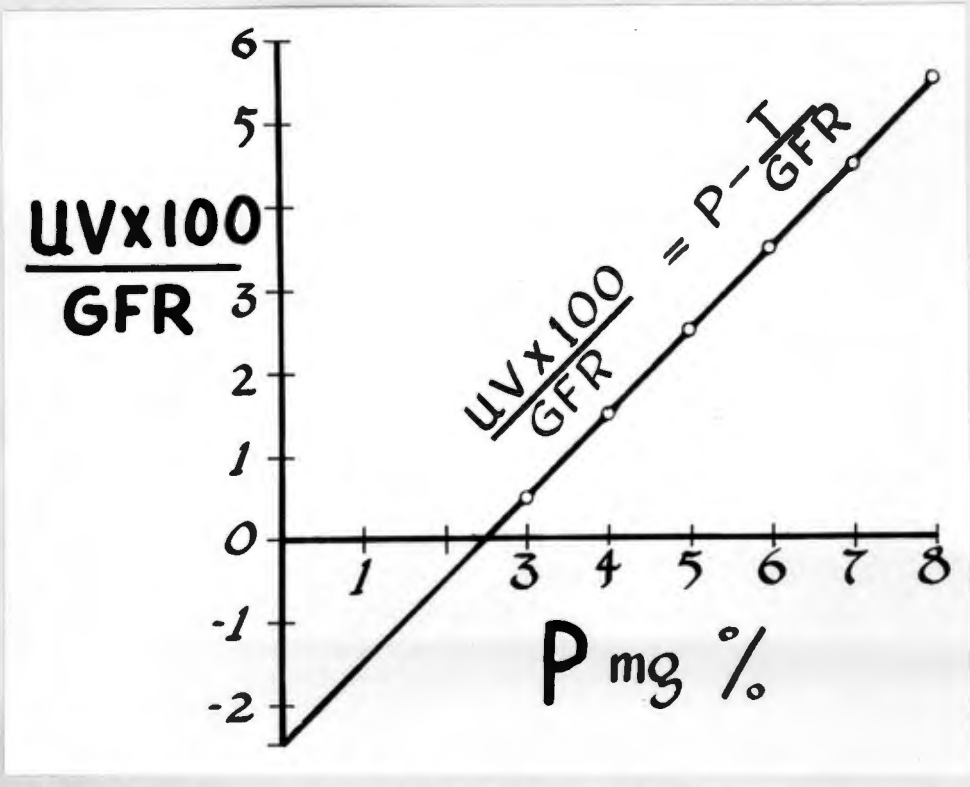

Fig. 3(c):- The regression coefficient is unity.
The line intercepts the ordinate and the abscissa at the threshold value of 2.5
The points lie on a straight line with good correlation.

Under ideal circumstances, therefore, where the glomerular filtration rate and the rate of tubular transport remain constant, there will be good correlation and the points will lie on a straight line no matter which co-ordinates are chosen to depict the results. The expected regression co-efficients and intercepts for the various co-ordinates will be found.

EXPERIMENT B.

Here let the glomerular filtration rate vary but let the maximal rate of tubular reabsorption remain constant at 3.0 mg./min.as is shown in Table B.

TABLE B.

PERIOD	GFR (ml./min)	P_p (mg.%)	F_p (mg./min)	T_p (mg./min)	UV_p (mg./min)	$T_p/100$ / GFR	$UV_p \times 100$ / GFR
I	110	3	3.3	3	0.3	2.73	0.27
II	90	4	3.6	3	0.6	3.33	0.66
III	120	5	6.0	3	3.0	2.50	2.5
IV	130	6	7.8	3	4.8	2.30	3.7
V	80	7	5.6	3	2.6	3.75	3.25
VI	100	8	8.0	3	5.0	3.00	5.0

Using the same three pairs of co-ordinates as were used in Experiment A these results are plotted in figures 4(a), 4(b) and 4(c).

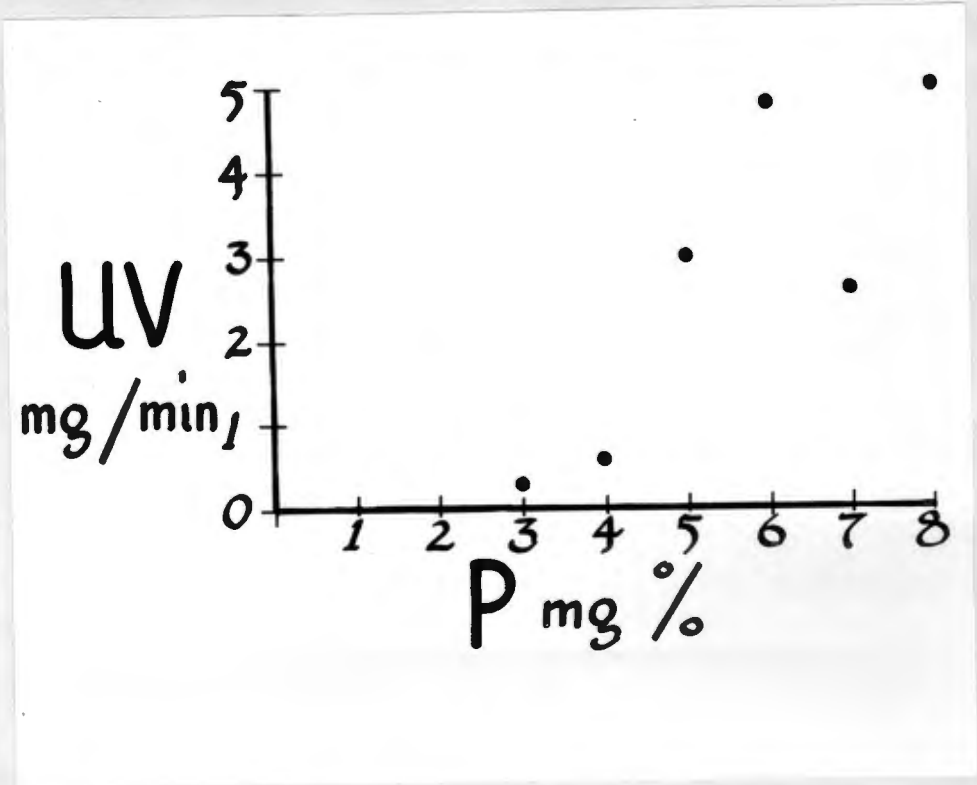

Fig. 4(a):-
The points are scattered and no indication is given of the glomerular filtration rate or the Tm.

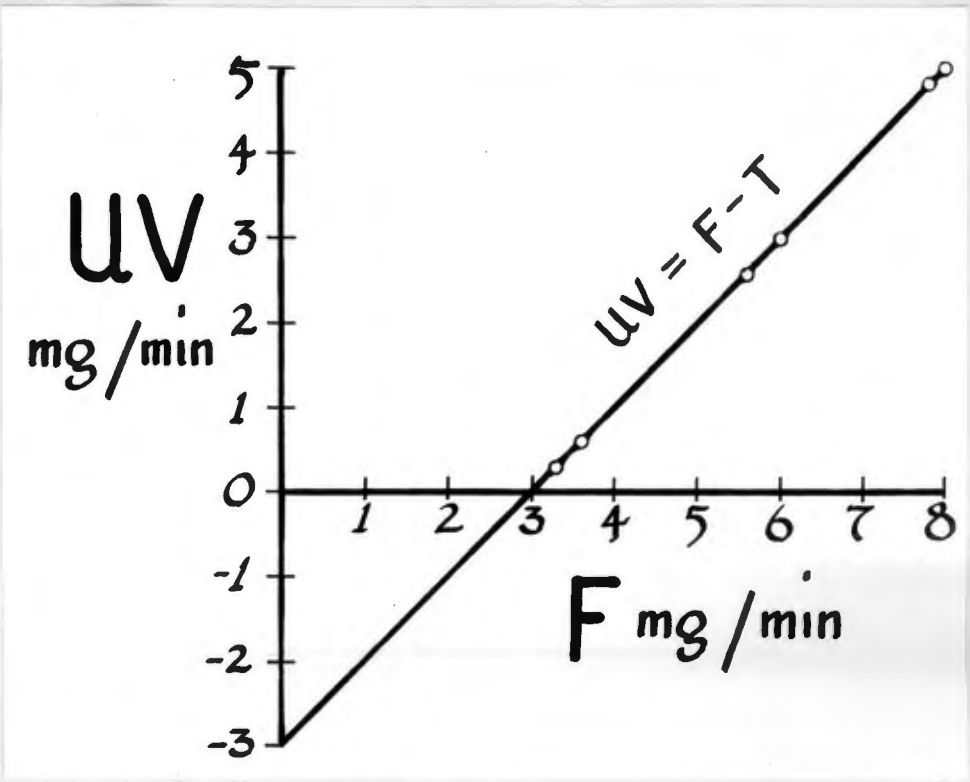

Fig. 4(b):- Correlation is good.
The slope is unity. The points lie on a straight line.
The rate of reabsorption is indicated by the intercept on the ordinate and the abscissa.

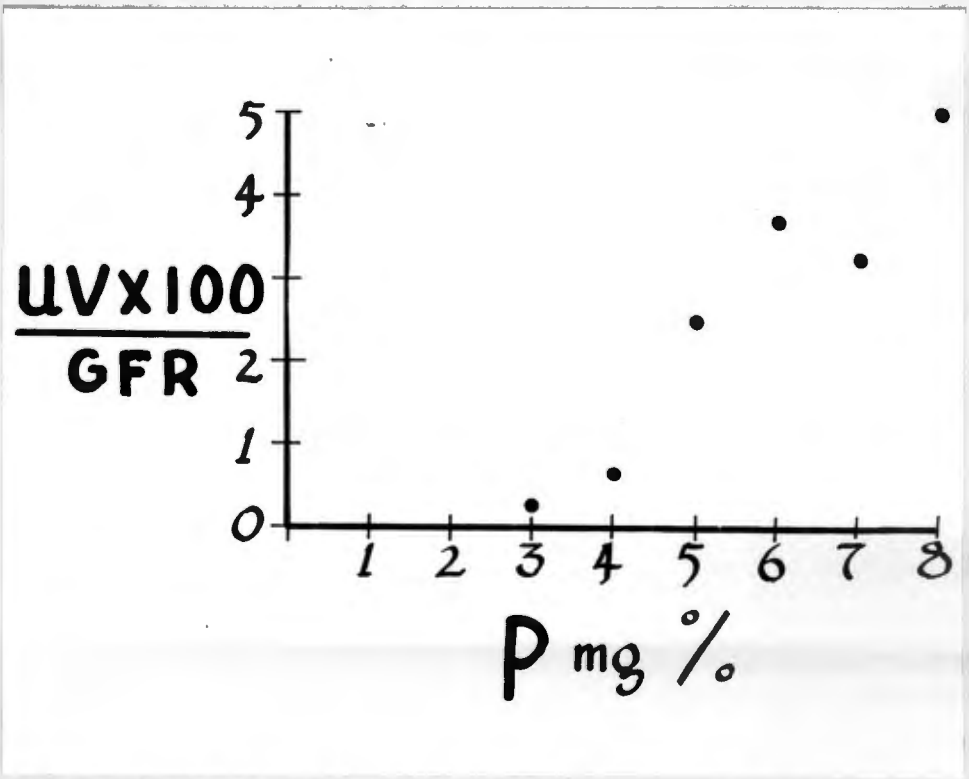

Fig.4(c):- The points are scattered, and no accurate estimate is given of the glomerular filtration rate or the rate of reabsorption.

Under ideal circumstances therefore, where the glomerular filtration rate is made to vary and where the absolute rate of reabsorption remains constant, a linear plot with good correlation will only be obtained when the rate of excretion is plotted against the filtered load.

EXPERIMENT C.

Here let the glomerular filtration rate vary as before but let the threshold($\frac{T_p}{GFR}$) remain constant at 3.0 mg./min./100ml. of filtrate formed, with consequent variance in the absolute rate of reabsorption as is shown in Table C.

TABLE C.

PERIOD	GFR (ml./min)	P_p (mg.%)	F_p (mg./min)	T_p (mg./min)	UV_p (mg./min)	$\frac{T_p}{GFR} \times 100$	$\frac{UV_p}{GFR} \times 100$
I	110	3	3.3	3.3	0	3.0	0
II	90	4	3.6	2.7	0.9	3.0	1
III	120	5	6.0	3.6	2.4	3.0	2
IV	130	6	7.8	3.0	3.9	3.0	3
V	80	7	5.6	2.4	3.2	3.0	4
VI	100	8	8.0	3.0	5.0	3.0	5

Using the same three pairs of co-ordinates as have been used previously these results are plotted in Figs. 5(a), 5(b) and 5(c).

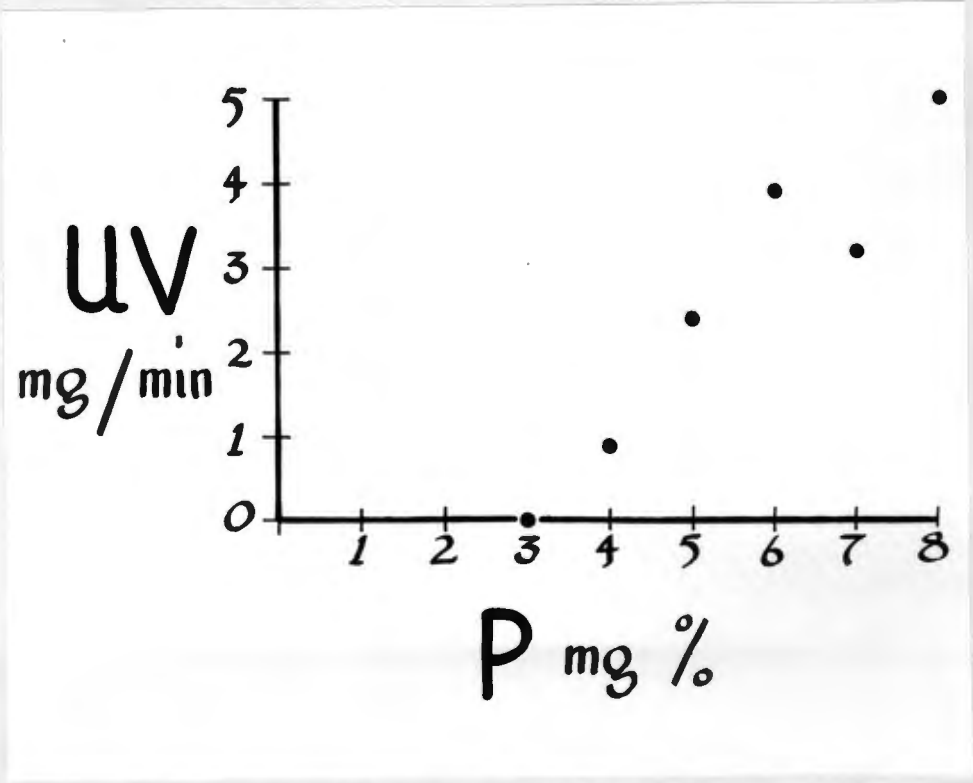

Fig. 5(a):- The points are scattered, and, while showing a trend, do not lie on a straight line.

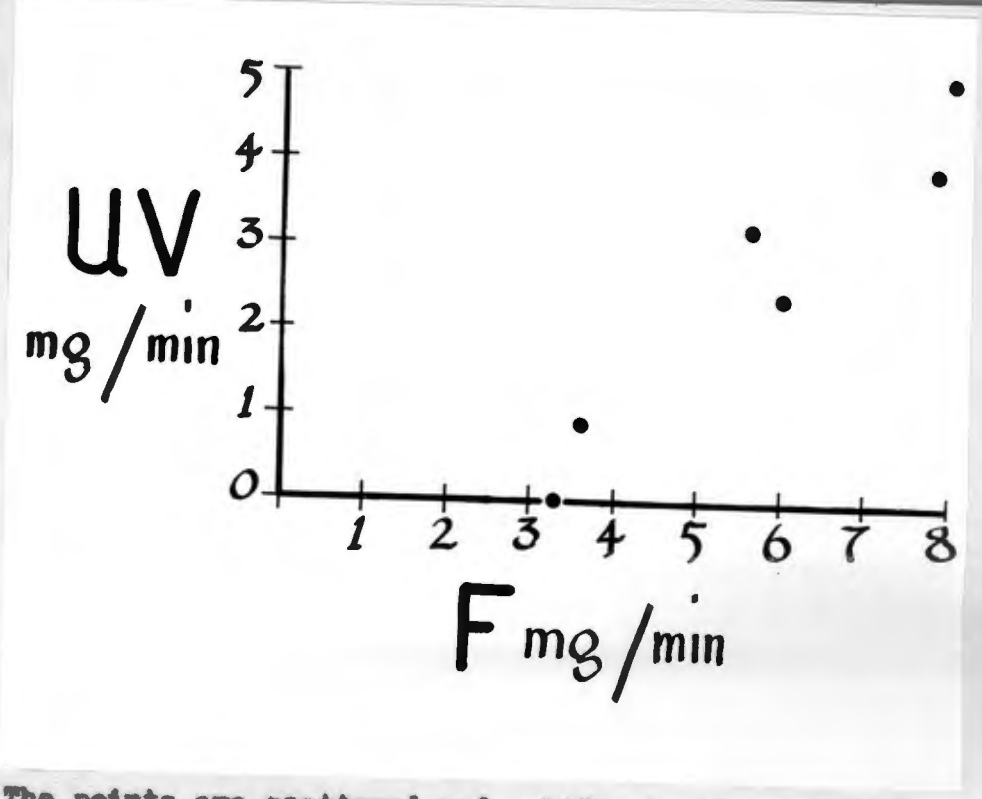

Fig. 5(b):- The points are scattered and, while showing a trend, do not lie on a straight line.

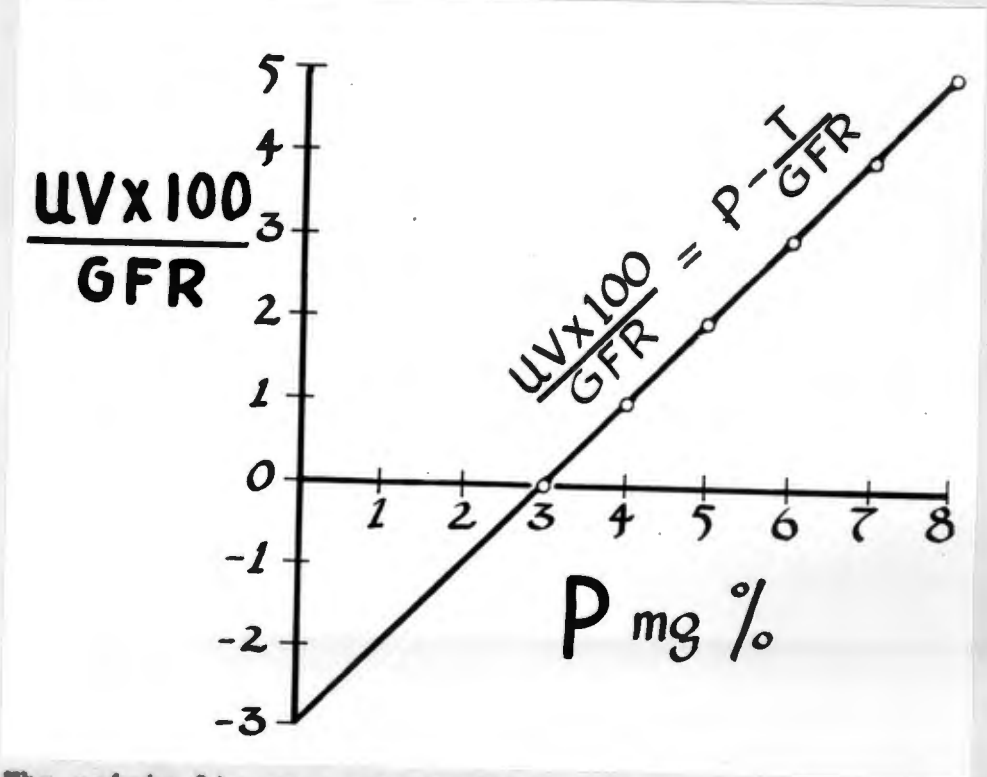

Fig. 5(c):- The points lie on a straight line with good correlation. The regression coefficient is unity and the threshold is indicated by the intercept on the ordinate. There is an error in the equation for the line which should read $\frac{UVx100}{GFR} = P - \frac{Ix100}{GFR}$

Under ideal circumstances, therefore, where the glomerular filtration rate varies and there is a directly proportional change in the rate of tubular transport (i.e. $\frac{Tp}{GFR}$ remains constant) a linear plot will only be obtained when the rate of excretion divided by the glomerular filtration rate is plotted against the plasma concentration.

It is apparent from these three theoretical models that the degree of correlation between variables that is obtained when different co-ordinates are used will provide a measure of the validity of the theories of renal function discussed previously.

The presence of a threshold and the validity of the theory in general will be most readily apparent from data collected while the glomerular filtration rate remains constant - as in Experiment A.

If there is a maximal limiting rate of tubular transport of inorganic phosphorus that is unaffected by changes in glomerular filtration rate this will be revealed by a greater degree of correlation of the points when the rate of excretion in the urine and the filtered load are plotted as co-ordinates than with any other co-ordinates. This is demonstrated in Experiment B.

If, however, the highest degree of correlation is obtained when the rate of excretion divided by the glomerular filtration rate is plotted against the plasma concentration, this would lend strong support to that body of opinion that holds that the threshold is more constant than the maximal reabsorptive capacity of the tubules. This is demonstrated in Experiment C.

Unhappily for the experimenter (and perhaps happily for the experimentee) the mathematically precise state of affairs that was conveniently prevalent during the theoretical experiments does not obtain in practice.

There are a number of known factors that may disturb the ideal relationship, and I shall discuss them individually.

1) Laboratory Errors.

In the hands of an experienced and fastidious technician, such as I was fortunate enough to have working with me, errors in the estimation of inorganic phosphorus and inulin are small and relatively unimportant. In addition, such laboratory errors as do exist are likely to be random rather than systematic provided such pitfalls as a wrongly calibrated pipette or a dirty colorimeter tube are avoided. Duplicate or triplicate analysis also does much to increase the accuracy of the procedures and to give an estimate of the inaccuracy of the techniques.

The laboratory, therefore, will be responsible for a minor degree of poor correlation in the eventual plot.

2) Incomplete bladder emptying and inaccurate timing of collection periods will result in poor correlation when UV is plotted against either F or P. Correlation will however be restored when the quotient of UVp and glomerular filtration rate is plotted against the plasma concentration.

3) Plasma phosphate concentration falling within the threshold range will result in a curvilinear relationship in all plots with an apparent diminution in the slope. The glomerular filtration rate

will be under-estimated when UVp is plotted against Pp and the regression coefficients of the other two graphs will be less than unity.

4) The formation of non-filterable phosphate complexes will affect the graph in either of two ways.

If, at all plasma concentrations a constant fraction of the plasma phosphate is in a non-filterable form, the slope will be decreased in all plots, but the linearity will not be disturbed.

If, however, the formation of non-filterable complexes occurs only at high plasma levels, the expected regression coefficients will be found until such time as "colloidal" phosphate is formed when the slope will be decreased with flattening of the curve and a disturbance of linearity.

5) A progressive increase in the rate of tubular reabsorption of phosphorus over the course of the experiment would diminish the slope of the regression lines in all plots. This effect is represented diagrammatically in Fig. 6.

Such a change in tubular activity could occur if the rate of reabsorption were affected by the concentration in the glomerular filtrate or if the experiment were done at a time of normally varying tubular activity.

If, as has been suggested[20,181], the rate of tubular reabsorption is diminished by prolonged infusion of phosphate, the opposite effect will be found with an increase in the slope of the lines.

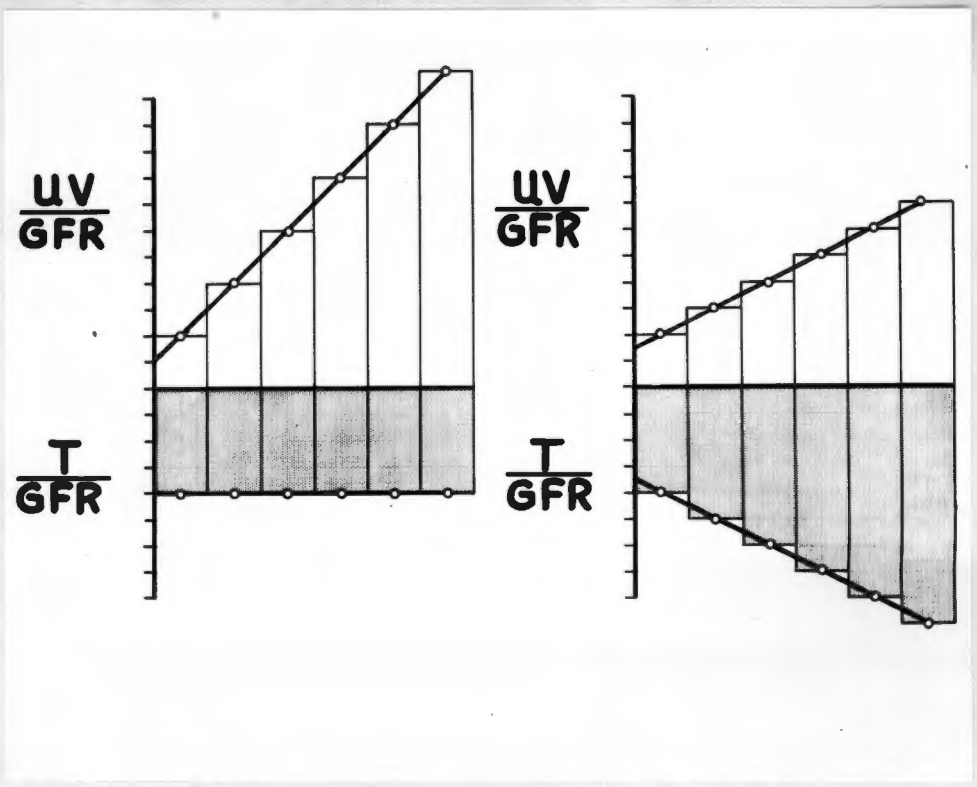

Fig. 6 :- In these two diagrams, where $\frac{T}{GFR}$ is taken as an index of tubular activity (indicated by shaded areas) the effect of a progressive increase in tubular activity upon the slope of the top line can be seen by comparing the right hand diagram with the left.

6) Renal delay time will, if reasonably constant, have no effect on the correlation of the points, the linearity of the plot or the slope of the line, but will exert a pronounced effect upon the threshold value and the rate of reabsorption of phosphate. This effect is shown diagrammatically in Fig. 7 where the line BB_1 is the apparent line and the line AA_1 is the real line with the discrepancy due to renal delay time.

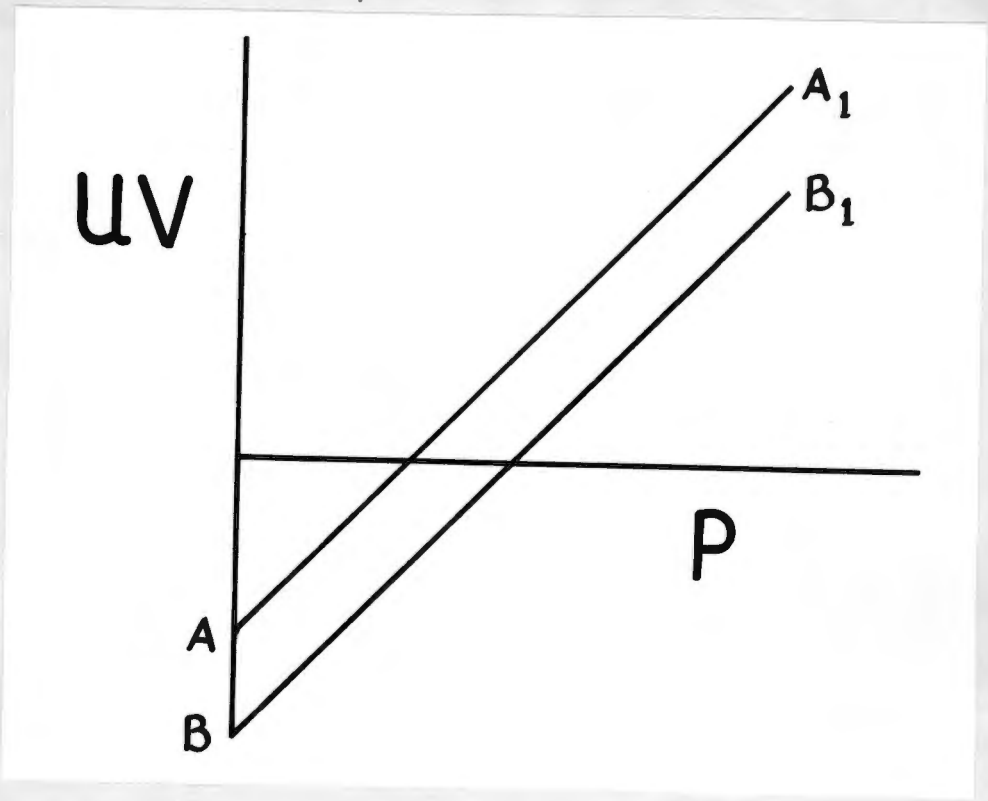

Fig. 7 :- Illustrates the effect of renal delay time.

One further point that needs emphasis is that the regression coefficient and the negative intercept on the ordinate cannot be considered separately. Owing to the fact that the line "pivots" around the lowest parameter, any factor which operates to diminish the slope will similarly diminish the negative value of the intercept.

The above disturbing factors are those which are known to apply in renal titration experiments. That there are probably others, I do not doubt.

SUMMARY.

In renal titration experiments, when the data are graphically represented by the three pairs of co-ordinates mentioned, and

(1) Poor correlation is found when UVp is plotted against Pp and when UVp is plotted against Fp but there is good correlation when $\frac{UVp}{GFR}$ is plotted against Pp, this is due to inconstancy of the glomerular filtration rate with proportional change in the phosphate reabsorption either real or apparent due to inaccurate timing of collection periods or incomplete bladder emptying.

(2) Poor correlation is found in all plots; this may be due to (a) errors in laboratory technique or (b) random variation in the rate of reabsorption of phosphate.

(3) Good correlation is found with poor linearity; this may be due to (a) plasma phosphate concentrations that fall within the threshold range, or (b) the formation of non-filterable phosphate complexes at high plasma concentration.

(4) The regression coefficients are lower than would be expected; this could be due to

(a) The presence of a constant proportion of the plasma phosphate in a non-filterable form.
(b) The superimposition of the experimental period upon a period of normally increasing tubular activity.
(c) Progressively increasing rates of tubular absorption with increasing filtered loads of phosphate, or
(d) the fact that the highest plasma concentration achieved has not exceeded the maximal threshold level.

Having defined the manner in which renal titration experiments may be interpreted I shall present for analysis my own experimental results.

An Analysis of thirteen renal titration experiments.

Material and Methods.

The subjects used in this study were all, with the exception of subject B.A., healthy male students recruited as volunteers from a University residence where I act in the capacity of medical officer.

The subjects were selected on the basis of prominence of arm veins to facilitate venipuncture during the course of the infusion. It was explained to them that they would be expected to pass urine at 20 minute intervals on demand for 3 hours, and they were requested to practise bladder voiding. All were imbued with a healthy competitive spirit, and competed eagerly for the prize I offered to the student with the largest minute volume of urine. Urine collections were made by spontaneous voiding, the cessation of flow being taken as the end-point of the period. The collection periods were timed by the simultaneous pressing of two stop watches (to start the one watch and to stop the other). Urine was passed directly into a measuring cylinder, and after recording the volume for championship purposes the urine was made up to a convenient volume with distilled water to facilitate subsequent dilution for analysis.

Blood was collected by veni-puncture without anti-coagulant approximately 2½ minutes before the midpoint of each period, and was separated by centrifugation after allowing 30 minutes for clot retractions to occur.

The experiments were all performed with the subjects in the fasting state and covered the three hours from 9 a.m. to 12 noon in all instances.

The experiments were performed with the subjects sitting in an armchair and rising only to pass urine. Smoking was prohibited during the experimental period.

A solution of 10% inulin made up to a convenient volume with 0.9% NaCl solution was administered by venoclysis at a constant rate calculated to maintain a plasma concentration of approximately 40 mg.% This infusion was started approximately one hour before the beginning of the first collection period, and was followed immediately by a priming injection of 10% inulin injected into the tubing of the administration set so that one hour was allowed for equilibration of the inulin before measurements of the glomerular filtration rate were commenced.

An infusion of sterile buffered phosphate similar to that used by Anderson[12] was administered through a second "push-in" at progressively increasing speeds from a constant infusion pump. The pump was reset every five minutes to give a greater speed of infusion, the setting being read off a graph drawn beforehand from the equation given by Anderson[12]. As can be seen from the tables, a reasonably linear rise in plasma phosphate concentration was obtained.

Subject B.A. was a coloured male of 42 years of age convalescent from a laparotomy performed for a stab wound in the abdomen. As B.A. was unable to pass urine spontaneously, urine was collected with an indwelling multi-eyed catheter and the periods terminated by washout with distilled water and air - the latter expressed by supra pubic compression.

Subject L.McK. had had a haemorrhoidectomy performed one week before the experiment.

Subject V.M.G. fainted during the equilibration period but recovered immediately and was able to continue the experiment.

A total of 18 experiments were done, but five were rejected for no other reason than that I did not think they had "gone well". Analyses were not done on the specimens obtained from these experiments.

Further details concerning the solutions used and their mode of administration will be found in Appendix B.

Details of times and comments on the conduct of experiments will be found in Appendix A.

The results are presented in tabular form followed by three graphs for each experiment. The first graph shows the data plotted with co-ordinates UVp and Pp, the second with co-ordinates UVp and Fp and the third with co-ordinates $\frac{UVp \times 100}{G.F.R.}$ and Pp.

A short comment on the data is given for each experiment.

EXPERIMENT : No. I Subject : T. le R.

Table No. 1

Period	Time (mins.)	Pp (mg.%)	G.F.R. (ml./min)	Fp (mg./min)	UVp (mg./min)	Tp (mg./min)	$\frac{Tp \times 100}{G.F.R.}$	$\frac{UVp \times 100}{G.F.R.}$
I	20.0	3.9	148.6	5.80	0.59	5.21	3.51	0.40
II	19.5	4.6	142.0	6.53	2.14	4.39	3.09	1.51
III	21.0	5.8	141.0	8.17	3.41	4.76	3.38	2.42
IV	20.0	6.4	139.6	8.94	3.91	5.03	3.60	2.80
V	20.0	6.9	140.1	9.67	4.72	4.95	3.53	3.37
VI	20.0	7.6	141.0	10.71	5.47	5.24	3.79	3.88
VII	20.0	8.3	138.9	11.53	6.54	4.99	3.59	4.71
VIII	19.0	8.9	140.3	12.49	7.93	4.56	3.25	5.65
IX	20.5	9.5	139.7	13.27	8.72	4.55	3.26	6.24

Pre-infusion plasma phosphorus concentration - 3.4 mg.%

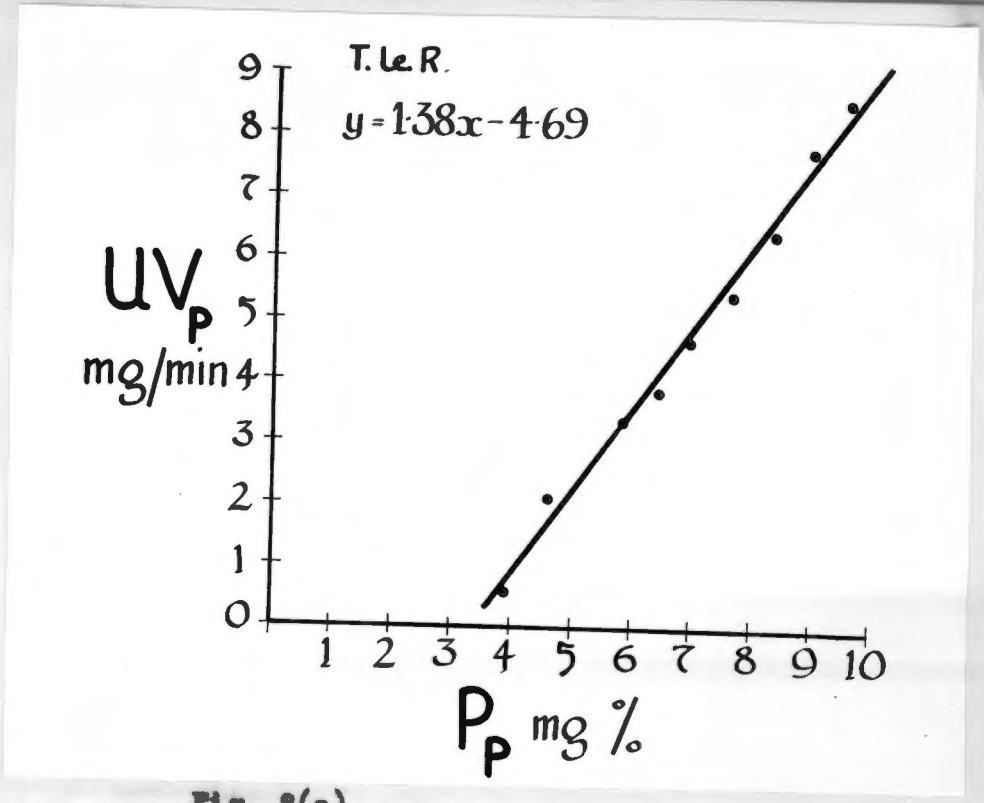
T. Le R.
y = 1·38x - 4·69
UVP mg/min
PP mg %
0
1
2
3
4
5
6
7
8
9
10

Fig. 8(a)

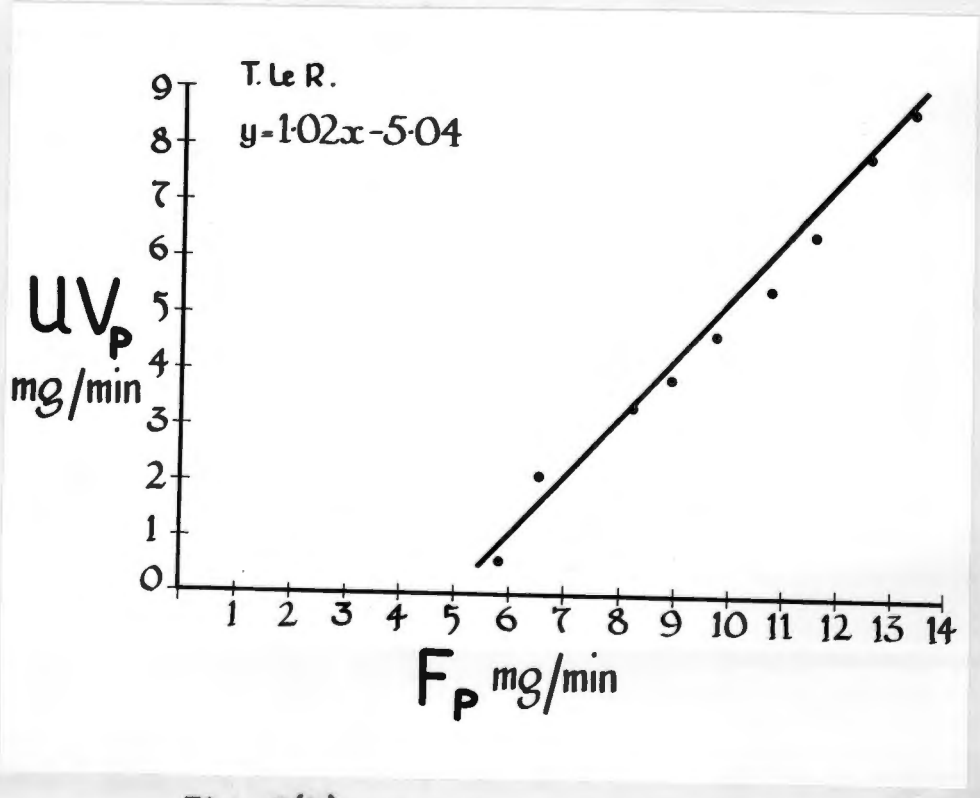
T. Le R.
y = 1·02x - 5·04
UVP mg/min
FP mg/min
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14

Fig. 8(b)

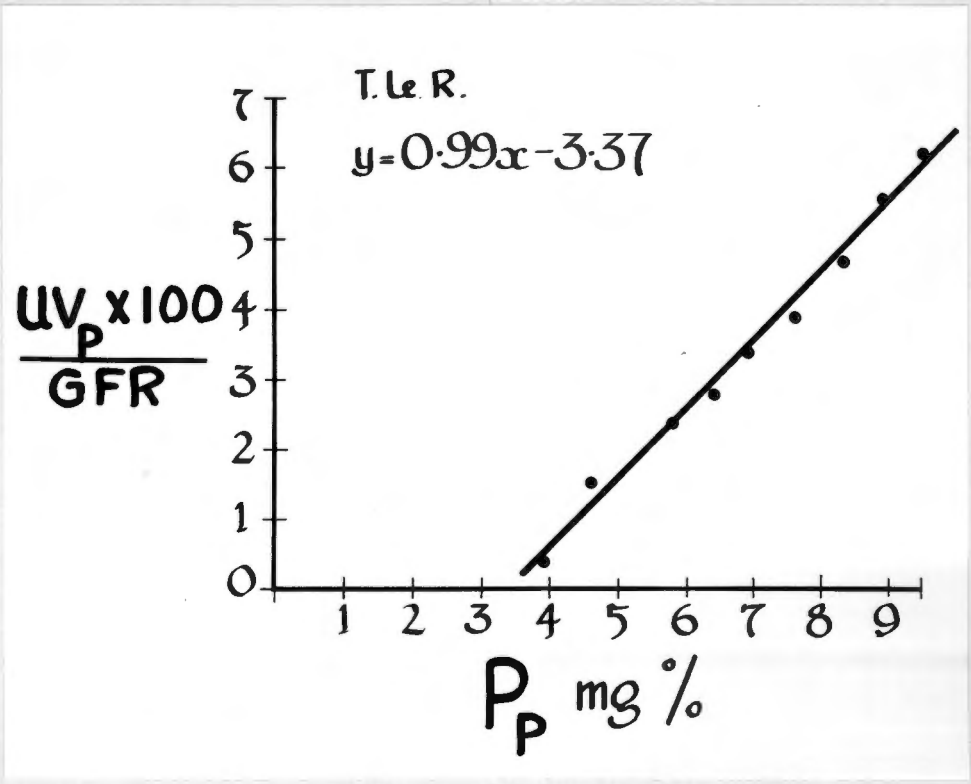

Fig. 8(c)

Comment :- Good correlation and linearity in all graphs.

EXPERIMENT : No. II Subject : V.M.G.

Table No.2

Period	Time (mins.)	Pp (mg.%)	G.F.R. (ml./min)	Fp (mg./min.)	UVp (mg./min.)	Tp (mg./min.)	$\frac{Tp \times 100}{G.F.R.}$	$\frac{UVp \times 100}{G.F.R.}$
I	19.0	5.1	138.0	7.04	1.20	5.84	4.23	0.87
II	20.5	5.3	136.3	7.23	1.44	5.79	4.24	1.06
III	21.0	5.9	132.4	7.83	1.55	6.28	4.74	1.17
IV	19.0	6.7	137.0	9.18	2.21	6.97	5.08	1.61
V	22.0	7.5	134.0	10.06	3.89	6.17	4.61	2.90
VI	20.0	8.3	134.1	11.13	7.42	3.71	2.77	5.53
VII	20.5	9.2	134.6	12.38	5.95	6.43	4.76	4.42
VIII	21.0	10.0	134.7	13.47	7.05	6.42	4.77	5.23
IX	22.0	10.6	134.0	14.22	7.33	6.89	5.14	5.47

Pre-infusion serum phosphorus concentration 4.4 mg.%

UVp for period VI is probably too high owing to technical error.

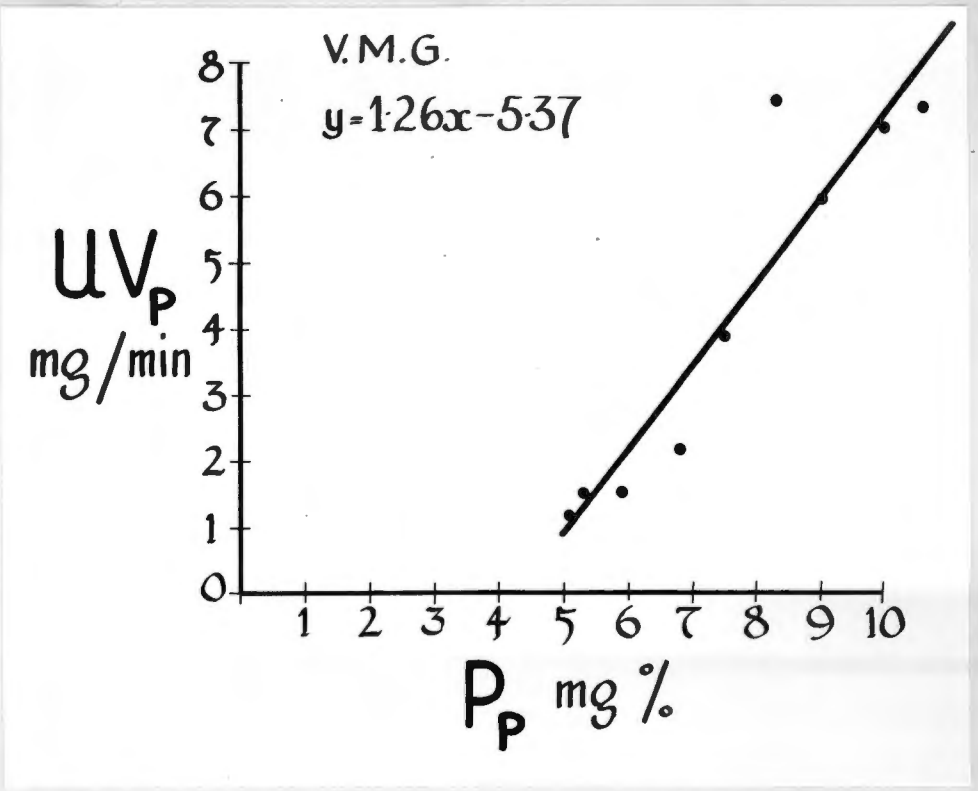
V.M.G.
y=1·26x-5·37
UVP mg/min
PP mg %
0
1
2
3
4
5
6
7
8
1 2 3 4 5 6 7 8 9 10

Fig. 9(a)

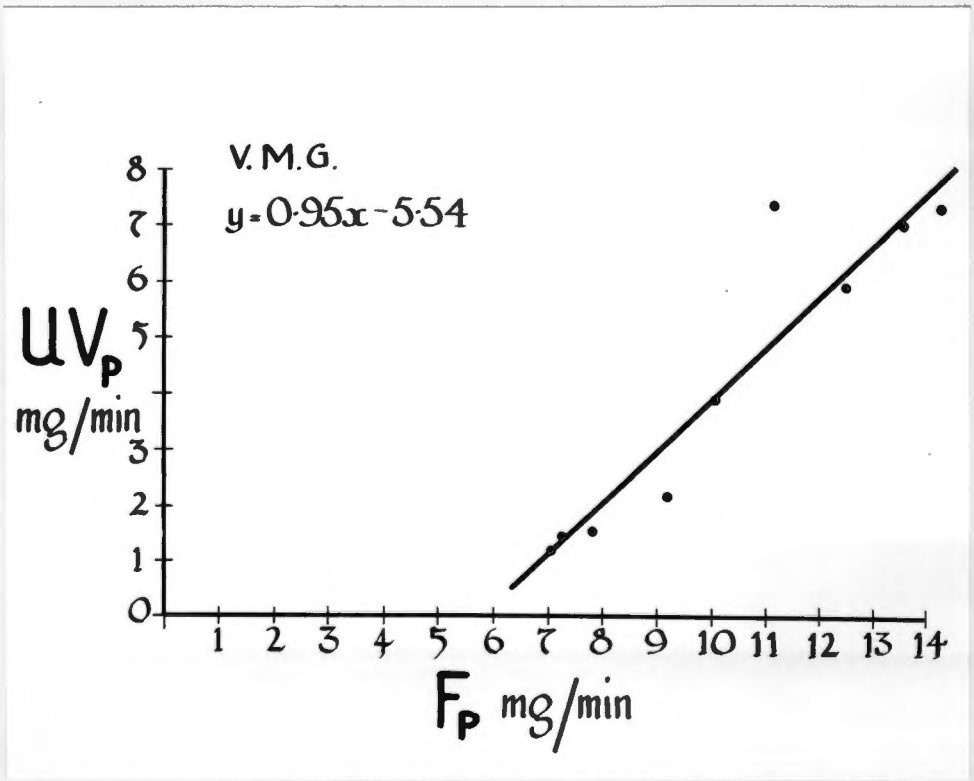
V.M.G.
y=0·95x-5·54
UVP mg/min
FP mg/min
0
1
2
3
4
5
6
7
8
1 2 3 4 5 6 7 8 9 10 11 12 13 14

Fig. 9(b)

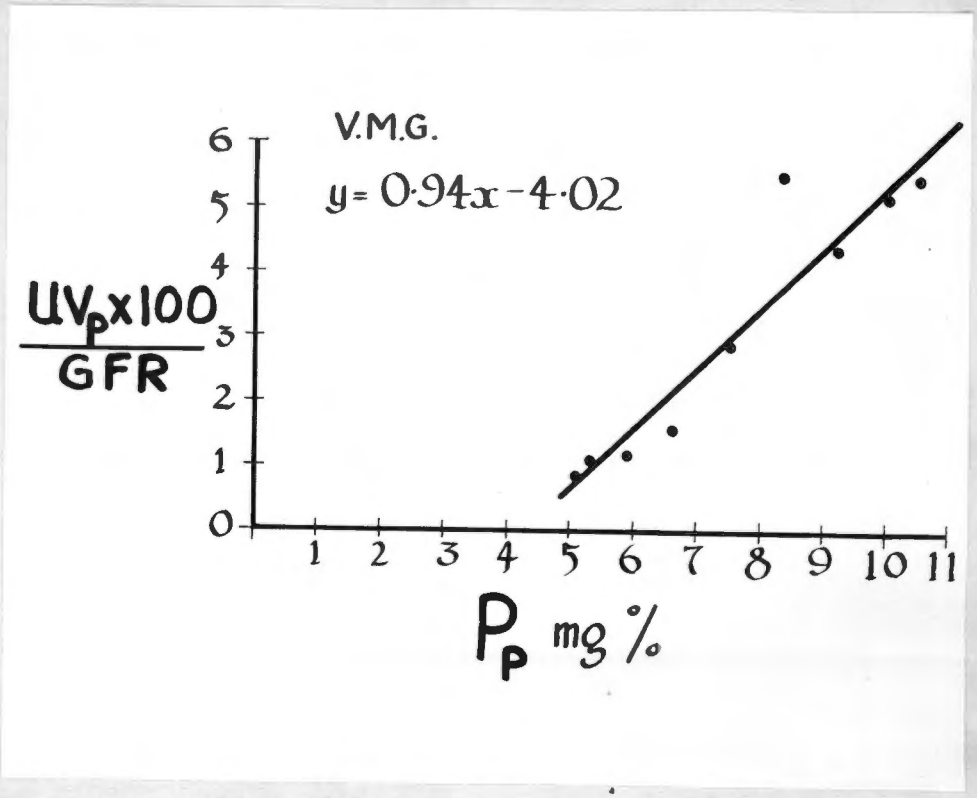

Fig. 9(c)

Comment:- Good correlation in all graphs with good linearity.

EXPERIMENT : No. III Subject : A.v.d.M.

Table No. 3.

Period	Time (mins.)	Pp (mg.%)	G.F.R. (ml./min)	Fp (mg./min.)	UVp (mg./min.)	Tp (mg./min.)	$\frac{Tp \times 100}{G.F.R.}$	$\frac{UVp \times 100}{G.F.R.}$
I	21.5	4.4	99.3	4.37	0.64	3.73	3.76	0.64
II	20.0	5.2	98.4	5.12	1.41	3.71	3.77	1.43
III	20.0	6.1	99.7	6.08	2.11	3.97	3.98	2.12
IV	19.5	6.9	100.1	6.91	2.91	4.00	3.99	2.91
V	18.0	7.6	98.7	7.50	3.67	3.83	3.88	3.72
VI	19.5	8.4	97.4	8.18	4.43	3.75	3.85	4.55
VII	20.0	9.3	102.0	9.48	5.48	4.00	3.92	5.37
VIII	21.0	10.2x	101.0x	10.30	6.66	3.64	3.61	6.60
IX	24.0	11.1	103.1	11.44	7.25	4.19	4.06	7.03

x As the blood specimen for this period was lost in centrifugation, interpolated values were used for Pp and for the plasma inulin concentration in calculating the G.F.R.

Pre-infusion serum phosphorus concentration - 3.7 mg.%

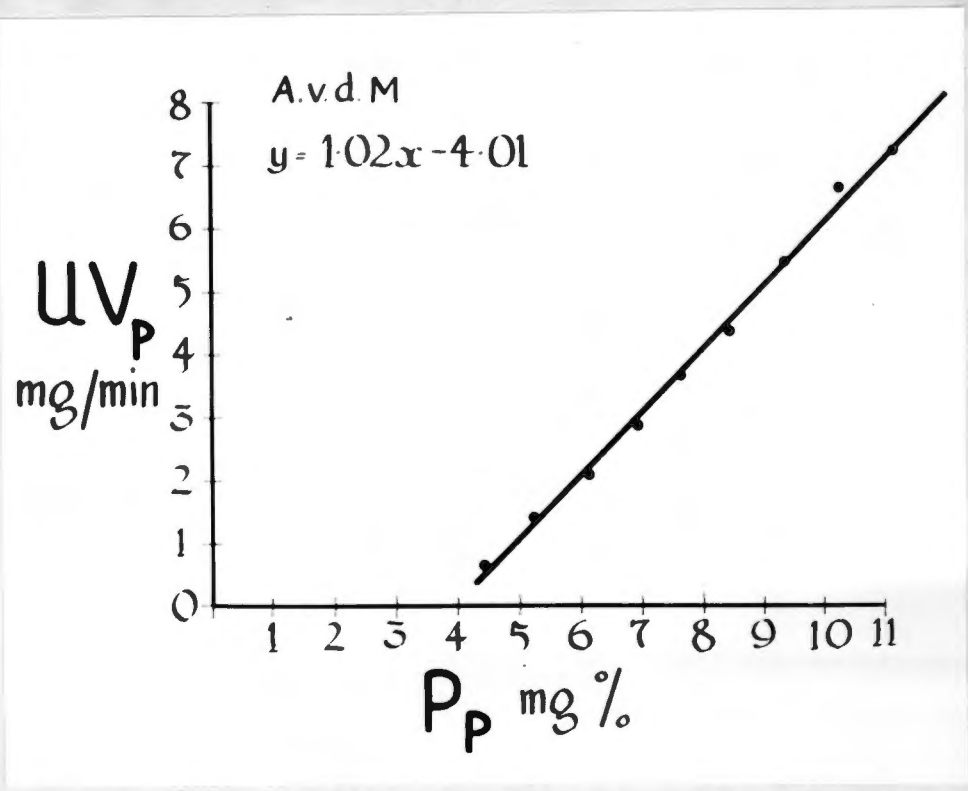

Fig. 10(a)

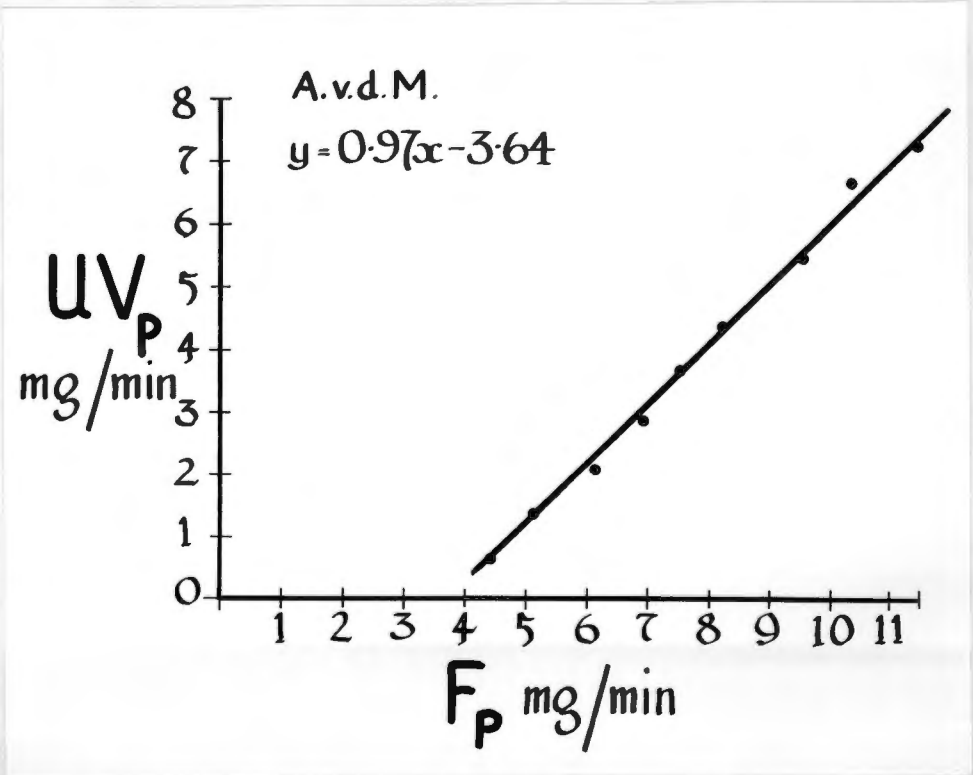

Fig. 10(b)

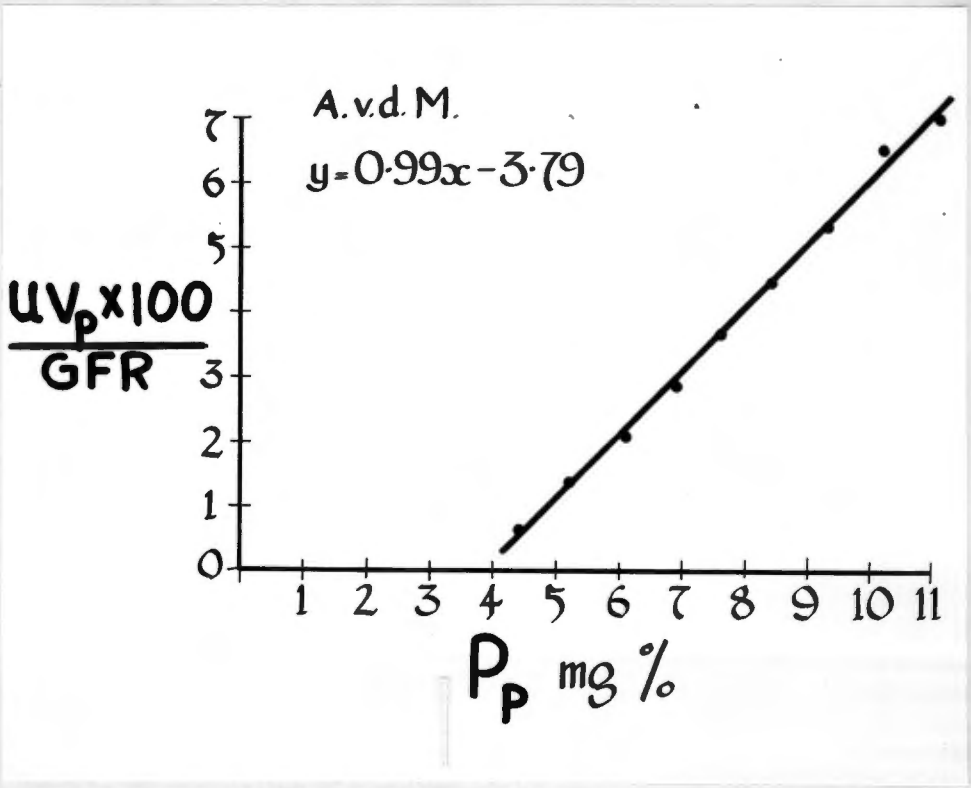

Fig. 10(c)

Comment :- High degree of linearity and correlation in all graphs.

EXPERIMENT : No. IV Subject : B.R.F.

Table No. 4.

Period	Time (mins.)	Pp (mg.%)	G.F.R. (ml./min.)	Fp (mg./min.)	UVp (mg./min.)	Tp (mg./min.)	$\frac{Tp \times 100}{G.F.R.}$	$\frac{UVp \times 100}{G.F.R.}$
I	19.5	5.3	104.8	5.55	0.94	4.61	4.40	0.90
II	20.0	6.2	105.1	6.52	1.83	4.69	4.46	1.74
III	21.0	7.2	104.7	7.53	2.85	4.68	4.48	2.72
IV	18.5	8.3	103.9	8.63	4.08	4.55	4.38	3.93
V	22.0	9.5	104.2	9.90	5.29	4.61	4.42	5.08
VI	20.0	10.3	105.0	10.82	5.73	5.09	4.84	5.46
VII	19.5	11.3	104.9	11.85	6.65	5.20	4.96	6.34
VIII	21.0	12.4	105.3	13.06	7.76	5.30	5.02	7.37
IX	19.5	13.8	103.7	14.31	9.14	5.17	4.99	8.81

Pre-infusion serum phosphorus concentration - 4.6 mg.%

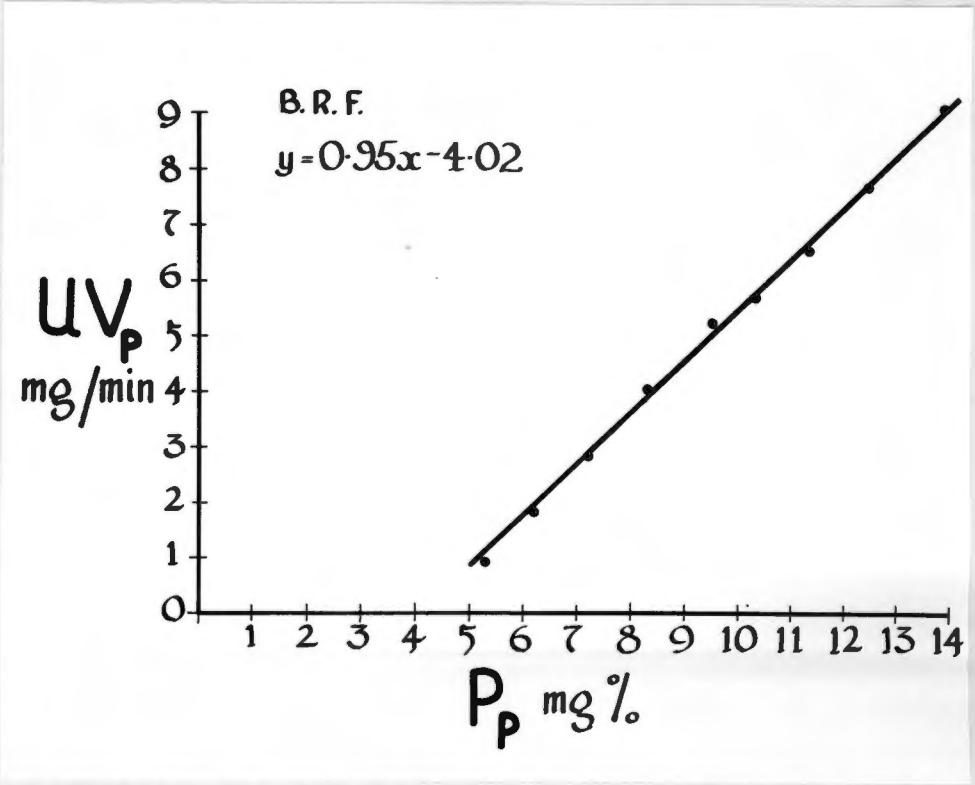

Fig. 11(a)

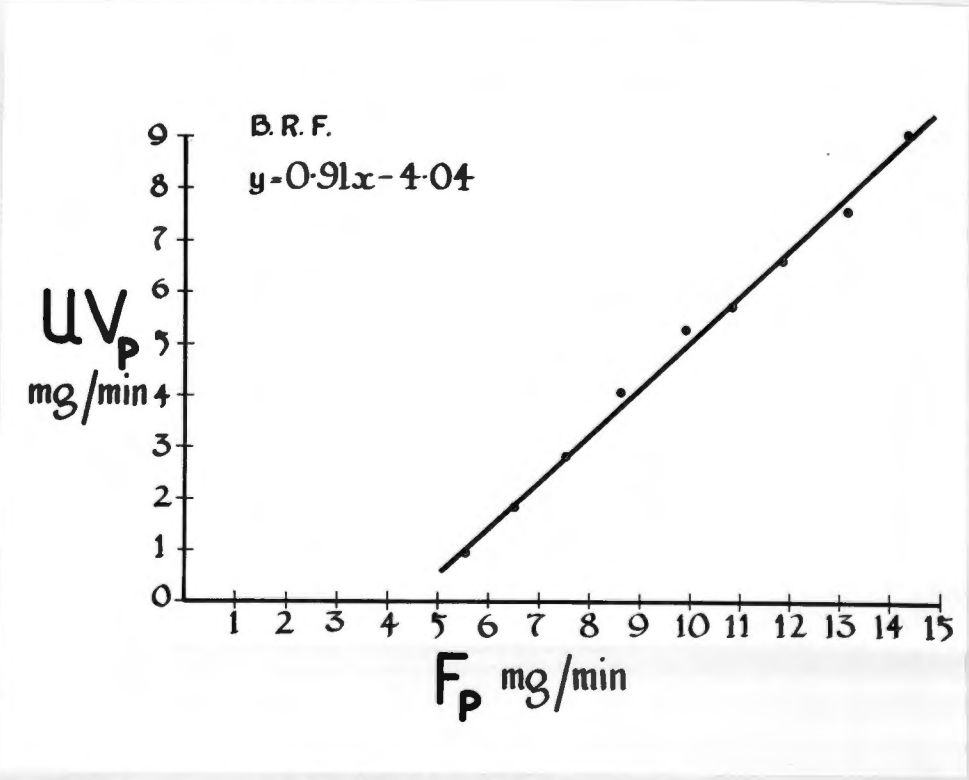

Fig. 11(b)

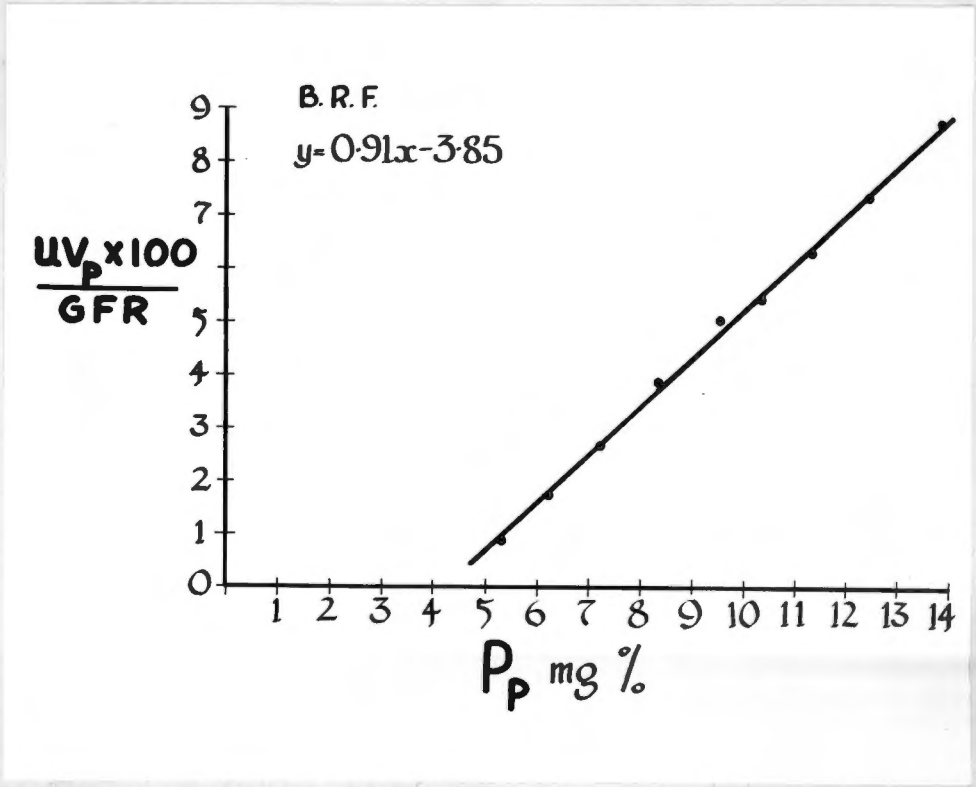

Fig. 11(c)

Comment :- There is a high degree of correlation in all graphs with good linearity.

EXPERIMENT : No. V Subject : L.A.E.

Table No. 5

Period	Time (mins.)	Pp (mg.%)	G.F.R. (ml./min.)	Fp (mg./min.)	UVp (mg./min.)	Tp (mg./min.)	$\frac{Tp \times 100}{G.F.R.}$	$\frac{UV \times 100}{G.F.R.}$
I	19.0	3.7	104.8	3.88	0.84	3.04	2.9	0.80
II	21.0	4.9	106.0	5.19	1.14	4.05	3.82	1.08
III	22.0	6.1	105.2	6.42	2.15	4.27	4.05	2.04
IV	18.5	7.2	104.9	7.55	2.75	4.80	4.57	2.62
V	19.5	7.9	104.9	8.29	3.33	4.96	4.71	3.17
VI	22.0	8.7	105.4	9.17	4.11	5.06	4.80	3.90
VII	20.0	9.3	107.0	9.95	5.44	4.51	4.21	5.08
VIII	19.0	9.9	106.3	10.53	5.52	5.01	4.71	5.19
IX	19.5	10.8	105.2	11.36	6.34	5.02	4.76	6.03

Pre-infusion serum phosphorus concentration - 3.4 mg.%

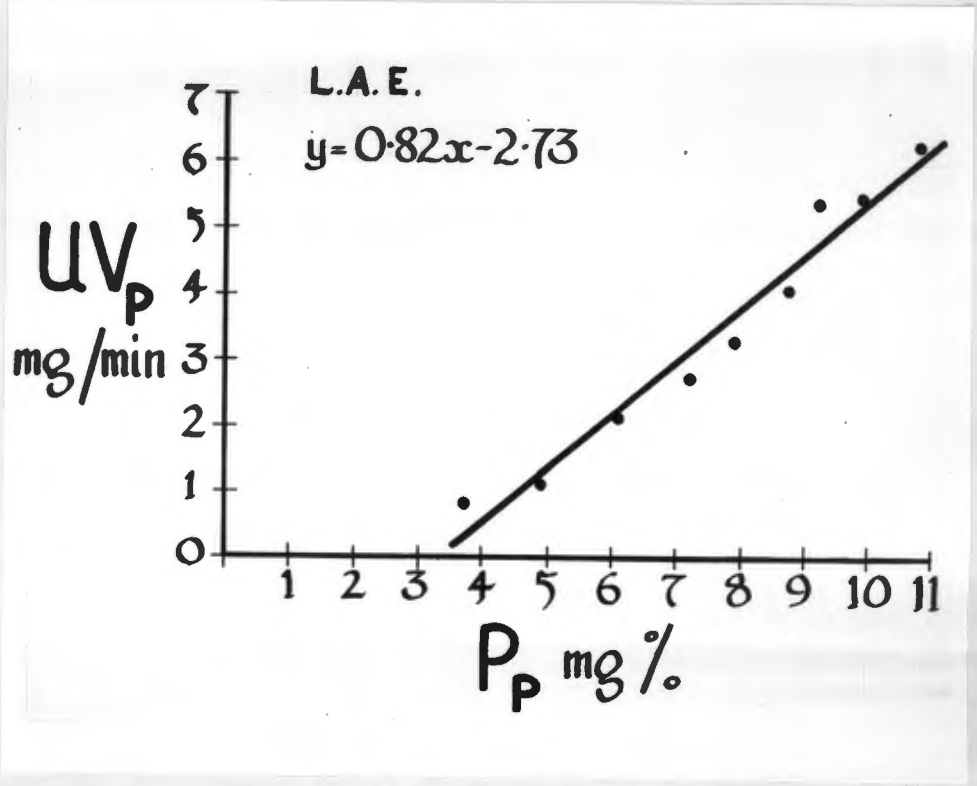

Fig. 12(a)

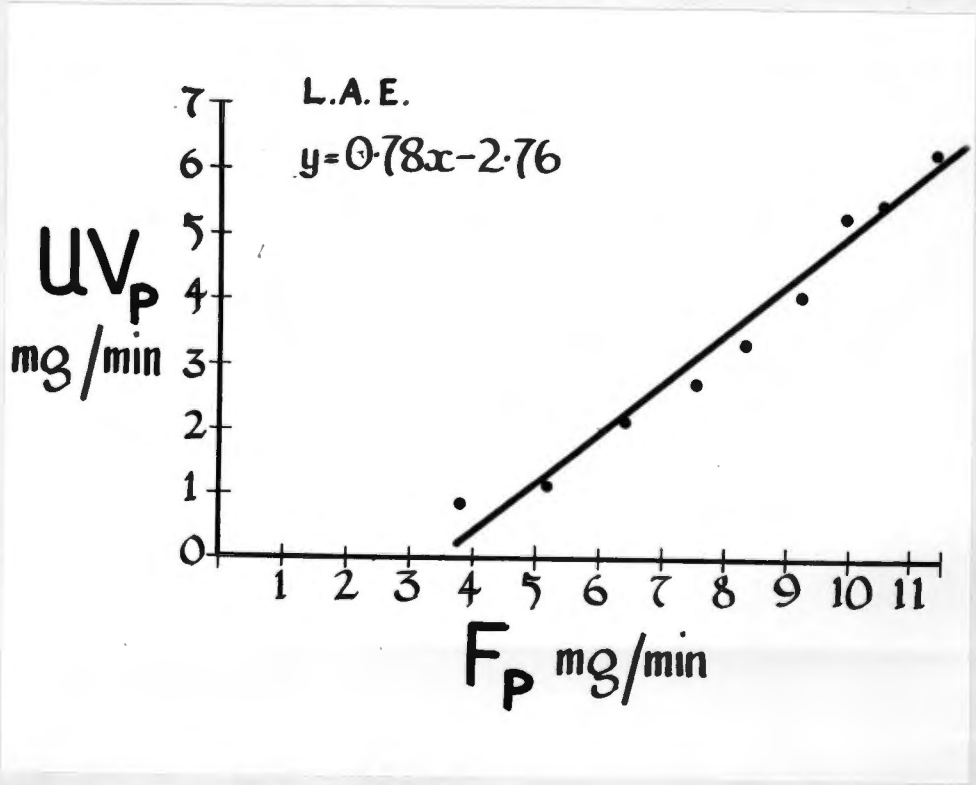

Fig. 12(b)

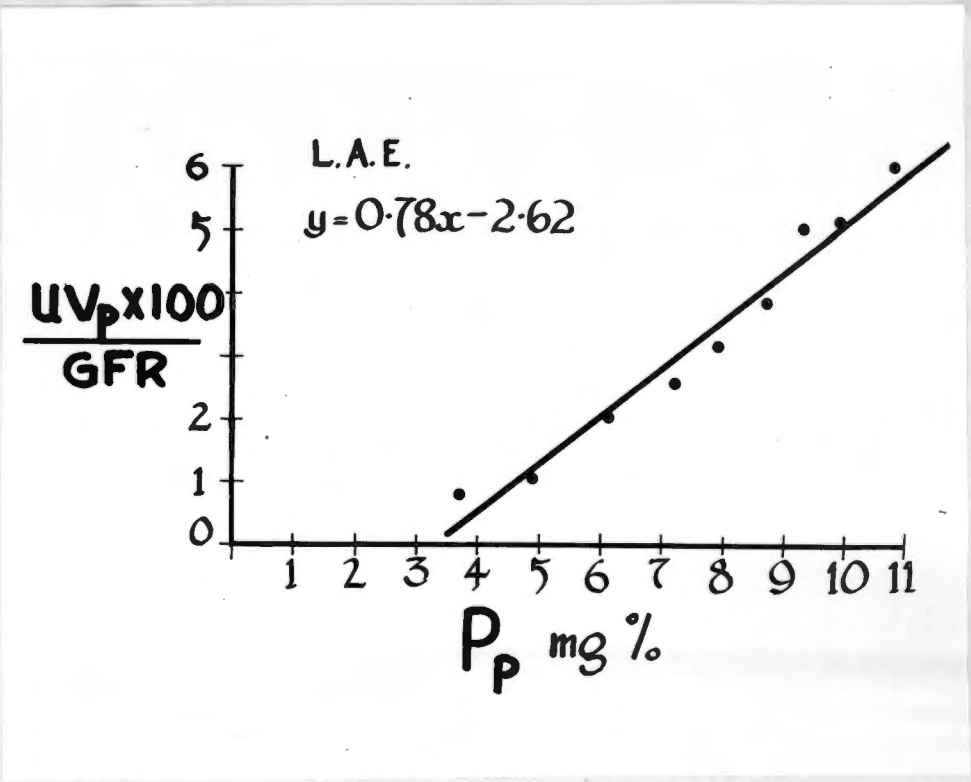

Fig. 12(c)

Comment :- There is good correlation and linearity in all plots.

EXPERIMENT : No. VI Subject : B.A.

Table No. 6

Period	Time (mins.)	Pp (mg.%)	G.F.R. (ml./min.)	Fp (mg./min.)	UVp (mg./min.)	Tp (mg./min.)	$\frac{Tp \times 100}{G.F.R.}$	$\frac{UVp \times 100}{G.F.R.}$
I	20.5	4.3	117.3	5.05	0.45	4.60	3.92	0.38
II	21.0	4.9	116.8	5.72	0.60	5.12	4.27	0.51
III	19.0	5.6	118.0	6.61	1.26	5.35	4.54	1.07
IV	20.5	6.4	117.6	7.52	1.45	6.07	5.16	1.23
V	19.0	7.2	117.3	8.44	2.39	6.05	5.15	2.04
VI	21.0	8.0	115.9	9.27	3.77	5.50	4.75	3.25
VII	22.0	8.9	117.3	10.44	4.91	5.53	4.72	4.19
VIII	18.0	9.5	117.9	11.20	4.01	7.19	6.1	3.40
IX	19.0	10.3	117.6	12.12	5.22	6.90	5.87	4.44

Pre-infusion serum phosphorus concentration 4.7 mg.% (slightly haemolysed).

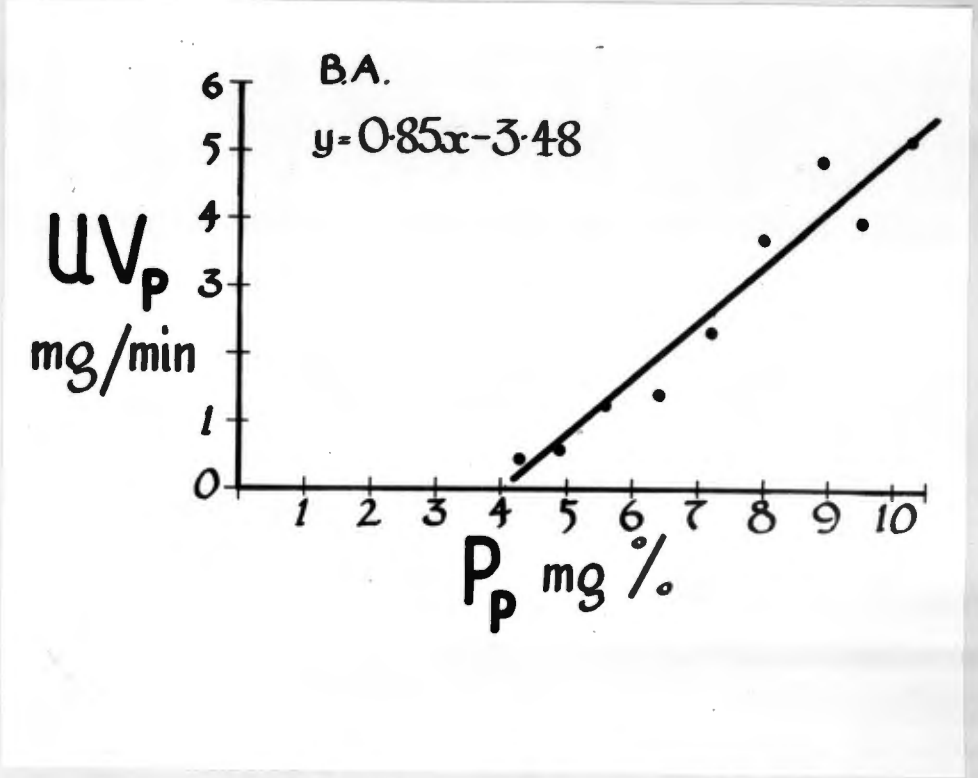

Fig. 13(a)

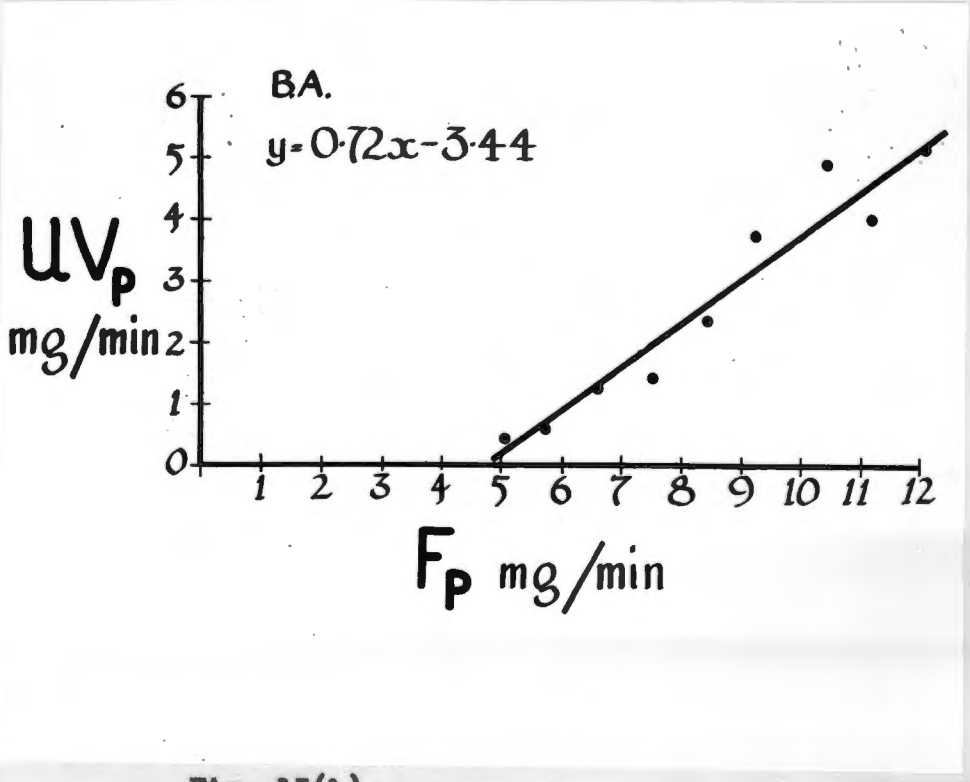

Fig. 13(b)

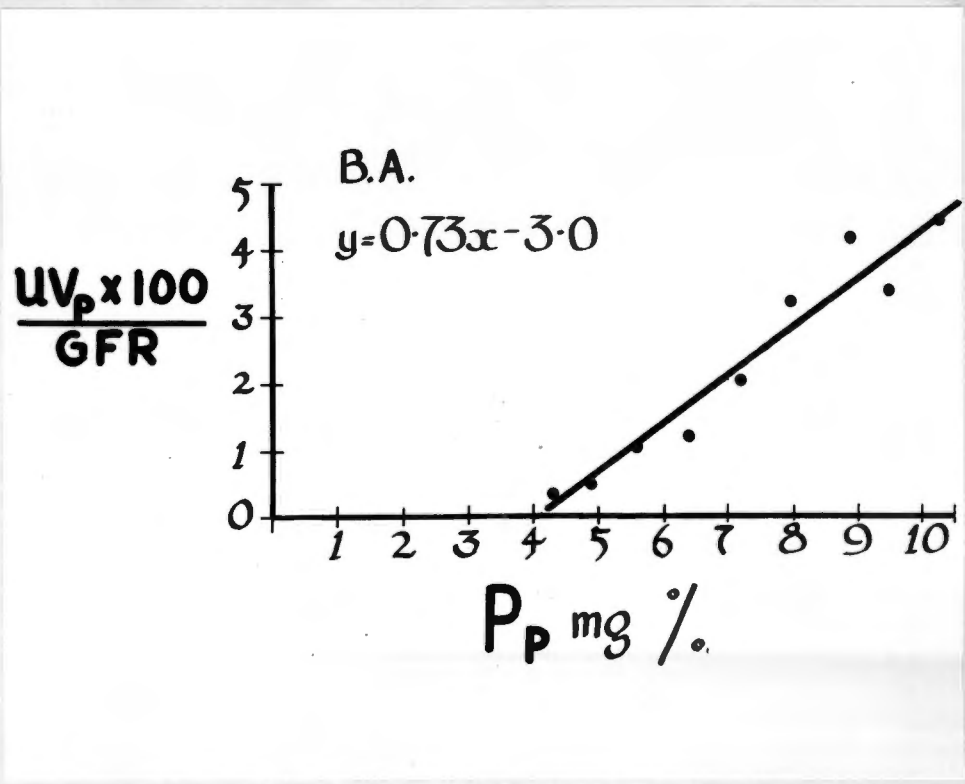

Fig. 13(c)

Comment :- There is reasonably good correlation in all plots with good linearity.

EXPERIMENT : No. VII Subject : L. G.

Table No. 7

Period	Time (mins.)	Pp (mg.%)	G.F.R. (ml./min.)	Fp (mg./min.)	UVp (mg./min.)	Tp (mg./min.)	Tp x 100 / G.F.R.	UVp x 100 / G.F.R.
I	20.5	4.9	159.0	7.79	1.21	6.58	4.14	0.76
II	20.0	5.4	151.0	8.15	1.28	6.87	4.55	0.85
III	18.0	6.0	153.2	9.20	1.73	7.47	4.88	1.13
IV	20.0	6.7	156.0	10.45	2.29	8.16	5.23	1.47
V	22.0	7.2	155.4	11.19	3.58	7.61	4.89	2.30
VI	19.5	7.7	152.3	11.72	3.99	7.73	5.08	2.62
VII	20.5	8.0	158.6	12.45	4.51	7.94	5.01	2.90
VIII	19.0	8.4	154.7	13.00	5.49	7.51	4.85	3.55
IX	22.0	8.9	153.2	13.64	6.48	7.16	4.67	4.23

Pre-infusion plasma phosphorus concentration - 3.8 mg.%

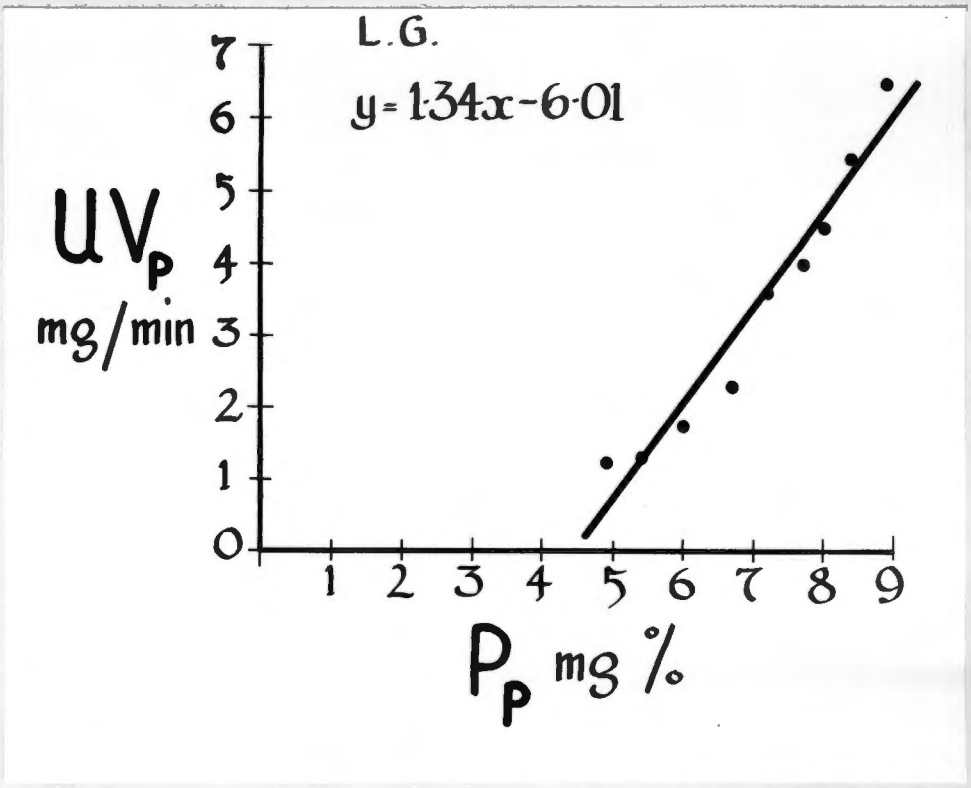

Fig. 14(a)

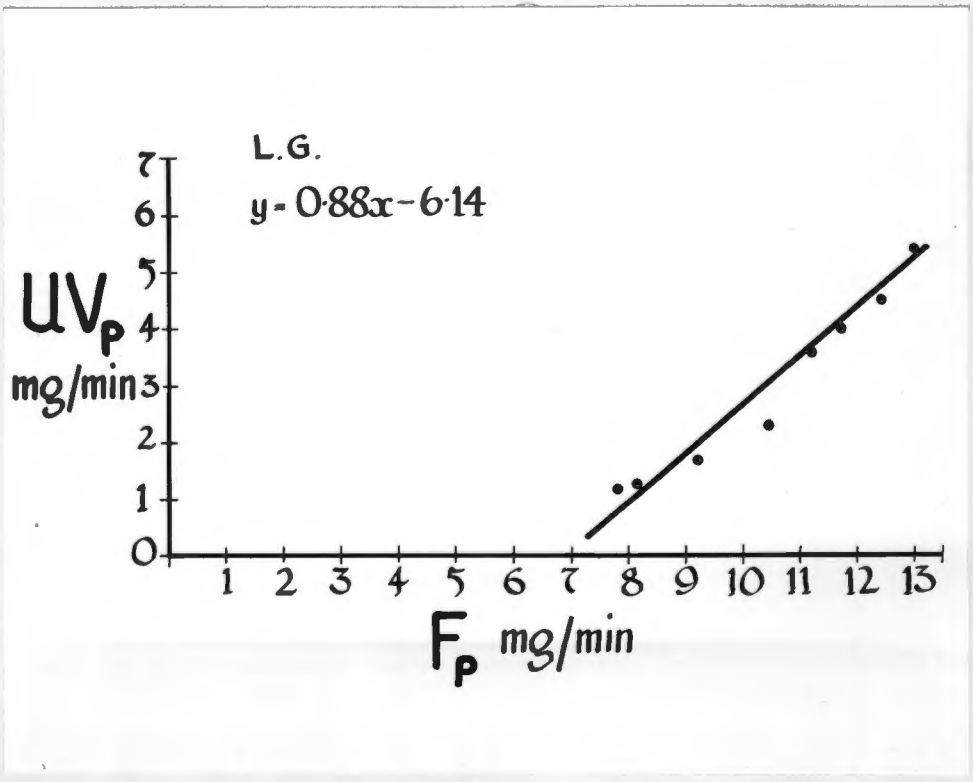

Fig. 14(b)

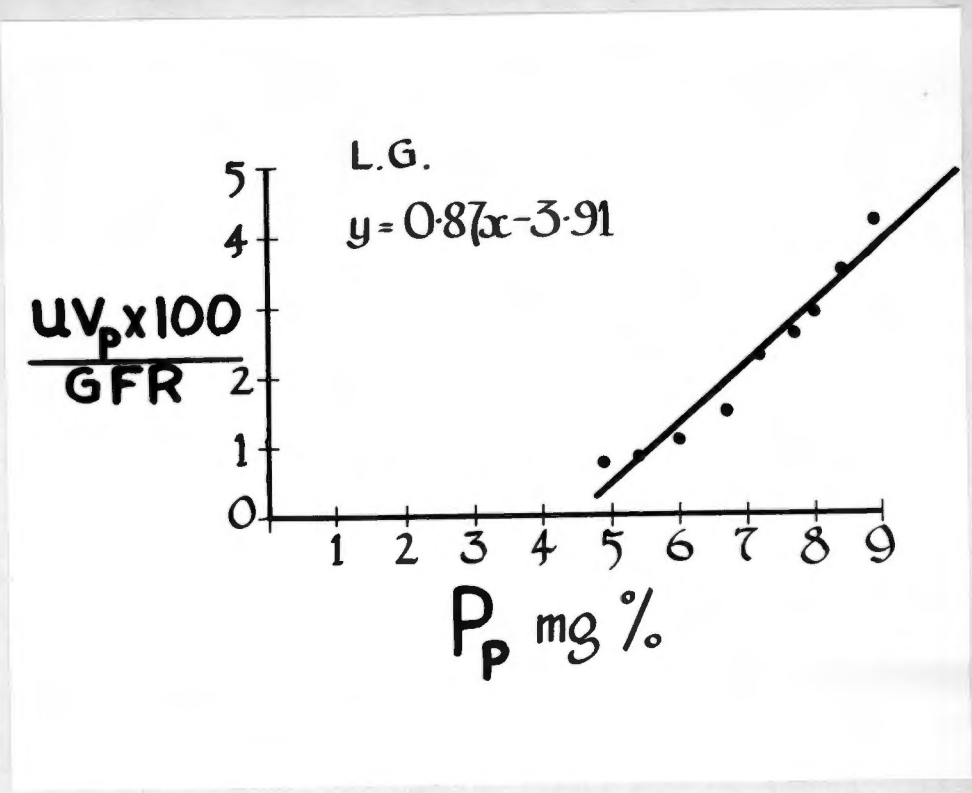

Fig. 14(c)

Comment :- Good correlation in all graphs.

Regression coefficients in Figs. (b) and (c) less than unity.

Curved line would fit points better than straight line.

EXPERIMENT : No. VIII Subject : C.E.

Table No. 8

Period	Time (mins.)	Pp (mg.%)	G.F.R. (ml./min.)	Fp (mg./min.)	UVp (mg./min.)	Tp (mg./min.)	$\frac{Tp \times 100}{G.F.R.}$	$\frac{UVp \times 100}{G.F.R.}$
I	21.0	5.6	124.2	6.96	2.00	4.96	3.99	1.61
II	20.0	5.9	123.8	7.31	2.35	4.96	4.00	1.90
III	19.0	6.6	124.9	8.24	2.49	5.75	4.60	1.99
IV	19.5	7.4	125.0	9.25	2.75	6.50	5.20	2.20
V	20.5	8.1	127.0	10.29	3.06	7.23	5.69	2.41
VI	19.5	8.8	123.6	10.87	3.79	7.08	5.74	3.07
VII	18.5	9.5	122.9	11.67	4.55	7.12	5.79	3.70
VIII	20.0	10.2	123.3	12.58	5.70	6.88	5.58	4.62
IX	20.0	11.0	127.0	13.96	6.36	7.60	5.97	5.01

Pre-infusion serum phosphorus concentration 4.9 mg.%

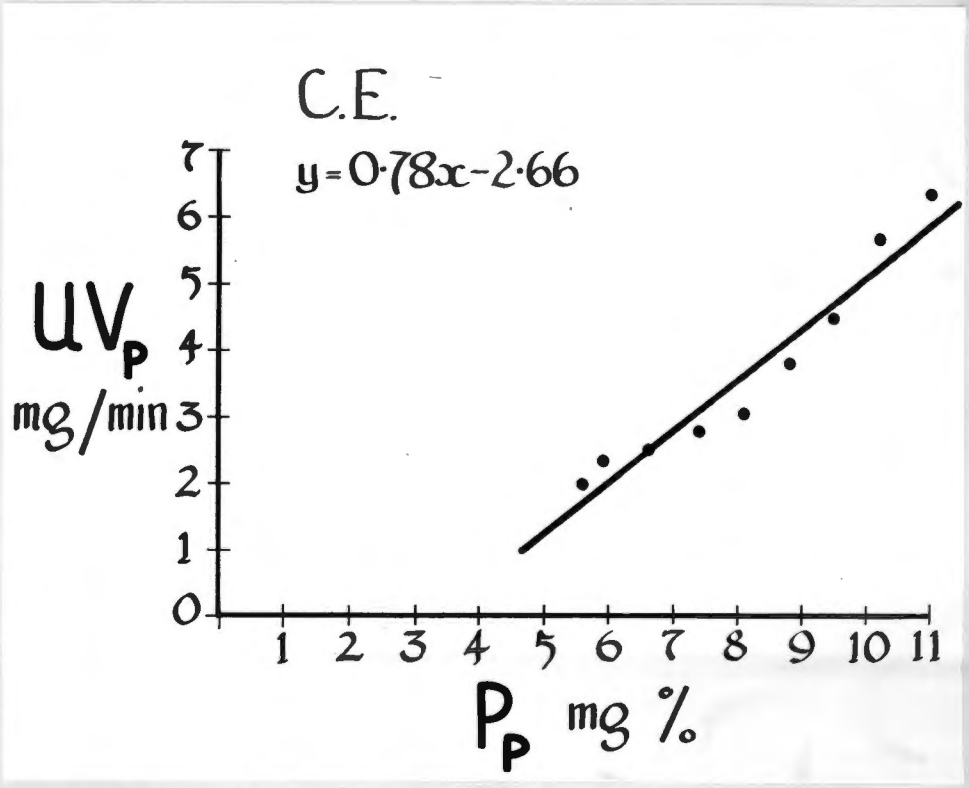

Fig. 15(a)

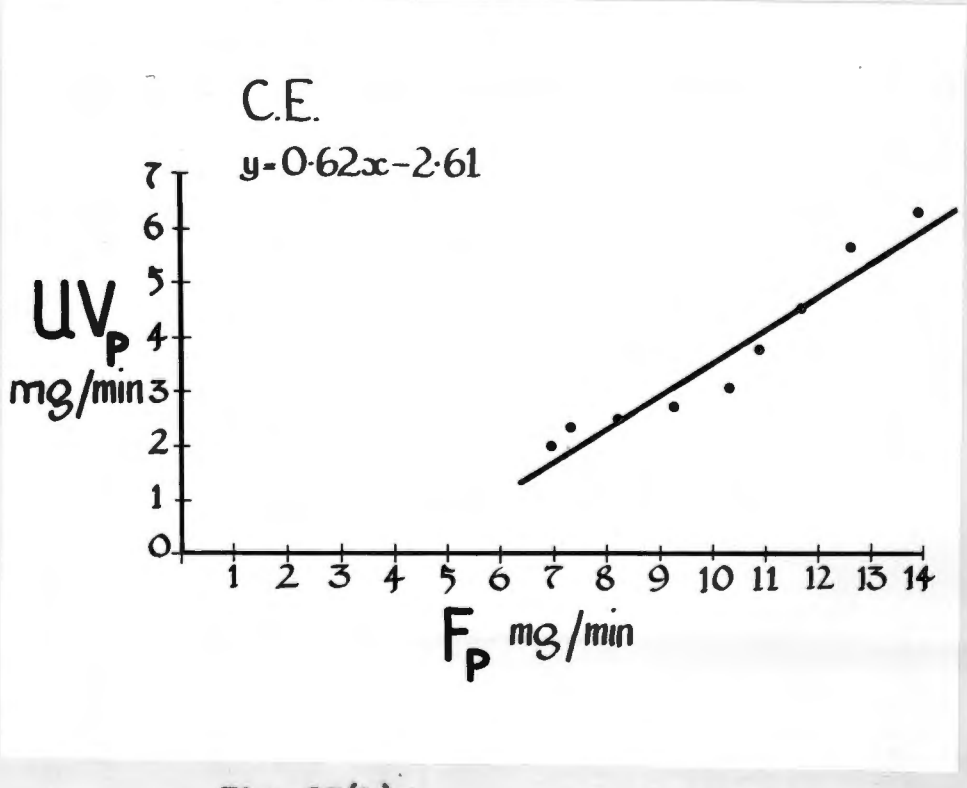

Fig. 15(b)

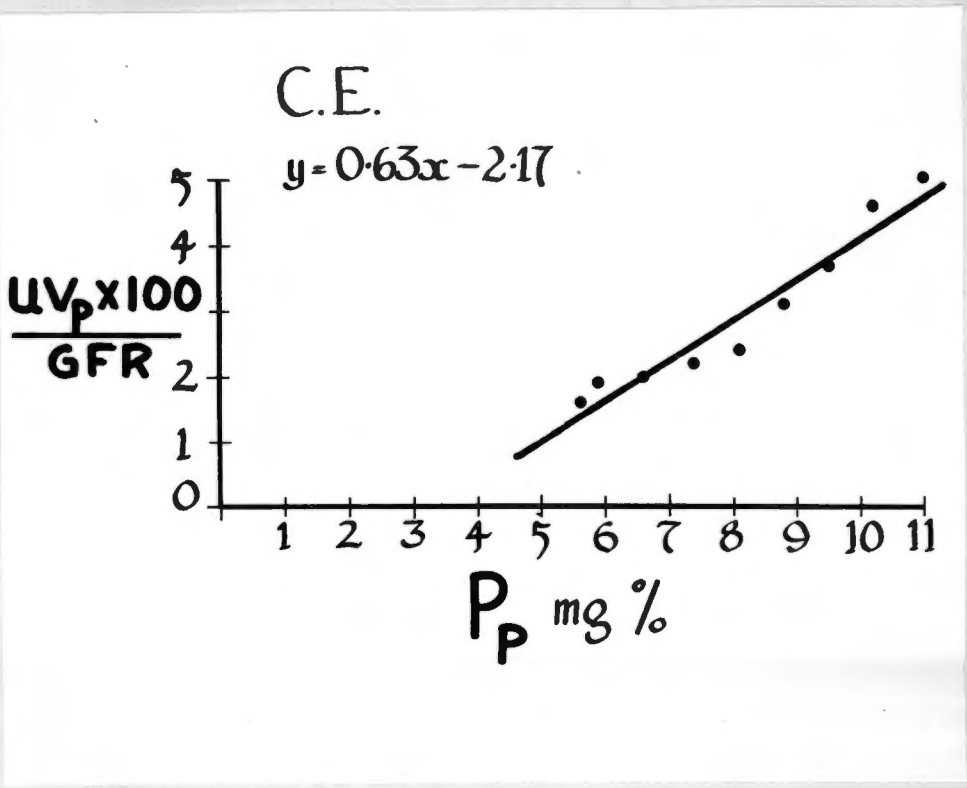

Fig. 15(c)

Comment:- Good correlation in all graphs.
Slightly curved line would fit points better than straight line.

EXPERIMENT : No. IX Subject : A.O.

Table No. 9

Period	Time (mins.)	Pp (mg.%)	G.F.R. (ml./min.)	Fp (mg./min.)	UVp (mg./min.)	Tp (mg./min.)	$\frac{Tp \times 100}{G.F.R.}$	$\frac{UVp \times 100}{G.F.R.}$
I	18.5	5.5	90.7	4.99	0.25	4.74	5.21	0.28
II	19.5	6.0	154.6	9.27	1.70	7.57	4.90	1.10
III	20.0	6.9	110.7	7.63	2.11	5.52	4.98	1.91
IV	20.0	7.8	130.8	10.20	3.56	6.64	5.07	2.72
V	21.0	8.8	115.6	10.17	4.16	6.01	5.20	3.60
VI	20.0	9.6	185.3	17.79	8.17	9.62	5.18	4.41
VII	22.0	10.4	100.7	10.47	5.09	5.38	5.20	5.05
VIII	19.0	11.4	100.8	11.49	6.38	5.11	5.08	6.33
IX	20.0	12.3	84.7	10.42	5.96	4.46	5.26	7.04

Pre-infusion serum phosphorus concentration - 5.0 mg.%

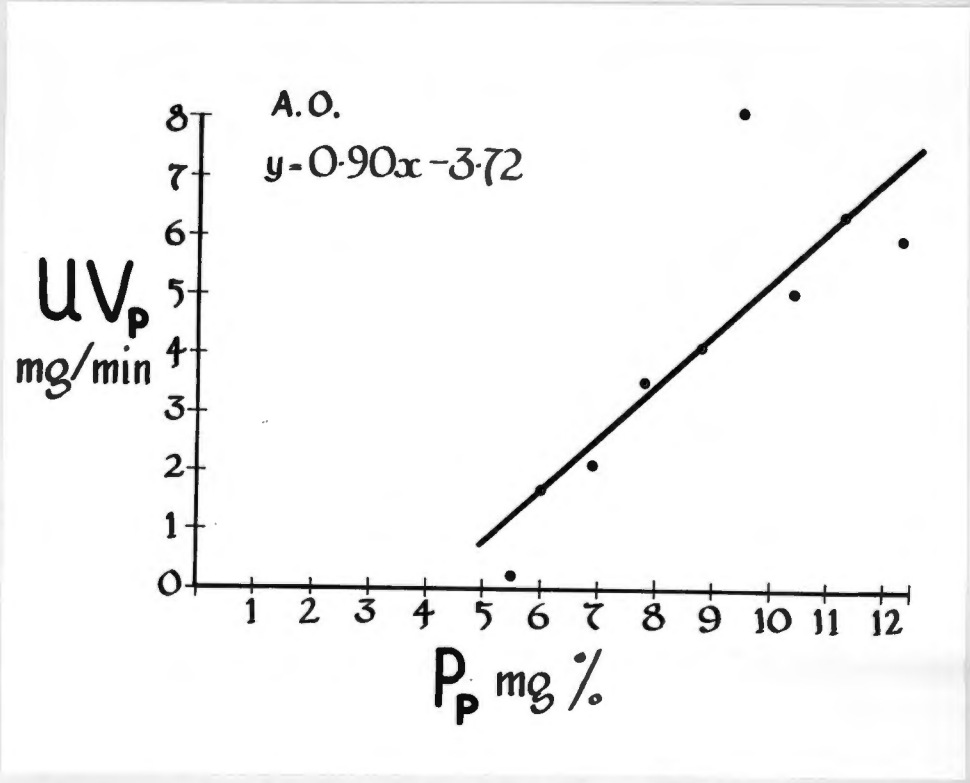

Fig. 16(a)

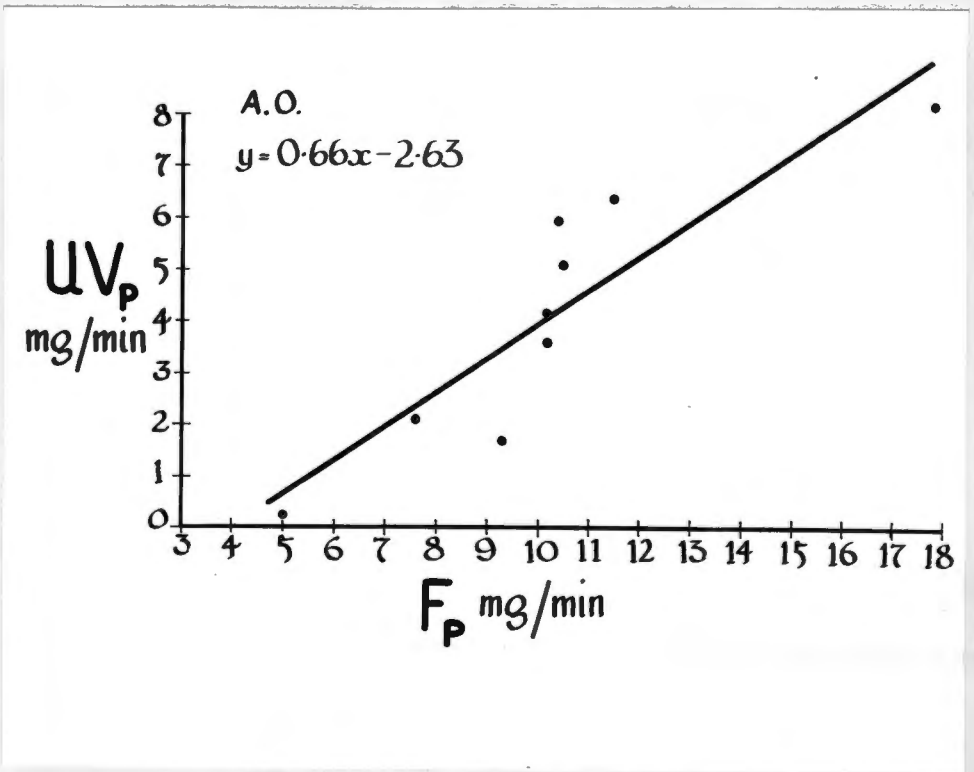

Fig. 16(b)

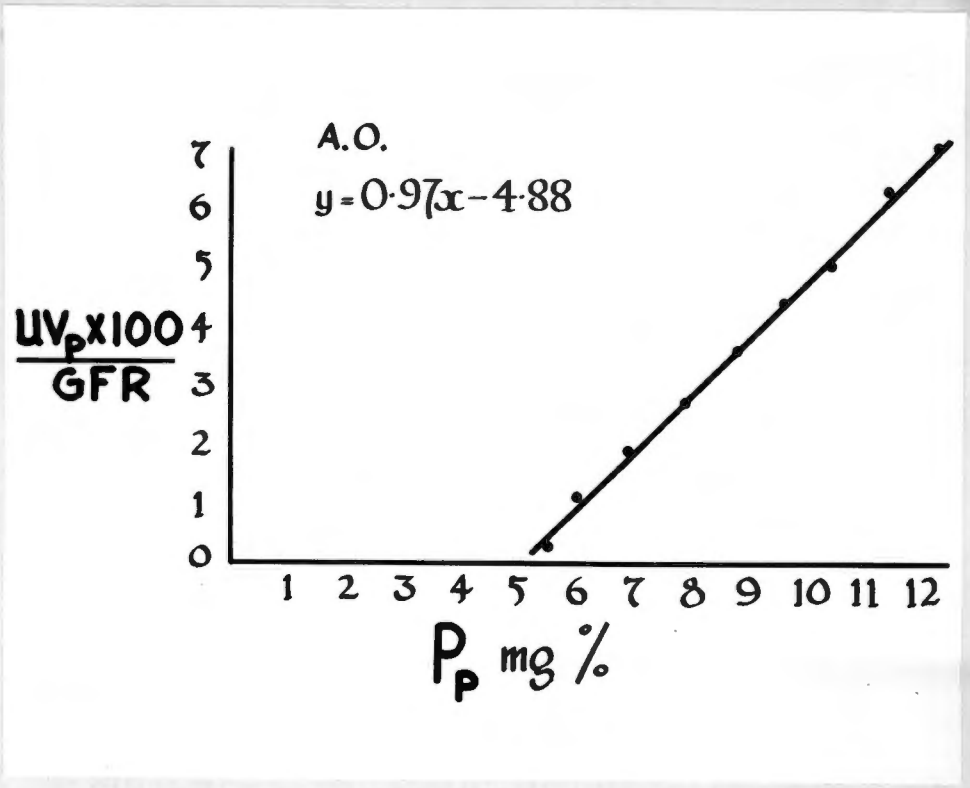

Fig. 16(c)

Comment:- Poor correlation in Fig.(a) that is worse when the co-ordinates in Fig.(b) are used. Fig.(c), however, shows excellent correlation and linearity.

EXPERIMENT : No. X Subject : G.M.

Table No.10

Period	Time (mins.)	Pp (mg.%)	G.F.R. (ml./min.)	Pp (mg./min.)	UVp (mg./min.)	Tp (mg./min.)	$\frac{Tp \times 100}{G.F.R.}$	$\frac{UVp \times 100}{G.F.R.}$
I	18.5	4.2	148.3	6.23	0.43	5.80	3.91	0.29
II	19.0	5.0	137.2	6.86	1.80	5.06	3.69	1.31
III	21.0	5.8	112.6	6.53	2.31	4.22	3.75	2.05
IV	20.0	6.7	140.4	9.40	3.66	5.74	4.09	2.61
V	20.0	7.3	108.3	7.91	3.39	4.52	4.17	3.13
VI	19.0	7.9	97.2	7.67	3.63	4.04	4.16	3.73
VII	21.0	8.5	139.4	11.84	5.91	5.93	4.25	4.24
VIII	20.5	9.2	108.6	9.99	5.50	4.49	4.13	5.06
IX	19.5	10.1	152.3	15.38	8.71	6.67	4.38	5.72

Pre-infusion serum phosphorus concentration - 3.4 mg.%

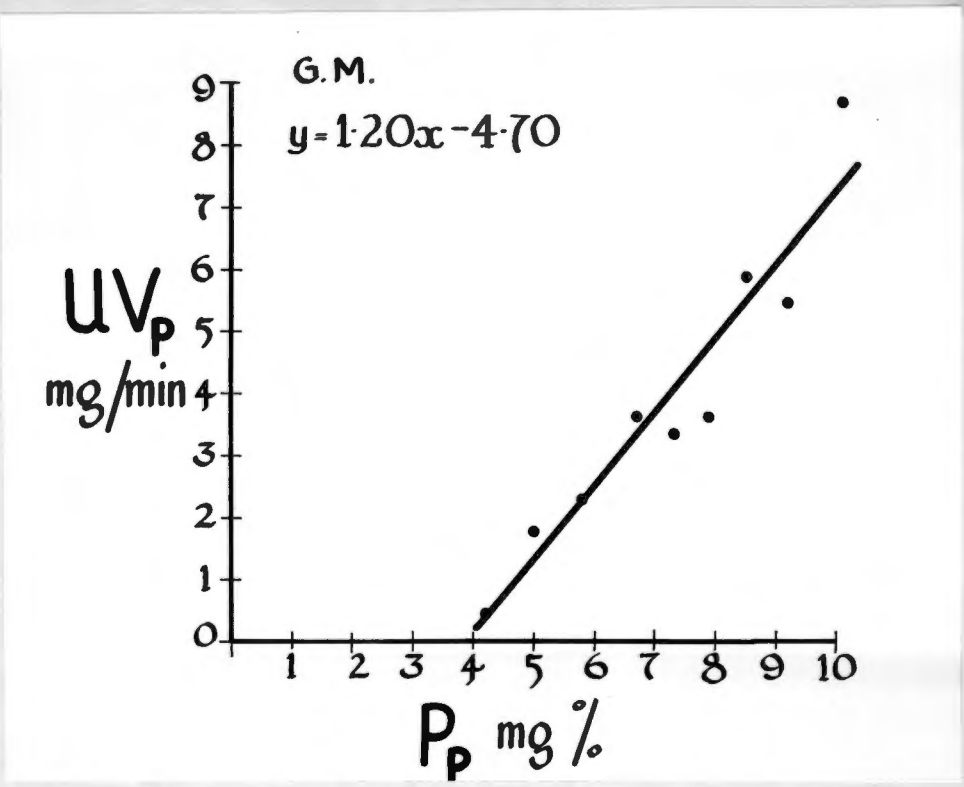

Fig. 17(a)

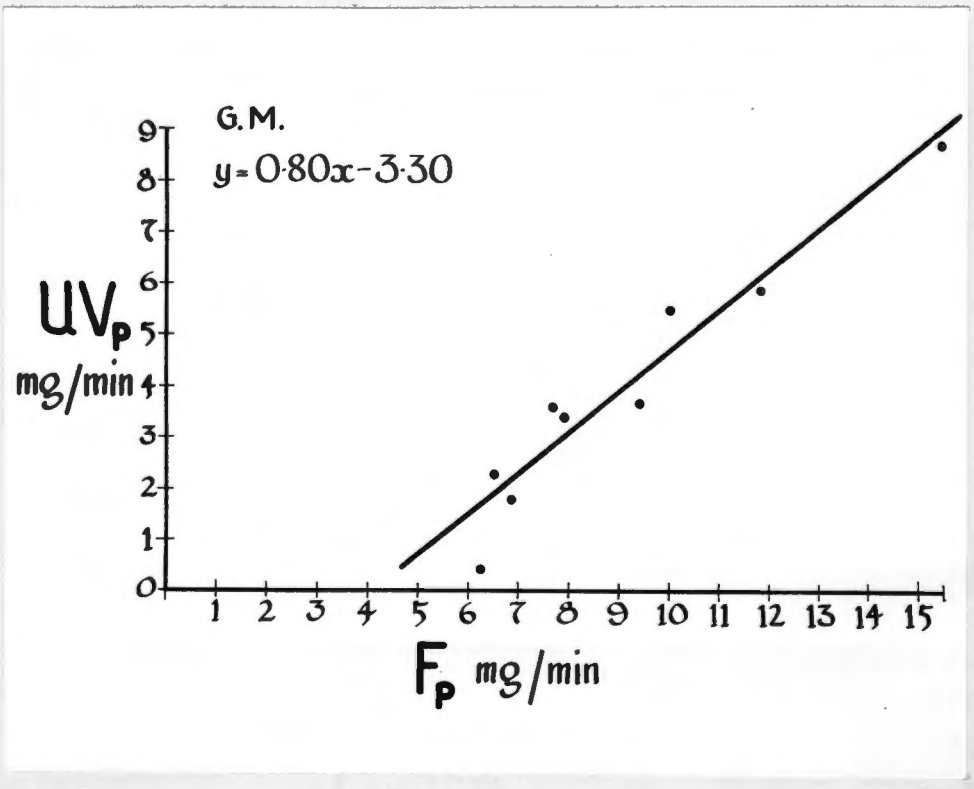

Fig. 17(b)

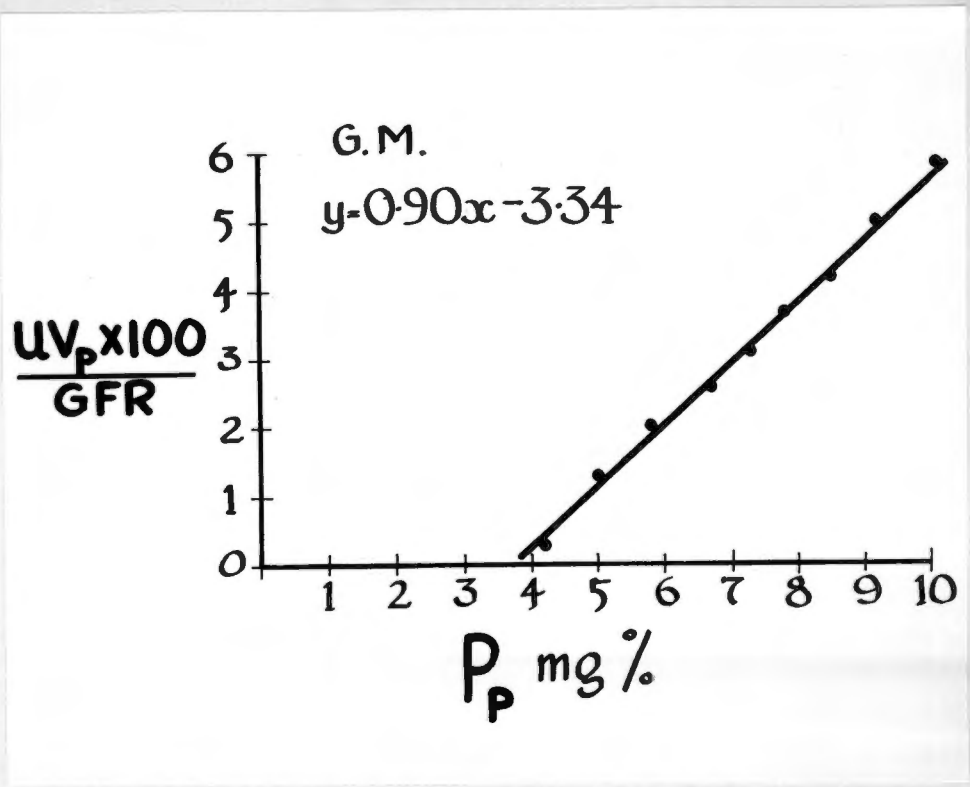

Fig. 17(c)

Comment :- Poor correlation in Figs. (a) and (b), with excellent correlation in Fig. (c).

EXPERIMENT : No. XI Subject : B.J.B.

Table No.11

Period	Time (mins.)	Pp (mg.%)	G.F.R. (ml./min.)	Fp (mg./min.)	UVp (mg./min.)	Tp (mg./min.)	$\frac{Tp \times 100}{G.F.R.}$	$\frac{UVp \times 100}{G.F.R.}$
I	21.5	5.3	159.7	8.46	0.80	7.66	4.79	0.50
II	20.0	5.9	100.3	5.92	0.87	5.05	5.03	0.87
III	23.0	6.7	105.6	7.08	1.80	5.28	5.00	1.71
IV	21.0	7.5	132.3	9.93	3.21	6.72	5.08	2.43
V	19.5	8.1	98.2	7.95	2.85	5.10	5.19	2.90
VI	18.5	8.8	104.2	9.17	3.87	5.30	5.09	3.71
VII	22.0	9.7	147.8	14.34	6.53	7.81	5.28	4.42
VIII	26.0	10.6	83.2	8.81	4.60	4.21	5.05	5.53
IX	18.0	11.4	168.7	19.24	10.29	8.95	5.31	6.10

Pre-infusion serum phosphorus concentration - 4.3 mg.%

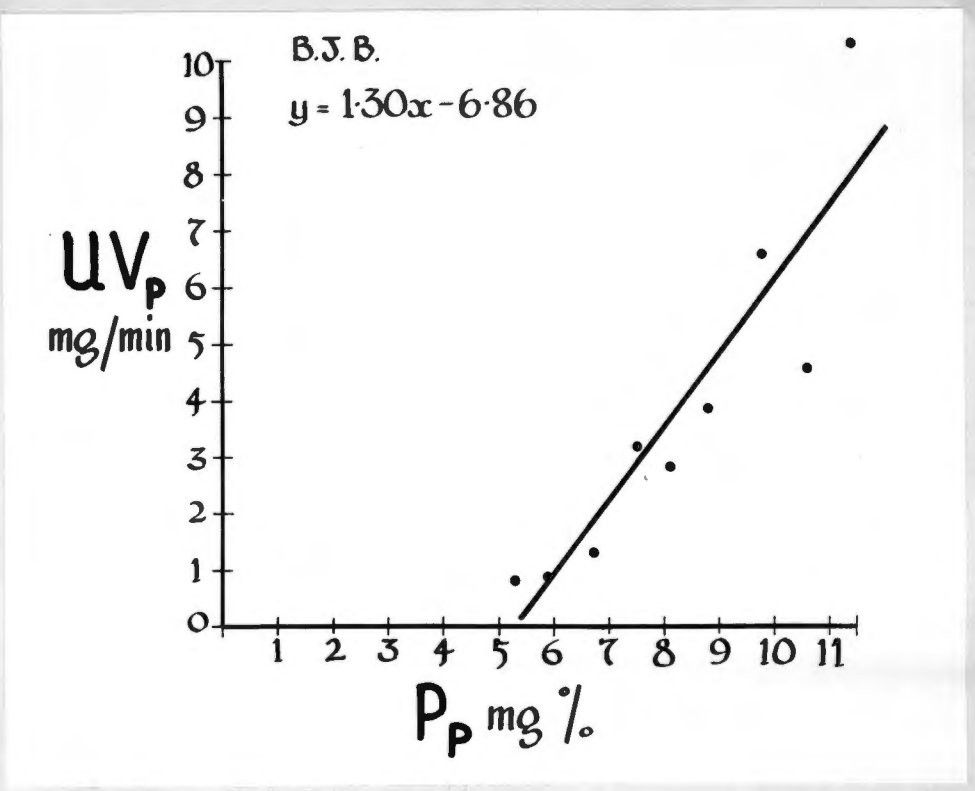

Fig. 18(a)

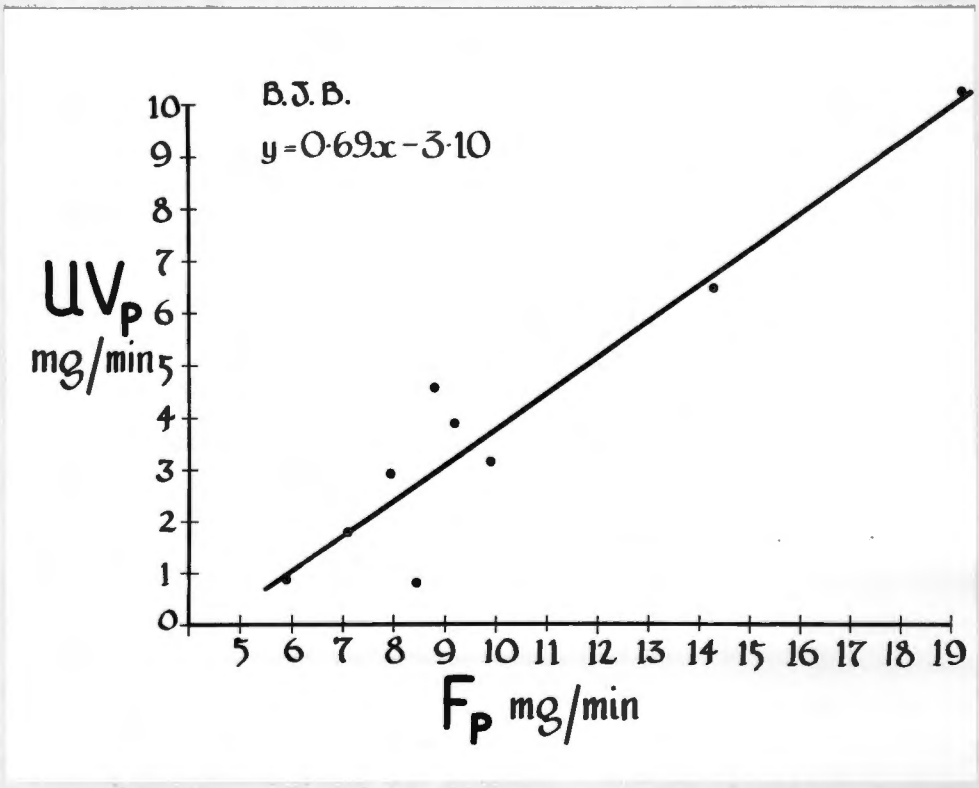

Fig. 18(b)

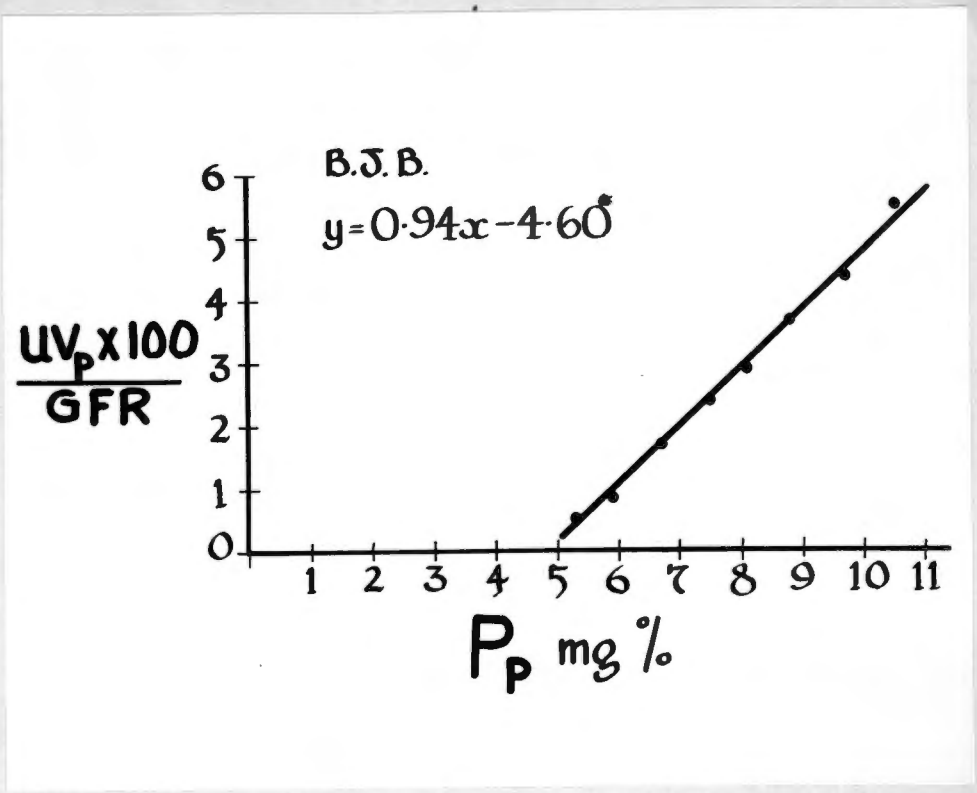

Fig. 18(c)

Comment :- Poor correlation in Figs. (a) and (b) with excellent correlation in Fig. (c).

Linearity in Fig. (c) excellent.

EXPERIMENT : No. XII Subject : B.A.B.

Table No. 12

Period	Time (mins.)	Pp (mg.%)	G.F.R. (ml./min.)	Fp (mg./min.)	UVp (mg./min.)	Tp (mg./min.)	$\frac{Tp \times 100}{G.F.R.}$	$\frac{UVp \times 100}{G.F.R.}$
I	20.0	4.0	154.6	6.18	0.29	5.89	3.81	0.19
II	20.0	4.2	168.3	7.07	0.64	6.43	3.82	0.38
III	20.0	5.0	142.0	7.10	0.88	6.22	4.38	0.62
IV	20.0	5.5	130.0	7.15	1.34	5.81	4.47	1.03
V	21.0	6.3	119.3	7.51	2.28	5.23	4.38	1.91
VI	19.0	7.0	121.0	8.47	3.05	5.42	4.48	2.52
VII	18.0	7.6	118.2	8.98	3.59	5.39	4.56	3.04
VIII	23.0	8.6	148.6	12.78	6.08	6.70	4.51	4.09
IX	19.0	9.4	145.4	13.66	6.80	6.86	4.72	4.68

Pre-infusion serum phosphorus concentration - 3.1 mg.%

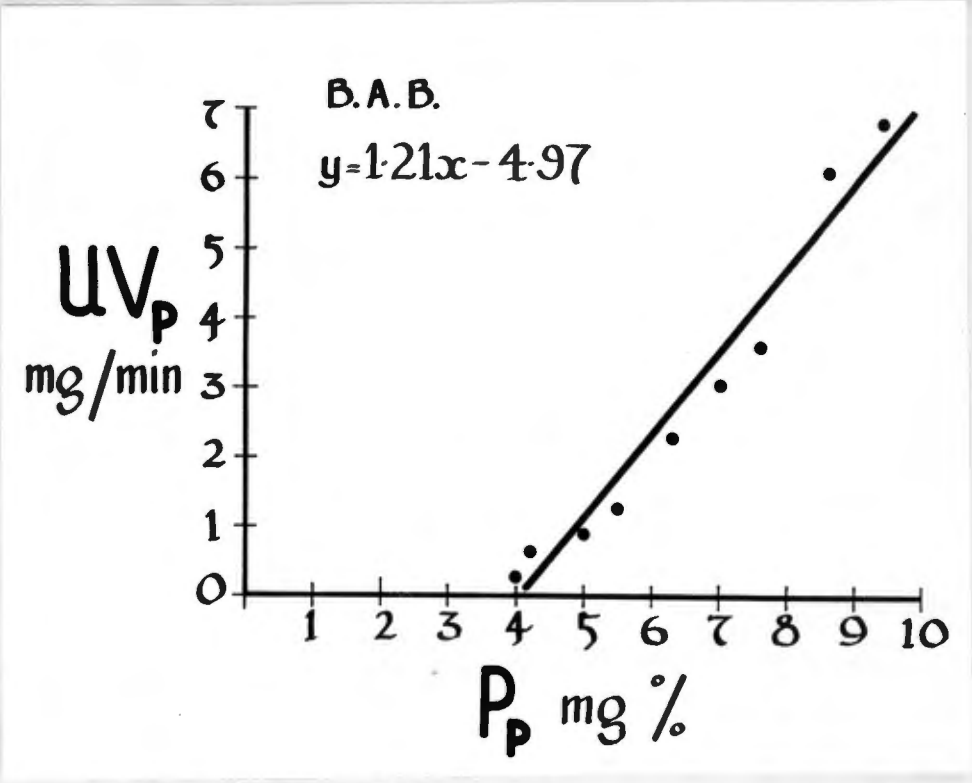

Fig. 19(a)

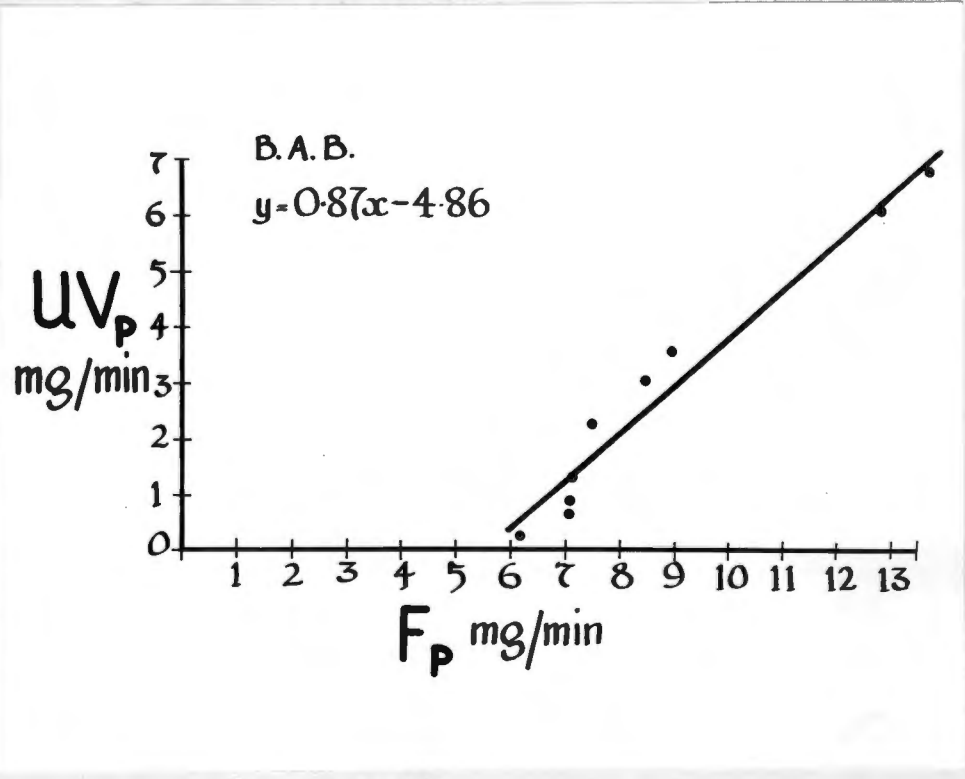

Fig. 19(b)

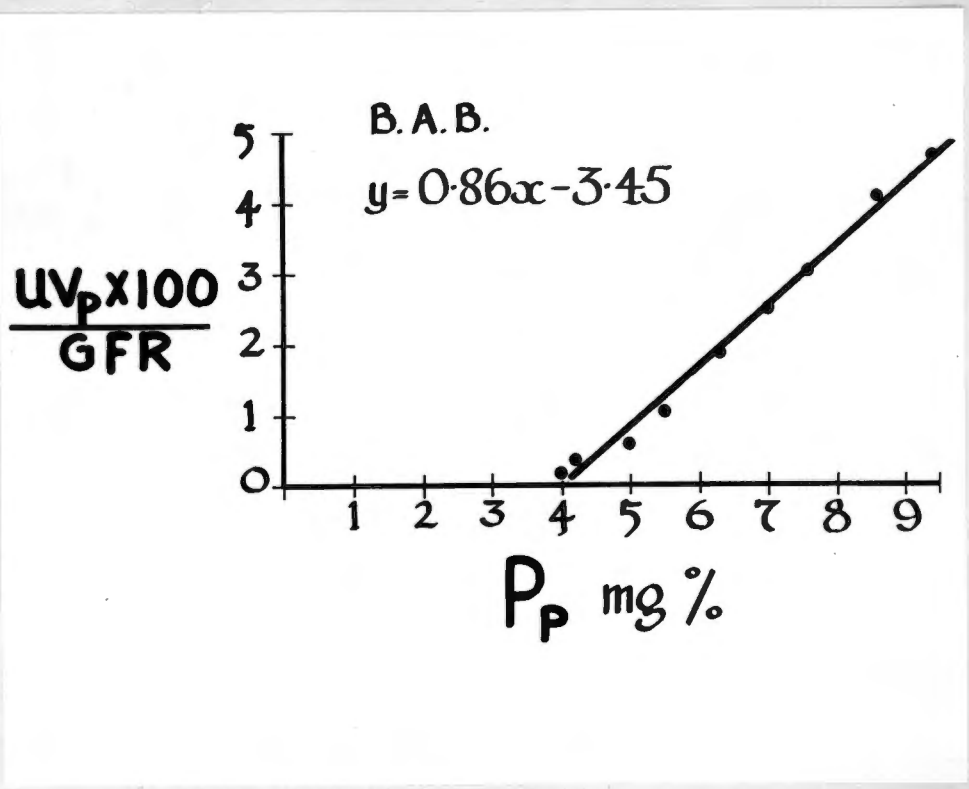

Fig. 19(c)

Comment :- Excellent correlation and linearity in Fig.(c) as opposed to that in Figs. (a) and (b).

If the co-ordinates for period 1 and 2 had been omitted from the graph, the regression coefficient would have been nearer unity and the linearity slightly improved.

EXPERIMENT : No. XIII Subject : L.McK.

Table No. 13

Period	Time (mins.)	Pp (mg.%)	G.F.R. (ml./min.)	Fp (mg./min.)	UVp (mg./min.)	Tp (mg./min.)	$\frac{Tp \times 100}{G.F.R.}$	$\frac{UVp \times 100}{G.F.R.}$
I	20.0	5.4	124.8	6.74	0.21	6.53	5.24	0.17
II	21.5	6.3	136.4	8.59	0.40	8.19	6.00	0.29
III	19.0	7.3	97.8	7.14	0.37	6.77	7.29	0.38
IV	21.0	8.5	100.7	8.55	1.63	6.92	6.86	1.62
V	19.5	9.4	120.5	11.33	2.46	8.87	7.35	2.04
VI	20.0	11.0	84.9	9.34	1.96	7.38	8.70	2.31
VII	18.5	12.8	99.6	12.75	3.57	9.18	9.22	3.58
VIII	22.0	14.0	100.5	14.65	4.64	10.01	9.97	4.62
IX	19.5	15.1	130.7	19.74	6.85	12.89	9.87	5.24

Pre-infusion serum phosphorus concentration - 5.1 mg.%

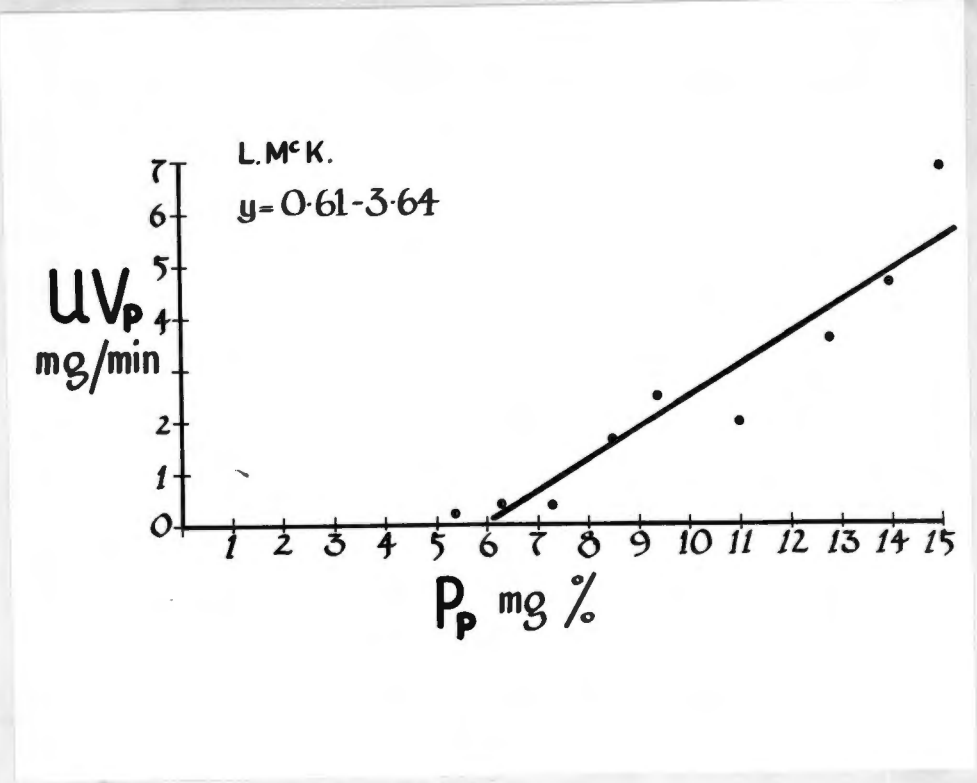

Fig. 20(a)

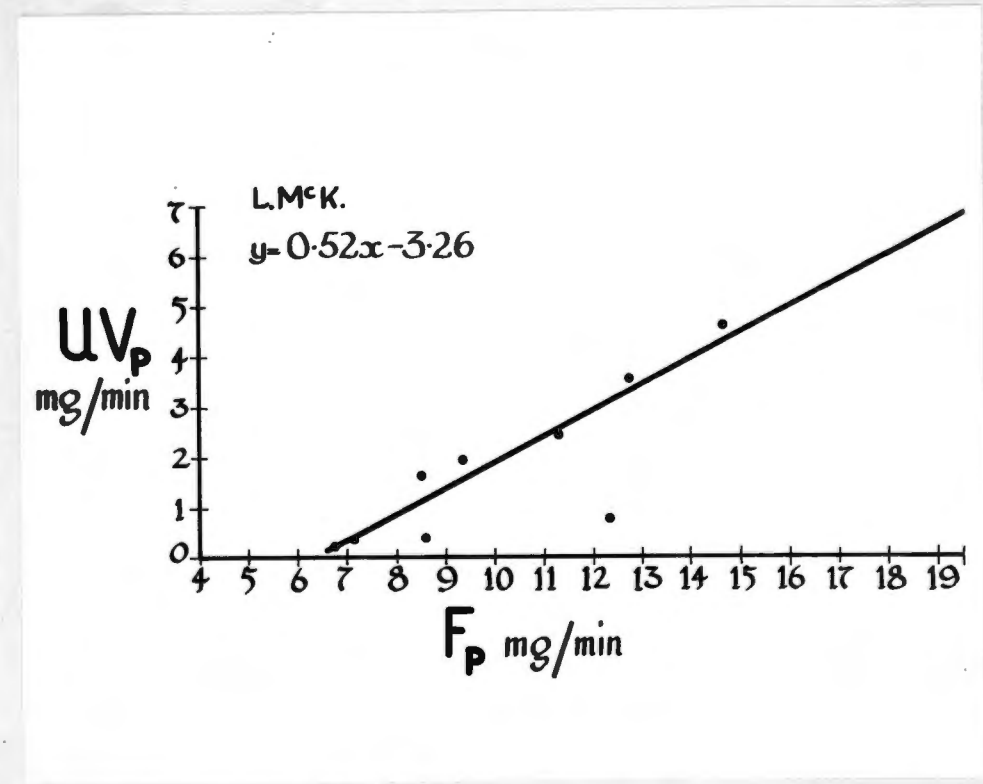

Fig. 20(b)

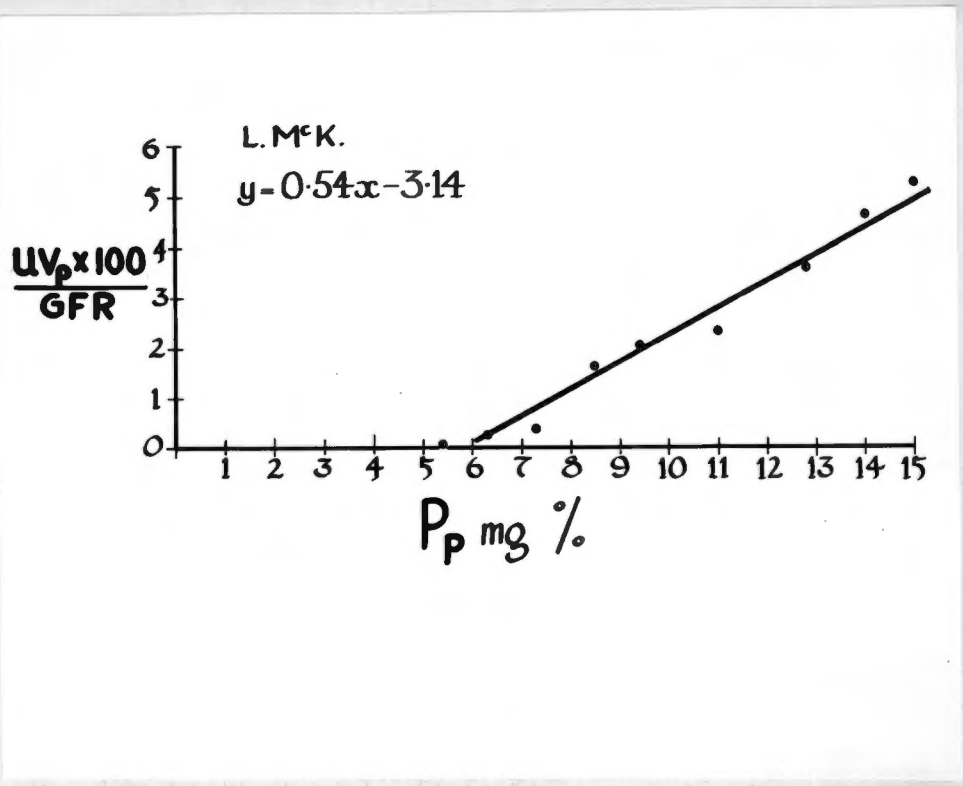

Fig. 20(c)

Comment :- Poor correlation in Figs. (a) and (b).
Good correlation in Fig.(c).

RESULTS AND DISCUSSION.

I have the following comments to make on these experimental results :-

1. In eight cases the glomerular filtration rate remained constant (Experiments I - VIII inclusive). In these there was good correlation no matter which co-ordinates were chosen to illustrate the relationship between the variables.

2. In two cases (Experiments VII and VIII) there was a curvilinear relationship between the variables at the lower plasma phosphate concentrations. This, I suspect, was due to the fact that the lower plasma phosphate concentrations fell within the threshold range with resultant "heeling of the curve".

3. In 5 cases (Experiments IX - XIII inclusive) the glomerular filtration rate varied over the course of the experiment. This variation may have been apparent, due to incomplete bladder emptying and inaccurate timing, or real due to changes in renal haemodynamics. I can offer no more acceptable evidence in favour of the latter alternative than the weight of my opinion. In subject L.McK. who had undergone a haemorrhoidectomy one week previously, I accept that incomplete bladder emptying may have been a factor. In no other case, however, did I have any reason to suspect that bladder emptying was incomplete.

4. In these 5 cases there was poor correlation between the plasma phosphate concentration and rate of excretion in the urine, and between filtered load and rate of excretion in the urine. In all cases, however, there was excellent correlation between

plasma phosphate concentration and the quotient of rate of excretion and glomerular filtration rate. Allowing the supposition that the variation in glomerular filtration rate was real, this indicates that a rise in glomerular filtration rate is accompanied by a rise in rate of tubular transport of phosphorus. This observation confirms the observation of others[14,15,166,115]. The easiest way of explaining the relationship between glomerular and tubular activity would be to suggest that intermittent glomerular shutdown would deprive those nephrons of filtrate and so mechanically decrease tubular reabsorption of phosphate. Glomerular intermittence, however, while accepted as occurring in amphibia[267,219] is not thought to occur in the mammalian kidney. Direct visual techniques employing injection of indian ink into the renal artery have yielded varying results[269,246], and although Homer Smith[239] has pointed out that there is no acceptable evidence to suggest that it does occur, there is, using the same argument, even less acceptable evidence to suggest that it does not occur. Handley et al.[107,153,188] have interpreted the relationship that exists between glucose or PAH transport and glomerular filtration rate as indicating the existence of glomerular intermittence. It is, to me, strange that no one has doubted the fact that changes in glomerular filtration rate may occur yet there is so little certainty regarding the nature of the mechanism operating to bring about these changes. The techniques of Lamport[158,159,160] are too empirical to be acceptable as methods for measuring the intricate

haemodynamics of efferent and afferent arteriolar resistance or the part that such factors may play in regulating the rate of glomerular filtration.

If one does not allow the supposition that real changes in glomerular filtration rate may occur during a short experimental period then the relationship that does exist between Tp and glomerular filtration rate is artefactual. My experience with experiments of this nature has taught me that it is only rarely that one can feel confident that the urinary minute volume has been accurately measured even with the aid of catheters, bladder washouts and suprapubic compression. An impasse has been reached for which I see no solution.

In my opinion, glomerular intermittence does occur in man and the mean threshold level is independent of changes in glomerular filtration rate.

5. The regression coefficients for the different plots are seen to deviate in practically all subjects from the expected values. As good correlation was only obtained in all cases when the regression of $\frac{UVp}{GFR}$ on Pp was plotted, the deviation of this coefficient from the expected value of unity will be discussed without reference to the other graphs.

In Table 14 the regression coefficients for $\frac{UVp}{GFR}$ on Pp are given for each subject with the level of probability at which the hypothesis that each regression coefficient is equal to unity can be rejected.

TABLE 14

SUBJECT	EXPERIMENT NO.	REGRESSION COEFFICIENT FOR $\frac{UVp}{GFR}$ upon Pp (a)	P ≡
T.le R.	I	0.99	Accepted
V.M.G.	II	0.94	Accepted
A.v.d.M.	III	0.99	Accepted
B.R.F.	IV	0.91	0.002 (S)
L.A.E.	V	0.78	0.01 (S)
B.A.	VI	0.73	0.01 (S)
L.G.	VII	0.87	0.20
C.E.	VIII	0.63	.001 (S)
A.O.	IX	0.97	0.2
G.M.	X	0.90	0.015 (S)
B.J.B.	XI	0.94	0.02 (S)
B.A.B.	XII	0.86	0.015 (S)
L.McK.	XIII	0.54	.001 (S)

≡ P - the level of probability at which the hypothesis that the observed value of a is equal to unity can be rejected.

(S) denotes a significant level.

The manner in which the level of probability was determined is described in Appendix B.

It will be seen that in eight experiments, a highly significant deviation of the observed regression coefficient from unity was found.

The following explanations for this observation offer themselves.

(a) In one subject (C.E. Experiment VIII) a portion of the heel of the curve was included in the graph, the effect of which would be to alter the overall slope of the line.

(b) The period of experimental study may have superimposed upon a period of normally increasing tubular activity. A diurnal variation in phosphate excretion unrelated to plasma level has been repeatedly described[75,198], but it is hardly likely that it would have increased so regularly in all the cases observed.

(c) There may have been a progressive increase in renal delay time. I doubt if this was the case as the rise in plasma phosphate concentration was approximately linear and a good diuresis was maintained throughout the experiment.

(d) If, as suggested by Crawford et al.[49], the rate of tubular reabsorption of phosphate is accelerated by increasing the concentration in the glomerular filtrate, such a low value for the regression coefficient would have been expected.

(e) A constant fraction of the plasma inorganic phosphorus may have been present in a non-filterable form. I attempted to test the validity of this explanation with in vitro ultrafiltration through a collodion membrane. I was, unfortunately, not sufficiently au fait with the techniques of ultra filtration to be able to produce conclusive results.

Of the two most likely explanations (c) and (d) I incline towards the latter, and hope to develop in vitro and in vivo techniques to answer for my inclination.

6. There is nothing in these experimental results to confirm the observation of others[181,20] that prolonged infusion of phosphate will in itself exhaust the tubular mechanism for reabsorption with a fall in Tmp. In none of these cases was supplementary potassium added for its alleged effect in correcting this "exhaustion".

It is relevant to review at this stage certain aspects of two papers that have appeared recently on this subject.

In a critical and excellent series of experiments with phosphate infusion in normals, Longson, Mills, Thomas and Yates[166] found results similar to mine in that in the experiments where the glomerular filtration rate remained constant a good plot was obtained whereas variations in the glomerular filtration rate (which they felt were real) caused a scattering of the points. They found a regression coefficient for Pp on Fp that was significantly lower than unity in several cases, and attributed this to progressive increase in glomerular filtration rate over the course of the experimental period.

Nassim, Saville and Mulligan[191] using renal titrations to test the effect of stilboestrol on the Phosphate Tm, were obliged in 2 cases (Cases I and IV) to correct the urinary phosphate values for changes in the amount of creatinine appearing in the urine before they were able to obtain a linear plot.

The low figures they obtained for the glomerular filtration rate (as determined by the regression coefficient of UVp upon Pp) in cases 2, 3 and 4 of their Table I make me suspect that similar factors were operating in their cases to those that were operating in cases IV, V, VI, VIII, X, XI, XII and XIII of my series of experiments.

7. In each case a negative value was obtained for the intercept on the ordinate indicating that tubular secretion of phosphate did not occur. To my mind, the values of these intercepts even in the cases where the expected slope was found, are meaningless as accurate measurements of the Tm or the mean threshold level. I say this because I had no estimate of the duration of renal delay time in these cases, and, in all probability, the 2½ minutes allowed for this was insufficient. I am not prepared to accept the statement that renal delay time is not important when dealing with changing plasma levels, and I think a rough idea of the extent to which renal delay time can influence the value of the intercept can be obtained from the values for $\frac{Tp}{GFR}$ in subject L.McK. (Table 13 column 7) where there was a rapid rise in plasma phosphorus concentration. These values indicate the presence of a mean threshold level some 50% higher than the resting inorganic phosphorus concentration. L.McK. it should be emphasised, is strappingly euparathyroidic.

SUMMARY.

(i) In a series of thirteen renal titration experiments on thirteen normal individuals, 8 cases were encountered where the

glomerular filtration rate remained constant during the experiment and in the remaining 5 cases it varied. This variation may have been apparent or real - I suspect it was real.

(ii) In those cases where the glomerular filtration rate varied the highest degree of graphic correlation was obtained where $\frac{UVp}{GFR}$ and Pp were chosen as co-ordinates. This indicates that the threshold ($\frac{Tp}{GFR}$) is more constant than the absolute amount reabsorbed (Tm) which varies proportionally with changes in the glomerular filtration rate.

(iii) The regression coefficient for $\frac{UVp}{GFR}$ on Pp was found, in eight cases, to be significantly lower than the expected value of unity. This may have been due to any of several factors. It is suggested that the presence of a non-filterable moiety of the plasma inorganic phosphorus is the most likely explanation.

(iv) The values for the intercepts are discussed, and it is concluded that their interpretation is vitiated by renal delay time.

THE NORMAL RENAL THRESHOLD FOR PHOSPHORUS IN MAN.

The inaccuracy introduced by renal delay time into techniques for measuring the renal threshold where changing plasma concentrations are used led me to develop this simpler technique.

With the subject fasting, a priming dose of inulin and of phosphate solution is administered. This is done most conveniently by mixing 1% buffered phosphate solution and 10% inulin in the same vacolitre in amounts calculated to raise the extracellular fluid concentrations by 5 to 10 mg.% for phosphorus and 40 mg.% for inulin. This mixture is allowed to run in intravenously and as soon as it is empty the vacolitre is replaced by another containing a sustaining mixture of inulin, phosphate and saline. The rate of flow of this mixture is kept constant at a speed calculated to maintain the desired level of phosphorus and inulin in the serum. A period of one hour is allowed for equilibration after which four urinary collections are made at 20 minute intervals with mid-period blood samples.

The first urine specimen is discarded. The remaining 3 urine specimens and the 3 blood samples are analysed for phosphorus and inulin. From these estimations the value for $\frac{Tp \times 100}{G.F.R.}$ can easily be calculated as $\frac{Tp \times 100}{G.F.R.} = Pp - \frac{Up \times Pin}{Uin}$

where Pp = Plasma phosphorus concentration in mg.%

Up = Urine phosphorus concentration in mg.%

Uin = Urine inulin concentration in mg.%

Pin = Plasma inulin concentration in mg.%

It will be seen that the factor V (minute volume) does not enter into the equation for calculating the threshold so that accurate bladder emptying and timing of periods is not of great importance.

The maintenance of constant plasma concentrations of inulin and phosphorus is, however, of importance and can be assured by allowing at least one hour for equilibration to take place and by the use of the simple and inexpensive constant infusion apparatus described in Appendix B.

I have found this method most satisfactory and present the results of the threshold value for 12 normal individuals determined by this technique in Table 15.

By virtue of the fact that constant plasma levels are used, this technique, I feel, gives a better estimate of the threshold level than does that of Anderson[12] where renal delay time must be a most important source of error.

The use of the term $\frac{Tp \times 100}{G.F.R.}$ to express renal tubular activity, as opposed to the Tm, affords a means of comparing results on patients of different sizes inasmuch as it refers the amount of phosphorus reabsorbed to a constant glomerular filtration rate of 100 ml./min.

If then, the tubular reabsorptive capacity for phosphate is to be measured at all, let it, I suggest, be measured with constant plasma levels of inulin and phosphate and let it be expressed in terms of amount reabsorbed in mg./min. from each 100 ml. of filtrate formed.

TABLE 15

Name	Race	Sex	Age	$\frac{Tp}{GFR}$
E.B.D.	E.	M.	26	4.4
L.F.	E.	M.	24	3.8
M.A.	B.	F.	30	4.6
F.B.	C.	F.	2	5.7
W.T.	B.	F.	1½	6.2
S.T.	E.	M.	37	3.6
S.S.	E.	M.	24	3.8
W.v.W.	E.	M.	30	3.1
E.K.	C.	F.	18	4.4
H.M.	B.	M.	25	4.9
M.D.	C.	F.	59	3.7
G.P.	C.	M.	48	3.0

The value given for $\frac{Tp}{GFR}$ in each case is the mean of three readings. In the two children in whom this measurement was made it can be seen that the $\frac{Tp}{GFR}$ was higher than in adults.

The technique I have described for estimation of the phosphorus threshold could very easily be adapted for routine use. It does not inconvenience the patient unduly and the cost in materials and time is not excessive.

For all this, I doubt very much the value of this of any other similar technique as an investigational tool. My reasons for harbouring this doubt are these.

In the first instance, we are, in measuring the phosphate threshold implicitly assuming that the human organism does not give a sufficiently accurate measure of this entity in the resting serum inorganic phosphorus concentration. I submit, humbly and perhaps unscientifically, that this is an impudent assumption.

In the second instance the widespread use of such a measurement could easily lead to the blind acceptance and stultifying entrenchment of the theories of renal function upon which it is based. I should accept as evidence of a low renal threshold the presence of a low serum phosphorus with a high urine phosphorus far more readily than evidence based on clearance analysis. Of all the methods employed to estimate the threshold level, that of Chambers et al.[38], where the effect of phosphate deprivation upon plasma and urinary phosphorus is noted, is physiologically the most acceptable.

In many of the diseased states in which an estimate of the phosphorus threshold is required, the interpretation of the estimate obtained is complicated by co-existing renal disease. The best example of this is hyperparathyroidism.

Other indices of renal tubular activity for phosphate have been described and I shall discuss these briefly :-

(i) Clearance of Phosphate divided by the glomerular filtration rate (Cp/Cin or Cp/Ccr)

(ii) The amount of phosphorus reabsorbed expressed as a percentage of the filtered load

$$\left(\frac{Tp \times 100}{Pp \times GFR}\right)$$

(iii) The clearance of phosphorus.

These three indices of renal tubular function all have the disadvantage that their values are affected by the plasma phosphorus concentration, irrespective of any change in renal tubular activity.

The ratio Cp/Cin will approach unity as an asymptote as the plasma phosphorus concentration rises; the second index will approach 100% as an asymptote and Cp will approach the glomerular filtration rate as an asymptote.

As these indexes have all been devised to measure the renal threshold for phosphorus, I see no reason for using them when the renal threshold can be measured directly as $\frac{Tp}{GFR}$.

Dowdle, Jackson and Hoffenberg[63] criticised the use of Cp/Ccr by Nordin and Fraser[196] and in reply to this criticism Fraser[77] offered yet another index of renal tubular activity which virtually "corrected" Cp/Ccr for variations in plasma phosphorus concentration.

As it is in the wards that measurements of renal tubular activity find their application, I suggest that the familiar "threshold"is more acceptable for practical usage than indices involving complicated hyperbolic relationships.

SECTION II.

The Renal Handling of Calcium.

THE RENAL HANDLING OF CALCIUM.

Despite the many syndromes in which abnormalities of urinary calcium excretion occur, knowledge regarding the mechanisms governing the renal excretion of this cation is fragmentary and inconclusive. Review articles dealing with the subject devote a paragraph or two to the physiology of calcium excretion and pages to such subjects as nephrocalcinosis and renal calculi. This lamentable lack of physiological knowledge is due to the fact that contemporary renal research, modelled as it is upon the "filtration - reabsorption" theory of renal function, requires to know with certainty the plasma concentration of filterable calcium before it can give any opinions on "renal mechanisms". Although a variety of techniques have been devised to estimate the concentration of this moiety in the plasma, workers have, I suspect, been loath to accept these estimations as necessarily applicable to the glomerular membrane. Two papers of interest have appeared, however, and I shall discuss them in some detail.

Wolf and Ball[278], experimenting with dogs, gave calcium solutions of known concentrations at known constant rates and observed the rate of excretion in the urine relative to the rate of infusion of calcium. From their observations they were able to conclude that :-

i) There is no non-limiting or limiting isorrhoeic quantity for the renal excretion of calcium.

ii) Owing to (i), infusion of calcium will build up a progressively increasing "load" or "positive balance" in the body

without reaching a state of equilibrium where the rate of infusion is equal to the rate of excretion of the cation.

iii) There is, however, a minimal isorrhoeic quantity for calcium, or "threshold of appearance". When this quantity is exceeded, progressively increasing loads will be accompanied by an increase in the rate of excretion of calcium, but this rate will never reach a level where it compensates adequately for the positive load.

Couched as it is in unfamiliar terms and concepts, the reasoning of the authors is not easy to understand or to follow. I think the conclusions reached by these authors may be summarised by saying that the kidney is an inefficient organ in eliminating excessive loads of calcium. By twisting this summary slightly, and translating it into popular terms, it can be said that the kidney knows more about the conservation of calcium than it knows about its excretion. I hope to apply this conclusion to the interpretation of my own studies.

The other paper, using a more orthodox approach to the subject, is by Chen and Neuman[40]. These workers, using dogs, estimated the concentration of diffusible plasma calcium by ultrafiltration through a cellophane membrane. They assumed that the concentration so obtained was the same as that in the glomerular filtrate, and, by measurement of the creatinine clearance and the rate of excretion of calcium in the urine were able to calculate the rate of tubular reabsorption of calcium.

From their results at normal and elevated calcium levels, and from the effects of citrate and versenate administration, they concluded that the diffusible calcium existed in two forms - ionised and non-ionised. Both were filtered through the glomerular membrane, the former was quantitatively reabsorbed by the tubules and the latter was rejected for excretion in the urine. In a second paper following immediately upon this, the same authors (41) were able to demonstrate inhibition of the calcium reabsorptive mechanisms of the tubules by phlorizin, dinitrophenol, sodium azide and the simultaneous reabsorption of strontium. Diamox was found to have no effect on tubular reabsorption. These authors record that single injections of calcium caused prolonged elevation of the serum calcium concentration and that even at these high levels 98% of the filtered calcium was reabsorbed - an observation that would support the contention of Wolf and Ball regarding the excretory "inefficiency" of the kidney with regard to calcium.

The acceptance of these results requires only the acceptance of the two assumptions made, namely that the in vitro estimate of the diffusible calcium is an accurate estimate of the concentration of calcium in the glomerular filtrate, and that the kidney handles calcium by a process of filtration and reabsorption. Allowing these two assumptions it is immediately apparent that no true Tm for Calcium exists, but that increasing amounts of calcium are absorbed as the concentration in the glomerular filtrate is increased.

Without having read either of these papers, and without having given much thought to the problems involved, I became interested in studying the renal mechanisms governing calcium excretion in man, and thought that the techniques of clearance analysis used by Hardwicke and Squire[109] to study the relationship between serum protein concentration and urinary excretion of protein in the nephrotic syndrome could be admirably adapted to serve this interest.

By "titrating" the kidney with calcium in a manner similar to that used in the phosphate experiments, I imagined that the relationship between plasma calcium concentration and rate of excretion of calcium in the urine should give an estimate both of the extent to which the plasma calcium is available for glomerular filtration and of the rate of reabsorption of calcium by the tubules according to the following mathematical formulation.

Let P_{Ca} denote total plasma calcium in mg.%

Let P''_{Ca} denote the concentration of calcium in a diffusible form.

Let UV_{Ca} denote the rate of excretion of calcium in the urine in mg./min.

Let T_{Ca} denote the rate of tubular reabsorption of calcium in mg./min.

Let G.F.R. denote the glomerular filtration rate in ml./min.

Then :-

$$\frac{G.F.R. \times P''_{Ca}}{100} = UV_{Ca} + T_{Ca} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

This equation can also be written

$$\frac{UV_{Ca} \times 100}{G.F.R.} = P''_{Ca} - \frac{T_{Ca} \times 100}{G.F.R.} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(2)$$

Now, for the time being, the acceptance of three hypotheses is required. These hypotheses are :-

i) That the amount of calcium in the plasma in a diffusible form is a constant proportion of the total plasma calcium at all levels of the latter. This has been suggested by the in vitro work of Howard et al.[129,131,132,133] and is apparent from the figures of Chen and Neuman[40].

ii) That the amount of calcium reabsorbed by the tubules from every 100 ml. of glomerular filtrate remains constant.

iii) That the calcium that is infused during the course of an experiment is incorporated naturally and rapidly into the diffusible and non-diffusible compartments of the plasma calcium.

If the first hypothesis is allowed, $P''_{Ca} = P_{Ca} \times k$ where k is a constant denoting that fraction of the plasma calcium in a diffusible form.

Substituting in Equation (2) we have:-

$$\frac{UV_{Ca} \times 100}{G.F.R.} = P_{Ca} \times k - \frac{T_{Ca} \times 100}{G.F.R.} \quad \ldots\ldots\ldots\ldots\ldots (3)$$

Accepting hypothesis (ii), it can be seen that this is a linear equation, and that the regression coefficient of $\frac{UV_{Ca} \times 100}{G.F.R.}$ on P_{Ca} would give, when P_{Ca} is made to change by intravenous infusion, an estimate of the filterability of the plasma calcium and the negative intercept upon the ordinate would give an estimate of the amount of calcium reabsorbed from each 100 ml. of filtrate.

The linearity of the plot would test the hypothesis that a constant fraction of the serum calcium is in a diffusible form at all plasma levels. The degree of correlation that

exists between the variables would test the hypothesis that the negative value of the intercept does not vary in a haphazard manner.

Having conceived the technique in theory, I applied it to six normal volunteers (Experiments XIV - XIX inclusive) in the following manner. The subjects were all fasting at the time of the experiments which were conducted with the patients lying supine. Calcium gluconate was infused at progressively increasing speeds (by the method described in Appendix B) over a period of 3 hours. During this time frequent urine collections were made and samples of blood were withdrawn $2\frac{1}{2}$ minutes before the midpoint of each urine collection period. These were centrifuged immediately and estimated for calcium concentration by the E.D.T.A. method. This method allowed a rapid estimation to be done so that a close check could be kept on the plasma calcium concentration. The glomerular filtration rate was measured with inulin.

In addition to the data from these experiments, I was able to collect satisfactory data from 3 experiments done 18 months previously. In these experiments (XX - XXII inclusive) calcium was infused at a constant rate over a short period of time and periods were selected from those where the calcium concentration was falling. Details of these experiments are presented in Appendix A.

Results.

The results are presented in Tables 16 to 24 inclusive, and in Figs. 21 to 29 inclusive.

Beneath each Table appears a value for four symbols. These are the four statistics used for each case in analysing the results.

In each case x denotes Poa in mg.% and y denotes $\frac{UVca \times 100}{G.F.R.}$

$$Sxx = \sum x^2 - \frac{(\sum x)^2}{n}$$

$$Syy = \sum y^2 - \frac{(\sum y)^2}{n}$$

$$Sxy = \sum xy - \frac{\sum x . \sum y}{n}$$

n = number of observations.

a = regression coefficient.

The slope of the line is given by Sxy/Sxx.

In the legend to each figure the 95% confidence interval of the regression coefficient is given as calculated from a pooled estimate of the error variance.

EXPERIMENT No. XIV Subject: D.M.

Table No: 16

Period	Pca mg.%	UVca mg./min.	G.F.R. ml./min.	UVca x 100 / G.F.R.
1	10.48	0.07	124.4	0.06
2	10.84	0.25	153.3	0.16
3	11.52	0.54	132.0	0.41
4	11.92	0.58	138.0	0.42
5	12.32	0.78	151.5	0.51
6	13.04	- ¤	149.2	- ¤

¤ Specimen insufficient for calcium determination due to partial loss after measurement of volume.

Sxx = 2.2899
Syy = 0.1471
Sxy = 0.1405
a = 0.248

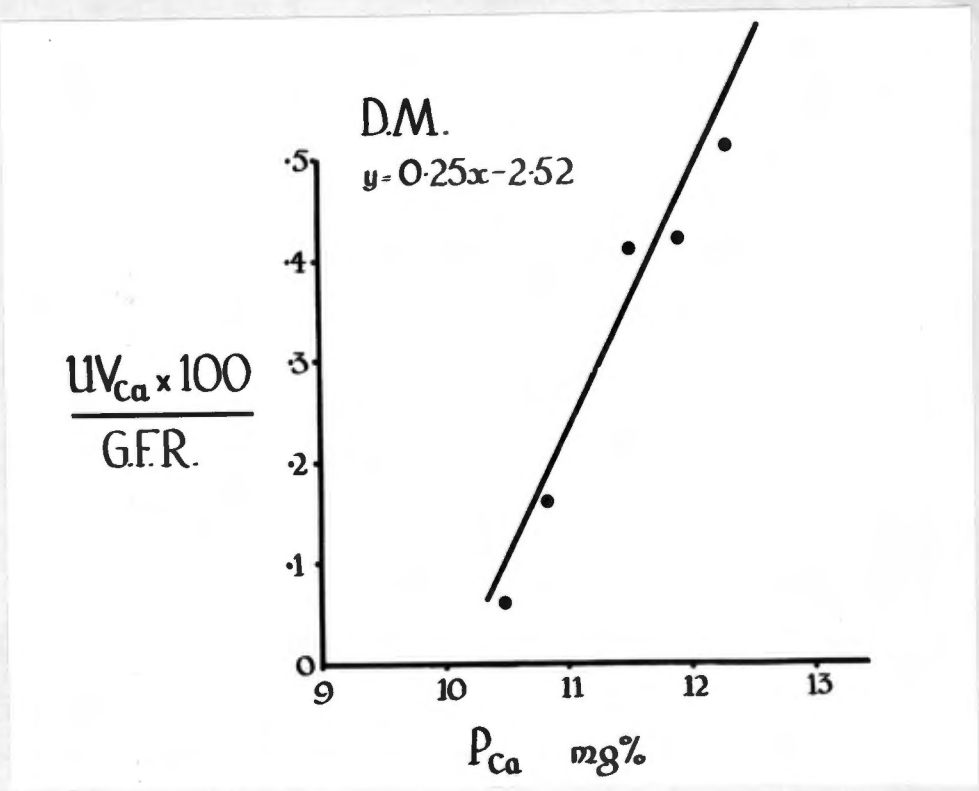

Fig. 21

Showing regression line for subject D.M. (Expt.XIV) calculated from data given in Table 16.

95% confidence interval of the regression coefficient a = 0.248 is from 0.063 to 0.433.

EXPERIMENT No. XV

Subject: E.P.

Table No: 17

Period	Pca mg.%	UVca mg./min.	G.F.R. ml./min.	UVca x 100 / G.F.R.
1	11.20	0.14	103.3	0.14
2	11.20	0.19	112.2	0.17
3	11.05	0.36	101.9	0.35
4	12.00	0.66	123.8	0.53
5	13.28	0.95	108.5	0.88
6	14.95	1.28	111.5	1.15
7	17.60	1.98	125.8	1.57
8	18.40	2.18	109.8	1.99
9	17.45	2.26	127.5	1.77

Sxx = 73.8840
Syy = 3.9842
Sxy = 16.9394
a = 0.229

The high value for Sxx is due to the high levels of plasma calcium that were achieved and to the preponderance of points at the extremes of the plot.

The effect of this large value for Sxx is to narrow the confidence interval of the regression coefficient and so increase the certainty with which the true slope α_i can be estimated.

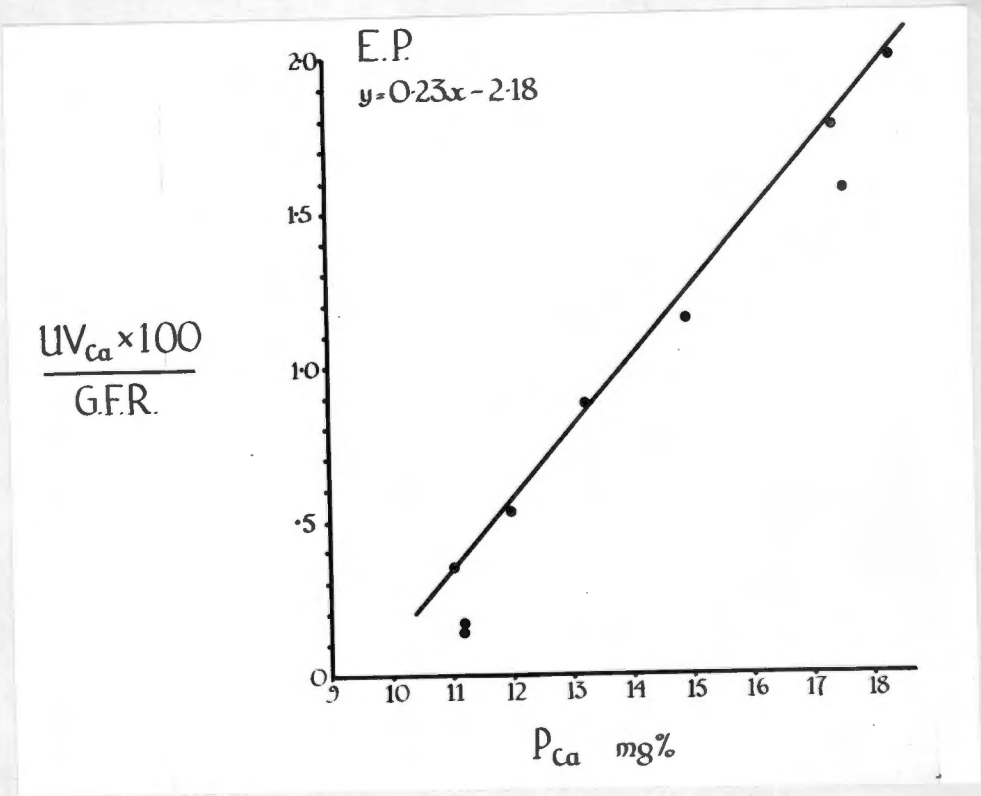

Fig. 22

Showing regression line for subject E.P. (Experiment XV) calculated from data given in Table 17.

95% confidence interval of the regression coefficient a = 0.229 is from 0.196 to 0.262.

EXPERIMENT No. XVI

Subject: S.A.

Table No: 18

Period	Pea mg.%	UVea mg./min.	G.F.R. ml./min.	UVea x 100 / G.F.R.
1	9.44	0.07	103.9	0.07
2	9.20	0.09	125.0	0.07
3	9.84	0.16	112.8	0.14
4	9.84	0.29	119.1	0.24
5	10.88	0.43	117.1	0.37
6	11.60	0.70	112.9	0.62
7	12.00	0.71	116.3	0.61
8	12.80	0.90	107.8	0.83
9	13.70	0.95	114.4	0.83

Sxx = 20.2592

Syy = 0.7706

Sxy = 3.8706

a = 0.191

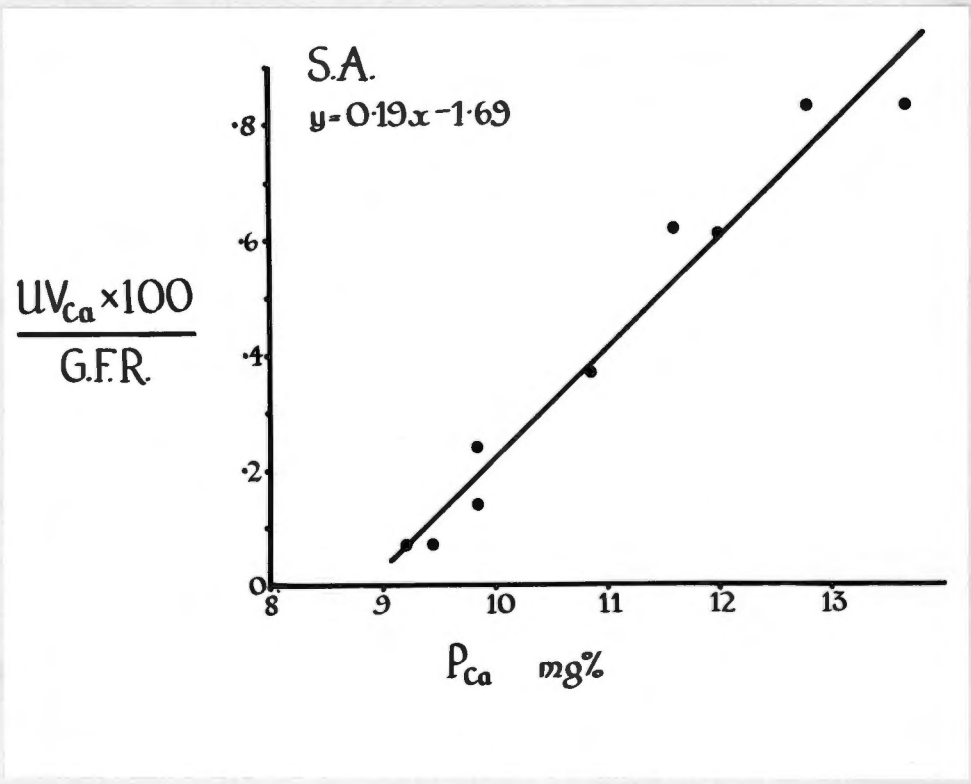

Fig. 23

Showing regression line for subject S.A.(Experiment XVI) calculated from data given in Table 18.

95% confidence interval of the regression coefficient a = 0.191 is from 0.129 to 0.253

EXPERIMENT No: XVII Subject: S.L.

Table No: 19

Period	Pca mg.%	UVca mg./min.	G.F.R. ml./min.	UVca x 100 G.F.R.
1	10.72	0.05	115.8	0.04
2	11.20	0.14	104.2	0.13
3	11.76	0.26	111.3	0.23
4	12.48	0.37	112.3	0.33
5	13.04	0.28	107.8	0.26
6	13.52	0.54	111.2	0.49

Sxx = 5.8720
Syy = 0.2446
Sxy = 0.7856
a = 0.134

The value for UVca for period 5 is probably too low owing to technical error in estimation. Unfortunately the specimen was discarded before a repeat analysis could be done.

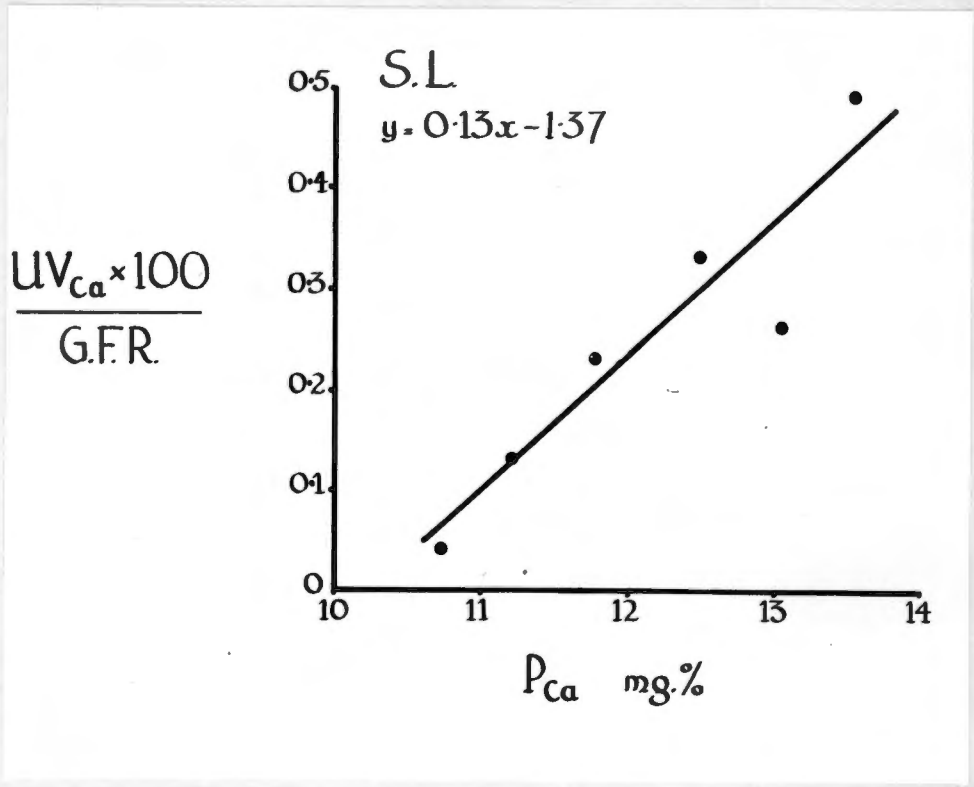

Fig. 24

Showing regression line for subject S.L.(Experiment XVII) calculated from data given in Table 19.

95% confidence interval of the regression coefficient a = 0.134 is from 0.018 to 0.250.

EXPERIMENT No. XVIII Subject: W.G.

Table No: 20

Period	Pca mg.%	UVca mg./min.	G.F.R. ml./min.	UVca x 100 / G.F.R.
1	10.88	-	117.3	-
2	11.04	0.001	107.1	0.01
3	11.92	0.270	109.7	0.25
4	12.64	0.380	119.6	0.32
5	13.76	1.200	126.5	0.95
6	14.48	0.900	102.2	0.88

Sxx = 10.6064
Syy = 0.8739
Sxy = 2.9371
a = 0.277

Urine Specimens U.1. and U.2. were virtually free of calcium.

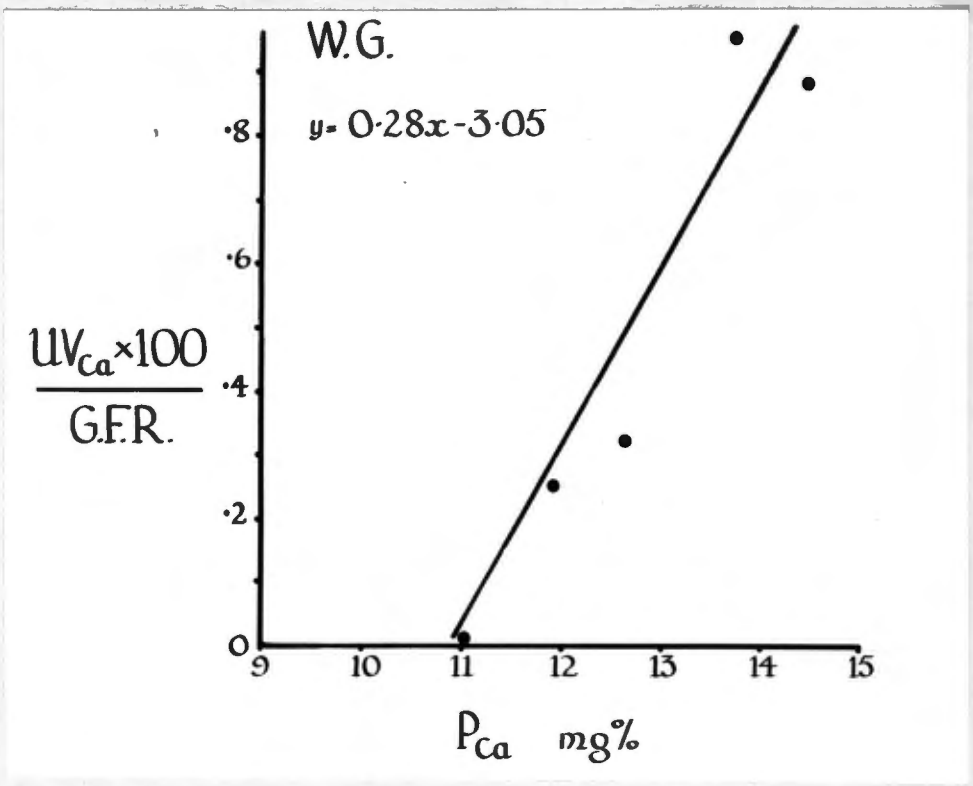

Fig. 25

Showing regression line for subject W.G.(Experiment XVIII) calculated from data given in Table 20.

95% confidence interval of the regression coefficient a = 0.277 is from 0.191 to 0.363.

EXPERIMENT No: XIX

Subject: F.S.

Table No: 21

Period	P_{Ca} mg.%	$U_{Ca}V$ mg./min.	G.F.R. ml./min.	$\frac{U_{Ca}V \times 100}{G.F.R.}$
1	10.56	0.06	76.5	0.08
2	10.88	0.07	90.6	0.08
3	11.52	0.18	95.0	0.19
4	12.00	0.46	91.6	0.50
5	12.64	0.87	95.2	0.91
6	13.36	1.34	107.8	1.24

$S_{xx} = 5.6373$

$S_{yy} = 1.1646$

$S_{xy} = 2.4928$

$a = 0.4422$

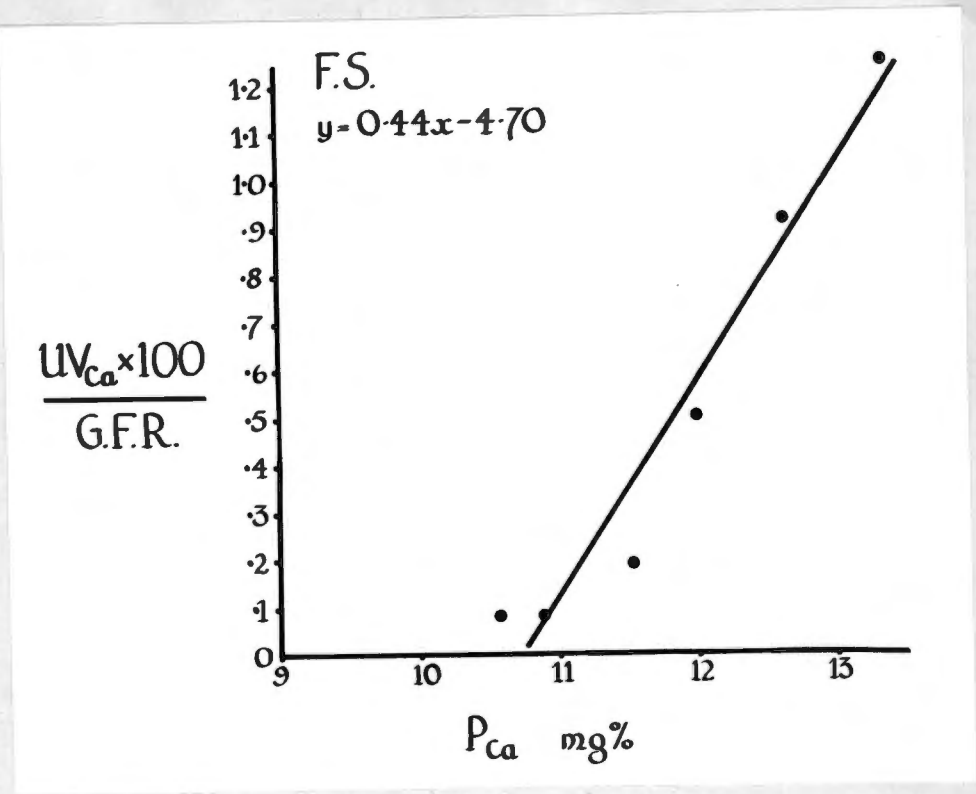

Fig.26

Showing regression line for subject F.S.(Experiment XIX) calculated from data presented in Table 21.

95% confidence interval for regression coefficient a = 0.442 is from 0.324 to 0.560.

EXPERIMENT No: XX Subject: H.A.

Table No: 22

Period	Pca mg.%	UVca mg./min.	G.F.R. ml./min.	UVca x 100 / G.F.R.
6	19.7	1.84	99.3	1.85
7	18.6	1.41	84.4	1.66
8	17.6	1.23	92.5	1.33
9	16.6	0.82	104.6	0.79
10	16.0	0.49	95.8	0.51
11	16.2	0.53	75.9	0.69

The data presented in this and the following two Tables were extracted from earlier experiments designed to study the metabolic effects of intravenous calcium. The parameters were estimated with a falling plasma calcium concentration.

$$S_{xx} = 10.79$$
$$S_{yy} = 1.5325$$
$$S_{xy} = 3.9980$$
$$a = 0.371$$

Despite the low value for n there is a reasonably high value for Sxx in this experiment owing to the preponderance of the points at the extremes of the plot. This has produced a fairly narrow confidence interval.

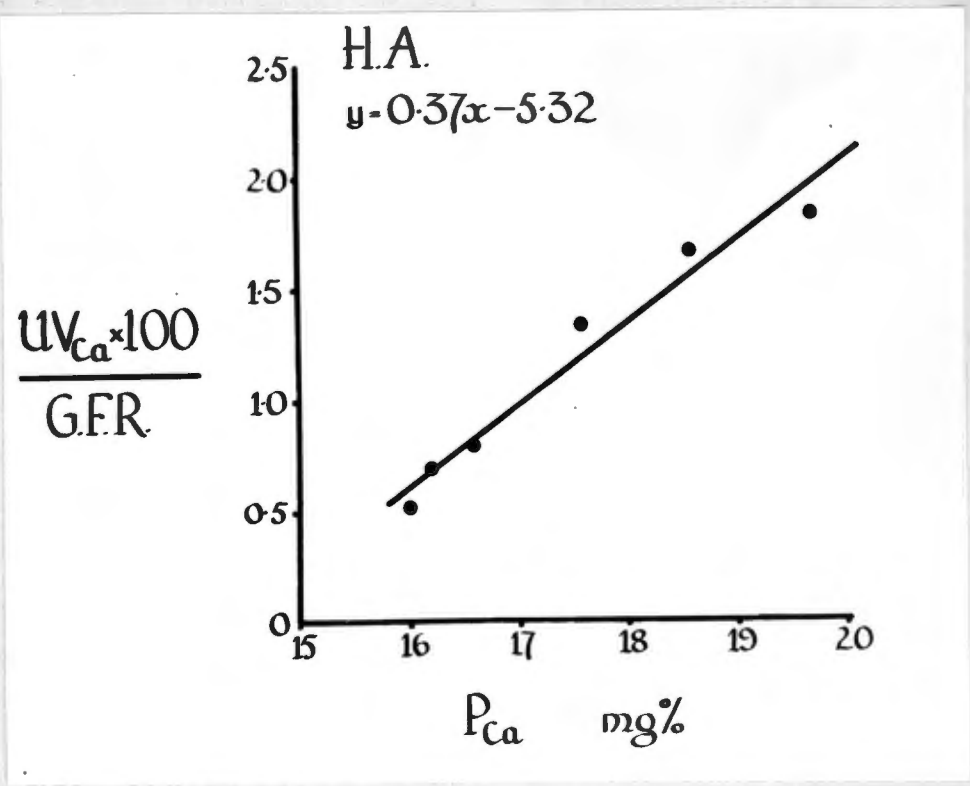

Fig. 27

Showing regression line for subject H.A.(Experiment XX) calculated from data presented in Table 22.

95% confidence interval for regression coefficient a = 0.371 is from 0.286 to 0.456.

It can be seen in this and the following two figures that the correlation with falling blood levels is not as good as it is with controlled rising blood levels.

EXPERIMENT No: XXI Subject: A.A.

Table No: 23

Period	P_{Ca} mg.%	$U_{Ca}V$ mg./min.	G.F.R. ml./min.	$\frac{U_{Ca}V \times 100}{G.F.R.}$
6	18.7	1.27	81.5	1.56
7	16.9	0.52	83.8	0.62
8	17.5	0.62	79.5	0.78
9	16.8	0.49	78.4	0.62
10	15.2	0.32	79.4	0.40
11	14.4	0.22	99.3	0.22

$S_{xx} = 12.15$

$S_{yy} = 1.0792$

$S_{xy} = 3.314$

$a = 0.2728$

Here the fairly high value for Sxx is attributable to the wide range of plasma calcium concentration.

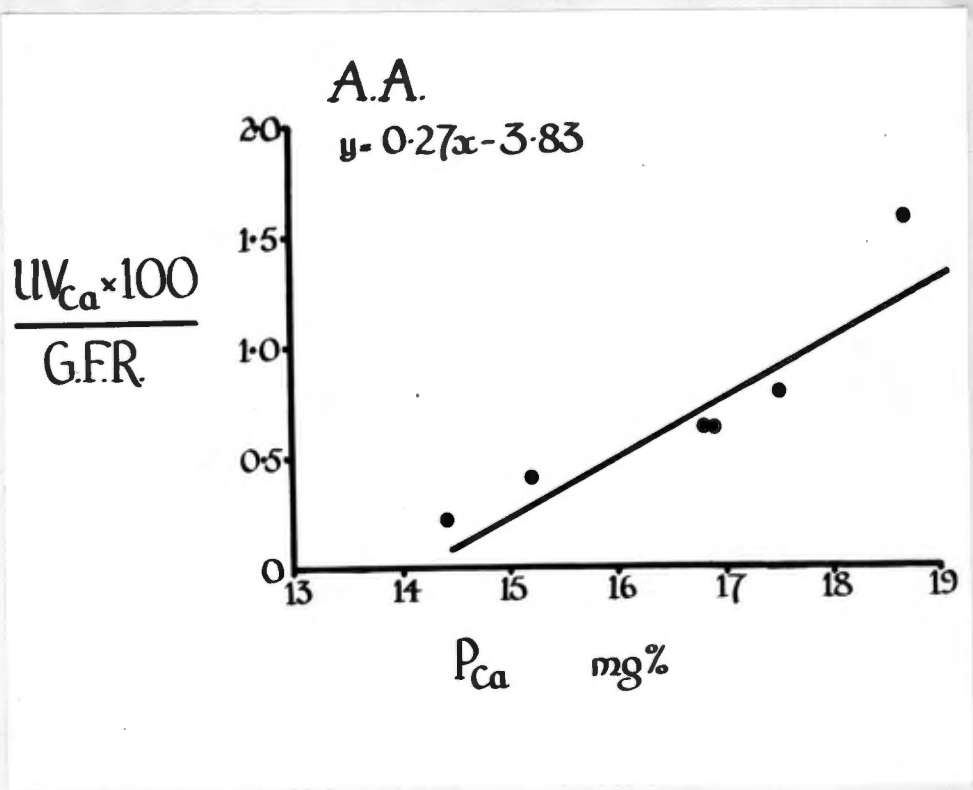

Fig. 28

Showing regression line for subject A.A.(Experiment XXI) calculated from data presented in Table 23.

95% confidence interval for regression coefficient a = 0.273 is from 0.193 to 0.353.

EXPERIMENT No: XXII

Subject: S.M.

Table No: 24

Period	Pca mg.%	UVca mg./min.	G.F.R. ml./min.	UVca x 100 / G.F.R.
6	18.4	1.01	127.6	0.79
7	18.4	1.14	101.0	1.13
8	16.7	1.05	111.6	0.94
9	17.3	0.82	109.4	0.75
10	15.8	0.63	128.1	0.49
11	15.8	0.35	107.5	0.33

$S_{xx} = 6.95$

$S_{yy} = 0.4253$

$S_{xy} = 1.352$

$a = 0.195$

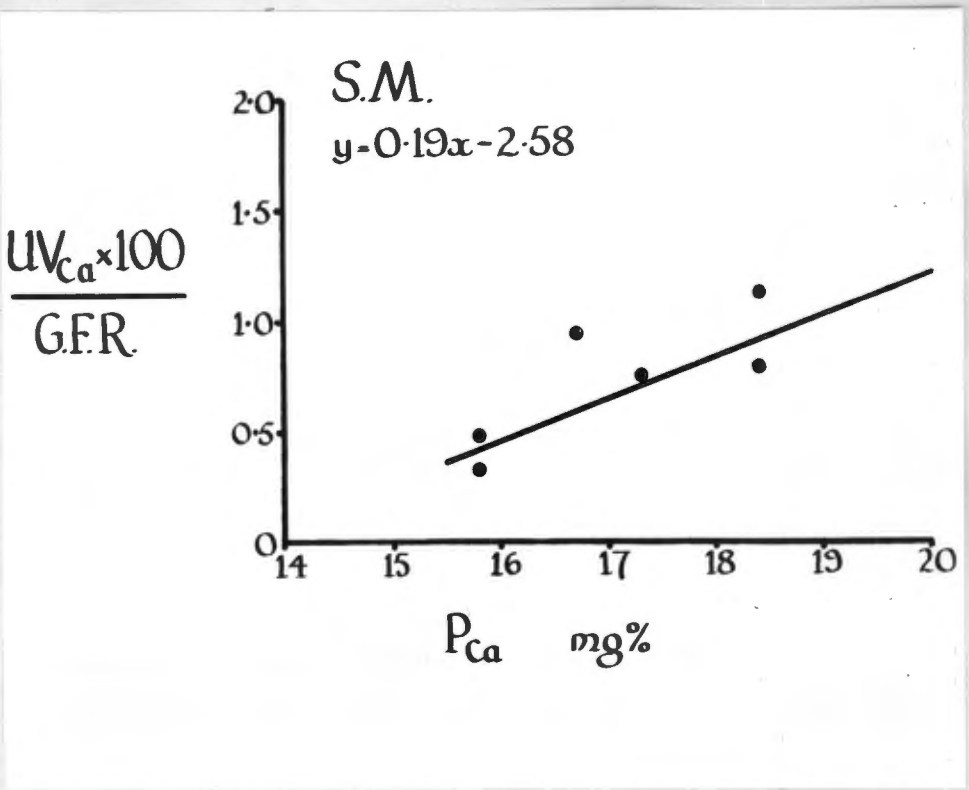

Fig. 29

Showing regression line for subject S.M.(Experiment XXII) calculated from data presented in Table 24.

95% confidence interval for regression coefficient a = 0.195 is from 0.089 to 0.301.

In this manner, 9 regression coefficients for $\frac{UVca \times 100}{G.F.R.}$ on Pca were obtained with 9 values for the negative intercept upon the ordinate. According to the theory upon which this technique was formulated, these parameters should be equal in each case to the proportion of the serum calcium in a filterable form and the amount of calcium reabsorbed from each 100 ml. of glomerular filtrate, respectively..

I shall discuss the technique and the values obtained by answering the questions that occurred to me when I came to interpreting the results. Before doing so, however, I would like to explain, but not to apologise for, the liberal use of statistics in this section.

The techniques for estimating calcium, unlike those for estimating inorganic phosphorus, are lengthy and tedious - both factors which have a pronounced effect on their accuracy. This fact has introduced an element of uncertainty into the results that was negligible with the phosphate experiments but is appreciable here. I have used statistics to measure the degree of certainty with which my conclusions may be drawn.

The questions that arose were these -

1. <u>How accurate is the technique for individual patients, and what measures can be taken to improve the accuracy</u> ?

As an indication of the inaccuracy of the technique I have given the 95% confidence interval for each regression coefficient, arguing that the wider the interval the more inaccurate is the method.

It will be seen that the correlation, and hence the

certainty with which the regression coefficient can be estimated, is increased when the technique employs rising blood calcium concentrations.

The wider the range of calcium concentration achieved, and the further these values are from their total mean value, the greater the degree of certainty with which the slope can be measured. This can be seen from the narrow confidence interval obtained when the variance of Pca was large. In practice, therefore, this should mean that one should aim for as high a serum calcium concentration as is possible and not harmful, and that the urinary collection periods should be shorter and more frequent at the lower and higher concentrations.

Using $\frac{UVca}{GFR}$ as the ordinate does, to a large extent, eliminate incomplete bladder emptying as a source of error by referring the rate of excretion of calcium to a constant glomerular filtration rate.

2. Was the rise in serum calcium concentration linear or not ?

This is an important question, as upon its answer depends the extent to which the results of analyses performed on the mid-period blood samples can be considered representative of the average plasma concentration during the urinary collection periods.

I have not used statistics to provide this answer. Examination of the line drawn for Pca against time in Figs 30 - 35 inclusive indicates that this question can reasonably be answered in the affirmative.

2a. Is the performance of the test in any way harmful to the patient ?

In no case where the plasma calcium concentration was kept below 15 mg.% were any unpleasant symptoms experienced. Concentrations in excess of this figure usually caused symptoms of nausea. E.C.G. changes were not looked for, but there were no changes in blood pressure or pulse rate to suggest significant effect on cardiac function. Muscular power, as judged by hand grip and ability to dorsiflex the foot against resistance, was unaffected. Dr.Jackson, Staff Nurse Schoonraad and myself, were convinced, on clinical impression, that psychogenic symptoms were often markedly improved by the procedure.

Even in the cases where the plasma calcium concentration did rise to very high levels, I feel certain that no lasting or dangerous ill-effects resulted.

3. Has the assumption that the diffusible fraction of the plasma calcium is a constant fraction of the total plasma calcium been substantiated ?

The good linearity of most of the plots has, I think, lent considerable support to this assumption. From the results of Hastings and McLean[171,172] I would have expected the value for "k" to increase as the plasma calcium concentration rose, with a resulting curvilinear plot for each case, and I am grateful to Dr. E.N.Keen of the Anatomy Department, for pointing out that a truly curvilinear relationship could be changed to apparent linearity by corresponding changes in the rate of reabsorption of calcium by the tubules. To use a very flimsy line of reasoning, there was no evidence to suggest that this had occurred so I shall presume

that it did not. In any event the net result was a straight line in most cases with a constant slope.

4. Are the observed differences between the regression coefficients for each normal subject significant or due to sampling error ?

This, to my mind, is a most important question that needs to be answered with certainty before an estimate of the "normal range" for these regression coefficients can be obtained. If the differences between patients is due solely to error in technique, the range becomes very wide and the test would lose any claim it may have to providing a parameter that will distinguish normal from abnormal.

The most satisfactory way of answering this question is to test, by means of an analysis of covariance and F Test, the hypothesis that all of the values for a are equal. A table of covariance to test this hypothesis was accordingly drawn up and is presented as Table 25. The details of the procedure used for drawing up this Table are presented in Appendix B.

	D.f.	Sums of Squares	Mean Sum of Squares
Differences between individual slopes a_i	8	0.5778	0.07223
Deviations within subjects from their own slopes, a_i	41	0.7885	0.01923
Total - Deviation within patients from average slope, $\bar{a}$	49	1.3663	

$F_{8.41}$ = 3.756. Significant at 1% level.

Table 25. Analysis of covariance to test the hypothesis that a_i are equal.

It is readily apparent from this Table that the hypothesis can be rejected with a very high degree of certainty.

It can be concluded that the differences between subjects are real and significant and that the technique is sufficiently accurate to be able to provide a parameter for distinguishing indivicuals.

5. Having decided that the parameter is reliable, what is the normal range for the regression coefficient ?

The standard procedure used in medical statistics to define a "normal range" for any parameter such as height, weight, or blood-pressure is to take numerous observations of that parameter from subjects judged by other criteria to be normal. The hypothesis that the values so obtained are distributed normally about some average value is tested. If this hypothesis is found to be acceptable, the standard deviation of the sample is used to compute a confidence interval for the entire population.

I am, in this instance, unable to follow this procedure because firstly I have insufficient data, secondly I have no authority for the assumption that the estimated regression coefficients are distributed normally in a large population, and thirdly I have no reasonably certain estimate of the true average slope.

All that I am able to do, therefore, is to say that

(i) I found the average slope in those 9 subjects to be 0.244.

(ii) In all cases, with the exception of F.S., I am fairly certain that the true value for the regression coefficient was below 0.5.

7. What proportion of the plasma calcium is generally accepted as being in a diffusible form ?

By far the major portion of the work that has been done on this problem has employed the principle of ultrafiltration under positive or negative pressure of serum through collodion or cellophane

membranes. The techniques employing this principle vary in the extent to which refinements are added, the most commonly used being that of Lavietes[161]. Grollman[94] has reviewed the techniques of ultrafiltration and their interpretation and concluded that the results could be affected by changes in pH, pore size and amount of pressure employed.

Using this method different workers have obtained different results[51,24,131,40,133,129,132,195,194]. As Chen and Neuman[40] have indicated, these discrepancies may be due to differences in analytical methods. As an average figure one can take it that some 50-60% of the serum calcium is filterable by this technique.

Compensation dialysis through artificial membranes has yielded similar results to the above whether applied in vitro as by Rona and Takahasi[221] or in vivo as by Greene and Power[89,213].

Inferences regarding the partitioning of plasma calcium have been drawn from its concentration in cerebro-spinal fluid[103] and in collections of oedema fluid and other transudates. Observations on the latter by Salvesen and Linder[224] indicate that the concentration of calcium in these fluids is approximately 55% of that in the plasma, rising to 75% as the protein content of the fluid increases.

The physico-chemical form in which the diffusible fraction exists is also a matter of some dispute. Some of the earliest work on the subject was done by Brinkman and van Dam[29] who with a turbidimetric technique using oxalate solution on an ultrafiltrate of plasma concluded that this contained some 17 - 25% of calcium in an ionised form. Neuhausen and Marshall,[193] measuring calcium ion

concentration in human plasma with a calcium amalgam electrode found that 15 - 25% of the plasma calcium was in an ionised form. As they point out themselves, however, this technique suffers from the disadvantage that the calcium amalgam forms an unstable electrode in solutions containing other cations.

Benjamin and Hess[23,24] were able to distinguish two fractions in an ultrafiltrate of plasma on the grounds of adsorbability by barium sulphate. As they found that barium sulphate did not absorb ionised calcium in pure solution they concluded that there were two distinct moieties in the ultrafiltrate, an ionised and a non-ionised. The latter could also be adsorbed by epiphyseal cartilage. They concluded that some 50% of the total plasma calcium was in a diffusible form, and this was equally divided into ionised and non-ionised fractions.

McLean and Hastings[171,172] from their classical studies with a frog heart preparation concluded that 50% of the plasma calcium existed in an ionised form. They extended their studies to show that the extent to which calcium was ionised depended upon the serum protein concentration so confirming the suggestion first made by Pribram[214] in 1871 and subsequently supported by others[224,164, 221,173].

Ignoring for the moment physico-chemical issues involved, this brief review can be summarised by saying that 50% is, if anything, a slightly conservative estimate of the proportion of ultrafilterable calcium in the plasma.

8. How do the values obtained for the 9 normal subjects in this series compare with the generally accepted value for the proportion of calcium in a filterable form ?

Taking 0.5 as the generally accepted value, and the regression coefficient for each patient, a_i, as the estimated value, I felt that the best way of answering this question would be to test by means of Students' t, the hypothesis that a_i was equal to 0.5. The results are given in Table 26.

Table 26.

Subject	Experiment No.	Regression Coefficient (a_i)	P ≡
D.M.	XIV	0.248	0.01
E.P.	XV	0.229	0.001
S.A.	XVI	0.191	0.001
S.L.	XVII	0.134	0.001
W.G.	XVIII	0.277	0.001
F.S.	XIX	0.442	0.25
H.A.	XX	0.371	0.01
A.A.	XXI	0.279	0.001
S.M.	XXII	0.195	0.001

P in each case refers to the level of probability at which the hypothesis that $a_i = 0.5$ can be rejected. This level of probability was calculated from the pooled error variance.

It is apparent from Table 26 therefore, that it can be said with a very high degree of certainty that, with the exception of subject F.S. my estimate of the diffusible calcium concentration is lower than that generally accepted.

9. How can this discrepancy best be explained ?

I can think of three possible explanations.

(i) The first, which I mention only to dismiss, is that the whole army is out of step with the exception of myself.

(ii) The second is that the tubules selectively reabsorb only the ionised fraction of the calcium in the glomerular filtrate and reject the remainder which is in a diffusible but non-ionic form. If this were the case the regression coefficient would estimate only the non-ionised diffusible calcium concentration. This explanation has the support of the experimental results of Chen and Neuman[40] who observed a rise in urine calcium after intravenous administration of sodium citrate. In addition, my regression coefficients agree fairly well with the estimated concentration of the non-ionised diffusible calcium of Benjamin and Hess[23,24].

(iii) The third explanation, and the one which I favour, is that the slope of the regression line has been diminished in each case by the fact that the tubules reabsorb calcium at a greater rate as the filtered load is increased by elevation of the plasma calcium concentration. The effect of increased tubular activity on the slope of the line was discussed in connection with the phosphate experiments and is diagrammatically shown in Fig. 6. The following facts, I think, lend indirect support to this explanation.

(a) The observations of Wolf and Ball, and Chen and Neuman quoted earlier regarding the "inefficiency" of the kidney as an excretory organ with regard to calcium. Clearly, if the tubules reabsorbed a fixed amount of calcium and rejected any excess the

kidneys would excrete a load far more rapidly than they would if the rate of conservation of calcium by tubular reabsorption increased with the filtered load.

(b) Most substances for which a "Tm" exists are handled principally by the proximal tubules. In the case of calcium where reabsorption, as I envisage it, is more of a "facultative" process one would expect the brunt of the responsibility for conservation to fall upon the distal tubules. That this is in fact the case is suggested by the fact that calcium "competes" with sodium for tubular reabsorption and by the micro dissection studies of Darmady and Stranack[54] which showed that metastatic calcification in a case of nephrocalcinosis was maximal in the distal tubules.

I hope to test the validity of this explanation by a series of experiments using clearance analysis and renal vein catheterisation. I have not, however, at the time of writing, marshalled my ideas into any decisive plan of action and am regretfully compelled to leave this important question unanswered for the moment.

10. Was there any correlation between the estimate of diffusibility and the serum protein concentration in these cases ?

Total and fractional serum protein estimations were done on the first six patients, and no inverse correlation, as would be expected, could be found.

11. What is the significance of the values obtained for the negative intercepts on the ordinates ?

The negative value of the intercept for each subject is, by virtue of the fact that it is dependent on the slope and is affected by renal delay time, meaningless.

Summary.

An experimental procedure is described that was devised to give an estimate of the proportion of the plasma calcium that is diffusible and of the rate of tubular reabsorption of calcium. When this procedure was used in a series of normal subjects, an estimate of the filterability of the plasma calcium was obtained that was below the generally accepted figure.

To explain this discrepancy I have suggested that the rate of tubular reabsorption of calcium is accelerated by increasing the concentration of calcium in the glomerular filtrate.

Statistical analysis of the data from these experiments indicates that the technique provides a parameter that will distinguish individuals. This parameter is the regression coefficient for $\frac{UV_{Ca} \times 100}{G.F.R.}$ on P_{Ca}.

The value for this parameter is normally below 0.5.

S E C T I O N III.

The Metabolic Effects of Intravenous Calcium Infusion.

The Effect of Calcium Infusion on Urine Sodium and Potassium Excretion.

THE METABOLIC EFFECTS OF INTRAVENOUS CALCIUM INFUSION.

The sequence of metabolic events taking place after or during the intravenous infusion of calcium salts has been studied for a variety of reasons. The earlier studies of Salvesen[223] were designed to elucidate the diuretic action of calcium salts. Schilling and Laszlo[228] used the retention of calcium following intravenous administration as an index of bone metabolism, and others have used the procedure to study parathyroid function. It is with the results and conclusions of this third group of investigators that this section is concerned.

The first effect of artificial elevation of the plasma calcium concentration is a rise in plasma inorganic phosphorus concentration. This was observed first by Salvesen et al. in 1927[223] and has been repeatedly confirmed since by many other workers.[132,84,196,156,40] Although Salvesen was the first to draw attention to the phenomenon, it is evident from the data given by Brull and Nicholz in the appendix to a paper published in 1925[34]. This rise, it is generally agreed, is not the result of transient parathyroid suppression with resulting increase in the rate of tubular reabsorption of phosphate, for the following reasons. Firstly, the rise is too rapid to be explained in this way; secondly, the extent of the rise would necessitate a tubular contribution to extracellular phosphate far in excess of that actually made by increased reabsorption; and, thirdly, the rise is also observed in hypoparathyroid patients. The increase in inorganic phosphate is therefore considered to be due to a non-specific transfer of phosphorus from the intra- to the extra-cellular fluid compartments.

Chen[40], by doing simultaneous estimations on red cell and serum phosphorus, was able to conclude that the rise in serum inorganic phosphorus was accompanied by fall in the concentration of organic ester phosphate in the cells, and so lent support to this explanation. Chambers et al.[38] have defined the "normal" rise that can be expected after a standard dose of calcium, and have interpreted the "subnormal" rise in patients with hyperparathyroidism as evidence of depleted intracellular phosphate stores.

The second effect that has been studied extensively has been that on the rate of excretion of phosphorus in the urine. With few exceptions most authors have reported a fall in urine phosphate, which, in conjunction with the rise in serum phosphate, has been taken to indicate an increased rate of phosphate reabsorption by the tubules owing, in turn, to parathyroid suppression.

Howard et al.[132], observed a fall in normal patients, a rise in patients with hypoparathyroidism and no change in patients with hyperparathyroidism.

Nordin and Fraser[196] found a similar effect, and added the observation that a greater phosphate diuresis could be produced by parathormone when given after a calcium infusion than when given normally.

Kyle, Schaaf and Erdman[156] found that calcium infusion "usually" caused an immediate fall in the rate of excretion of phosphate in the urine that was more apparent when the total 24 hour specimen was considered.

It is interesting to note in Figs. 1.A. and 3 of this paper that there was a marked rise in the rate of excretion of phosphorus following the infusion.

Milne[183] has published a paper recording his observations on the mode of action of parathormone, and it is apparent from Fig. 3 of that paper that Calcium injection caused a rise in the rate of appearance of phosphorus in the urine.

Baylor et al.[22], in a long term experiment in which intravenous calcium was administered as a daily supplement to oral calcium, noted a fall in urine phosphorus as a result of this.

Brull and Eichols[34] gave details of experiments with intravenous calcium chloride experiments in dogs in an appendix to a paper published in 1925. It is apparent from these data that small doses of $CaCl_2$ caused a rise in phosphate secretion whereas large doses caused a fall.

Levitt et al.[163] noted a rise in urine phosphorus with calcium infusion that reached a maximum one hour after the infusion had been discontinued.

As I was unable to find any record in the literature of an investigation into the effects of calcium infusion on the renal handling of phosphate using inulin clearances as a measure of the glomerular filtration rate, I embarked on a series of experiments on normal subjects using this method.

The results of these experiments are recorded in Tables 27 to 37 inclusive and are graphically shown in Figs. 30 to 40 inclusive.

In the first 6 cases calcium was infused at a progressively increasing rate throughout the experiment. In the last 5 cases it was infused over a short period of time after a control period had elapsed. In these last five cases estimations were continued for up to eight hours after the infusion had ended. The diagrams and tables are self-explanatory, although I should explain my choice of $\frac{UVp \times 100}{G.F.R.}$ and $\frac{Tp \times 100}{G.F.R.}$ to indicate the rate of excretion of phosphorus in the urine and the rate of tubular reabsorption of phosphorus respectively. My main reason for this choice was to correct these values for changes in glomerular filtration rate that may have been due to incomplete bladder emptying. If absolute values are preferred they may be found in the tables. Further details regarding these experiments will be found in Appendix A.

EXPERIMENT No. XIV

Subject: D.M.

Table No: 27

Period	Time (mins)	Pea (mg.%)	Pp (mg.%)	G.F.R. ml./min.	Fp (mg/min)	UVp (mg/min)	Tp (mg/min)	Tp x 100 / G.F.R.	UVp x 100 / G.F.R.
1	29.0	10.48	4.12	124.4	5.13	0.03	5.10	4.10	0.02
2	31.7	10.84	4.23	153.3	6.48	0.14	6.34	4.14	0.09
3	31.7	11.52	4.28	132.0	5.65	0.23	5.42	4.11	0.17
4	29.3	11.92	5.04	138.0	6.96	0.24	6.72	4.87	0.17
5	31.1	12.32	5.50	151.5	8.33	0.38	7.95	5.25	0.25
6	28.0	13.04	6.24	149.2	9.31	0.59	8.72	6.84	0.40

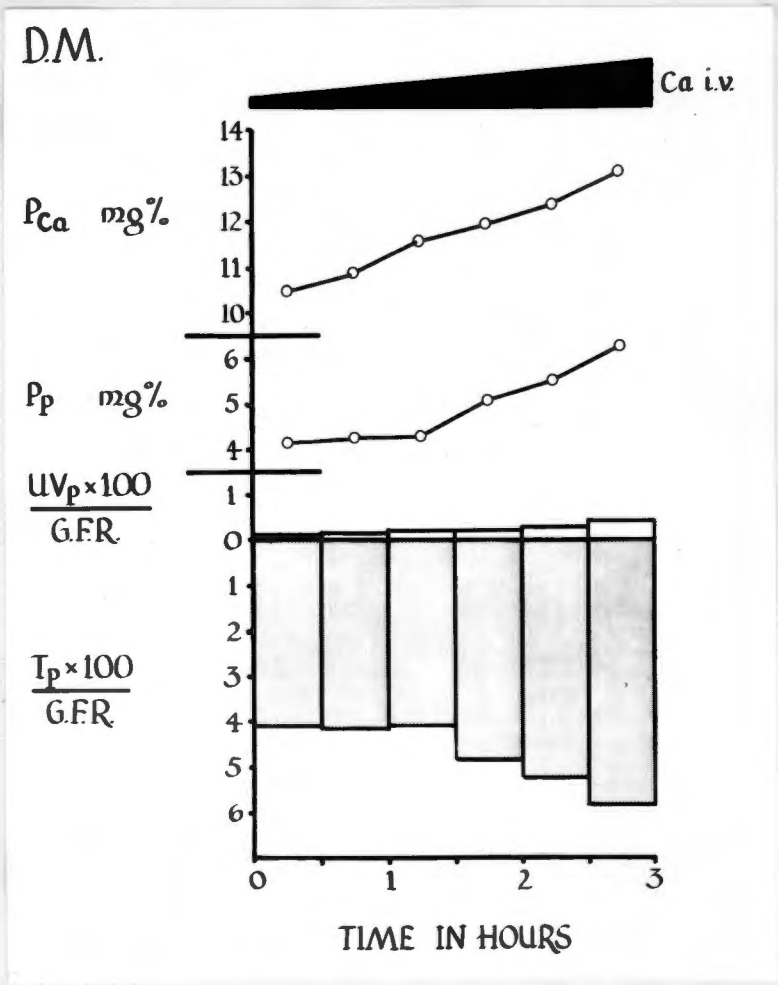

Fig. 30.

In this and all subsequent figures in this section the effects of intravenous calcium gluconate upon serum calcium concentration(P_{Ca}), Serum inorganic Phosphorus concentration (P_p), the rate of excretion of phosphate in the urine in mg./min./100 cc. glomerular filtrate (UVp x 100/G.F.R.) and the rate of tubular reabsorption of phosphorus in mg./min./100 mls. filtrate (Tp x 100/G.F.R.) are shown.

The values for UVp x 100/G.F.R. are represented by clear blocks extending upwards from the base line.

The values for Tp x 100/G.F.R. are represented by shaded blocks plotted downwards from the base line.

EXPERIMENT No. XV

Subject: E.P.

Table No: 28

Period	Time (mins)	Poa (mg.%)	Pp (mg.%)	G.F.R. ml./min.	Fp (mg/min)	UVp (mg/min)	Tp (mg/min)	Tp x 100 / G.F.R.	UVp x 100 / G.F.R.
1	20.1	11.20	3.87	103.3	4.00	0.34	3.66	3.54	0.33
2	19.9	11.20	4.10	112.2	4.60	0.33	4.27	3.81	0.29
3	20.8	11.05	4.08	101.9	4.16	0.38	3.78	3.71	0.37
4	19.8	12.00	4.33	123.8	5.36	0.52	4.84	3.91	0.42
5	23.6	13.28	4.65	108.5	5.05	0.60	4.45	4.10	0.55
6	21.1	14.95	5.07	111.5	5.65	0.71	4.94	4.43	0.64
7	19.5	17.60	5.57	125.8	7.01	1.34	5.67	4.51	1.06
8	16.5	18.40	5.67	109.8	6.23	1.49	4.74	4.32	1.35
9	18.2	17.45	5.90	127.5	7.52	1.95	5.57	4.39	1.53

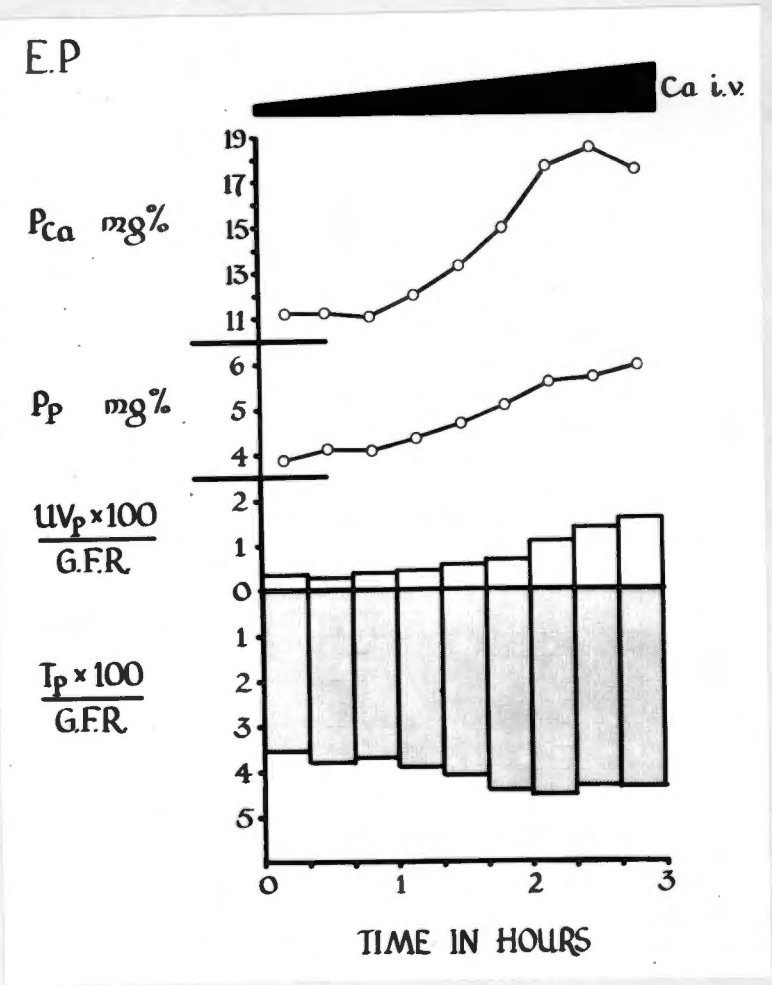
E.P
Ca i.v
P_{Ca} mg%
P_P mg%
$\frac{UV_P \times 100}{G.F.R.}$
$\frac{T_P \times 100}{G.F.R.}$
19
17
15
13
11
6
5
4
2
1
0
1
2
3
4
5
0
1
2
3
TIME IN HOURS

Fig. 31.

EXPERIMENT No: XVI

Subject: S.A.

Table No: 29

Period	Time (mins)	Pca (mg.%)	Pp (mg.%)	G.F.R. ml./min.	Pp (mg/min)	UVp (mg/min)	Tp (mg/min)	Tp x 100 / G.F.R.	UVp x 100 / G.F.R.
1	23.1	9.44	3.59	103.9	3.73	0.12	3.61	3.47	0.12
2	22.0	9.20	3.80	125.0	4.75	0.18	4.57	3.66	0.14
3	21.0	9.84	4.05	112.8	4.57	0.25	4.32	3.83	0.22
4	20.8	9.84	4.15	119.1	4.94	0.55	4.39	3.69	0.46
5	21.7	10.88	4.38	117.1	5.13	0.49	4.64	3.96	0.42
6	19.9	11.60	4.55	112.9	5.14	0.72	4.42	3.91	0.64
7	22.0	12.00	5.02	116.3	5.84	0.87	4.97	4.27	0.75
8	19.5	12.80	5.07	107.8	5.47	1.09	4.38	4.06	1.01
9	20.5	13.70	5.32	114.4	6.09	1.10	4.99	4.36	0.96

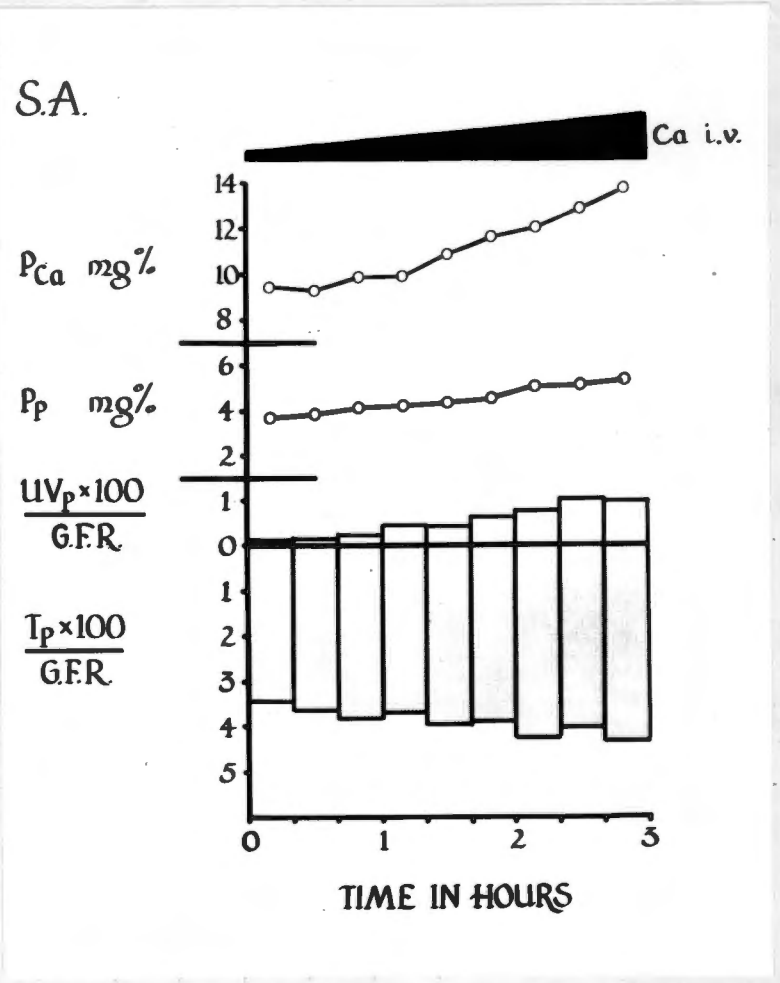
S.A.
Ca i.v.
P_{Ca} mg%
P_P mg%
$\frac{UV_P \times 100}{G.F.R.}$
$\frac{T_P \times 100}{G.F.R.}$
14
12
10
8
6
4
2
1
0
1
2
3
4
5
0
1
2
3
TIME IN HOURS

Fig. 32.

EXPERIMENT No: XVII

Subject: S.L.

Table No: 30

Period	Time (mins)	Pea (mg.%)	Pp (mg.%)	G.F.R. ml./min.	Fp (mg/min)	UVp (mg/min)	Tp (mg/min)	Tp x 100 / G.F.R.	UVp x 100 / G.F.R.
1	30.5	10.72	4.64	115.8	5.37	0.37	5.00	4.32	0.32
2	30.2	11.20	4.92	104.2	5.13	0.30	4.83	4.64	0.28
3	31.5	11.76	4.83	111.3	5.38	0.36	5.02	4.51	0.32
4	29.1	12.48	5.15	112.3	5.78	0.35	5.43	4.84	0.31
5	33.2	13.04	5.26	107.8	5.67	0.32	5.35	4.96	0.30
6	24.5	13.52	5.80	111.2	6.45	0.44	6.01	5.40	0.40

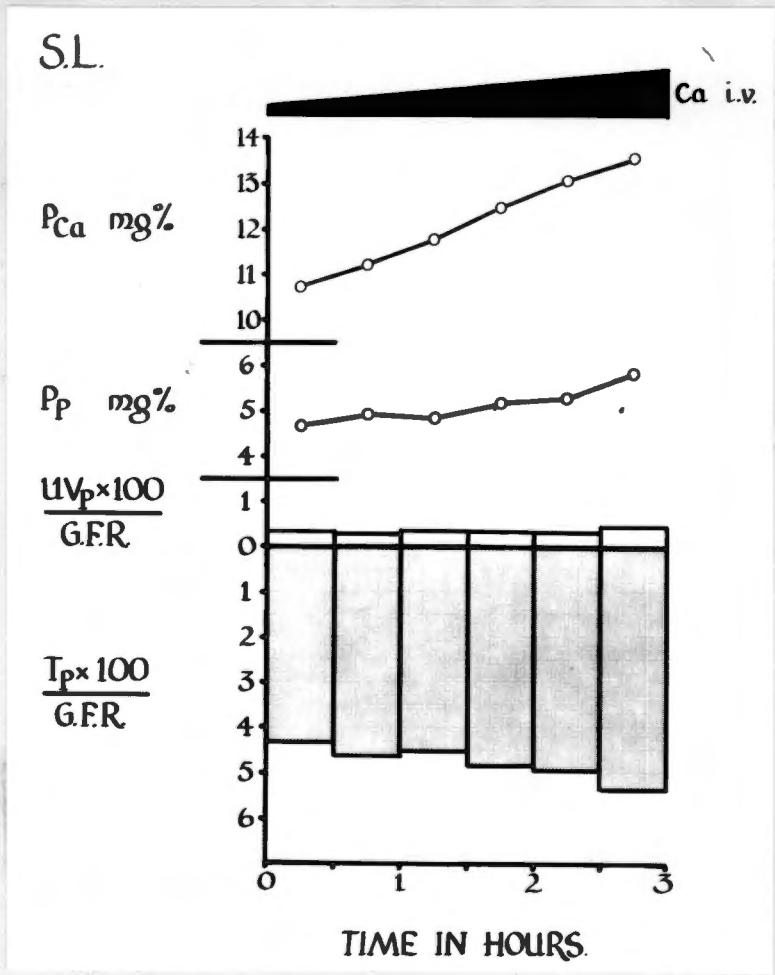
S.L.
Ca i.v.
P_{Ca} mg%
P_P mg%
$\frac{UV_P \times 100}{G.F.R.}$
$\frac{T_P \times 100}{G.F.R.}$
14
13
12
11
10
6
5
4
1
0
1
2
3
4
5
6
0
1
2
3
TIME IN HOURS.

Fig 33.

EXPERIMENT No: XVIII

Subject: W.G.

Table No: 31

Period	Time (mins)	Pca (mg.%)	Pp (mg.%)	G.F.R. ml./min)	Fp (mg/min)	UVp (mg/min)	Tp (mg/min)	Tp x 100 / G.F.R.	UVp x 100 / G.F.R.
1	28.5	10.88	4.85	117.3	5.69	0.21	5.48	4.67	0.18
2	31.0	11.04	5.22	107.1	5.59	0.28	5.31	4.96	0.26
3	30.0	11.92	5.40	109.7	5.92	0.40	5.52	5.03	0.37
4	30.7	12.64	5.64	119.6	6.75	0.56	6.19	5.18	0.46
5	32.0	13.76	6.13	126.5	7.75	0.69	7.06	5.58	0.55
6	27.2	14.48	6.15	102.2	6.29	0.77	5.52	5.40	0.75

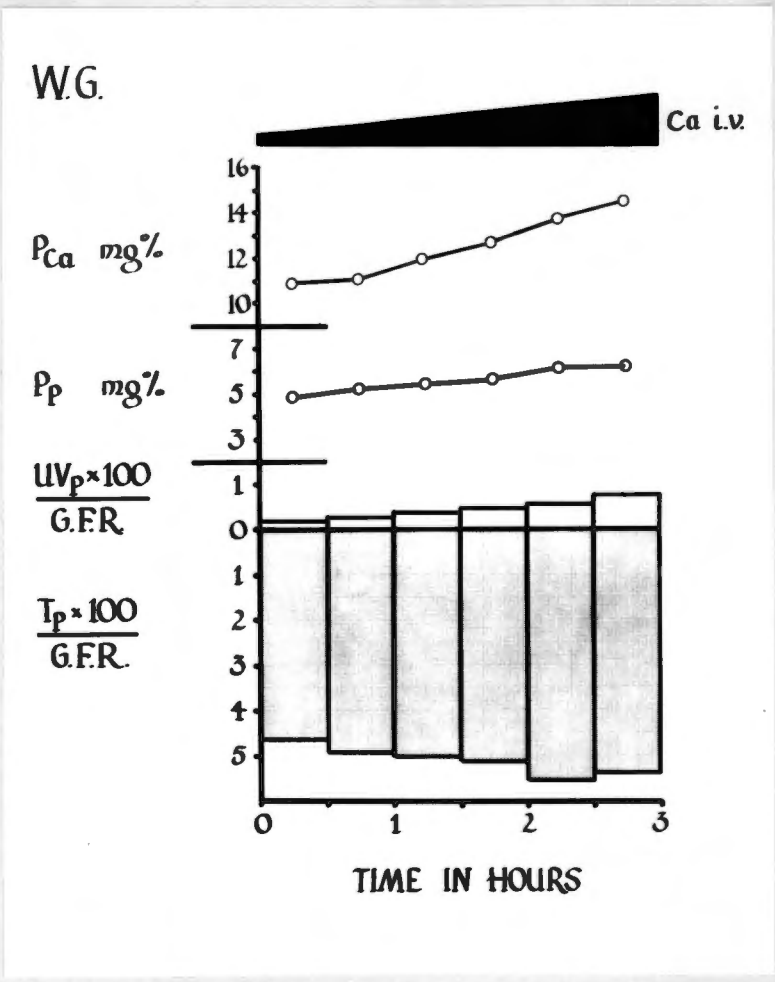
W.G.
Ca i.v.
P_{Ca} mg%
P_P mg%
$\frac{UV_P \times 100}{G.F.R.}$
$\frac{T_P \times 100}{G.F.R.}$
16
14
12
10
7
5
3
1
0
1
2
3
4
5
0
1
2
3
TIME IN HOURS

Fig. 34.

EXPERIMENT No: XIX

Subject: F.S.

Table No: 32

Period	Time (mins)	Pca (mg.%)	Pp (mg.%)	G.F.R. ml./min.	Fp (mg/min)	UVp (mg/min)	Tp (mg/min)	Tp x 100 / G.F.R.	UVp x 100 / G.F.R.
1	30.5	10.56	3.76	76.5	2.88	0.40	2.48	3.24	0.52
2	30.0	10.88	3.86	90.6	3.50	0.52	2.98	3.29	0.57
3	30.6	11.52	4.18	95.0	3.97	0.72	3.25	3.42	0.76
4	30.5	12.00	4.46	91.6	4.09	0.87	3.22	3.52	0.94
5	32.9	12.64	4.87	95.2	4.64	1.23	3.41	3.58	1.29
6	24.0	13.36	4.98	107.8	5.37	1.59	3.78	3.51	1.47

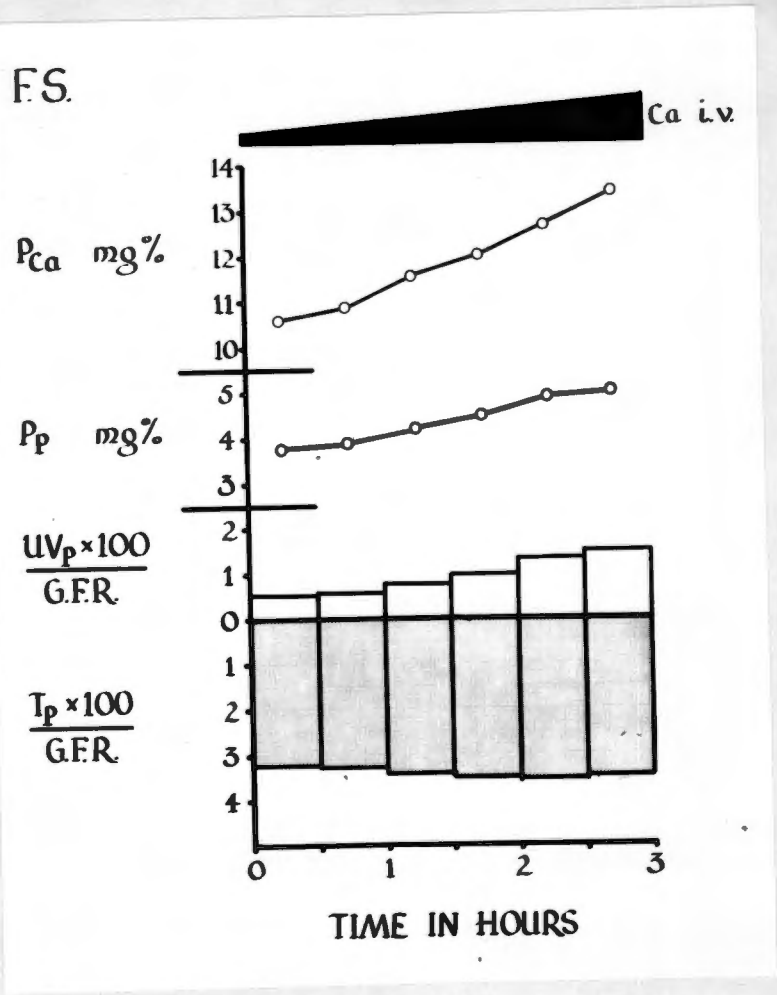
F.S.
Ca i.v.
P_{Ca} mg%
P_p mg%
$\frac{UV_p \times 100}{G.F.R.}$
$\frac{T_p \times 100}{G.F.R.}$
14
13
12
11
10
5
4
3
2
1
0
1
2
3
4
0
1
2
3
TIME IN HOURS

Fig. 35.

EXPERIMENT No: XX

Subject: H.A.

Table No: 33

Period	Time (mins)	Pea (mg.%)	Pp (mg.%)	G.F.R. ml./min.	Fp (mg/min)	UVp (mg/min)	Tp (mg/min)	Fp x 100 / G.F.R.	UVp x 100 / G.F.R.
1	20.5	10.1	3.60	102.7	3.69	0.38	3.31	3.22	0.37
2	25.0	10.1	3.65	127.7	4.66	0.46	4.20	3.29	0.36
3	20.5	15.8	3.87	37.8	1.45	0.08	1.38	3.65	0.21
4	21.5	17.1	4.59	125.4	5.64	0.98	4.66	3.72	0.78
5	22.0	20.1	5.06	90.7	4.54	1.02	3.52	3.88	1.12
6	28.0	19.7	5.65	99.3	5.63	2.00	3.63	3.66	2.01
7	25.0	18.6	5.95	84.4	5.01	1.93	3.08	3.65	2.29
8	32.0	17.6	5.01	92.5	4.70	1.41	3.29	3.56	1.52
9	61.0	16.6	4.74	104.6	4.98	1.12	3.86	3.69	1.07
10	59.0	16.0	4.66	95.8	4.47	0.68	3.79	3.96	0.71
11	94.0	16.2	3.72	75.9	2.94	0.55	2.39	3.15	0.72

Calcium administered during periods 3, 4 and 5.

Period 11 is not shown in Fig. 36.

Note marked fall in glomerular filtration rate during Period 3, accompanied by vomiting and sweating.

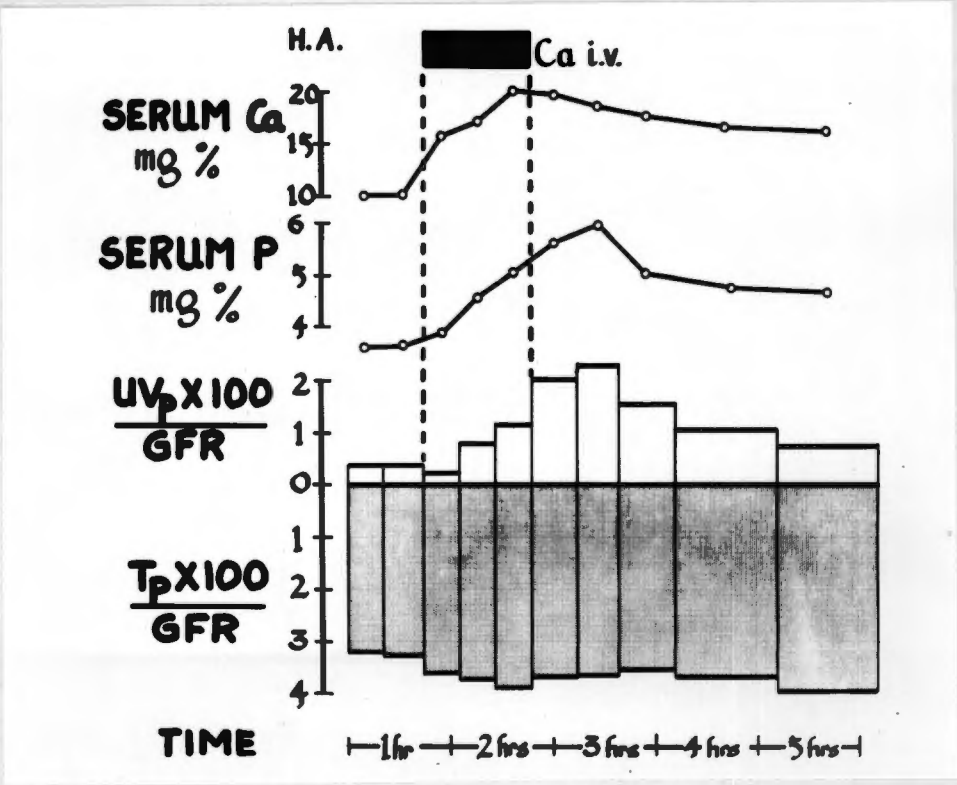
H.A.
Ca i.v.
SERUM Ca
mg %
20
15
10
SERUM P
mg %
6
5
4
UVp X 100
GFR
2
1
0
TpX100
GFR
1
2
3
4
TIME
1hr
2 hrs
3 hrs
4 hrs
5 hrs

Fig. 36.

EXPERIMENT No: XXI

Subject: A.A.

Table No: 34

Period	Time (mins)	Pca (mg.%)	Pp (mg.%)	G.F.R. ml./min.	Fp (mg/min)	UVp (mg/min)	Tp (mg/min)	Tp x 100 / G.F.R.	UVp x 100 / G.F.R.
1	23.0	10.2	2.5	113.8	2.84	0.19	2.65	2.33	0.16
2	24.0	10.7	2.6	112.5	2.92	0.17	2.75	2.45	0.15
3	20.5	12.3	2.7	119.8	3.22	0.30	2.92	2.44	0.25
4	21.5	13.3	3.1	101.8	3.12	0.48	2.64	2.59	0.47
5	21.0	16.8	3.8	77.9	2.90	0.88	2.02	2.59	1.13
6	21.0	18.7	4.5	81.5	3.60	1.01	2.59	3.18	1.24
7	21.0	16.9	4.9	83.8	4.06	0.98	3.08	3.68	1.17
8	60.0	17.5	4.9	79.5	3.90	0.77	3.13	3.94	0.97
9	60.0	16.8	5.2	78.4	4.07	0.50	3.57	4.55	0.64
10	60.5	15.2	5.3	79.4	4.21	0.36	3.85	4.85	0.45
11	119.5	14.4	5.5	99.3	5.46	0.26	5.20	5.24	0.26

Calcium infused at constant rate during Periods 3, 4 and 5.

Values for Period 11 are not shown in Fig. 37

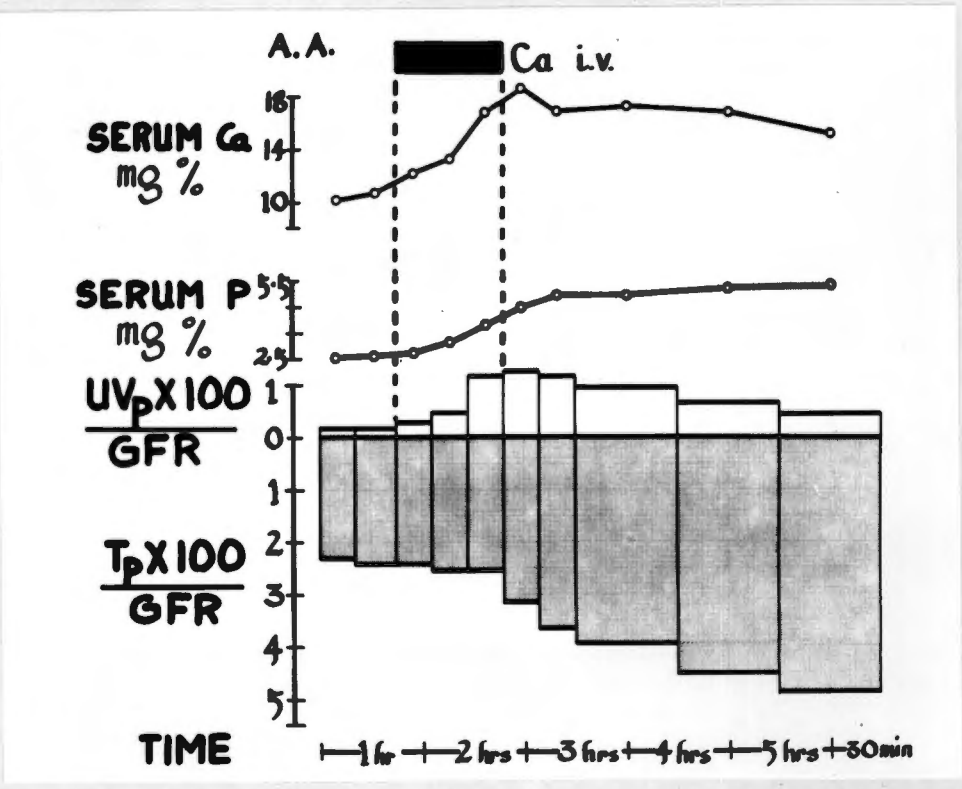
A.A.
Ca i.v.
SERUM Ca
mg %
16
14
10
SERUM P
mg %
5.5
2.5
UVp X 100
GFR
1
0
TpX100
GFR
1
2
3
4
5
TIME
1 hr
2 hrs
3 hrs
4 hrs
5 hrs
30 min

Fig. 37.

EXPERIMENT No: XXII

Subject: S.M.

Table No: 35

Period	Time (mins)	Pca (mg.%)	Pp (mg.%)	G.F.R. ml./min.	Fp (mg/min)	UVp (mg/min)	Tp (mg/min)	Tp x 100 / G.F.R.	UVp x 100 / G.F.R.	UVns (mg/min)
1	21.0	10.6	3.4	112.1	3.79	0.38	3.41	3.04	0.34	0.19
2	27.0	10.5	3.5	110.0	3.87	0.44	3.43	3.12	0.40	0.20
3	20.0	14.0	3.7	152.0	5.59	0.93	4.66	3.07	0.61	0.97
4	20.5	15.9	4.2	149.6	6.19	1.19	5.00	3.34	0.80	1.40
5	22.0	16.7	4.6	129.8	5.93	1.40	4.53	3.49	1.08	1.60
6	21.0	18.4	5.0	127.6	6.20	1.38	4.82	3.78	1.08	1.40
7	20.0	18.4	5.2	101.0	5.21	1.54	3.67	3.64	1.52	1.60
8	30.0	16.7	5.2	111.6	5.80	1.37	4.43	3.97	1.23	1.20
9	30.5	17.3	5.3	109.4	5.79	1.12	4.67	4.27	1.02	0.91
10	30.0	15.8	5.4	128.1	6.90	0.92	5.98	4.67	0.72	0.70
11	120.0	15.8	5.3	107.5	5.70	0.43	5.27	4.90	0.40	0.26

Calcium infused at constant rate during periods 3, 4 and 5.

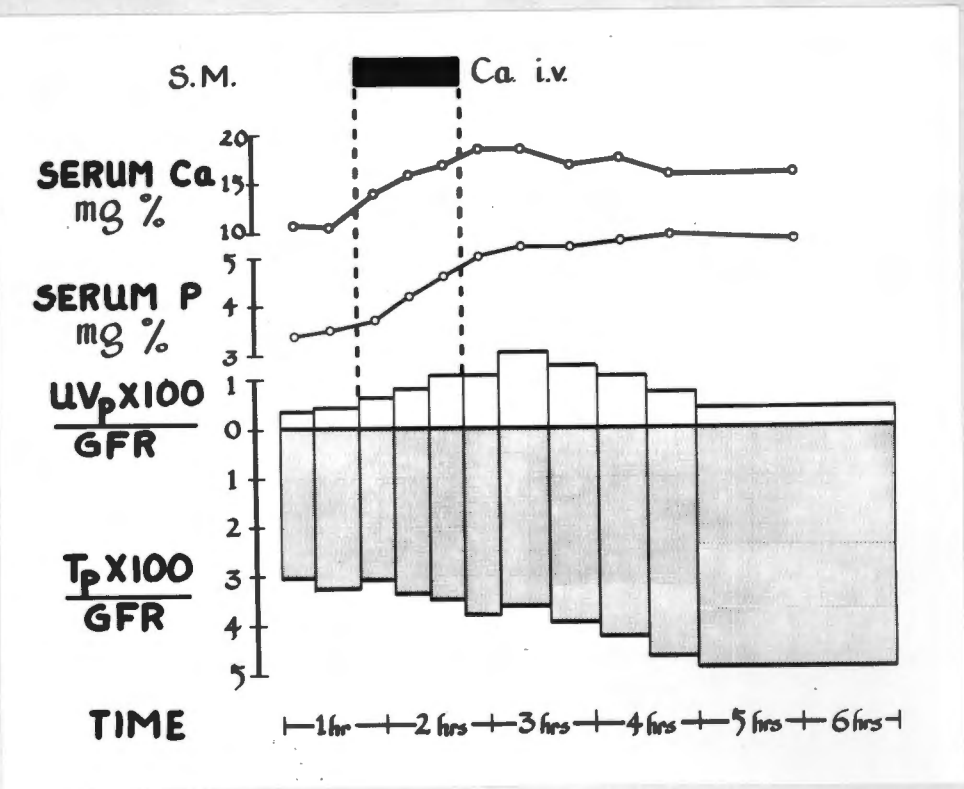
S.M.
Ca. i.v.
SERUM Ca
mg %
20
15
10
SERUM P
mg %
5
4
3
UVp X100
GFR
1
0
1
2
Tp X100
GFR
3
4
5
TIME
1 hr
2 hrs
3 hrs
4 hrs
5 hrs
6 hrs

Fig. 38.

EXPERIMENT No: XXIII

Subject: O.L.

Table No: 36

Period	Time (mins)	Pca (mg.%)	Pp (mg.%)	G.F.R. ml./min.	Fp (mg/min)	UVp (mg/min)	Tp (mg/min)	Tp x 100 / G.F.R.	UVp x 100 / G.F.R.	UVna (mg/min)
1	20.5	10.6	3.6	116.4	4.18	0.10	4.08	3.51	0.09	0.07
2	22.0	10.6	3.7	89.6	3.31	0.12	3.19	3.56	0.13	0.06
3	20.0	13.9	4.0	112.9	4.45	0.19	4.26	3.78	0.17	0.10
4	20.5	15.8	5.2	108.6	5.30	0.61	4.70	4.32	0.56	0.26
5	20.0	17.1	5.9	81.2	4.68	0.76	3.92	4.83	0.94	0.33
6	22.0	20.0	6.9	97.9	6.61	1.10	5.52	5.63	1.12	0.51
7	32.5	18.4	7.3	82.8	6.02	1.66	4.36	5.26	1.12	1.44
8	31.0	17.8	7.1	82.6	5.87	1.26	4.61	5.58	2.00	1.05
9	31.5	18.2	6.6	80.1	5.29	0.71	4.59	5.73	1.53	0.49
10	60.0	16.8	6.6	84.8	5.60	0.45	5.15	6.07	0.89	0.15
11	60.0	-	6.9	81.1	5.55	0.34	5.21	6.43	0.53	0.04
12	60.0	15.2	7.0	77.1	5.39	0.35	5.04	6.54	0.45	0.07

Calcium infused at constant rate during Periods 4, 5 and 6.

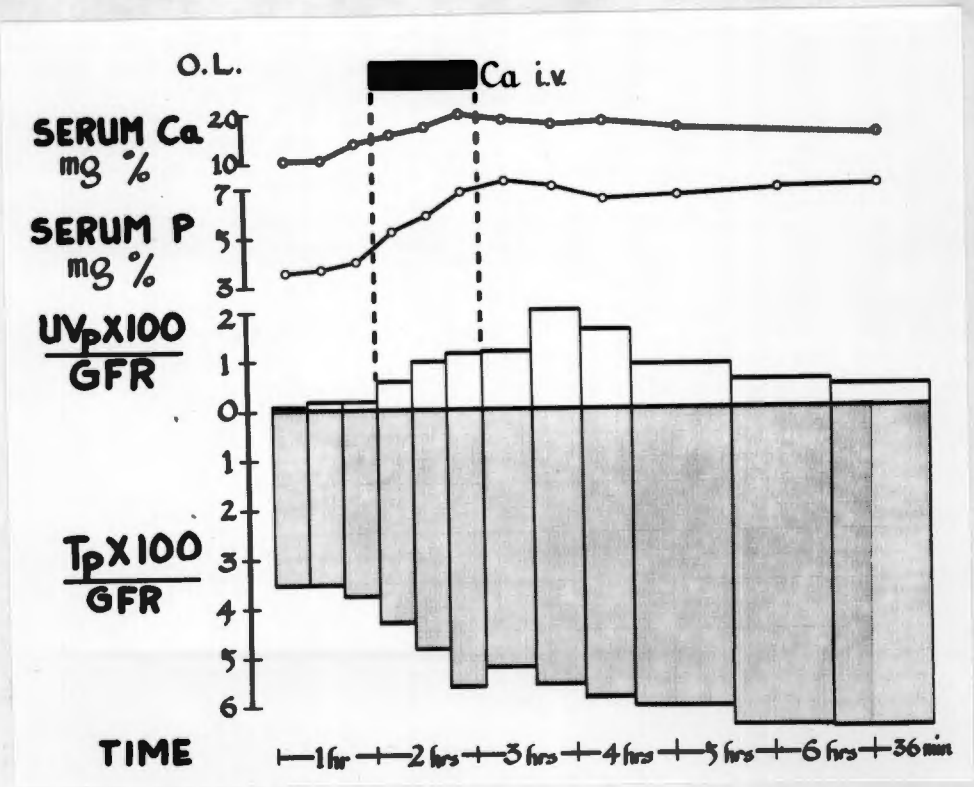
O.L.
Ca i.v.
SERUM Ca
mg %
20
10
SERUM P
mg %
7
5
3
UVpX100
GFR
2
1
0
1
2
TpX100
GFR
3
4
5
6
TIME
1 hr
2 hrs
3 hrs
4 hrs
5 hrs
6 hrs
36 min

Fig. 39.

EXPERIMENT No: XXIV

Subject: L.B.

Table No: 37

Period	Time (mins)	Poa (mg.%)	Pp (mg.%)	G.F.R. ml./min.	Fp (mg/min)	UVp (mg/min)	Tp (mg/min)	Tp x 100 / G.F.R.	UVp x 100 / G.F.R.
1	20.5	10.6	3.1	120.7	3.73	0.11	3.62	3.00	0.09
2	21.0	10.6	3.2	120.5	3.84	0.13	3.71	3.08	0.11
3	21.0	10.5	3.3	133.1	4.38	0.21	4.18	3.14	0.16
4	22.0	13.6	3.5	139.7	4.75	0.52	4.24	3.04	0.37
5	20.0	17.3	4.8	143.6	6.62	1.07	5.56	3.87	0.75
6	21.5	20.1	5.6	92.6	5.10	1.01	4.09	4.42	1.09
7	21.0	19.7	7.0	107.3	7.41	1.56	5.85	5.45	1.45
8	20.5	17.8	6.8	136.8	9.34	2.45	6.89	5.04	1.79
9	30.5	16.9	6.6	147.7	9.76	2.18	7.58	5.13	1.48
10	60.5	15.9	5.9	136.8	8.11	1.60	6.51	4.76	1.17
11	50.5	15.0	5.9	127.2	7.51	1.32	6.19	4.87	1.04

Calcium infused at constant rate during Periods 4, 5 and 6.

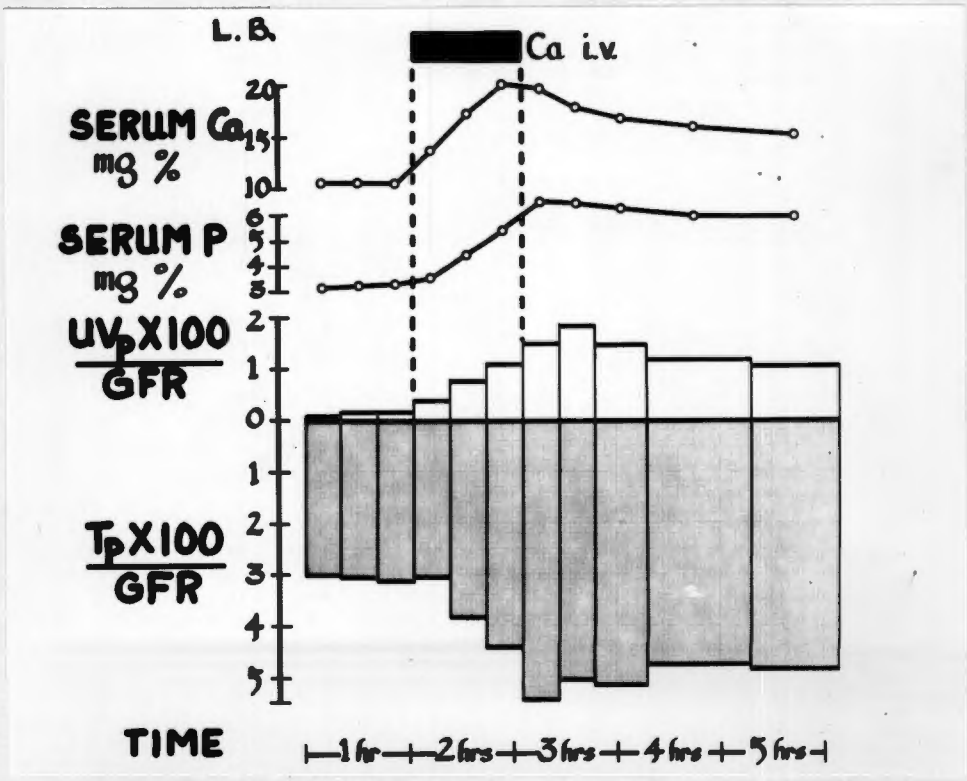
L.B.
Ca i.v.
SERUM Ca mg %
20
15
10
SERUM P mg %
6
5
4
3
UVpX100 / GFR
2
1
0
TpX100 / GFR
1
2
3
4
5
TIME
1 hr
2 hrs
3 hrs
4 hrs
5 hrs

Fig. 40.

Results and discussion.

The following effects of intravenous calcium administration on phosphorus metabolism are readily apparent from these experiments.

i) There was a marked and sustained rise in serum inorganic phosphorus concentration in each case. This I suspect, was due to a shift of intracellular phosphorus into the extracellular fluid. I have attempted to measure the total phosphorus content of the blood by the method described in Appendix B in the hope that I would be able to demonstrate a change which would indicate the source of the increase in serum inorganic phosphorus. Unfortunately the technique has not proved sufficiently accurate in my hands to enable me to draw definite conclusions.

To find out whether this effect was reproducible in vitro, I added to samples of my own blood and that of Dr. Jackson sufficient calcium gluconate solution to raise the calcium concentration well above the concentration encountered in the experiments described. This blood was then incubated at 37 degrees C. for three hours and the phosphorus content compared with that of a control sample to which no calcium had been added.

The results were as follows :-

Serum inorganic phosphorus concentration:-	W.P.U.J.	E.B.D.D.
a) Immediately after venesection	2.45 mg%	4.26 mg%
b) After three hours incubation with 0.2 ml. 0.9% NaCl solution	2.47 mg%	4.32 mg%
c) After three hours incubation with 0:2 ml. Calcium gluconate solution	2.45 mg%	4.30 mg%

This, I think, indicates that the rise in serum inorganic phosphorus concentration that follows calcium infusion is an in vivo phenomenon

ii) There was in each case a rise in the calculated value for both the absolute amount of phosphorus reabsorbed per minute and for the amount reabsorbed from each 100 ml. of filtrate formed.

This may be due to the suppression of parathyroid activity by the hypercalcaemia with resulting increase in the rate of tubular transport of phosphorus or to the formation of non-diffusible calcium phosphate complexes. I have attempted to settle this question by in vitro ultrafiltration of samples of the serum at different concentrations of calcium, but have found the technique unsatisfactory in my hands. I hope to devote more time to this problem.

It could also be argued that this rise in the rate of tubular reabsorption of phosphate was due to the fact that at the low levels of plasma phosphate obtaining before the infusion the tubular reabserptive mechanism was not saturated, and that the Tm was only reached when the serum inorganic phosphorus rose high enough. That this was not the case, however, is indicated by the fact that the rate of tubular transport of phosphorus continued to increase after the infusion despite no further increase in plasma phosphate concentration.

iii) There occurred, in every case, a significant rise in the urinary output of phosphorus following calcium infusion. This was transient and maximal during and immediately after the infusion, subsiding slowly to pre-infusion levels. In no case was a fall encountered to below pre-infusion levels, but, had the period of observation been prolonged, this may have been observed. It can be seen that the rise in serum phosphorus concentration was more prolonged than the rise in the rate of excretion of phosphorus.

SUMMARY.

In eleven normal adults artificial elevation of the plasma calcium concentration was accompanied by the following events.

(i) A rise in the plasma inorganic phosphorus concentration. This was thought to be due to a shift of phosphorus out of the cells into the extracellular fluid.

(ii) A rise in the renal threshold for phosphorus. This may have been due to parathyroid suppression or to the formation of non-diffusible phosphorus complexes.

(iii) A rise in the rate of excretion of phosphorus in the urine.

The effect of Calcium Infusion on Urine Sodium and Potassium Excretion.

In six of the normal subjects just described, the urine specimens were analysed for sodium and potassium content and the rate of excretion of these two cations in the urine was calculated. The results for sodium are given in Table 38 and Fig. 41. As there was no significant effect on potassium excretion the figures are not given here. They can be found in Appendix A. It will be seen that a prompt diuresis of sodium followed almost immediately upon calcium infusion, the rate of excretion of sodium and of calcium correlating quite well.

This observation confirms that of others who have noted a similar phenomenon[223,278,163].

In all subjects, accompanying this rise in sodium output, there was a simultaneous water diuresis. Unfortunately I have no figures for urine volumes as the urine specimens were made up to a volume convenient for subsequent analysis immediately after being passed. In addition the subjects were encouraged to drink copiously during the experiment to minimise errors that may result from low minute volumes.

The reason for this outpouring of sodium is obscure, as is the nature of the diuresis provoked. I doubt if sufficient calcium was filtered through the glomerular membrane to exercise any appreciable osmotic effect, and would prefer to regard the sodium lyuresis as indicating a form of "competition" for sodium transport through the distal tubular epithelium by divalent calcium ions.

Table 38.

Q.L.		S.A.		S.L.		S.M.		L.B.		V.C.	
UVca (mg/min)	UV_{Na^+} (mEq/min)	UVca (mg/min)	UV_{Na^+} (mEq/min)	UVca (mg/min)	UV_{Na^+} (mEq/min)	UVca (mg/min)	UV_{Na^+} (mEq/min)	UVca (mg/min)	UV_{Na^+} (mEq/min)	UVca (mg/min)	UV_{Na^+} (mEq/min)
0.04	0.07	0.07	.160	0.05	.163	0.30	0.19	0.11	0.25		.1302
0.04	0.06	0.09	.193	0.14	.29	0.20	0.20	0.12	0.25	.001	.205
0.12	0.10	0.16	.298	0.26	.38	0.71	0.97	0.12	0.27	.270	.345
0.51	0.26	0.29	.366	0.37	.48	1.01	1.40	0.75	0.59	380	.366
0.75	0.33	0.43	.422	0.28	.412	1.21	1.60	1.58	0.85	1.200	.512
0.64	0.51	0.70	.502	0.54	.593	1.01	1.40	1.26	0.60	0.900	.784
1.85	1.44	0.71	.543			1.14	1.60	0.67	0.03		
1.28	1.05	0.90	.616			1.05	1.20	1.34	0.84		
0.62	0.49	0.95	.633			0.82	0.91	1.58	1.27		
0.30	0.15					0.63	0.70	1.08	0.73		
0.15	0.04					0.35	0.26	0.62	0.28		
0.26	0.07										

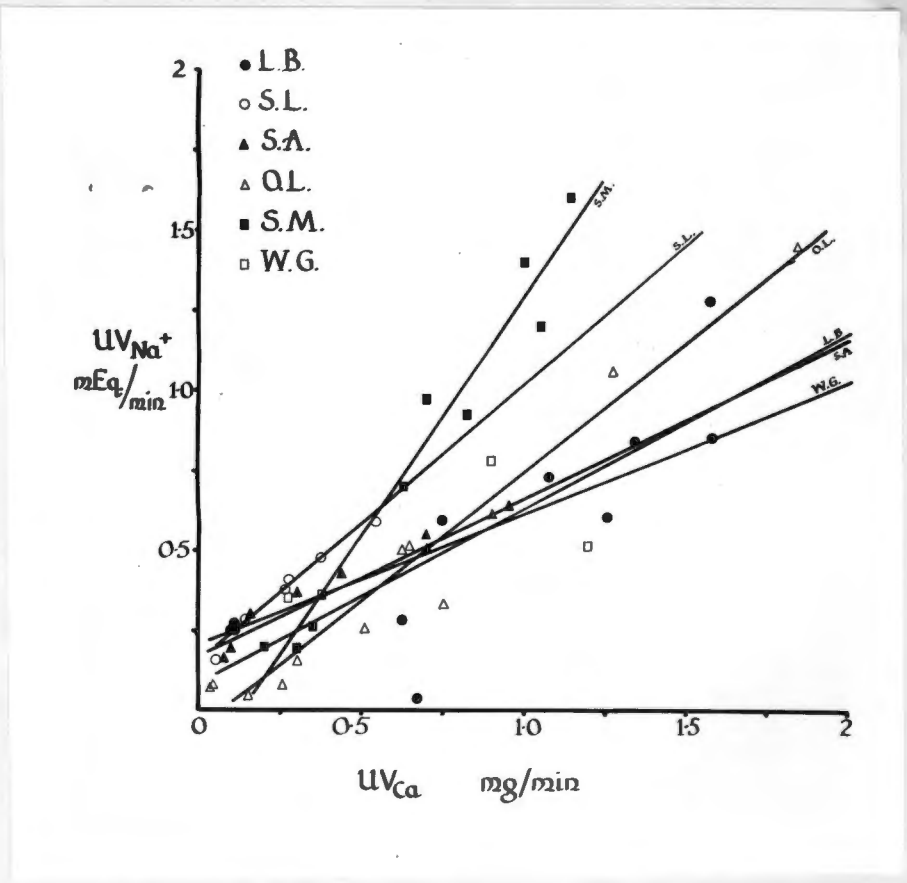

Fig. 41.

In this figure the combined plots of six normal subjects are shown with the regression lines for each individual fitted. In each case the rate of excretion of sodium in the urine increased with that of Calcium. The six regression equations are given below with, in parentheses, the 95% confidence interval for each individual regression coefficient.

L.B.	y = 0.54x + 0.09	(0.28 - 0.80)
S.L.	y = 0.86x + 0.16	(0.30 - 1.41)
S.A.	y = 0.49x - 0.17	(- - 1.84)
O.L.	y = 0.79x - 0.05	(0.67 - 0.91)
S.M.	y = 1.51x - 0.21	(1.30 - 1.72)
W.G.	y = 0.40x + 0.21	(0.03 - 0.76)

Wolf and Ball[278] did not think the sodium lyuresis following calcium administration could be definitely ascribed to the calcium, as they were able to produce a similar phenomenon with glucose infusion. As I have had no experience with experimental work on sodium homeostasis I am unable to criticise this opinion and prefer to leave the matter in abeyance.

The question is of importance, however, in that an answer will help to elucidate the pathogenesis of the "diabetes insipidus-like" syndrome that is frequently found in patients with syndromes involving hypercalcuria.

SECTION IV.

The Mode of Action of Parathyroid Hormone.

THE MODE OF ACTION OF PARATHYROID HORMONE

If I were to succumb to the temptation to introduce each section of this thesis with a pithy quotation, I think I should choose for this section a statement made by Professor Gibberd some eight years ago in the course of a lecture delivered while he was the Sims travelling Professor in South Africa. He said, in essence, "If I were to claim that orange peel was a cure for cancer of the breast, it would take twenty years of intensive research to prove me wrong!" This is the reductio ad absurdum of the state of affairs that has prevailed in parathyroid hormone research for the past two decades. In a paper published in 1934 from the research laboratories of Fuller Albright[69] it was suggested that the effects of parathyroid hormone were mediated solely through the primary effect this substance had in provoking a phosphate diuresis. This hypothesis resulted in a complete re-direction of research into experimental channels designed to prove or refute its validity, with comparatively little in the literature since 1934 to indicate any original line of approach to the study of parathyroid function.

Before reviewing the voluminous literature that has accumulated around this hypothesis of the Albright team, let me review the knowledge of parathyroid function at the time at which it was elaborated.

Largely owing to the independent observations of Sandström and Gley[19] at the end of the 19th century, the existence of the parathyroids and their association with tetany

had been known for forty years. Greenwald[90], in 1924, drew attention to the effects of parathyroidectomy on the plasma calcium and phosphorus concentrations, and in 1926[91,92] published his observations on the effects of parathyroid extract on urinary calcium and phosphorus excretion in dogs. In 1925 Collip and Clark[45,46] published results of their experiences with an extract of beef parathyroid tissue and noted that it could be used to maintain the serum calcium concentration in parathyroidectomised dogs and would, when administered to normal animals, produce an abnormal elevation of plasma calcium concentration. The histological changes wrought in bone by excess of endogenous parathyroid hormone were well known from the earlier studies of von Recklinghausen and Askenazy[19], and the first parathyroid exploration had been successfully attempted for osteitis fibrosa generalisata (the case of Mandl quoted by[9]). Holt, la Mer and Chown[127,128] had published their conclusions on the physical chemistry of calcium-phosphate solutions.

From these studies the following facts had accumulated :-

i) Deficiency of parathyroid hormone causes a fall in plasma calcium concentration, a rise in plasma phosphate concentration and a fall in the rate of excretion of phosphorus in the urine.

ii) Excess of parathyroid hormone causes a rise in plasma calcium concentration, bone changes with a typical histological features ("osteitis fibrosa") and a rise in the rate of excretion of phosphorus in the urine.

iii) Parathyroid hormone causes an acceleration in the rate of urinary excretion of calcium.

iv) The fall in plasma calcium concentration after parathyroidectomy is not due to increased excretion in the urine.

v) The product of Calcium and Phosphate concentrations in the plasma exceeds the solubility product of Calcium orthophosphate, so that some mechanism exists for keeping these ions in a supersaturated solution.

With this knowledge in hand Albright et al. embarked on a series of investigations into the mechanism of parathyroid function using parathyroid extract in both normal and hypoparathyroid subjects. Firstly the phosphate diuretic effect of the parathyroid hormone was described[4] with a paper in the same year[7] describing a similar effect in patients with hypoparathyroidism. In this paper it was concluded that, in the absence of any demonstrable inability of the hypoparathyroid kidney to excrete a phosphate load, the phosphate diuresis that followed parathormone administration must be due to some alteration in the plasma inorganic phosphorus whereby it was made more readily available for urinary excretion. In 1932, however, Ellsworth[69] accepted that the phosphate diuretic effect of parathyroid hormone was a result of lowering of the renal threshold, and, because of the sequence of events observed following injection of parathyroid extract, stated in crystallised form the theory of parathyroid function favoured by Albright and himself. This theory was restated in 1934[70] in a paper describing the phosphorus

diuretic effect of parathyroid extract in normal human subjects. The loss of phosphate in the urine, it was claimed, led to a fall in plasma phosphorus with resulting disturbance in the normally maintained solubility product of calcium and phosphorus. The low product of the concentrations of these two substances was restored to normal by mobilisation of calcium from the bones with resulting hypercalcaemia, hypercalcuria and hyperphosphaturia.

Implied or expressed in this postulated mechanism were the following assertions with which subsequent research has busied itself.

1) <u>Parathyroid hormone will cause an increased rate of excretion of phosphate in the urine</u>.

This effect of parathyroid extract has been convincingly demonstrated for man and a variety of experimental animals[11,165,92,142,58,183,150,196,4,7,69,36,125,70,59,33,248]. Others [55,182] have said that the effect varies directly with the concentration of plasma phosphate at the time of injection, but Goadby and Stacey[82] were able to demonstrate an effect even at the very low plasma phosphorus levels produced by carbohydrate feeding.

No such effect was seen in cats[11]. Dent[62] was able to find only a variable and insignificant effect of parathyroid extract on urinary phosphorus and suggested that it may be apparent rather than real and due to either the crude nature of the extract or the normal diurnal variation in rate of excretion of phosphate.

Despite these two dissenters, and because it is well known that patients with hyperparathyroidism have a high urinary output of phosphorus, one must accept this assertion as valid.

2) Parathyroid hormone, by virtue of the fact that it acts primarily upon the kidneys, will have no effect when administered to a nephrectomised animal.

Tweedy et al.[256], in a poorly conceived and interpreted experiment supported this assertion by showing that parathyroid hormone had no calcium raising power when given to nephrectomised dogs, and that nephrectomy "protected" these animals from the adverse effects of massive parathormone dosage. Further support came from Neufeld and Collip[192] who, in a more acceptable series of of experiments, demonstrated with a variety of animals that the serum calcium did not rise when parathormone was given to animals made anuric by nephrectomy, overdosage with posterior pituitary extract or ureteral ligation. In one animal cutting of the ureteral ligatures was followed by an immediate rise in plasma calcium concentration.

Ingalls, Donaldson and Albright[136], however, showed that nephrectomy did not abolish the effect of parathyroid extract in producing an osteoclastic proliferation in bone, and this was confirmed by Collip et al.[47]

Monahan and Freeman [184] were able to produce an elevation of plasma calcium in nephrectomised dogs by administering parathyroid extract, and a fall in plasma calcium by parathyroidectory.

Grollman[97] showed that the parathyroid control of plasma calcium concentration was independent of renal mediation by producing hypocalcaemia in nephrectomised dogs by parathyroidectomy, and restoring the serum calcium concentration to normal with parathyroid extract.

By the use of an ingeniously conceived "oxalate tolerance test" Stewar and Bowen[244] were able to show that the extra-renal actions of endogeneous parathyroid hormone were in no way compromised by nephrectomy.

On the balance of evidence, therefore, this assertion must be declared invalid.

3) Parathyroid hormone causes a fall in plasma phosphorus concentration before it causes a rise in plasma calcium concentration.

Although this sequence of events was found by the original proponents of the theory[4,69] the results of several other workers[253,96,165,125] have indicated that this is not the case. Moreover Jacob[140] has shown that artificial elevation of the plasma phosphorus will not interfere with the calcium raising effect of parathormone. It is, however, well known that parathyroidectomy will cause a rise in plasma phosphorus[351] and that clinical hyperparathyroidism is usually associated with a fall in plasma inorganic phosphorus.

The assertion that parathyroid excess will cause a fall in plasma phosphate is therefore probably valid. The assertion that this fall is a necessary prerequisite for a rise in plasma calcium is unacceptable.

4) There exists, in the body, a mechanism operating to ensure that the product of the calcium and phosphorus concentration will remain constant, so that a fall in one will be compensated by a rise in the other.

Phosphate infusion has been known to cause tetany in experimental animals[240,210,1]. There is little information on

this score however, for man. Sirota[238] has reported a fall in plasma calcium following phosphate infusion to a patient with hyperparathyroidism, and Peters and Eiserson,[205] using the results from routine laboratory investigation, were able to show an inverse relationship between plasma calcium and phosphorus concentration. In none of the many reports on phosphate infusion in man has there been anything to suggest a lowered serum calcium concentration.

It is well known that artificial raising of the plasma calcium concentration will cause a rise in plasma phosphorus, and Thompson and Pugsley[253] were able to show that insulin injection, while it lowered the plasma phosphorus, did not cause a rise in plasma calcium.

This assertion can be said to be unsubstantiated.

5) <u>The potent principle in parathyroid extract is a single pure hormone with an effect principally on the renal threshold for phosphate</u>.

The validity of this assertion was questioned first by Handler, de Maria and Cohn[106,105] who showed that there was an immediate rise in the rate of urinary phosphate excretion following intravenous injection of parathyroid extract that was accompanied by a rise in the rate of tubular reabsorption of phosphate, the glomerular filtration rate and the renal plasma flow. Although no figures were given to enable me to calculate the threshold, they state that the rise in the rate of reabsorption was in "a manner rather less than proportional to the amount filtered".

In a subsequent paper[104] Handler and Cohn confirmed their previous results and were able to show that the factors present which raised the glomerular filtration rate and the plasma calcium concentration were similar in that they were both non-dialysable and could be inactivated by peptic digestion. The factor, however, which raised the blood pressure in rats was disalysable and could be digested by pepsin. Parathyroid hormone, when administered subcutaneously, caused a marked rise in plasma calcium without change in glomerular filtration rate or renal plasma flow. From this they concluded, very reasonably, that the haemodynamic effect of parathyroid extract was of no importance in raising the plasma calcium and that there were probably two distinct hormones.

Stewart and Bowen[245] showed that a phosphate diuresis could be provoked with parathyroid extract that had been inactivated by formaldehyde or with an extract from spleen and thymus, and concluded that this effect of parathyroid extract was an artefact due to the crudity of the preparation.

Davis and Gordon[56] found that the "phosphate diuretic" factor of parathyroid hormone was dialysable through a cellophane membrane whereas the hypercalcaemic factor was not and postulated a second hormone. A rise in glomerular filtration rate following intravenous parathyroid extract has been recorded by several other workers [141,150,36,126,182].

From this work two facts emerge. Firstly, the assertion as it stands is invalid, and secondly, injections of parathyroid hormone may cause a rise in glomerular filtration rate.

The theory of Albright then comes out rather badly and one is forced to accept that the effects of parathyroid hormone on bone and on serum calcium are independent of the effects on the urinary elimination of phosphate. Albright and Reifenstein[9] in an impartial review of this subject, accept an action on bone and concede that the renal action is the less important. They cling tenaciously however, to the "solubility product" and return once more to the theory that parathyroid hormone alters the phosphate in the serum in such a way as to render it more readily available for urinary excretion and so to disturb the solubility product. This, it will be seen, represents a return to the original suggestion of the Albright school, that the renal threshold for phosphorus is not maintained solely by renal tubular action, but is, in part if not in whole, an expression of the "availability" of the plasma phosphate for urinary excretion. Translated into terms of accepted theory, this would mean that the phosphorus exists in the plasma in a largely non-filterable form.

It is interesting to examine the reasons for the great deal of attention that the Albrightian theory has received. I think that these reasons are twofold.

Firstly the theory came from a very reputable school of research, and secondly it is the only theory of parathyroid action that has attempted to explain the fact that hyperparathyroidism may exist without bone changes. The drain of calcium from the bones, it was argued, would only produce bone lesions if uncompensated for by supplies of calcium from the bowel.

Hence it was to be expected that only a minority of cases would show bone changes. Why it is, however, that these bone changes take the form of osteitis fibrosa and not osteomalacia, has not been explained. Albright himself has said that primary hyperparathyroidism in children does not cause epiphyseal changes.(6)

In this thesis I have concerned myself principally with the renal effects of parathyroid hormone. I have been handicapped by the apparent scarcity of cases of clinical hyperparathyroidism at Groote Schuur Hospital, and the investigations that I have done have been on normal subjects and on two cases (H.W. and E.B.) of surgical hypoparathyroidism.

In the normal subjects my studies were confined to the action of parathyroid extract on the renal handling of phosphate at normal and elevated plasma levels, and in the Hypoparathyroid subjects to the measurement of phosphate threshold and to the effects of calcium infusion.

The effect of Parathyroid extract on the renal handling of inorganic phosphate in normal subjects.

A number of reports on the effect of parathyroid extract on the tubular reabsorption of phosphate as measured by clearance analysis have appeared in the literature.

Working with dogs, Fay et al.[73] and Jahan and Pitts[143] were unable to demonstrate any convincing effect of parathyroid extract injection on the rate of tubular transport of phosphate. Analysis of the data given by Pitts, however, shows that in the six animals who received parathyroid hormone, while there was a rise in Tp there was a fall in $\frac{Tp}{GFR}$ from a mean preinjection level of 0.131 to a mean post-injection level of 0.125. It was, in addition only some 16 - 24 hours after injection that the clearances of creatinine and phosphorus were measured.

Harrison and Harrison [114] demonstrated a convincing fall in $\frac{Tp}{GFR}$ following parathormone injection, with a slight rise in Tp.

Hogben and Bollman in 1949[125] ascribed the phosphate diuresis following parathormone injection to rise in plasma phosphorus concentration and in 1951[126] to rise in glomerular filtration rate. In neither instance would they agree to a direct action of parathyroid hormone on the tubular mechanism for reabsorption of phosphate.

Handler et al.[105] confirmed the fact that the glomerular filtration rate may rise after parathormone injection.

Experiments upon human subjects have yielded similarly conflicting results.

Michie and Shorey[182] ascribed the rise in UVp to the increase in glomerular filtration rate.

Others, however, while admitting that the glomerular filtration rate may rise, have said that an effect on tubular activity can also be shown.[36,141,149,150] Of all of the experimental work on man with parathyroid extract, only that of Jacobs and Verbunck[141] has used simultaneous infusion of phosphate.

The original supposition that parathormone does have an effect on the kidneys is supported by the cross-transfusion experiments of Brull[33] and the observations of Goadby and Stacey[81] and Kleeman and Cooke[149] who noted absence of a phosphate diuretic response to parathormone in patients with acute nephritis that returned after recovery.

To summarise this short review of the pertinent literature it can be said that parathyroid extract may or may not cause a rise in glomerular filtration rate, and it may or may not cause a change in the rate of renal tubular reabsorption of phosphate. The important question that requires an answer before justifiable conclusions can be drawn is this: To what extent will a rise in glomerular filtration rate cause a rise in urine phosphorus excretion ?

If the classical Tm hypothesis for phosphate be true, a rise in glomerular filtration rate will cause a rise in filtered phosphorous, which, by virtue of the fact that the absolute amount reabsorbed remains constant, will be sufficient to cause a rise in the amount of phosphorus in the urine.

If, however, as I have suggested earlier, the absolute rate of reabsorption is proportional to the glomerular filtration rate, a rise in the latter will be accompanied by a directly proportional change in the former so that the increase in the glomerular filtration rate will not cause a rise in urinary phosphorus excretion.

If, moreover, the threshold is more constant than the rate of tubular reabsorption, and this threshold is largely determined by renal tubular activity, an increase in urinary phosphorus, in the absence of a rise in plasma phosphorus, must indicate a change in renal tubular activity.

It was with a knowledge of this problem that I undertook the following experiments to study the effect of parathormone on the renal handling of phosphorus.

A total of 7 experiments were performed on 5 subjects judged to have normal renal function. In each case, the glomerular filtration rate was measured with inulin, and accurately timed urine collections were made at 20 minute intervals. Blood samples representative of each urine collection period were analysed for inulin, phosphorus and calcium, and similar estimations were carried out on the urine. After three control periods 400 units of "Parathormone" (Lilly) were injected intravenously and the effects studied for 3 hours after the injection. The experiments are recorded in greater detail in Appendix A.

The results are presented in Tables 39 to 45 inclusive and are graphically depicted in Figs. 42 to 48 inclusive. A short comment is given in each case.

In two cases, J.J. and H.R., the test was performed at normal levels of serum phosphorus and was repeated subsequently with the serum phosphorus concentration artificially elevated by intravenous infusion of sterile buffered phosphate.

In one case (T.M.) the test was only done once and then at elevated plasma levels.

In the diagrams I have chosen $\frac{UVp \times 100}{G.F.R.}$ and $\frac{Tp \times 100}{G.F.R.}$ to represent the rate of excretion of phosphate and the rate of reabsorption respectively. The former is represented, for each urinary collection period, by clear blocks extending upwards from the base line and the latter by shaded blocks extending downwards from the base line.

The effect of choosing these indices is to correct both the absolute rate of tubular reabsorption and the rate of urinary excretion of inorganic phosphorus for changes in glomerular filtration rate that may have been real or due to incomplete bladder emptying. These indices are expressed in mg./min./100 ml. of filtrate formed. If absolute values are preferred they can be found in the relevant Tables.

As there was no effect of parathyroid extract on plasma or urine calcium, I have not presented these figures in the Tables in this section. They can be found in the summaries of the experiments that appear in Appendix A.

EXPERIMENT No: XXV

Subject: U.G.

Table No: 39

Period	Time (mins.)	Pp (mg.%)	G.F.R. (ml./min.)	Fp (mg./min.)	UVp (mg./min.)	Tp (mg./min.)	$\frac{Tp \times 100}{G.F.R.}$	$\frac{UVp \times 100}{G.F.R.}$
1	21.0	3.90	50.3	1.89	0.20	1.69	3.36	0.40
2	20.0	3.71	53.7	2.02	0.21	1.81	3.82	0.39
3	20.5	3.83	54.6	2.05	0.24	1.81	3.31	0.44
4	20.0	3.59	49.7	1.87	0.45	1.42	2.86	0.91
5	20.0	3.76	47.1	1.77	0.69	1.08	2.30	1.46
6	20.5	3.76	52.8	1.99	0.64	1.35	2.56	1.21
7	19.0	3.76	-	-	0.62	-	-	
8	22.0	3.76	55.8	2.10	0.56	1.54	2.77	1.00
9	19.0	3.76	60.8	2.29	0.58	1.71	2.81	0.95
10	20.0	3.76	69.2	2.60	0.41	2.19	3.16	0.59
11	20.0	3.76	52.7	1.98	0.39	1.59	3.10	0.74
12	20.0	3.76	59.0	2.22	0.40	1.82	3.09	0.68

Parathormone 400 units given intravenously at end of period 3.
Note no change in glomerular filtration rate. Fall in Tp and $\frac{Tp}{GFR}$.
Marked rise in urine phosphorus.

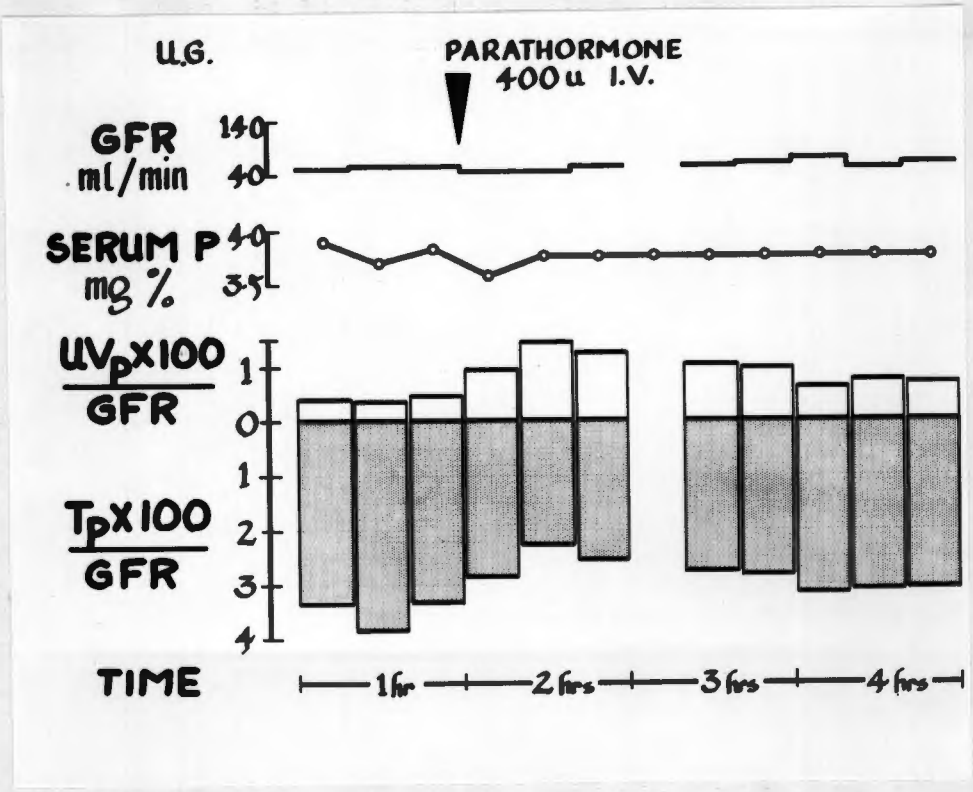

Fig 42

Showing effect of parathormone administration to subject U.G. (Experiment XXV).

Note the marked rise in urinary phosphorus and fall in tubular reabsorption of phosphorus.

Data presented in Table 39.

EXPERIMENT No: XXVI

Subject: A.F.

Table No: 40

Period	Time (mins.)	Pp (mg.%)	G.F.R. (ml./min.)	Fp (mg./min.)	UVp (mg./min.)	Tp (mg./min.)	Tp x 100 / G.F.R.	UVp x 100 / G.F.R.
1	49.0	3.26	115.3	3.90	0.22	3.68	3.19	0.19
2	20.0	3.01	103.8	3.12	0.15	2.97	2.86	0.15
3	21.0	3.14	108.0	3.39	0.35	3.04	2.82	0.32
4	20.0	3.35	103.3	3.46	0.79	2.67	2.59	0.76
5	22.0	3.38	84.0	2.84	0.70	2.14	2.55	0.83
6	20.0	3.19	79.5	2.54	0.60	1.94	2.44	0.75
7	20.0	3.76	120.0	4.27	0.64	3.63	3.03	0.53
8	20.0	-	-	-	0.61	-	-	-
9	20.0	-	124.0	-	0.83	-	-	0.67
10	20.0	3.87	112.3	4.35	0.88	3.47	3.09	0.78
11	20.0	3.82	117.2	4.48	0.91	3.51	4.84	0.78

Parathormone 400 units administered intravenously at end of period 3.

Note marked rise in UVp.

No obvious effect upon glomerular filtration rate.

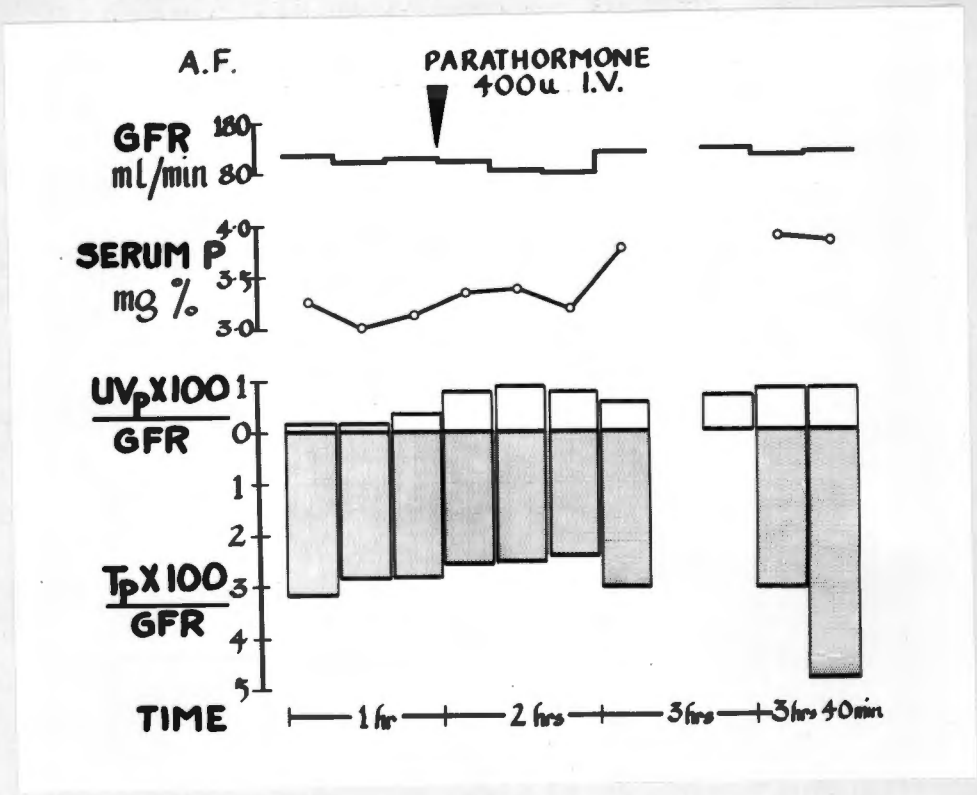

Fig. 43

Showing effect of parathormone administration to subject A.F. (Experiment XXVI).

Note slight fall in $\frac{Tp}{GFR}$. Rise in $\frac{UVp}{GFR}$.

Data presented in Table 40.

The blank periods are due to technical difficulty in obtaining blood samples.

EXPERIMENT No: XXVII

Subject: T.M.

Table No. 41

Period	Time (mins.)	Pp (mg.%)	G.F.R. (ml./min.)	Fp (mg./min.)	UVp (mg./min.)	Tp (mg./min.)	$\frac{Tp \times 100}{G.F.R.}$	$\frac{UVp \times 100}{G.F.R.}$
1	20	15.56	117.8	18.33	14.65	3.68	3.12	12.44
2	20	14.56	114.8	16.71	13.80	2.91	2.53	12.02
3	19	13.33	114.0	15.20	12.79	2.41	2.11	11.21
4	21	12.48	190.7	23.80	18.57	5.23	2.74	9.74
5	20	12.15	132.7	16.12	12.95	3.17	2.39	9.76
6	27	11.36	110.3	12.53	10.85	1.68	1.52	9.84
7	18	11.32	104.6	11.84	9.33	2.51	2.40	8.92
8	21	10.64	116.8	12.43	10.43	2.00	1.71	8.93
9	18.5	10.31	121.4	12.52	10.24	2.28	1.88	8.43
10	19.5	10.03	121.7	12.21	9.72	2.49	2.05	7.99

Parathormone 400 units given intravenously after period 3.

Note abrupt and marked rise in glomerular filtration rate following parathormone injection accompanied by simultaneous rise in Tp.

There is no obvious effect upon $\frac{Tp}{GFR}$.

The effect on UVp is obscured by simultaneous phosphate infusion.

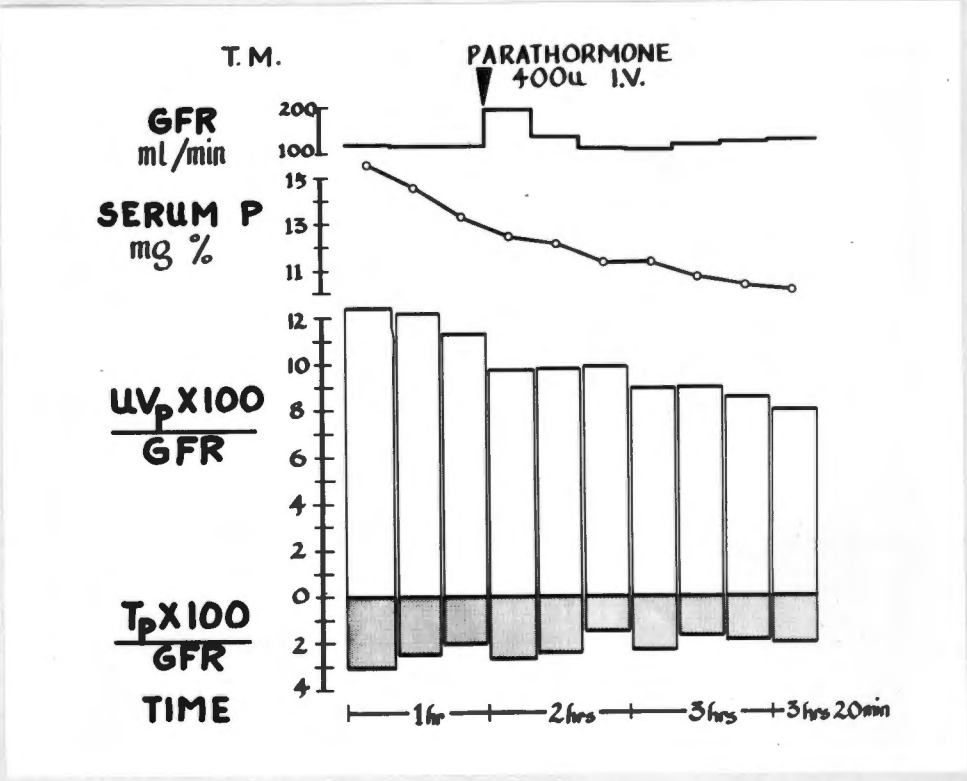

Fig 44

Showing effect of parathormone administration to Subject T.M. (Experiment XXVII).

Note marked rise in glomerular filtration rate following parathormone administration. No convincing effect of parathormone upon rate of reabsorption of phosphorus. Effect on urinary phosphorus obscured by simultaneous phosphate infusion.

EXPERIMENT No: XXVIII

Subject: J.J.

Table No: 42

Period	Time (mins.)	Pp (mg.%)	G.F.R. (ml./min.)	Fp (mg./min.)	UVp (mg./min.)	Tp (mg./min.)	$\frac{Tp \times 100}{G.F.R.}$	$\frac{UVp \times 100}{G.F.R.}$
1	19.5	3.45	162.2	5.60	0.29	5.31	3.28	0.18
2	20.5	3.50	155.5	5.44	0.31	5.13	3.30	0.20
3	20.0	3.70	134.1	4.96	0.29	4.67	3.49	0.22
4	20.0	3.56	154.4	5.50	0.51	5.00	3.24	0.33
5	20.0	3.51	149.8	5.26	0.84	4.42	2.95	0.56
6	20.0	3.66	164.3	6.01	0.92	5.09	3.10	0.56
7	20.0	3.74	156.9	5.87	0.83	5.05	3.22	0.53
8	20.0	3.55	159.7	5.67	0.77	4.90	3.07	0.48
9	21.0	3.60	187.9	6.76	0.72	6.04	3.22	0.38
10	21.0	3.63	169.1	6.14	0.70	5.45	3.22	0.41
11	25.0	3.68	172.2	6.34	0.85	5.49	3.19	0.49
12.	20.0	3.90	169.4	6.61	0.71	5.90	3.48	0.42

Parathormone 400 units given intravenously at the end of period 3.

Note slight fall in $\frac{Tp}{GFR}$ following parathormone.

No obvious effect upon glomerular filtration rate.

No obvious effect upon Tp.

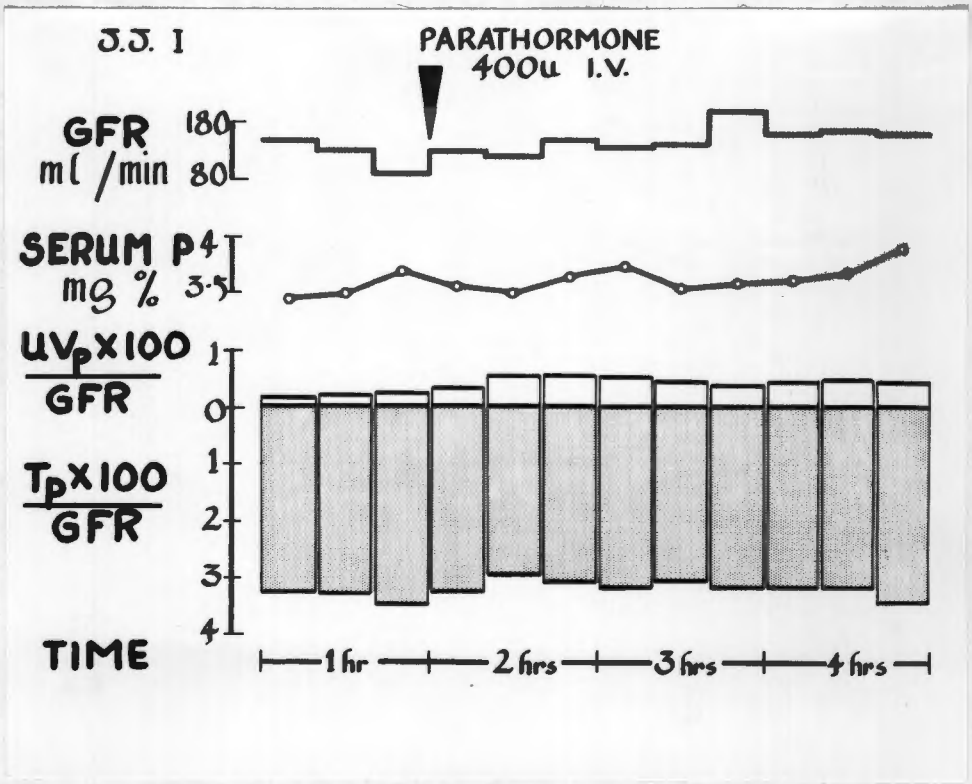

Fig. 45

Showing effect of parathormone administration to Subject J.J. (Experiment XXVIII).

Data presented in Table 42.

Normal plasma phosphate levels maintained throughout.

EXPERIMENT No: XXIX

Subject: J.J.

Table No: 43

Period	Time (mins.)	Pp (mg.%)	G.F.R. (ml./min.)	Tp (mg./min.)	UVp (mg./min.)	Tp (mg./min.)	Tp x 100 / G.F.R.	UVp x 100 / G.F.R.
1	20.0	6.84	149.8	10.25	3.66	6.59	4.40	2.44
2	20.0	7.38	151.6	11.19	4.06	7.13	4.70	2.68
3	20.0	7.48	139.3	10.42	3.63	6.79	4.87	2.61
4	20.0	6.98	161.6	11.28	5.23	6.05	3.74	3.24
5	20.5	6.90	185.7	12.81	5.98	6.83	3.68	3.22
6	20.0	6.96	159.7	11.12	4.89	6.23	3.90	3.06
7	24.0	7.03	123.4	8.68	4.17	4.51	3.65	3.38
8	20.0	6.96	130.6	9.09	3.87	5.22	4.00	2.96
9	20.0	6.90	142.8	9.85	3.07	6.78	4.75	2.15
10	20.0	7.45	112.1	8.35	3.70	4.65	4.15	3.30
11	20.0	7.30	124.7	9.10	3.78	5.32	4.27	3.03
12	20.0	7.14	128.0	9.14	4.28	4.86	3.80	3.34

Parathormone 400 units given intravenously at end of period 3.

Note sharp rise in glomerular filtration rate following parathormone with simultaneous rise in Tp but fall in $\frac{Tp}{GFR}$.

Same subject as in Experiment No. XXVIII but with plasma phosphate concentration artificially elevated by infusion of buffered phosphate.

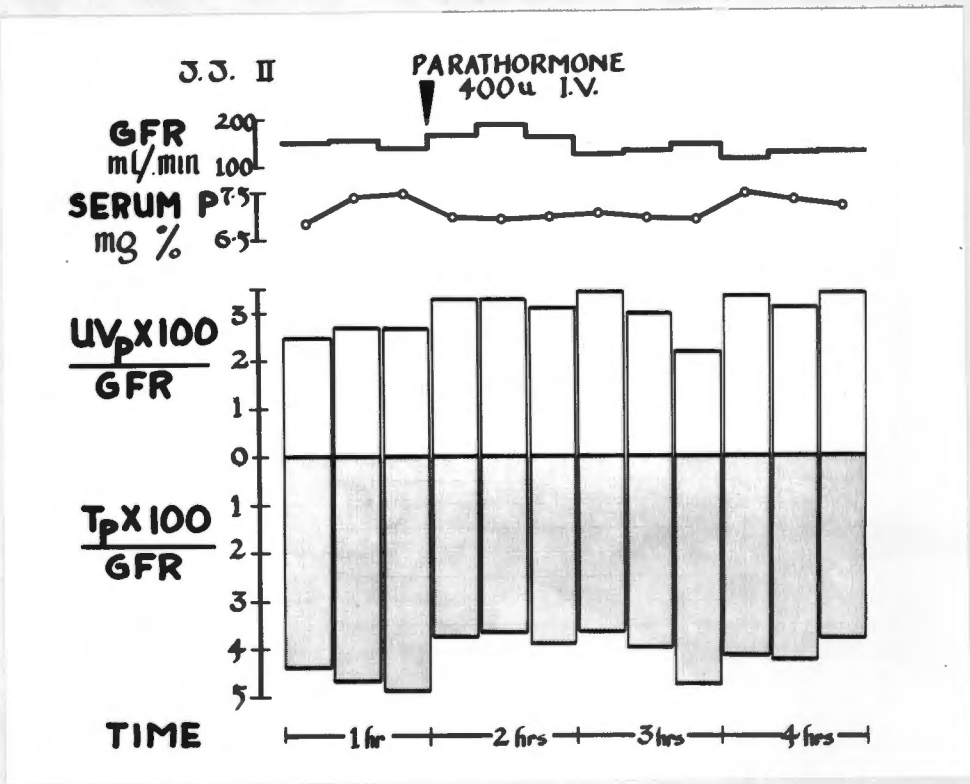

Fig. 46

Showing effect of parathormone administration to Subject J.J. (Experiment XXIX), this time combined with phosphate infusion.

Data presented in Table 43.

The fall in $\frac{T_P}{GFR}$ is more apparent at high plasma levels, (cf. Fig. 45).

EXPERIMENT No: XXX

Subject: H.R.

Table No: 44

Period	Time (mins.)	Pp (mg.%)	G.F.R. (ml./min.)	Fp (mg./min)	UVp (mg./min.)	Tp (mg./min.)	$\frac{Tp \times 100}{G.F.R.}$	$\frac{UVp \times 100}{G.F.R.}$
1	20.0	4.50	137.0	6.16	1.11	5.05	3.69	0.81
2	20.0	5.13	118.6	6.08	0.74	5.34	4.50	0.62
3	20.0	5.03	115.4	5.81	0.80	5.01	4.34	0.69
4	20.0	4.96	115.1	7.69	1.18	6.51	4.20	1.03
5	20.0	5.27	188.6	9.94	1.51	8.43	4.47	0.80
6	20.0	4.39	169.9	7.46	1.26	6.20	3.65	0.74
7	20.0	4.78	183.1	8.75	1.39	7.36	4.02	0.76
8	20.0	5.06	124.9	6.32	1.03	5.29	4.24	0.82
9	20.0	4.78	96.1	4.59	0.79	3.80	3.95	0.82
10	20.0	4.98	127.6	6.35	0.87	5.48	4.30	0.68
11	20.5	4.85	144.2	6.99	0.91	6.08	4.22	0.63
12	19.5	5.42	125.7	6.81	0.79	6.02	4.79	0.63

Parathormone 400 units administered intravenously at end of period 3.

Note sharp rise in glomerular filtration rate during periods 5,6,7 with corresponding rise in Tp.

The urine phosphorus excretion has remained more or less unchanged. No convincing effect on $\frac{Tp}{GFR}$.

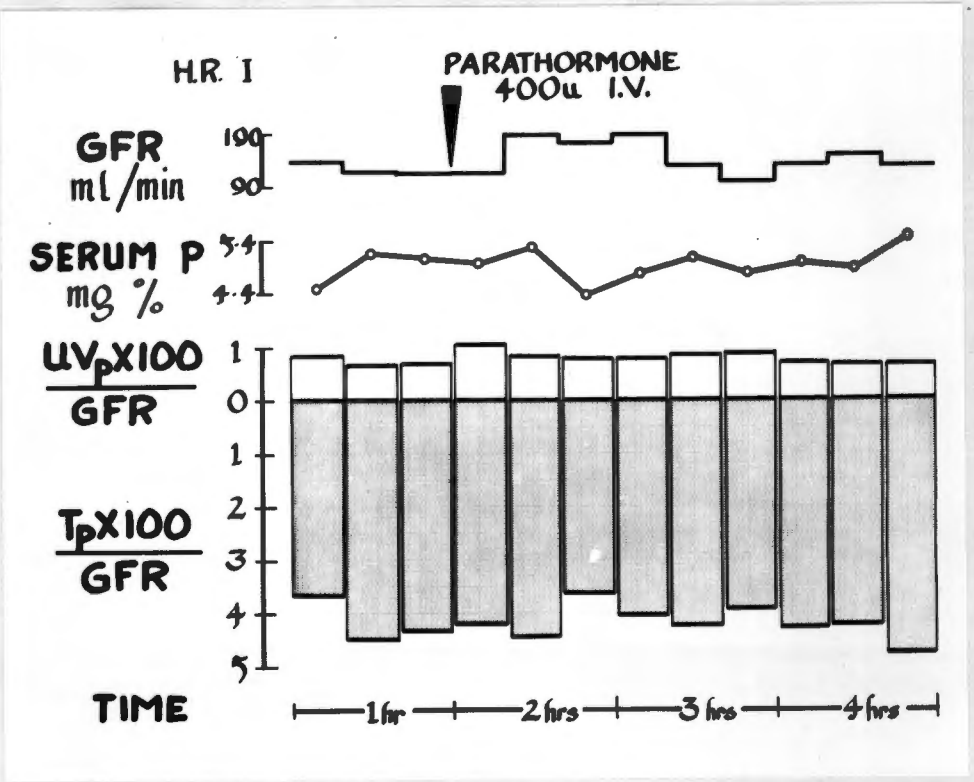

Fig. 47

Showing effect of parathormone administration to Subject H.R. (Experiment XXX).

Data presented in Table 44.

Normal plasma phosphate concentration.

EXPERIMENT No: XXXI

Subject: H.R.

Table No:45

Period	Time (mins.)	Pp (mg.%)	G.F.R. (ml./min.)	Fp (mg./min.)	UVp (mg./min.)	Tp (mg./min.)	Tp x 100 / G.F.R.	UVp x 100 / G.F.R.
1	20.0	7.28	135.6	9.87	3.45	6.42	4.74	2.55
2	20.0	7.45	126.8	9.45	3.13	6.32	4.98	2.47
3	20.0	7.54	134.3	10.13	3.46	6.67	4.97	2.58
4	20.0	7.34	157.9	11.59	4.42	7.17	4.54	2.80
5	20.0	7.40	141.2	10.45	4.28	6.17	4.37	3.03
6	20.0	7.29	105.0	7.65	3.23	4.42	4.21	3.08
7	20.0	7.39	82.8	6.12	2.51	3.61	4.36	3.03
8	20.0	7.30	187.8	13.71	6.38	7.33	3.90	3.40
9	20.0	7.46	111.1	8.29	3.54	4.75	4.28	3.20
10	20.0	7.40	142.0	10.51	4.35	6.16	4.34	3.06
11	20.5	7.65	133.9	10.25	4.19	6.06	4.52	3.13
12	19.5	7.55	135.6	10.24	3.92	6.32	4.66	2.89

Parathormone 400 units administered intravenously at end of period 3.

Elevated plasma phosphorus concentration maintained by constant infusion of phosphate solution.

The marked rise in glomerular filtration rate during period 8 is probably due to incomplete bladder emptying as the glomerular filtration rate for the preceding period is abnormally low.

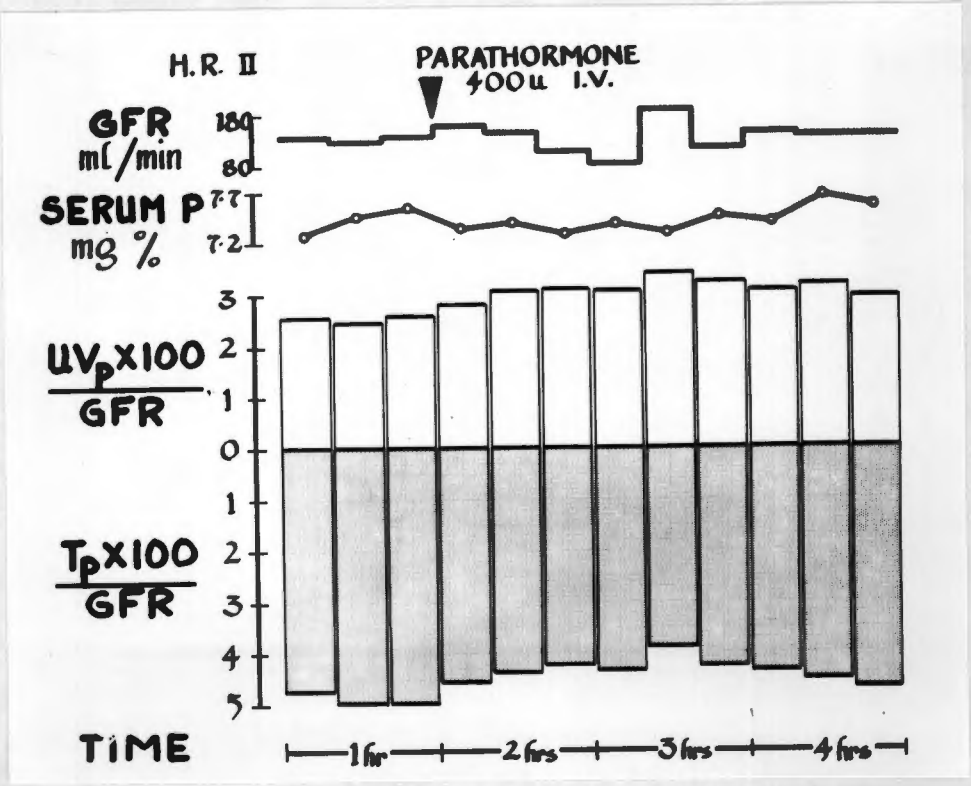

Fig. 48

Showing effect of parathormone administration to Subject H.R. (Experiment XXXI), this time combined with phosphate infusion.

Data presented in Table 45.

The fall in $\frac{T_P}{GFR}$ is more apparent at high plasma levels. (cf. Fig. 47).

Results.

The first conclusion which can be reached from these results is that, however categorical my commentaries may appear, the results are depressingly inconclusive. I think I am able to say that in three experiments parathyroid hormone caused an increase in the rate of excretion of phosphorus in the urine. The true relations of this increase makes me think it was significant, and due to the parathormone.

In three instances parathormone injection was followed by an increase in the glomerular filtration rate. In each case this increase in glomerular filtration rate was accompanied by a rise in the absolute rate of reabsorption of phosphate but a fall in the threshold. This lends support to my earlier contention that the rate of reabsorption of phosphate is affected by the glomerular filtration rate.

The unconvincing nature of the results was in part due to the fact that I was attempting to measure, with a crude technique, a small change in tubular function, and in part to the fact that the parathyroid extract was administered to normal individuals whose renal tubules were already subject to the action of endogenous parathyroid hormone. I feel certain that I could have demonstrated more convincing changes had I conducted similar experiments on hypoparathyroid patients. Unfortunately I have not done this, nor do I see much point in so doing.

I feel that the case for a phosphate diuretic effect of parathyroid hormone has been proved by others. If this phosphate diuresis takes place without a rise in the plasma concentration

(as it does) one may justifiably conclude that parathyroid hormone causes a lowering of the renal threshold. I do not think that the matter need be taken much further. My "observer bias" has led me to feel that this is due to suppression of renal tubular activity, but I accept that this feeling may be wrong.

No effect of Parathormone on Plasma or urine calcium is evident from the results.

I plead for a shift of emphasis in parathyroid research away from the renal aspect and on to the intricate physical chemistry of calcium and phosphorus homeostasis. It is only at this level that the problem of the mode of action of parathyroid hormone can be solved.

Experiments on Hypoparathyroid Subjects.

In two cases of surgical hypoparathyroidism, H.W. and E.B., the metabolic effects of intravenous calcium were studied, and in H.W. the relationship between $\frac{UVca}{G.F.R.}$ and Pca was studied.

In the case of H.W., the calcium was infused at a progressively increasing rate in the hope that a linear rise in plasma calcium could be achieved. In patient E.G., calcium gluconate was infused at a constant rate over a short period of time after a control period had elapsed and estimations were continued for 4 hours after the infusion had been discontinued.

The conduct of these two experiments was in all respects similar to that for the normals and is described in greater detail in Appendix A.

Case reports of these two patients may be found in Appendix A. It is important to state at this stage, however, that patient E.B. had been off all therapy for 4 weeks prior to the

EXPERIMENT No: XXXII

Subject: E.B.

Table No. 46

Period	Time (mins)	Pca. (mg.%)	Pp (mg.%)	G.F.R. (ml/min)	Fp (mg/min)	UVp (mg/min)	Tp (mg/min)	Tp x 100 / G.F.R.	UVp x 100 / G.F.R.
1	23.5	7.2	5.4	230.9	12.51	0.72	11.79	5.11	0.31
2	20.0	6.9	5.3	200.9	10.69	0.68	10.02	4.99	0.34
3	20.0	8.6	5.4	225.2	12.12	0.86	11.27	5.00	0.38
4	26.5	9.6	5.9	224.0	13.24	1.02	12.23	5.46	0.46
5	23.0	15.7	7.1	205.0	14.31	1.92	12.39	6.04	0.94
6	23.0	15.5	7.9	237.8	18.50	2.54	15.97	6.71	1.07
7	30.5	15.3	8.7	215.1	18.61	2.85	15.76	7.33	1.32
8	31.0	15.2	8.1	203.0	16.50	2.16	14.34	7.06	1.06
9	60.0	13.4	7.9	170.6	13.49	1.43	12.06	7.07	0.84
10	62.5	13.4	7.6	209.4	15.91	1.25	14.67	7.00	0.60

Calcium administered intravenously during periods 3 and 4.

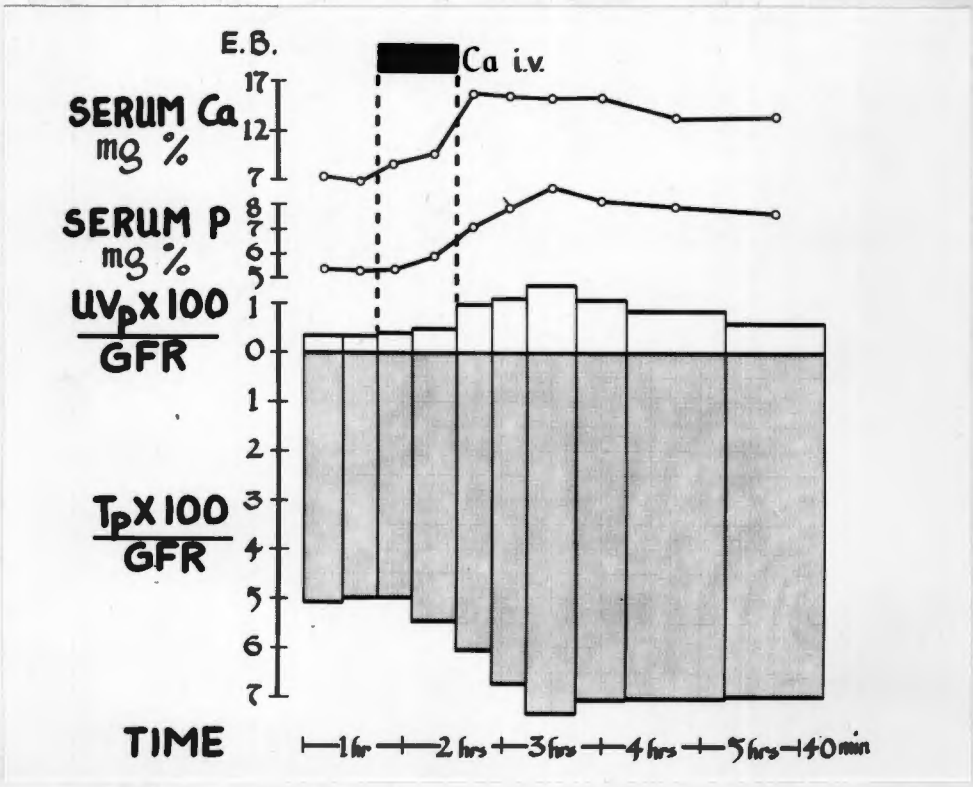

Fig. 49

Showing effects of intravenous calcium infusion on phosphorus metabolism in subject E.B. (Experiment XXXII).

Data presented in Table 46.

EXPERIMENT No: XXXIII Subject: H.W.

Table No. 47

Period	Time (mins)	Pca (mg.%)	Pp (mg.%)	G.F.R. (ml/min)	Tp (mg/min)	UVp (mg/min)	Tp (mg/min)	Tp x 100 / G.F.R.	UVp x 100 / G.F.R.
1	21.5	9.52	3.73	18.1	0.68	0.11	0.57	3.15	0.58
2	20.2	10.00	3.76	221.0	8.31	1.40	6.91	3.13	0.63
3	20.8	9.76	3.50	142.8	5.00	1.17	3.83	2.68	0.82
4	21.8	10.00	3.32	114.4	3.80	0.90	2.90	2.53	0.79
5	19.3	10.00	3.58	136.5	4.89	1.12	3.77	2.76	0.82
6	19.2	10.80	3.90	86.4	3.37	0.91	2.46	2.85	1.05
7	20.0	11.04	4.33	153.0	6.62	1.65	4.97	3.25	1.08
8	18.5	11.68	4.53	137.2	6.22	1.67	4.55	3.32	1.21
9	19.2	12.24	4.57	117.5	5.37	1.86	3.51	2.99	1.58

The marked variation in glomerular filtration rate is due to incomplete bladder emptying.

Pre-infusion plasma calcium concentration - 9.49 mg.%

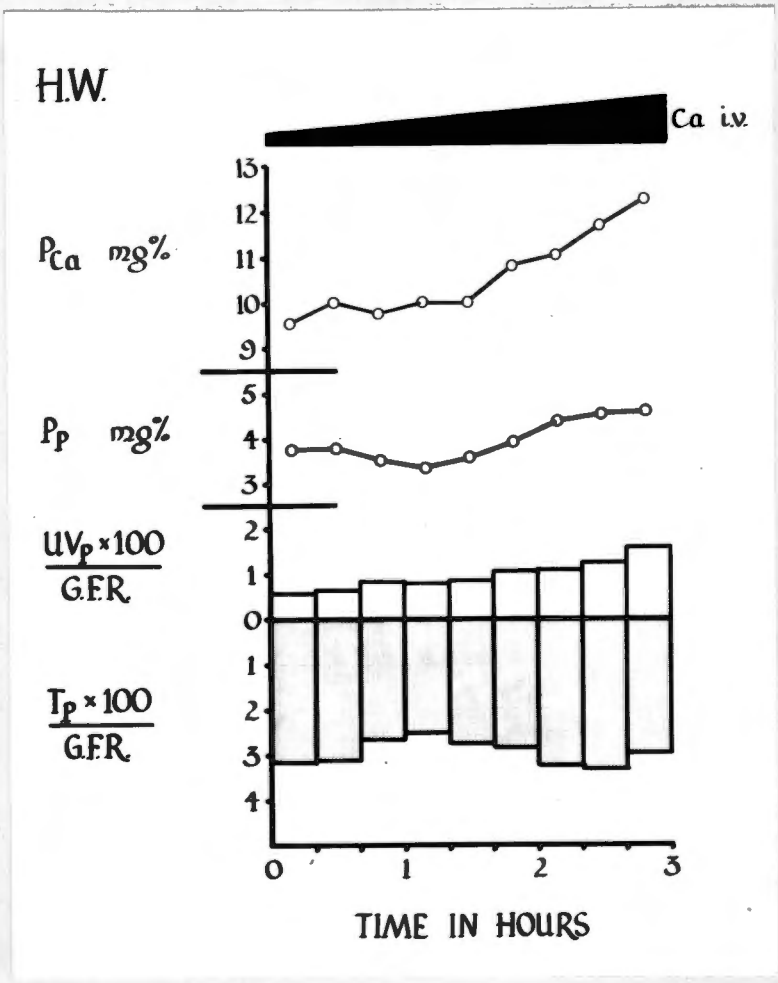

Fig.50

Showing effects of intravenous calcium infusion on phosphorus metabolism in subject H.W. (Experiment XXXIII). Data presented in Table 47.

performance of the experiment. Patient H.W. was on large doses of calciferol and prednisone at the time of the test, the latter given to correct exophthalmos.

(a) The metabolic effects of intravenous calcium.

The effects of calcium infusion on phosphorus metabolism in these two patients are shown in Fig. 49 and Table 46 for E.B. and in Fig. 50 and Table 47 for H.W.

The following points are apparent from these results :

(i) Subject E.B. had a low resting serum calcium concentration. This was associated with signs of latent tetany. Subject H.W. had a normal resting calcium concentration owing presumably to the calciferol therapy. It is interesting to note that the prednisone therapy had not apparently interfered with the ability of oral calciferol to maintain her plasma calcium concentration within normal limits.

(ii) In both instances the infusion of calcium resulted in a rise in plasma phosphorus concentration. This rise was not as marked in subject H.W. as it was in E.B. This may have been due to the fact that the extent of the rise in plasma calcium was not so great in H.W. as it was in E.B.

(iii) In both cases, calcium infusion resulted in a rise in the rate of excretion of phosphorus in the urine. This was more marked in subject E.B. than in H.W., and was probably due to the fact that the rise in serum phosphorus concentration was greater in E.B. than it was in H.W.

(iv) In patient E.B. there was a marked rise in the calculated rate of tubular transport of phosphorus both as judged by

the absolute value (Tp) or the threshold ($\frac{Tp}{GFR}$). As this patient is grossly hypoparathyroidic, it can scarcely be argued that this increase in the rate of reabsorption of phosphate was due to parathyroid suppression by the hypercalcaemia. An alternative and more acceptable explanation would be that the renal threshold for phosphate was increased not by any direct effect on the renal tubules but by the formation of a non-diffusible phosphorus complex. The presence of such a complex in the plasma would make the estimate of the rate of tubular reabsorption falsely high.

In patient H.W. a similar significant change in calculated rate of tubular reabsorption was not found. This may have been due to the fact that Vitamin D inhibits the formation of non-diffusible phosphorus complexes.

(v) The absolute amount of phosphate being reabsorbed and the threshold for phosphorus was high in E.B. during the control period before calcium infusion, but was normal in H.W. The resting serum inorganic phosphorus concentration was high in E.B. but was normal in H.W.

Assuming that these two cases are comparable, and that the differences in between them are due to calciferol, it may be concluded that Vit. D lowers the renal threshold for phosphate. This is not an altogether unreasonable assumption because it is well known that Vit. D will restore the plasma phosphorus concentration to normal in patients with hypoparathyroidism and will increase the rate of phosphate excretion in the urine[21].

An opposite effect of Vitamin D on the renal threshold for phosphorus is seen in rickets, where it causes a rise in renal threshold for phosphorus[116,113]. This has been explained by Albright[9] by suggesting that the increased absorption of calcium from the bowel following therapy in rickets elevates the plasma calcium concentration and so suppresses the secretion of parathyroid hormone that was, in the first instance, responsible for the low renal threshold. A "Parathormone-like" action of Vitamin D on the kidneys is suggested to explain the lowering of the phosphate threshold in hypoparathyroidism.

I suggest the alternative explanation that Vitamin D acts primarily on the plasma phosphorus in hypoparathyroidism rendering it diffusible and hence more readily available for renal excretion.

(vi) In case E.B., calcium infusion resulted in a prompt rise in the rate of sodium excretion in the urine. The figures are given in Appendix B.

(b) <u>The relationship between the rate of excretion of calcium in the urine and the plasma calcium concentration in Patient H.W.</u>

The results from Experiment XXXIII were used to study this relationship in a similar manner to that described for normal subjects (Experiments XIV to XIX inclusive.)

The relevant data are given in Table 48 and are depicted graphically in Fig. 51.

It will be seen that the value obtained for the regression coefficient of $\frac{UVca \times 100}{G.F.R.}$ on Pca is very much higher than that

EXPERIMENT No: XXXIII Subject: H.W.

Table No. 48

Period	Time (mins.)	Pca (mg.%)	UVca (mg./min.)	G.F.R. (ml./min.)	UVca x 100 / G.F.R.
1	21.5	9.52	0.08	18.1	0.44
2	20.2	10.00	0.82	221.0	0.37
3	20.8	9.76	0.81	142.8	0.57
4	21.8	10.00	0.91	114.4	0.80
5	19.3	10.00	1.45	136.5	1.05
6	19.2	10.80	2.54	86.4	2.94
7	20.0	11.04	2.95	153.0	1.93
8	18.5	11.68	2.96	137.2	2.16
9	19.2	12.24	3.17	117.5	2.70

Sxx = 7.0272
Syy = 9.0596
Sxy = 6.5415
a = 0.9309

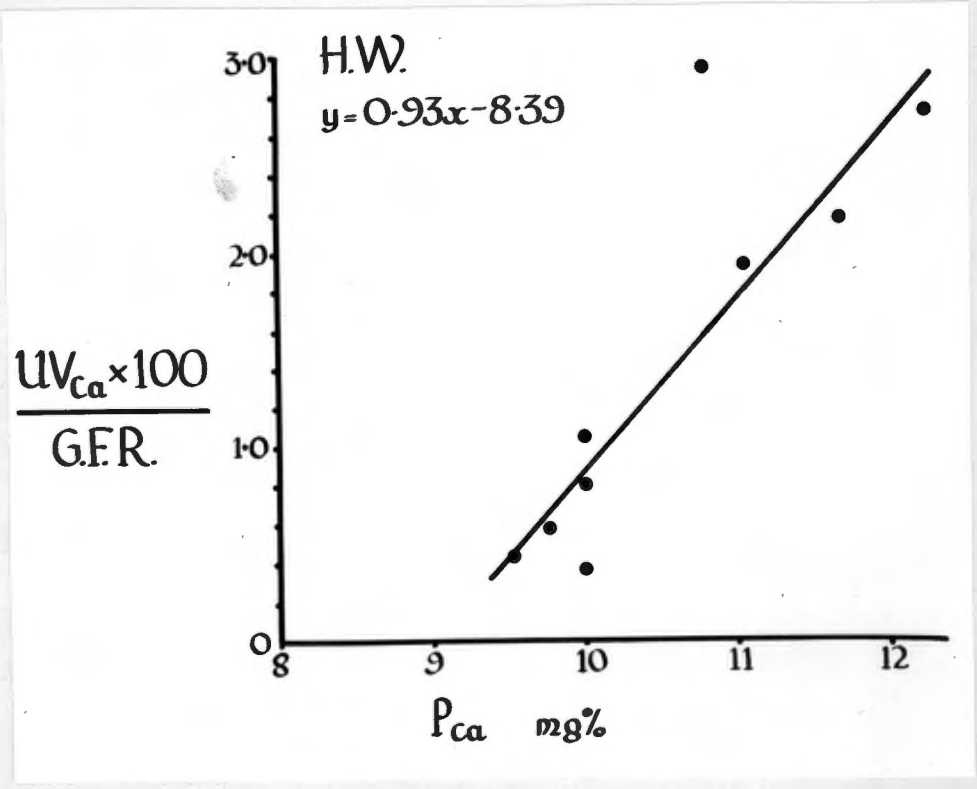

Fig. 51

Regression line for $\frac{UVca \times 100}{G.F.R.}$ on Pca in patient H.W. (Experiment XXXIII) calculated from data presented in Table 48.

The 95% confidence interval for the regression coefficient a = 0.931, as calculated from a pooled estimate of the variance of a, is from 0.825 to 1.037.

encountered for normals. There are three explanations for this discrepancy that suggest themselves to me.

(i) That the calcium in the plasma exists in an entirely filterable form. This, I think is highly unlikely.

(ii) That the calcium that was infused was not incorporated naturally into the various compartments of the plasma calcium and was consequently rejected quantitatively in the urine as a "foreign substance". This would imply that the mechanism for "binding" calcium was saturated and could accommodate none of the infused calcium. This explanation is supported by arguing conversely from the observations of Martin and Perkins[176,177] that the ability of serum albumin to bind protein is increased in patients with hyperparathyroidism and returns to normal after parathyroidectomy.

Goldman and Bassett[84] have made the astute observation that when the serum calcium concentration in patients with hypoparathyroidism is raised to normal levels by calcium infusion, the rate of excretion of calcium in the urine is greatly in excess of the normal rate of excretion, an observation that confirms my own on Subject H.W.

Talmadge et al.[251] noted that the fall in urine calcium following parathyroidectomy in rats was preceded by a sharp rise - a phenomenon that could easily be explained on the basis of sudden fall in the calcium binding capacity of the plasma as a result of parathyroidectomy.

(iii) Owing to the Vitamin D which the patient had been receiving there was, in her plasma, an excess of some substance that combined with the infused calcium to render it non-reabsorbable by the tubular epithelium, without affecting its diffusibility. If this were the case, the filtered calcium would behave in a manner similar to inulin and the regression coefficient would be equal to unity.

The interpretation of the results of the calcium titration experiment in this case is complicated by the fact that she was receiving prednisone and calciferol at the time. Cortisone has been shown by Roberts and Pitts[220] to affect the renal threshold for phosphate.

SUMMARY.

(i) The literature recording observations on the mode of action of parathyroid hormone is reviewed and it is concluded that parathyroid hormone exercises an effect on bone and on the renal threshold for phosphorus. These two effects are independent of each other.

(ii) The results of seven acute experiments with parathyroid extract are recorded and the following conclusions are tentatively drawn :

(a) Parathyroid extract may increase the glomerular filtration rate. This is not, however, an invariable response.

(b) Parathyroid extract may, by causing an increase in the glomerular filtration rate, cause an increase in

the the rate of tubular reabsorption of phosphorus. The amount of phosphorus reabosrbed from each 100 ml. of filtrate formed is, however, reduced by administration of parathyroid extract.

(c) Parathyroid extract causes no apparent change in plasma calcium concentration or rate of excretion of calcium in the urine over the three hour period following its administration.

(iii) The results of calcium infusion experiments on two cases of surgical hypoparathyroidism are discussed. One case was receiving no therapy while the other was receiving calciferol and prednisone at the time of the experiment.

Calcium infusion caused an increase in the renal threshold for phosphorus in the case on no therapy but had no effect on the case receiving prednisone and Vitamin D.

The renal mechanism for excreting calcium in case H.W. was found to be abnormal.

SECTION V.

The Renal Complications of Sarcoidosis.

THE RENAL COMPLICATIONS OF SARCOIDOSIS

Since Harrell and Fisher[112] first recorded the occurrence of hypercalcaemia in six of eleven cases of Sarcoidosis, the association between Sarcoid and abnormalities of Calcium homeostasis has been repeatedly observed.

Horton, Lincoln and Pinner[130] described metastatic deposits of calcium in the kidneys of a case of Sarcoid at post mortem, and in 1946 Klinefelter and Salley[151] described a case of sarcoidosis that had presented with predominantly renal manifestations. They attributed the signs of renal failure in their case to massive granulomatous infiltration of the kidneys with sarcoid tissue, but this was not proved by histology.

Klatskin and Gordon[148] have recently published an excellent review of the renal complications in sarcoid. From four of their own cases and from an additional five which they were able to cull from the literature, they came to the acceptable conclusion that nephrocalcinosis is the principal renal lesion in sarcoidosis and that non-caseating tubercles are rarely found and probably do not give rise to symptoms. In all of the cases analysed by Klatskin and Gordon, hypercalcaemia was present, and it was to this and the resulting hypercalcuria that they attributed the renal lesions. From their study one other fact emerges, and that is that the kidney lesions in sarcoidosis produce symptoms of tubular rather than glomerular insufficiency. Thus hyposthenuria, polyuria and polydipsia are found with relative frequency, while azotaemia and hypertension are uncommon. This pure picture may be obscured by pyelonephritis complicating the renal calculi which are also frequently found.

I have, thanks to the cooperation of Professor Forman and Dr. Jackson, had an opportunity to study the nature of the renal abnormality in two cases of Sarcoidosis. As they both illustrate some important features of this aspect of the disease I shall present them in some detail.

Case 1: N.N. a Bantu Male of 29 years of age, presented at the Dermatology outpatient department in January 1955 complaining of painless skin nodules which were discretely scattered over the trunk and upper limbs and had been present for 3 weeks.

These skin nodules had appeared over a period of 5 days. He had no other symptoms at the time, nor has he complained of any other symptoms since. He has repeatedly denied having polyuria, polydipsia or local symptoms referable to the urinary tract.

He was admitted to the dermatology wards where a biopsy was taken from one of the skin nodules. Histological examination of this tissue revealed the presence of numerous non-caseating tubercle follicles throughout the corium.

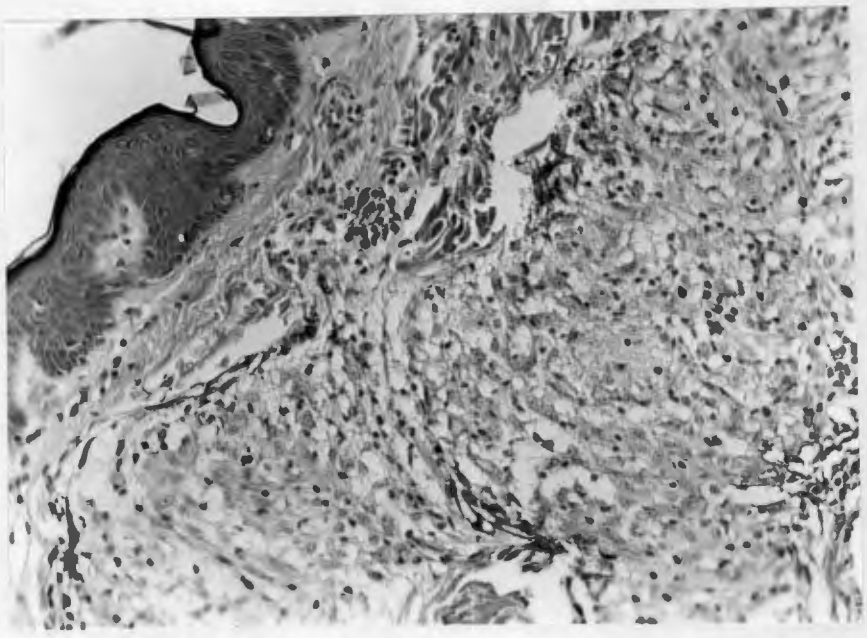

Fig. 52.

Skin biopsy from patient N.N. showing extensive infiltration of the corium with granulomatous tissue.

Physical examination at that time revealed no abnormality save for the presence of the skin lesions and posterior cervical lymph node enlargement. An ophthalmological investigation disclosed the presence of quiescent uveitis in the right eye which had not given rise to symptoms.

The serum calcium concentration at that stage was found to be 10.4 mg.%, and the urinary calcium output 650 mg./24 hours. The serum protein concentrations were albumin 3.6 grammes %, globulin 3.9 grammes %; no radiological abnormalities could be demonstrated in the chest or in the bones of the hands and feet.

Treatment with oral cortisone brought about prompt subsidence of the skin lesions, and the patient was discharged with the advice to maintain a high fluid intake. The cortisone was withdrawn on discharge, and 3 weeks later the skin nodules recurred.

In March 1955 he was admitted to the metabolism ward for investigation and was treated with a further course of cortisone while in hospital. The skin nodules disappeared once more and have not recurred since.

While in the metabolism ward daily estimations of urine calcium were done in the course of a metabolic balance, and these were consistently above 500 mg./24 hours. Frequent estimations of serum calcium concentration yielded a normal result. The urine at this stage was normal save for the presence of marked crystalluria.

The patient was discharged in June of 1955 and has attended the outpatient department regularly since. During the period of time that elapsed between June of 1955 and the beginning of 1957 no further symptoms have developed and the patient has been able to continue his work as a manual labourer without inconvenience.

In October 1956 his parotids became enlarged and have remained so since. This was not noticed by the patient and did not give rise to symptoms.

On clinical examination in January of 1957 I found him to be generally healthy. There were numerous slightly depigmented discrete scars in the skin over the trunk and upper limbs. Both parotids were moderately enlarged and were diffusely granular on palpation. The optic fundi were normal. The blood pressure was 115/70 mm. of mercury.

The following investigations were conducted by myself at this time.

Urine: No protein, sugar or bile pigments. No abnormal formed elements in urinary sediment. No red cells or pus cells.

Serum protein concentration:

Albumin - 3.4 grammes %

Globulin - 4.4 grammes %

Urine concentration test: The patient was able to produce a urine specimen with a specific gravity of 1.026 after 15 hours of fluid deprivation.

Percutaneous renal biopsy: (Figs. 53, 54, 55)

The specimen of renal tissue obtained revealed the presence of normal and hyalinised glomeruli. There were two fairly well defined non-caseating tubercle follicles in the specimen. Occasional tubules showed hyaline droplet degeneration. There was no metastatic calcification in the specimen examined.

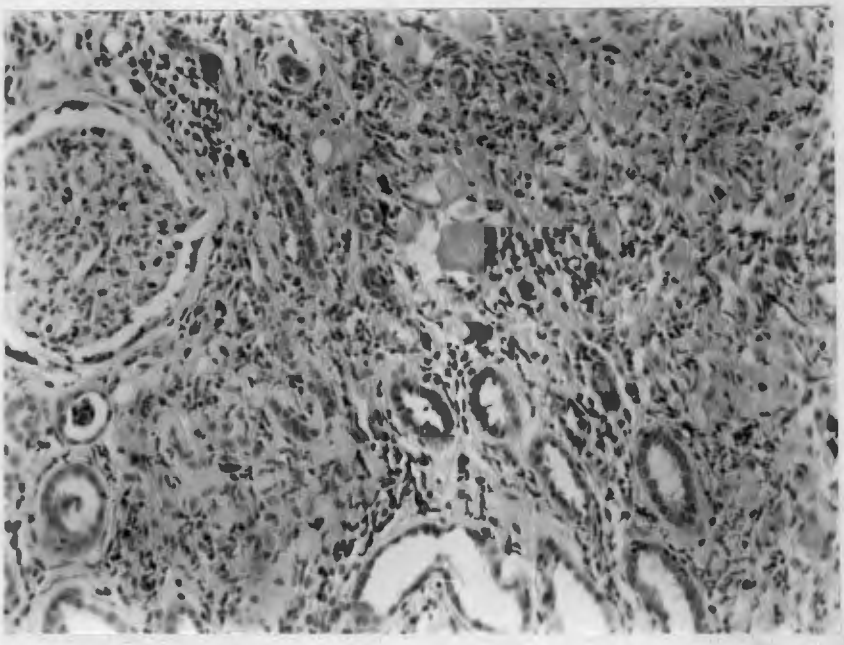

Fig. 53.
Low power view of the renal biopsy specimen obtained from N.N. stained with Haematoxylin and Eosin. On the left a normal glomerulus can be seen. In the centre is a giant cell with, extending upwards and to the right, a large non-caseating follicle.

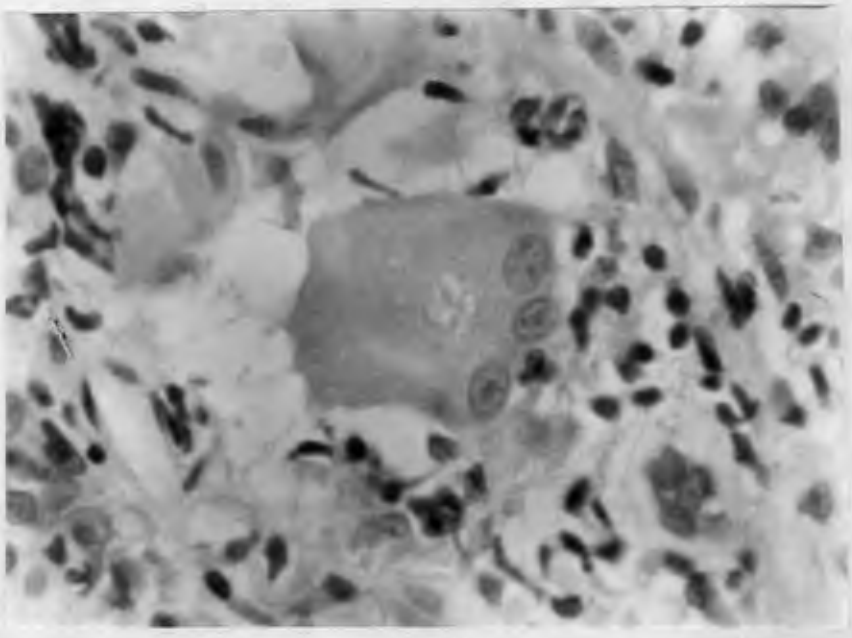

Fig. 54.
High power view of the giant cell seen in Fig. 53.

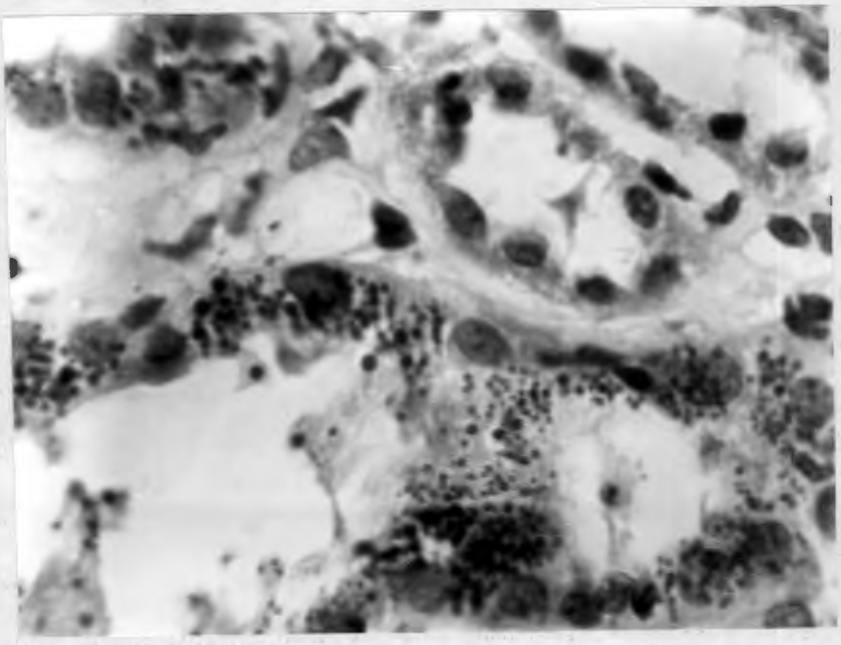

Fig. 55.

High power view of the tubules showing hyaline droplet degeneration. (Stained with Haematoxylin and Eosin; photographed with blue filter).

Calcium infusion experiment.

Calcium was infused at progressively increasing speeds over a period of three hours in a manner similar to that described for normals. The effects of artificial elevation of the plasma calcium concentration upon the renal handling of phosphate are shown and recorded in Table 49 and Fig. 56. In addition the relationship between $\frac{UVca \times 100}{G.F.R.}$ and Pca was studied and is recorded in Table 50 and Fig. 57.

Details of this experiment are given in Appendix A (Experiment XXXIV)

EXPERIMENT No: XXXIV

Subject: N.N.

Table No. 49

Period	Time (mins)	Pca (mg.%)	Pp (mg.%)	G.F.R. (ml/min)	Pp (mg/min)	UVp (mg/min)	Tp (mg/min)	Tp x 100 / G.F.R.	UVp x 100 / G.F.R.
1	20.5	10.08	3.13	92.8	2.90	0.32	2.58	2.78	0.35
2	23.0	10.64	3.13	82.6	2.59	0.45	2.14	2.59	0.54
3	19.0	10.80	3.17	84.2	2.67	0.47	2.20	2.61	0.56
4	20.6	11.28	3.52	91.4	3.22	0.56	2.66	2.91	0.61
5	20.5	11.52	3.55	89.0	3.16	0.86	2.30	2.58	0.97
6	18.5	12.08	3.74	94.6	3.53	1.25	2.28	2.41	1.33
7	20.7	12.48	3.87	97.5	3.77	1.45	2.32	2.38	1.49
8	18.5	12.72	4.26	98.2	4.18	1.76	2.42	2.46	1.80
9	19.3	13.04	4.74	103.3	4.90	1.85	3.05	2.95	1.79

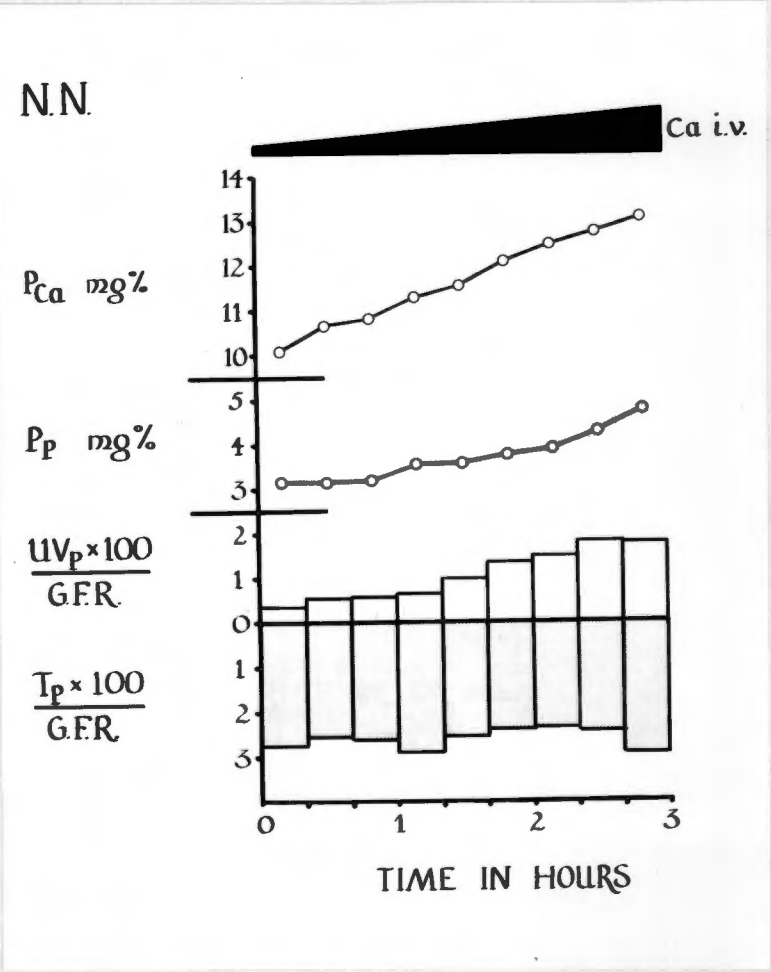

Fig. 56.

Showing the effect of intravenous calcium infusion on the renal handling of phosphate in patient N.N. (Experiment XXXIV).

Data presented in Table 49.

EXPERIMENT No: XXXIV Subject: N.N.

Table No: 50

Period	Time (mins.)	Pca (mg.%)	UVca (mg./min.)	G.F.R. (ml./min.)	UVca x 100 / G.F.R.
1	20.5	10.08	0.68	92.8	0.73
2	23.0	10.64	0.67	82.6	0.81
3	19.0	10.80	1.14	84.2	1.35
4	20.6	11.28	1.48	91.4	1.62
5	20.5	11.52	2.03	89.0	2.28
6	18.5	12.08	2.36	94.6	2.49
7	20.7	12.48	2.90	97.5	2.97
8	18.5	12.72	3.38	98.2	3.44
9	19.3	13.04	3.61	103.3	3.49

Sxx = 8.3072
Syy = 8.9943
Sxy = 8.5077
a = 1.0241

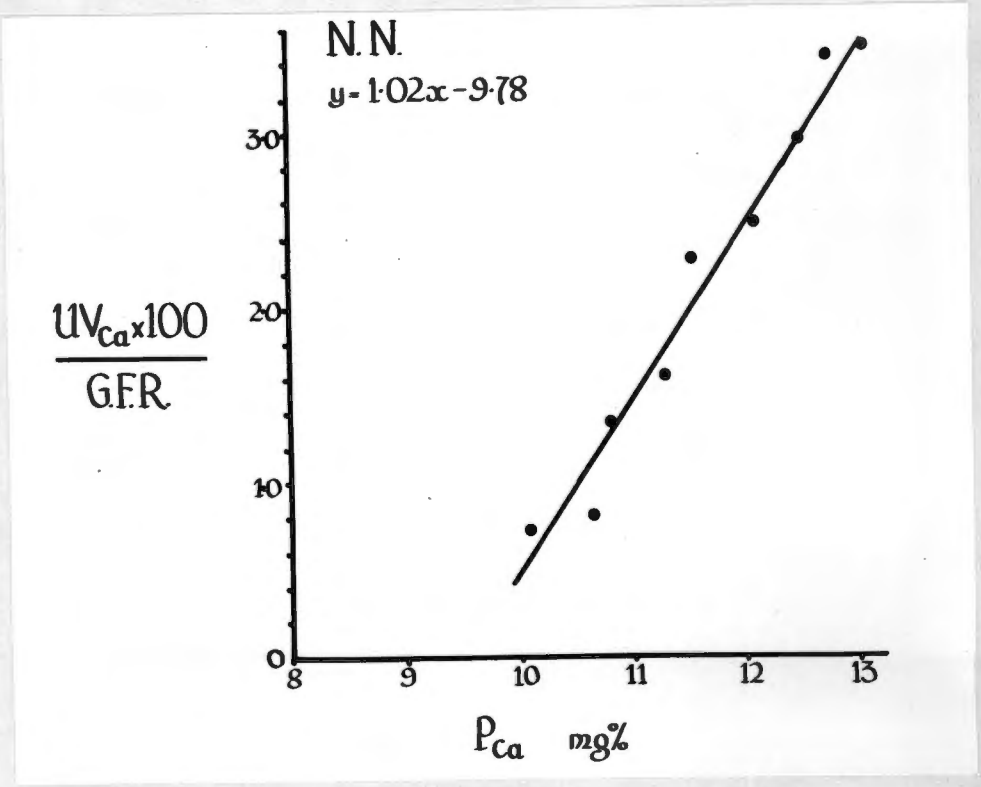

Fig. 57.

Showing the regression line for $\frac{UVca \times 100}{G.F.R.}$ upon Pca in patient N.N. (Experiment XXXIV) calculated from the data presented in Table 50.

The 95% confidence interval for the regression coefficient a = 1.024 is from 0.927 to 1.121.

Case 2: A.C. A Bantu female aged 26 years was seen first in January of 1956 when she presented at the gynaecological outpatient department complaining of lower abdominal pain, menstrual irregularity and productive cough which had been present for 4 months. She was admitted to hospital and a uterine curettage was performed which yielded normal endometrium. An X-Ray of the chest at this time showed diffuse irregular opacification. A diagnosis of pulmonary tuberculosis was made and she was discharged.

Three weeks after her discharge she presented at the ophthalmology outpatient department complaining of pain in the left eye. Iritis and pulmonary tuberculosis was diagnosed and the patient was referred to the Municipal tuberculosis clinic.

She was admitted to the Dr. Stals chest sanatorium in April of 1956. While in the tuberculosis hospital numerous examinations of the sputum for acid fast bacilli were made but these were consistently negative, and the patient was repeatedly negative to Mantoux testing. As she did not improve on a course of anti-tuberculous therapy, the diagnosis of sarcoidosis was suspected and she was transferred to Groote Schuur Hospital in November of 1956 for confirmation of this diagnosis.

In October of 1956, while in the sanatorium, she had an attack of pain lasting for 2 days and associated with "cold shivers". This pain was constant in nature and felt mainly in the right renal angle, radiating round the abdomen to the suprapubic area. Urine examination at the time revealed the presence of a heavy pyuria. No organisms were cultured and the attack subsided spontaneously leaving a residual mild proteinuria that has been present since.

Her serum proteins in October were found to be:
Albumin 3.2 grammes %
Globulin 6.4 grammes %

On admission to Groote Schuur Hospital she complained of weakness and occasional pain in the left loin after prolonged sitting or standing. She admitted to polyuria when questioned directly, but did not volunteer this symptom.

On examination she was an ill woman who had apparently lost weight.

There was total blindness in the left eye with opacification of the lower half of the cornea. Through the upper half of the cornea posterior synechiae were visible with nodules on the iris and lens opacities. The right eye was normal. (Fig. 58)

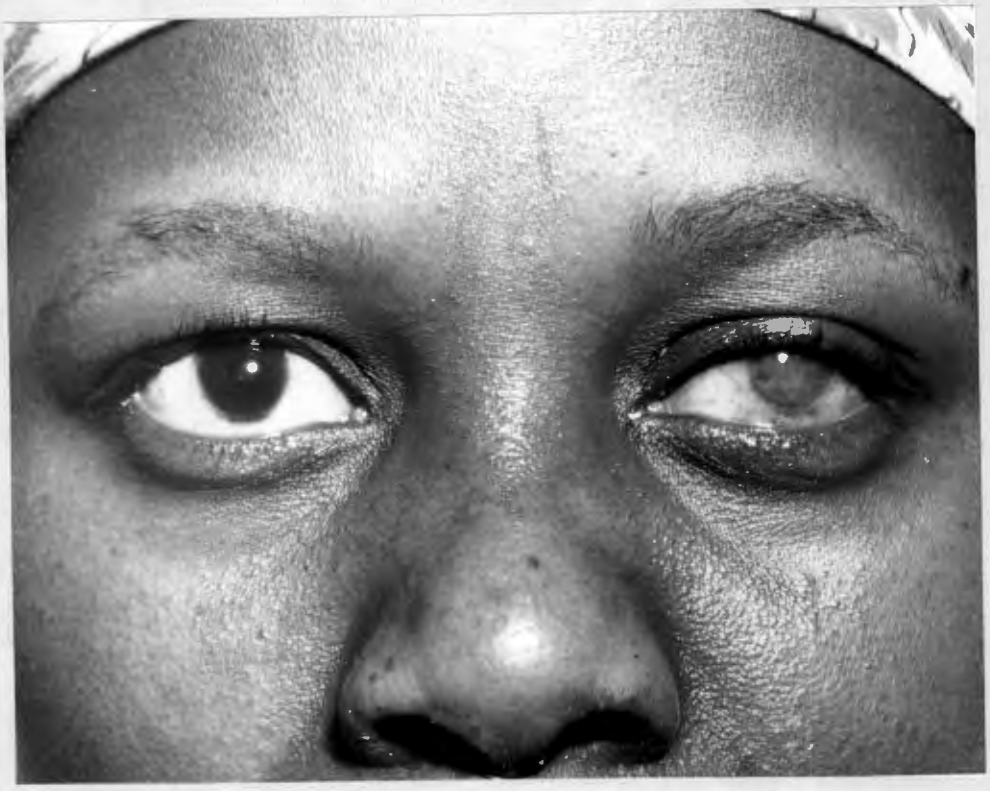

Fig. 58. Photograph of eyes of A.C. showing opacity of lower half of left cornea and oedema of the eyelids. The sharply defined upper edge of the corneal opacity can just be seen.

The blood pressure was 140/100.

No abnormal physical signs could be elicited on examination of the chest or other systems.

The urine was found to contain small amounts of protein (320 mg./24 hours). There were no red blood cells or pus cells in the urinary sediment.

The patient has been observed in hospital for 3 months and numerous investigations have been carried out. The results of these, in summary, is as follows :-

Chest X-Ray (Fig. 59) 7/1/57: Diffuse mottling of both lung fields maximal on the left and towards the hila of both lungs. Hilar lymph node enlargement was evident on the left side.

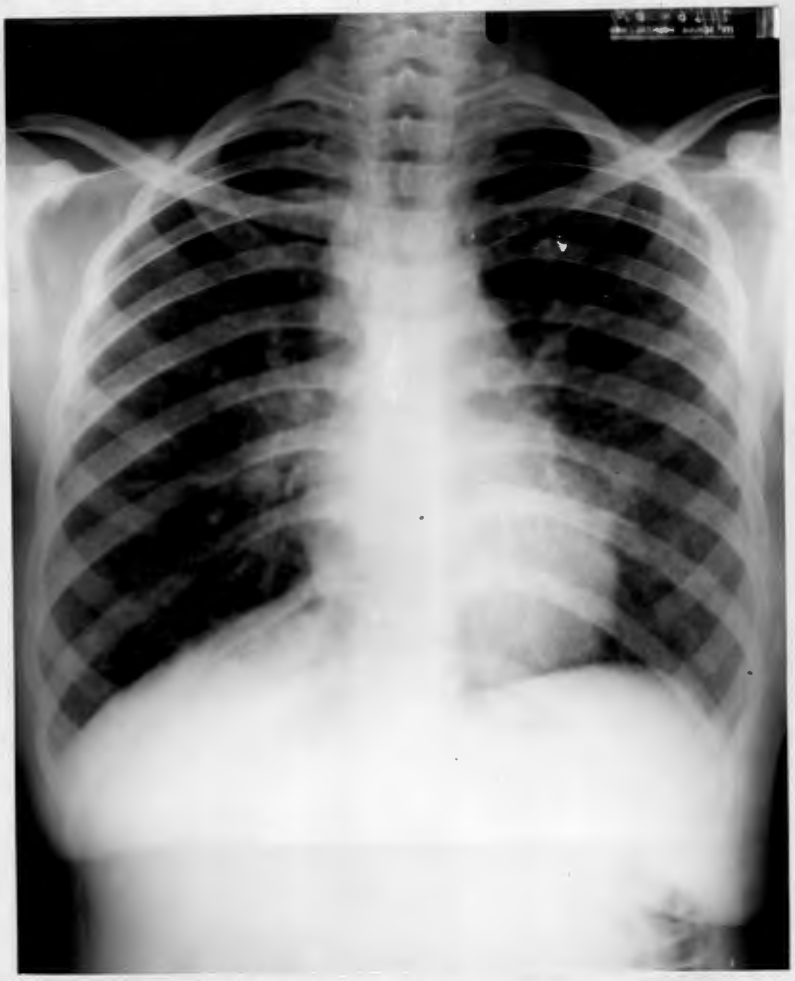

Fig 59. Chest X-Ray of patient A.C.

Intravenous pyelography (Fig.60). Both kidneys showed very poor concentration of dye with barely visible opacification of the kidneys after 30 minutes. No radio-opaque calculi were seen.

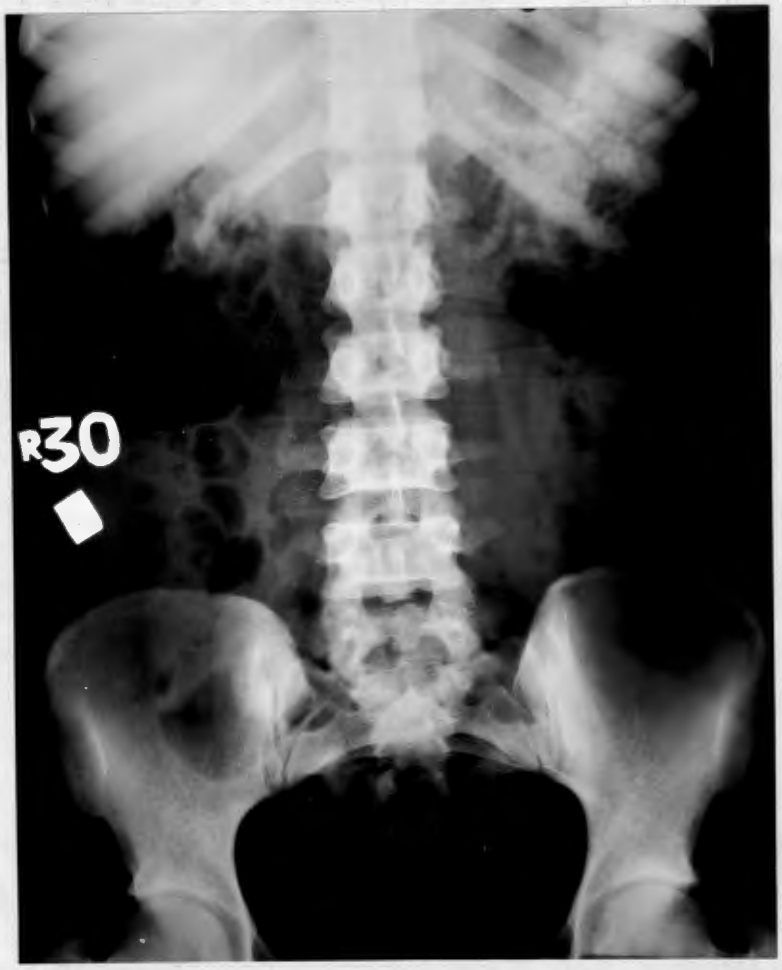

Fig. 60. Intravenous pyelogram of patient A.C. Taken 30 minutes after injection of contrast medium.

Blood urea concentration: This has varied between 60 and 200 mg.%

Urea clearance: 25.9% standard. 30.6% maximum.

Urine concentration test: The patient passed a specimen of urine of specific gravity 1.014 after 15 hours of fluid deprivation. At this time she complained of intense thirst.

Serum Protein.

Total 8.05 grammes %

Albumin 3.6 grammes %

α-1 - globulin 0.25 grammes %

α-2 - globulin 0.60 grammes %

β - globulin 0.60 grammes %

γ - globulin 3.00 grammes %

Serum Calcium Concentration. This has consistently been elevated above 11.5 mg.% on repeated estimations.

Urinary calcium output. 620 mg./24 hours.

Serum inorganic phosphorus concentration. Between 3.5 and 4.5 mg.% on repeated estimations.

Percutaneous Renal biopsy. 24/1/57). Figs. 61 and 62.

The salient histological feature of the tissue obtained on percutaneous biopsy of the kidneys were these :

(i) Dilatation of the capsular spaces surrounding the glomeruli, with normal glomerular tufts.

(ii) Dilatation of the tubules with atrophy.

(iii) Many small irregularly shaped deposits of calcium with surrounding chronic inflammatory reaction.

(iv) Focal areas of non-specific round cell infiltration.

(v) No sarcoid follicles could be found.

Calcium infusion experiment. Calcium was infused over a three hour period and the effects of this infusion on the renal handling of phosphate were studied. In addition the relationship between $\frac{UVca \times 100}{G.F.R.}$ and ^{P}Ca was studied. The results of this experiment are recorded in Tables 51 and 52 and are depicted graphically in Figs. 63 and 64.

This experiment was conducted along lines similar to those used in the normals and is recorded in greater detail in Appendix A (Experiment XXXV).

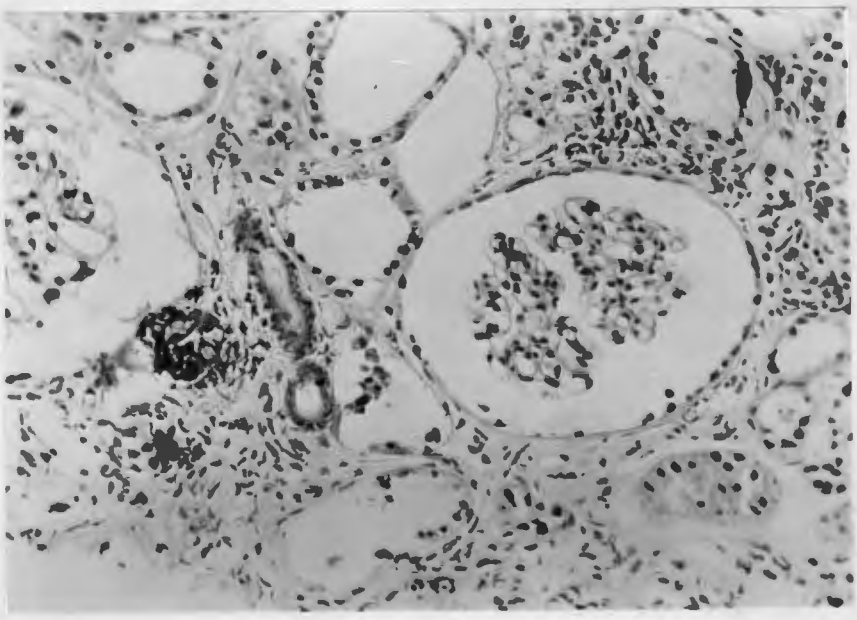

Fig. 61.
Low power view of renal biopsy specimen from patient A.C. The dilated capsular spaces surrounding the two glomerular tufts can be well seen. There is an area of metastatic calcification to the right of centre of the field shown, with widespread tubular dilatation and atrophy. A normal renal tubule can be seen lying between the glomerulus and the calcium deposit.

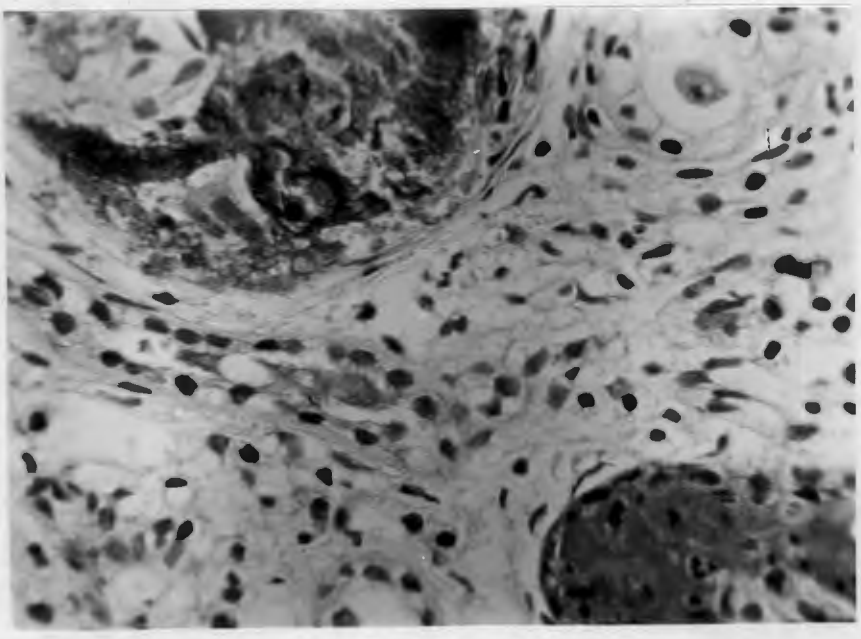

Fig. 62.
High power view of renal biopsy specimen from patient A.C. showing in the top left hand corner a deposit of calcium salts with surrounding fibroblastic proliferation. In the lower right hand corner is a tubule containing a protein cast.
(H. & E. photographed with blue filter).

EXPERIMENT No: XXXV Subject: A.C.

Table No. 51

Period	Time (mins)	Pca (mg.%)	Pp (mg.%)	G.F.R. (ml/min)	Fp (mg/min)	UVp (mg/min)	Tp (mg/min)	Tp x 100 / G.F.R.	UVp x 100 / G.F.R.
1	20.5	12.09	5.20	27.0	1.40	0.66	0.74	2.74	2.46
2	19.7	12.96	5.35	24.4	1.31	0.69	0.62	2.64	2.71
3	20.3	13.52	5.55	23.1	1.28	0.79	0.49	2.12	3.43
4	20.4	13.36	5.22	21.7	1.13	0.68	0.45	2.07	3.15
5	20.0	13.76	5.26	22.2	1.17	0.76	0.41	1.85	3.41
6	20.1	13.92	5.02	23.2	1.16	0.79	0.37	1.60	3.42
7	19.0	14.88	4.88	24.2	1.18	0.81	0.37	1.53	3.35
8	20.2	15.44	5.04	23.9	1.21	0.84	0.37	1.55	3.49
9	19.2	15.92	5.15	19.8	1.02	0.86	0.36	1.82	3.33

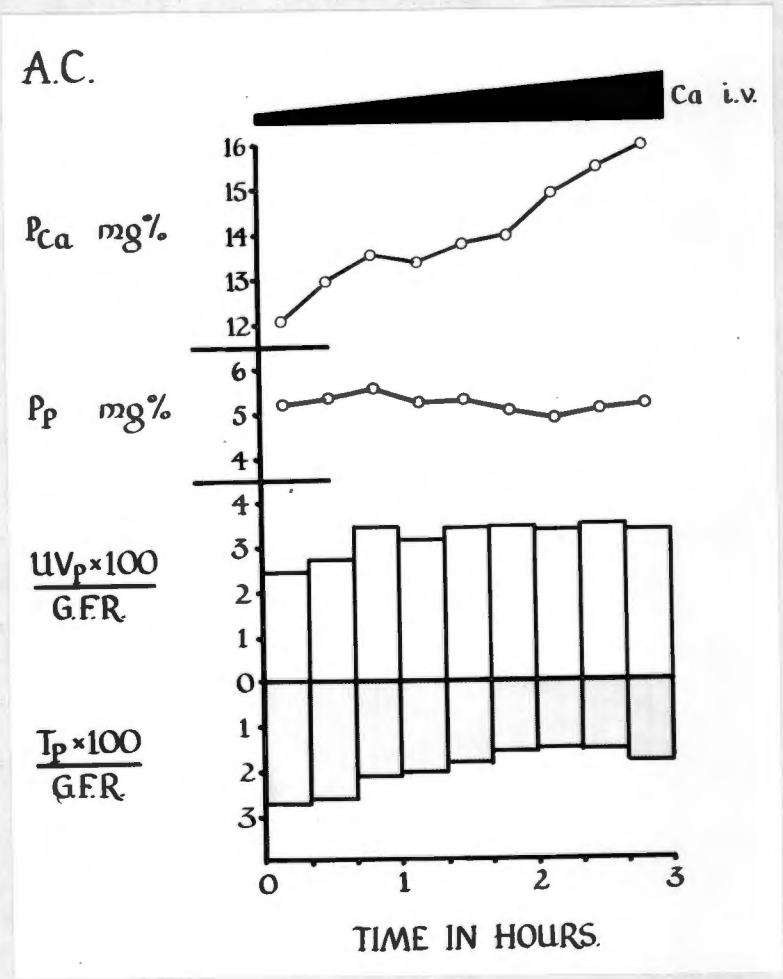

Fig. 63

Showing the effect of intravenous calcium infusion on the renal handling of phosphate in patient A.C. (Experiment XXXV).

The data are presented in Table 51.

EXPERIMENT No: XXXV

Subject: A.C.

Table No: 52

Period	Time (mins.)	Pea (mg.%)	UVea (mg./min.)	G.F.R. (ml./min.)	$\frac{UVea \times 100}{G.F.R.}$
1	20.5	12.09	0.35	27.0	1.30
2	19.7	12.96	0.41	24.4	1.68
3	20.3	13.52	0.47	23.1	2.03
4	20.4	13.36	0.55	21.7	2.53
5	20.0	13.76	0.69	22.2	3.11
6	20.1	13.92	0.83	23.2	3.58
7	19.0	14.88	0.93	24.2	3.84
8	20.2	15.44	0.95	23.9	3.97
9	19.2	15.92	1.04	19.8	5.25

Sxx = 11.9656
Syy = 12.8424
Sxy = 11.7742
a = 0.9840

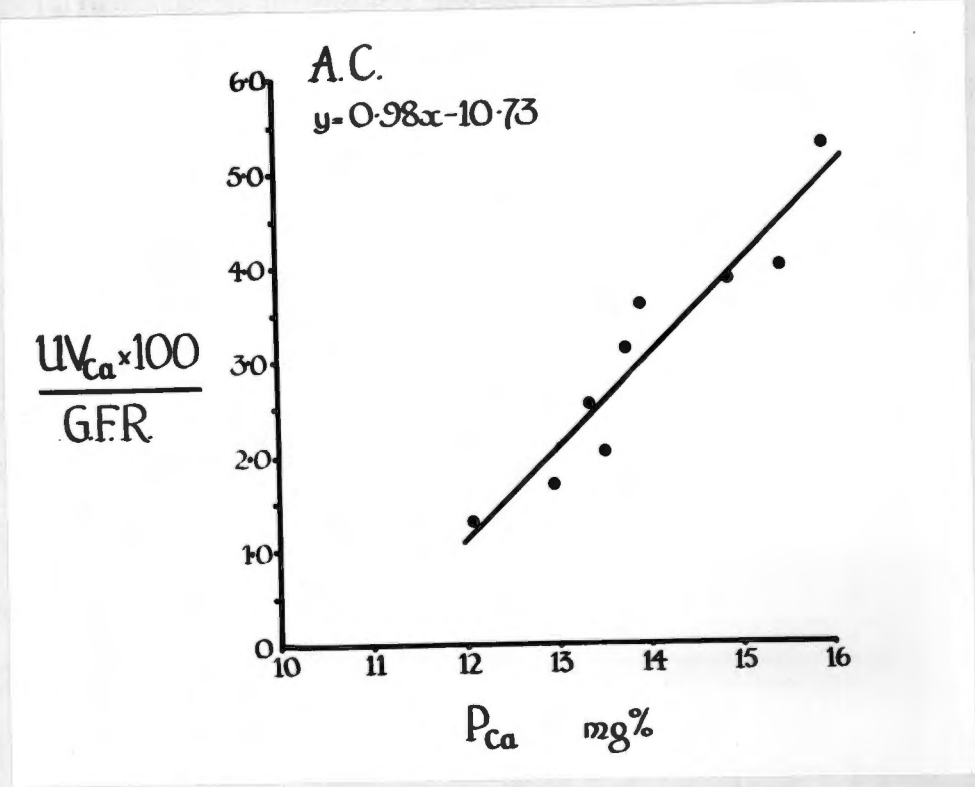

Fig. 64

Showing the regression line for $\frac{UVca \times 100}{G.F.R.}$ upon Pca in patient A.C. (Experiment XXXV) calculated from the data presented in Table 52.

The 95% confidence interval for the regression coefficient a = 0.984 is from 0.903 to 1.065.

Discussion.

In these two cases the diagnosis of Sarcoidosis was proved beyond reasonable doubt in the one and was extremely likely on clinical grounds in the other. I shall confine myself to a discussion of the renal abnormality in these two cases as it is with this aspect that this thesis is principally concerned. While acknowledging the danger of drawing far reaching conclusions from only two cases, I do think the following comments are justified.

1. In neither case did the renal lesion give rise to symptoms that formed a major part of the mode of presentation. This emphasises the necessity for recognising the occurrence of renal complications which may go undiagnosed unless looked for specifically

2. In the case of A.C. the dilated capsular spaces and renal tubules suggests the presence of hydronephrosis which I suggest was due to an intra-renal obstruction of the individual tubules by deposits of calcium and the fibrosis surrounding these deposits. I make this suggestion for two reasons. Firstly there were, in the renal biopsy specimen, relatively normal tubules indicating that the process was not entirely diffuse, and secondly there was no evidence of more distal obstruction by a large renal calculus. Had such a calculus been present I feel certain that it would have been visible radiologically.

3. It would appear from these two cases that signs of glomerular failure such as azotaemia and raised blood pressure are secondary to the obstructive tubular lesions rather than the

result of primary glomerular pathology. In case N.N. where there was evidence of glomerular damage azotaemia was not present, whereas in case A.C. azotaemia was marked despite the presence of normal glomerular tufts.

4. In case N.N. the presence of marked hypercalcuria was not associated with any other evidence of tubular disease. The tubules appeared normal histologically save for the presence of occasional areas of hyaline droplet degeneration, and there was no impairment of the ability of the kidneys to produce a concentrated urine. This, I think, lends support to the contention that the hypercalcuria is the consequence of some qualitative abnormality of plasma calcium rather than the result of decreased tubular reabsorption of filtered calcium.

5. The presence of hypercalcuria is not dependent on the presence of hypercalcaemia. This is well illustrated in the case of N.N. where the 24 hr. urinary calcium output was consistently above 500 mg. despite a serum calcium concentration that remained within a normal range. I suggest that the hypercalcuria in his case was due to the presence in the plasma of an abnormally high concentration of a non-reabsorbable calcium complex.

6. Despite the fact that both cases exhibited hypercalcuria of the same degree, there was marked metastatic calcification in the one case and none in the other. It is not easy from the available information to find an explanation for this discrepancy, and I do not think that any benefit can accrue from speculation. The most obvious difference between the two cases that could be

inculpated in explanation was the presence of hypercalcaemia in the case showing nephrocalcinosis. It is, however, well known that nephrocalcinosis may occur in other disease states without hypercalcaemia.(35,9).

There was nothing in these two cases to throw any light on the pathogenesis of nephrocalcinosis in Sarcoidosis. The source of the ectopic calcium may have been either the blood stream or the tubular reabsorbate. The site at which the calcification can be presumed to have occurred makes me suspect the latter as the probable source, but I accept that this is not a very strong argument. The predeliction for the kidney as the site of ectopic calcification can best be explained by the high concentration of phosphatases in this organ.

7. The results of calcium infusion in these two cases are most interesting. It will be seen in Fig. 56 and Fig. 63, that by comparison with the normals (Figs. 30 to 40) the extent of the rise in serum phosphorus concentration following calcium infusion was small in N.N. and insignificant in A.C. In the absence of any better explanation for this, I interpret it as indicating depleted intracellular phosphate stores in these two patients. Of more importance, however, is the fact that in neither case was there a rise in the calculated rate of tubular reabsorption of phosphorus or in the index $\frac{T_P}{GFR}$ and in the case of A.C. there was actually a significant fall in tubular activity with regard to phosphate. I do not think one can legitimately argue that this failure of the renal threshold to rise following calcium administration can be attributed to "non-responsiveness" of the parathyroids

to artificially induced hypercalcaemia. I put forward the following theory to explain this abnormal response to calcium injection.

(a) The rise in the renal threshold following calcium injection in normals is not mediated through suppression of parathyroid secretion and a consequent effect on tubular reabsorption, but is due to the formation of non-diffusible phosphorus complexes.

(b) In patients with sarcoidosis there is some substance present in the blood stream that prevents the formation of such non-diffusible phosphorus complexes.

In support of this theory I have the conclusions of Henneman et al.[122] that the abnormality of calcium metabolism in sarcoidosis is caused by "endogenous hypersensitivity to Vitamin D", and my own observations on case H.W. (Fig.47) whose renal response to calcium injection was similar to that of these two cases with sarcoidosis. H.W. it will be recalled, was a patient with surgical hypoparathyroidism who was on massive doses of calciferol at the time of the calcium infusion.

Against this theory I quote the observations of Brull[32] who found that colloidal phosphorus formation did not occur until very high levels of plasma calcium concentration had been reached.

The issues involved in this theory are very complex, however, and I have insufficient evidence to assess its validity at the moment.

I hope that a series of experiments to be undertaken in conjunction with Professor Linder and Dr. Jackson will throw some light on the problem posed by this observation.

8. A further point of interest emerges from the analysis of the relationship that existed in these cases between the rate of excretion of calcium in the urine and the plasma concentration. As can be seen from Figs. 57 and 64, the regression coefficient for $\frac{UVca \times 100}{G.F.R.}$ on P_{Ca} was approximately unity in each case whereas that in the normals was not above 0.5. There are several interpretations that can be placed on this observation.

i) The calcium in the plasma of patients with sarcoidosis exists in an entirely filterable form - I do not think this at all likely.

ii) The glomerular membrane in patients with sarcoidosis is abnormally permeable to calcium in the plasma with the result that all of the calcium is effectively diffusible through the glomerular membrane. The absence of obvious histological evidence to indicate such a glomerular lesion and the virtual absence of protein from the urine in these cases makes this unlikely.

iii) The "binding" mechanism for infused calcium is fully saturated in patients with sarcoidosis with the result that the infused calcium is excreted quantitatively in the urine as a foreign inert substance similar to inulin.

iv) There is an excess of some substance in the plasma of patients with sarcoidosis which binds infused calcium in the form of a complex that is diffusible but not reabsorbable. I suspect from the work of Chen[40] that this substance may be similar to citrate, and hope to embark on a series of experiments that will test the hypothesis that such a substance is present in excessive amounts in the plasma of patients with sarcoidosis.

9. Calcium infusion in these two cases caused a sodium lyuresis that was in no way different from that found in normal patients. The figures for sodium excretion appear in Appendix A.

Before concluding this section I should like to record the observations of Hardy[110] who has found hypercalcuria in patients with Berylliosis. In this condition the histological changes are remarkably similar to those found in patients with sarcoidosis and renal calculi are also frequently found. The principal renal lesion found in these cases is a granulomatous infiltration of the parenchyma with non-caseating follicles, and matastatic calcification is rare. I have suggested to Dr. Hardy that calcium infusion experiments similar to those described in this paper be conducted on patients with Berylliosis, and await her findings with interest.

SUMMARY.

Two cases of Boeck's Sarcoidosis are presented to illustrate the renal abnormality that may be encountered in this disease. Both cases showed

i) Hypercalcuria;
ii) an abnormal filure of the renal threshold for phosphate to rise following calcium infusion;
iii) an abnormally high regression coefficient for $\frac{UVca \times 100}{G.F.R.}$ upon Pca.

It is concluded that the three abnormalities listed are an expression of some obscure abnormality in the physico-chemical mechanisms entailed in calcium and phosphorus transport in the plasma.

In the one case the renal lesion took the form of metastatic calcification with intra-renal hydronephrosis. In the other there were sarcoid follicles in the kidney with scattered areas of tubular degeneration and occasional hyalinised glomeruli.

In neither case did the renal lesions give rise to presenting symptoms.

Symptoms of tubular insufficiency were only present in the case with nephrocalcinosis.

Hypercalcaemia is not a necessary concomitant of hypercalcuria in sarcoidosis.

A P P E N D I X A.

Details of Experiments I - XXXV.

EXPERIMENT No: I

Name: T.le R.

Date: 26.10.56 **Height:** 6' 2" **Weight:** 168 lbs.

Urine collected by: Spontaneous voiding.

Comments: Good diuresis maintained by oral hydration - up to 19 ml./min.

	Cumulative time. (mins.)	Plasma		Urine	
		Inulin (mg.%)	Phosphorus (mg.%)	Inulin (mg/min)	Phosphorus (mg/min)
Take UB.					
Take BB.			3.4		
Set up inulin infusion.					
Give inulin primer.	-60.0				
Start phosphate infusion.	-05.0				
U.D.	00.0				
B.1.	7.5	30.6	3.9		
U.1.	20.0			45.50	0.59
B.2.	28.0	31.2	4.6		
U.2.	39.5			44.30	2.14
B.3.	47.0	32.3	5.8		
U.3.	60.5			45.54	3.41
B.4.	68.5	33.0	6.4		
U.4.	80.5			46.08	3.91
B.5.	90.5	32.9	6.9		
U.5.	100.5			46.10	4.72
B.6.	108.5	33.5	7.6		
U.6.	120.5			47.21	5.47
B.7.	128.0	34.5	8.3		
U.7.	140.5			47.90	6.54
B.8.	149.0	33.6	8.9		
U.8.	159.5			47.14	7.93
B.9.	168.0	34.8	9.5		
U.9.	180.0			48.62	8.72

EXPERIMENT No: II

Name: V.M.G. European Male 22 Normal Control

Date: 30.10.56 Height: 5'11½" Weight: 162 lbs.

Urine collected by: Spontaneous voiding.

Comments: Good diuresis maintained. Up to 15 ml./min. Fainted after BB. but recovered and endured remainder of test without incident.

	Cumulative time. (mins.)	Plasma		Urine	
		Inulin (mg.%)	Phosphorus (mg.%)	Inulin (mg/min)	Phosphorus (mg/min)
Take UB.					
Take BB.			4.4		
Set up inulin infusion.					
Give inulin primer.	-60.0				
Start phosphate infusion.	-05.0				
U.B.	00.0				
B.1.	7.00	30.7	5.1		
U.1.	19.00			42.34	1.20
B.2.	27.00	31.0	5.3		
U.2.	39.5			42.25	1.44
B.3.	47.5	30.0	5.9		
U.3.	60.5			39.74	1.55
B.4.	68.0	29.8	6.7		
U.4.	79.5			40.83	2.21
B.5.	87.5	29.7	7.5		
U.5.	101.5			39.82	3.89
B.6.	109.5	29.9	8.3		
U.6.	121.5			40.12	7.42
B.7.	129.5	28.6	9.2		
U.7.	142.0			38.50	5.95
B.8.	149.5	27.2	10.0		
U.8.	163.0			36.64	7.05
B.9.	173.0	27.8	10.6		
U.9.	185.0			37.25	7.33

EXPERIMENT No. III

Name: A.v.d.M. European Male 21 Normal Control

Date: 19/10/56 Height: 5'8" Weight: 135 lbs.

Urine collected by: Spontaneous voiding.

Comments: Good urine output - up to 18 ml./min.

	Cumulative time. (mins.)	Plasma		Urine	
		Inulin (mg.%)	Phosphorus (mg.%)	Inulin (mg/min)	Phosphorus (mg/min)
Take UB.					
Take BB.					
Set up inulin infusion.			3.7		
Give inulin primer.	-60.0				
Start phosphate infusion.	-05.0				
U.D.	00.0				
B.1.	8.0	42.0	4.4		
U.1.	21.5			41.70	0.64
B.2.	29.0	41.8	5.2		
U.2.	41.5			41.12	1.41
B.3.	51.5	40.7	6.1		
U.3.	63.5			40.58	2.11
B.4.	70.5	41.0	6.9		
U.4.	83.0			41.03	2.91
B.5.	90.0	42.3	7.6		
U.5.	101.0			41.75	3.67
B.6.	109.0	41.8	8.4		
U.6.	120.5			40.72	4.43
B.7.	130.5	41.0	9.3		
U.7.	140.5			41.82	5.48
B.8.	Specimen lost				
U.8.	161.5			42.0	6.66
B.9.	171.5	42.0	11.1		
U.9.	185.5			43.30	7.25

EXPERIMENT No. IV

Name: B.R.F. Normal Control

Date: 15.10.56 Height: 5'11" Weight: 156 lbs.

Urine collected by: Spontaneous voiding

Comments: Good diuresis maintained - up to 14 ml./min.

	Cumulative time. (mins.)	Plasma		Urine	
		Inulin (mg.%)	Phosphorus (mg.%)	Inulin (mg/min)	Phosphorus (mg/min)
Take UB.					
Take BB.			4.6		
Set up inulin infusion.					
Give inulin primer.	-60.0				
Start phosphate infusion.	-05.0				
U.D.	00.0				
B.1.	7.5	41.0	5.3		
U.1.	19.5			42.97	0.94
B.2.	27.0	40.7	6.2		
U.2.	39.5			42.78	1.83
B.3.	47.5	45.3	7.2		
U.3.	60.5			47.42	2.85
B.4.	67.5	45.8	8.3		
U.4.	79.0			47.58	4.08
B.5.	87.0	46.0	9.5		
U.5.	101.0			47.93	5.29
B.6.	109.5	45.8	10.3		
U.6.	121.0			48.10	5.73
B.7.	128.0	46.2	11.3		
U.7.	140.5			48.50	6.65
B.8.	149.0	47.0	12.4		
U.8.	161.5			49.50	7.76
B.9.	169.0	47.1	13.8		
U.9.	181.0			48.86	9.14

EXPERIMENT No. V

Name: L.A.E. Normal Control

Date: 15.11.56 Height: 6'1" Weight: 180 lbs.

Urine collected by: Spontaneous voiding.

Comments: Good diuresis maintained - up to 12 ml./min.

	Cumulative time. (mins.)	Plasma		Urine	
		Inulin (mg.%)	Phosphorus (mg.%)	Inulin (mg/min)	Phosphorus (mg/min)
Take UB.					
Take BB.			3.4		
Set up inulin infusion					
Give inulin primer.	-60.0				
Start phosphate infusion.	-05.0				
U.D.	00.0				
B.1.	7.0	38.3	3.7		
U.1.	19.0			40.17	0.84
B.2.	29.0	38.7	4.9		
U.2.	40.0			41.02	1.14
B.3.	47.5	38.1	6.1		
U.3.	62.0			40.10	2.15
B.4.	70.0	38.4	7.2		
U.4.	80.5			40.30	2.75
B.5.	88.0	38.7	7.9		
U.5.	100.0			40.60	3.33
B.6.	108.0	37.3	8.7		
U.6.	122.0			39.35	4.11
B.7.	131.0	38.9	9.3		
U.7.	142.0			41.63	5.44
B.8.	150.0	39.2	9.9		
U.8.	161.0			41.70	5.52
B.9.	178.5	38.6	10.8		
U.9.	180.5			40.60	6.34

EXPERIMENT No: VI

Name: B.A. Non-European Male 42 Normal Control

Date: 6.11.56 Height: 6'1½" Weight: 158 lbs.

Urine collected by: Indwelling catheter. Bladder wash out with distilled water and air.

Comments: Refused oral fluid. Low urine output in neighbourhood of 2 - 4 mls./minute.
Marked renal delay.

	Cumulative time. (mins.)	Plasma		Urine	
		Inulin (mg.%)	Phospherus (mg.%)	Inulin (mg/min)	Phospherus (mg/min)
Take UB.					
Take BB.			4.7 ≡		
Set up inulin infusion.					
Give inulin primer.	-60.0				
Start phosphate infusion.	-05.0				
U.D.	00.0				
B.1.	0.75	32.6	4.3		
U.1.	20.5			38.23	0.45
B.2.	28.3	33.0	4.9		
U.2.	41.5			38.56	0.60
B.3.	49.0	32.9	5.6		
U.3.	60.5			38.82	1.26
B.4.	68.5	33.1	6.4		
U.4.	81.0			38.95	1.45
B.5.	89.0	33.0	7.2		
U.5.	100.0			38.72	2.39
B.6.	107.5	32.9	8.0		
U.6.	121.0			38.12	3.77
B.7.	129.0	32.9	8.9		
U.7.	143.0			38.48	4.91
B.8.	153.0	31.9	9.5		
U.8.	161.0			37.50	4.01
B.9.	168.5	30.7	10.3		
U.9.	180.0			36.12	5.22

≡ This specimen slightly haemolysed.

EXPERIMENT No: VII

Name: L.G. Normal Control

Date: 24.10.56 Height: 5'8" Weight: 153 lbs.

Urine collected by: Spontaneous voiding.

Comments: Good diuresis maintained - up to 21 ml./min.

	Cumulative time. (mins.)	Plasma		Urine	
		Inulin (mg.%)	Phosphorus (mg.%)	Inulin (mg/min)	Phosphorus (mg/min)
Take UB.					
Take BB.		3.8			
Set up inulin infusion.					
Give inulin primer.	-60.0				
Start phosphate infusion.	-05.0				
U.D.	00.0				
B.1.	8.0	32.0	4.9		
U.1.	20.5			50.90	1.21
B.2.	28.0	30.0	5.4		
U.2.	40.5			45.32	1.28
B.3.	47.0	31.0	6.0		
U.3.	58.5			47.49	1.73
B.4.	66.5	29.3	6.7		
U.4.	78.5			45.72	2.29
B.5.	88.5	28.7	7.2		
U.5.	100.5			44.60	3.58
B.6.	107.5	26.4	7.7		
U.6.	120.0			40.22	3.99
B.7.	128.0	25.8	8.0		
U.7.	140.5			40.15	4.51
B.8.	150.0	26.0	8.4		
U.8.	159.5			40.22	5.49
B.9.	169.5	25.9	8.9		
U.9.	181.5			39.70	6.48

EXPERIMENT No: VIII

Name: C.E. European Male 22 Normal Control.

Date: 5.11.56 Height: 6'0½" Weight: 175 lbs.

Urine collected by: Spontaneous voiding.

Comments: Good diuresis maintained with oral hydration - up to 19 ml./min.

	Cumulative time. (mins.)	Plasma		Urine	
		Inulin (mg.%)	Phosphorus (mg.%)	Inulin (mg/min)	Phosphorus (mg/min)
Take UB.					
Take BB.					
Set up inulin infusion.					
Give inulin primer.	-60.0				
Start phosphate infusion.	-05.0				
U.D.	00.0				
B.1.	7.5	38.2	5.6		
U.1.	21.0			47.42	2.00
B.2.	29.0	37.1	5.9		
U.2.	41.0			45.95	2.35
B.3.	48.5	38.3	6.6		
U.3.	60.0			47.82	2.49
B.4.	69.0	39.7	7.4		
U.4.	79.5			49.64	2.75
B.5.	87.0	40.1	8.1		
U.5.	100.0			50.93	3.06
B.6.	108.5	39.8	8.8		
U.6.	119.5			49.20	3.79
B.7.	127.5	40.0	9.5		
U.7.	138.0			49.17	4.55
B.8.	147.0	42.3	10.2		
U.8.	158.0			52.16	5.70
B.9.	164.0	40.0	11.0		
U.9.	178.0			50.81	6.36

EXPERIMENT No: IX

Name: A.O. European Male 23 Normal Control.

Date: 10.11.56 Height: 6'1" Weight: 162 lbs.

Urine collected by: Spontaneous voiding.

Comments: Good diuresis maintained, up to 15 ml./min. with oral hydration.

	Cumulative time. (mins.)	Plasma		Urine	
		Inulin (mg.%)	Phosphorus (mg.%)	Inulin (mg/min)	Phosphorus (mg/min)
Take UB.					
Take BB.			5.0		
Set up inulin infusion.					
Give inulin primer.	-60.0				
Start phosphate infusion.	-05.0				
U.D.	00.0				
B.1.	07.5	37.8	5.5		
U.1.	18.5			34.31	0.25
B.2.	26.0	35.4	6.0		
U.2.	38.0			54.78	1.70
B.3.	46.0	35.8	6.9		
U.3.	58.0			39.61	2.11
B.4.	65.5	36.2	7.8		
U.4.	78.0			47.34	3.56
B.5.	86.0	35.9	8.8		
U.5.	99.0			41.5	4.16
B.6.	106.5	36.3	9.6		
U.6.	119.0			67.24	8.17
B.7.	129.0	36.1	10.4		
U.7.	141.0			36.35	5.09
B.8.	150.0	36.0	11.4		
U.8.	160.0			36.28	6.38
B.9.	167.5	35.2	12.3		
U.9.	180.0			29.82	5.96

EXPERIMENT No. X

Name: G.M. European Male 29 Normal Control.

Date: 8.11.56 Height: 6'0" Weight: 175 lbs.

Urine collected by: Spontaneous voiding.

Comments: Good diuresis maintained with oral hydration (up to 23 ml./min.)

	Cumulative time. (mins.)	Plasma		Urine	
		Inulin (mg.%)	Phosphorus (mg.%)	Inulin (mg/min)	Phosphorus (mg/min)
Take UB.					
Take BB.			3.4		
Set up inulin infusion.					
Give inulin primer.	-60.0				
Start phosphate infusion.	-05.0				
U.D.	00.0				
B.1.	07.0	46.3	4.2		
U.1.	18.5			68.70	0.43
B.2.	26.0.	45.2	5.0		
U.2.	37.5			62.00	1.80
B.3.	45.5	44.9	5.8		
U.3.	58.5			50.53	2.31
B.4.	68.5	45.3	6.7		
U.4.	78.5			63.60	3.66
B.5.	87.5	43.8	7.3		
U.5.	98.5			47.40	3.39
B.6.	105.5	46.0	7.9		
U.6.	117.5			44.70	3.63
B.7.	127.5	44.8	8.5		
U.7.	138.5			62.44	5.91
B.8.	146.5	45.2	9.2		
U.8.	159.0			49.10	5.50
B.9.	166.5	44.3	10.1		
U.9.	178.5			67.46	8.71

EXPERIMENT No. XI

Name: B.J.B. European Male 24 Normal Control.

Date: 13.11.56 Height: 5'10" Weight: 150 lbs.

Urine collected by: Spontaneous voiding.

Comments: Good diuresis maintained with oral hydration - up to 18 ml./min.

	Cumulative time. (mins.)	Plasma		Urine	
		Inulin (mg.%)	Phosphorus (mg.%)	Inulin (mg/min)	Phosphorus (mg/min)
Take UB.					
Take BB.			4.3		
Set up inulin infusion.					
Give inulin primer.	-60.0				
Start phosphate infusion.	-05.0				
U.D.	00.0				
B.1.	8.0	37.9	5.3		
U.1.	21.5			60.53	0.80
B.2.	29.0	38.2	5.9		
U.2.	41.5			38.28	0.87
B.3.	51.5	39.4	6.7		
U.3.	64.5			41.61	1.80
B.4.	73.5	40.0	7.5		
U.4.	85.5			52.89	3.21
B.5.	92.5	40.8	8.1		
U.5.	105.5			40.05	2.85
B.6.	112.0	40.8	8.8		
U.6.	123.5			42.50	3.87
B.7.	133.5	41.2	9.7		
U.7.	145.5			60.90	6.53
B.8.	156.5	41.0	10.6		
U.8.	171.5			34.10	4.60
B.9.	179.5	40.7	11.4		
U.9.	189.5			68.70	10.29

EXPERIMENT No: XII

Name: B.A.B. European Male 21 Normal Control.

Date: 12.11.56 Height: 6'0" Weight: 165 lbs.

Urine collected by: Spontaneous voiding.

Comments: Good diuresis maintained with oral hydration (up to 19.5 ml./min.)

	Cumulative time. (mins.)	Plasma		Urine	
		Inulin (mg.%)	Phosphorus (mg.%)	Inulin (mg/min)	Phosphorus (mg/min)
Take UB.					
Take BB.			3.1		
Set up inulin infusion.					
Give inulin primer.	-60.0				
Start phosphate infusion.	-05.0				
U.D.	00.0				
B.1.	7.50	29.9	4.0		
U.1.	20.0			46.20	0.29
B.2.	28.0	31.2	4.2		
U.2.	40.0			52.50	0.64
B.3.	47.5	31.3	5.0		
U.3.	60.0			44.42	0.88
B.4.	68.5	30.0	5.5		
U.4.	80.0			39.00	1.34
B.5.	87.0	28.9	6.3		
U.5.	101.0			34.47	2.28
B.6.	108.5	30.1	7.0		
U.6.	120.0			36.40	3.05
B.7.	129.0	31.0	7.6		
U.7.	138.0			36.64	3.59
B.8.	148.0	31.4	8.6		
U.8.	161.0			46.65	6.08
B.9.	168.0	32.0	9.4		
U.9.	180.0			46.50	6.80

EXPERIMENT No: XIII

Name: L.McK. White male 28. Normal Control.

Date: 24.10.56 Height: 5'11" Weight: 149 lbs.

Urine collected by: Spontaneous voiding.

Comments: Good diuresis maintained with oral hydration.
Bladder emptying probably incomplete due to haemorrhoidectomy 1 week previously.
Rapid rise in Plasma phosphorus concentration.

	Cumulative time. (mins.)	Plasma		Urine	
		Inulin (mg.%)	Phosphorus (mg.%)	Inulin (mg/min)	Phosphorus (mg/min)
Take UB.					
Take BB.			5.1		
Set up inulin infusion.					
Give inulin primer	-60.0				
Start phosphate infusion.	-05.0				
U.D.	00.0				
B.1.	7.5	42.1	5.4		
U.1.	20.0			52.52	0.21
B.2.	28.0	41.5	6.3		
U.2.	41.5			56.62	0.40
B.3.	49.0	42.5	7.3		
U.3.	60.5			41.55	0.37
B.4.	69.5	42.5	8.5		
U.4.	81.5			42.80	1.63
B.5.	91.5	43.0	9.4		
U.5.	101.0			51.80	2.46
B.6.	108.0	43.1	11.0		
U.6.	121.0			36.60	1.96
B.7.	129.0	42.9	12.8		
U.7.	139.5			42.71	3.57
B.8.	147.0	43.0	14.0		
U.8.	161.5			43.20	4.64
B.9.	169.5	43.8	15.1		
U.9.	181.0			57.25	6.85

EXPERIMENT No. XIV

Name: D.M. Coloured Male 29. Eczema.

Date: 9.1.57 Height: 5' 7½" Weight: 129 lbs.

Calcium (885 mg.) infused at progressively increasing speeds from t = 0 to t = 180.6

Urine collected by: Catheter and bladder washout.

Inulin primer given approx. 1 hour before UD.

Total serum protein: 7.2 grammes %.

	Time (mins)	Plasma Calcium (mg.%)	Plasma Phosphorus (mg.%)	Plasma Inulin (mg.%)	Urine Calcium (mg/min)	Urine Phosphorus (mg/min)	Urine Inulin (mg/min)
BB.		10.20	3.6				
UD.	00.0						
B.1.	13.5	10.48	4.12	32.6			
U.1.	29.0				0.07	0.03	40.6
B.2.	41.5	10.8	4.23	28.6			
U.2.	60.7				0.25	0.14	43.8
B.3.	73.8	11.52	4.28	30.0			
U.3.	92.2				0.54	0.23	39.6
B.4.	104.7	11.92	5.04	27.8			
U.4.	121.5				0.58	0.24	38.4
B.5.	134.8	12.32	5.50	22.7			
U.5.	152.6				0.78	0.38	34.4
B.6.	165.1	13.04	6.24	22.0			
U.6.	180.6					0.59	32.8

EXPERIMENT No. XV

Name: E.P. Coloured Male 65. Osteoporosis.

Date: 2.11.56 Height: 5' 7½" Weight: 149 lbs.

Calcium (1398 mg.) infused at progressively increasing speeds from t = 0 to t = 179.5

Urine collected by: Catheter and bladder washout.

Inulin primer given approx. 1 hour before UD.

Total serum protein: 7.2 grammes %.

	Time (mins)	Plasma Calcium (mg.%)	Plasma Phosphorus (mg.%)	Plasma Inulin (mg.%)	Urine Calcium (mg/min)	Urine Phosphorus (mg/min)	Urine Inulin (mg/min)
BB.		10.2	3.8				
UD.	00.0						
B.1.	08.5	11.20	3.87	44.2			
U.1.	20.1				0.14	0.34	45.7
B.2.	28.6	11.20	4.10	43.6			
U.2.	40.0				0.19	0.33	49.2
B.3.	48.8	11.05	4.08	41.2			
U.3.	60.8				0.36	0.38	41.8
B.4.	69.6	12.00	4.33	39.0			
U.4.	80.6				0.66	0.52	48.4
B.5.	91.9	13.28	4.65	37.0			
U.5.	104.2				0.95	0.60	40.2
B.6.	113.0	14.95	5.07	33.4			
U.6.	125.3				1.28	0.71	37.2
B.7.	133.8	17.60	5.57	30.4			
U.7.	144.8				1.98	1.34	38.2
B.8.	151.3	18.40	5.67	29.8			
U.8.	161.3				2.18	1.49	32.7
B.9.	170.1	17.45	5.90	27.8			
U.9.	179.5				2.26	1.95	35.5

EXPERIMENT No. XVI

Name: S.A. Coloured Male 25 Eczema.

Date: 5.12.56 Height: 5' 8" Weight: 126 lbs.

Calcium (950 mg.) infused at progressively increasing speeds from t = 0 to t = 190.5

Urine collected by: Catheter and bladder washout.

Inulin primer given approx. 1 hour before UD.

Total serum protein: 6.9 grammes %.

	Time (mins)	Plasma			Urine				
		Ca. (mg.%)	P. (mg.%)	In. (mg.%)	Ca. (mg/min)	P. (mg/min)	In. (mg/min)	Na^+ mEq/min.	K^+ mEq/min.
BB.		9.22	3.60						
UD.	00.0								
B.1.	8.5	9.44	3.59	27.0					
U.1.	23.1				0.07	0.12	40.3	.160	.030
B.2.	31.9	9.20	3.80	37.0					
U.2.	45.1				0.09	0.18	46.3	.193	.037
B.3.	53.1	9.84	4.05	35.0					
U.3.	66.1.				0.16	0.25	39.5	.298	.040
B.4.	74.1	9.84	4.15	34.7					
U.4.	86.9				0.29	0.55	41.4	.366	.031
B.5.	95.4	10.88	4.38	34.0					
U.5.	108.6				0.43	0.49	39.8	.422	.027
B.6.	116.6	11.60	4.55	33.4					
U.6.	128.5				0.70	0.72	.502	.025	
B.7.	136.5	12.00	5.02	31.7					
U.7.	150.5				0.71	0.87	36.9	.543	.030
B.8.	158.5	12.80	5.07	32.1					
U.8.	170.0				0.90	1.09	.616	.033	
B.9.	178.8	13.70	5.32	32.1					
U.9.	190.5				0.95	1.10	26.7	.633	.027

EXPERIMENT No. XVII

Name: S.L. Coloured Female 31 Lupus vulgaris

Date: 16.1.57 Height: 5' 5½" Weight: 121 lbs.

Calcium (1100 mg.) infused at progressively increasing speeds from t = 0 to t = 179.0

Urine collected by: Catheter and bladder washout.

Inulin primer given approx. 1 hour before UD.

Total serum protein: 7.9 grammes %.

	Time (mins)	Plasma			Urine				
		Ca. (mg.%)	P. (mg.%)	In. (mg.%)	Ca. (mg/min)	P. (mg/min)	In. (mg/min)	Na^+ mEq/min.	K^+ mEq/min.
BB.		10.50	4.62						
UD.	00.0								
B.1.	12.5	10.72	4.64	37.1					
U.1.	30.5				0.05	0.37	43.0	.163	.055
B.2.	43.0	11.20	4.92	37.2					
U.2.	60.7				0.14	0.30	38.8	.29	.0504
B.3.	73.7	11.76	4.83	35.6					
U.3.	92.2				0.26	0.36	39.6	.38	.060
B.4.	104.7	12.48	5.15	34.0					
U.4.	121.3				0.37	0.35	38.2	.48	.061
B.5.	135.3	13.04	5.26	32.8					
U.5.	154.5				0.28	0.32	35.4	.412	.063
B.6.	164.5	13.52	5.80	37.4					
U.6.	179.0				0.54	0.44	41.6	.593	.078

EXPERIMENT No. XVIII

Name: W.G. Bantu Male 18 Lupus Vulgaris

Date: 10.1.57 Height: 5' 3" Weight: 112 lbs.

Calcium (1018 mg.) infused at progressively increasing speeds from t = 0 to t = 179.4

Urine collected by: Catheter and bladder washout.

Inulin primer given approx. 1 hour before UD.

Total serum protein: 7.4 grammes %.

	Time (mins)	Plasma			Urine				
		Ca. (mg.%)	P. (mg.%)	In. (mg.%)	Ca. (mg/min)	P. (mg/min)	In. (mg/min)	Na^+ mEq/min.	K^+ mEq/min.
BB.		10.62	4.81						
UD.	00.0								
B.1.	12.2	10.88	4.85	35.9					
U.1.	28.5				-	0.21	42.2	.1302	.128
B.2.	42.0	11.04	5.22	34.8					
U.2.	59.5				0.001	0.28	37.3	.205	.099
B.3.	73.5	11.92	5.40	33.0					
U.3.	89.5				0.270	0.40	36.2	.345	.079
B.4.	102.0	12.64	5.64	29.4					
U.4.	120.2				0.380	0.56	85.2	.366	.0885
B.5.	132.2	13.76	6.13	27.0					
U.5.	152.2				1.200	0.69	34.2	.512	.096
B.6.	162.7	14.48	6.15	27.8					
U.6.	179.4				0.900	0.77	28.4	.784	.119

EXPERIMENT No. XIX

Name: F.S. Bantu male 35 Convalescent empyema.

Date: 8.1.57 Height: 5' 10" Weight: 174 lbs.

Calcium (1118 mg.) infused at progressively increasing speeds from t = 0 to t = 178.5

Urine collected by: Catheter and bladder washout.

Inulin primer given approx. 1 hour before UD.

Total serum protein: 7.8 grammes %.

	Time (mins)	Plasma Calcium (mg.%)	Plasma Phosphorus (mg.%)	Plasma Inulin (mg.%)	Urine Calcium (mg/min)	Urine Phosphorus (mg/min)	Urine Inulin (mg/min)
BB.		10.02	3.85				
UD.	00.0						
B.1.	13.0	10.56	3.76	40.6			
U.1.	30.5				0.06	0.39	31.1
B.2.	44.5	10.88	3.86	35.0			
U.2.	60.5				0.07	0.52	31.7
B.3.	73.5	11.52	4.18	34.3			
U.3.	91.1				0.18	0.72	32.6
B.4.	104.1	12.00	4.46	33.7			
U.4.	121.6				0.46	0.87	30.9
B.5.	134.1	12.64	4.87	29.4			
U.5.	154.5				0.87	1.23	28.0
B.6.	167.5	13.36	4.98	25.3			
U.6.	178.5				1.34	1.59	27.3

EXPERIMENT No. XX

Name: H.A. Coloured Male 32. Hysterical paraplegia.

Date: 3.10.55 Height: 5' 10". Weight: 132 lbs.

Calcium (1350 mg.) infused at a constant rate between t = 41.5 and t = 106.5

Urine collected by: Catheter and bladder washout.

Inulin primer given approx. ½-hour before UD.

	Time (mins)	Plasma Calcium (mg.%)	Plasma Phosphorus (mg.%)	Plasma Inulin (mg.%)	Urine Calcium (mg/min)	Urine Phosphorus (mg/min)	Urine Inulin (mg/min)
BB.		10.0	3.63				
UD.	00.0						
B.1.	9.5	10.1	3.60	17.0			
U.1.	20.5				0.35	0.38	17.76
B.2.	30.5	10.1	3.65	15.0			
U.2.	45.5				0.29	0.46	19.28
B.3.	55.5	15.8	3.87	32.5			
U.3.	66.0				0.98	0.08	10.73
B.4.	77.0	17.1	4.59	21.9			
U.4.	87.5				0.69	0.98	28.84
B.5.	98.5	20.1	5.06	19.6			
U.5.	109.5				1.15	1.02	18.05
B.6.	119.5	19.7	5.65	20.0			
U.6.	137.5				1.84	2.00	19.86
B.7.	148.5	18.6	5.95	19.8			
U.7.	162.5				1.41	1.93	16.72
B.8.	178.5	17.6	5.01	18.1			
U.8.	194.5				1.23	1.41	16.84
B.9.	225.5	16.6	4.74	17.7			
U.9.	255.5				0.82	1.12	18.52
B.10.	284.5	16.0	4.66	18.4			
U.10.	314.5				0.49	0.68	17.63
B.11.	375.5	16.2	4.72	18.5			
U.11.	408.5				0.53	0.55	14.04

EXPERIMENT No. XXI

Name: A.A. Coloured Male 30. Psychoneurosis.

Date: 11.10.55 Height: 5' 10" Weight: 130 lbs.

Calcium (1350 mg.) infused at a constant rate between t = 47.0 and t = 110.0

Urine collected by: Catheter and bladder washout.

Inulin primer given approx. ½-hour before UD.

	Time (mins)	Plasma Calcium (mg.%)	Plasma Phosphorus (mg.%)	Plasma Inulin (mg.%)	Urine Calcium (mg/min)	Urine Phosphorus (mg/min)	Urine Inulin (mg/min)
BB.		10.20	2.7				
UD.	00.0						
B.1.	10.0	10.2	2.5	19.2			
U.1.	23.0				0.01	0.19	21.96
B.2.	35.0	10.7	2.6	17.9			
U.2.	47.0				0.01	0.17	20.13
B.3.	57.5	12.3	2.7	16.0			
U.3.	67.5				0.19	0.30	19.41
B.4.	78.0	13.3	3.1	17.0			
U.4.	89.0				0.05	0.48	17.21
B.5.	99.0	16.8	3.8	17.0			
U.5.	110.0				1.96	0.88	13.24
B.6.	120.0	18.7	4.5	18.1			
U.6.	131.0				1.27	1.01	14.67
B.7.	142.0	16.9	4.9	17.0			
U.7.	152.0				0.52	0.98	14.33
B.8.	181.5	17.5	4.9	19.0			
U.8.	212.0				0.62	0.77	15.03
B.9.	242.0	16.8	5.2	21.1			
U.9.	272.0				0.49	0.50	16.47
B.10.	302.0	15.2	5.3	19.5			
U.10.	332.5				0.32	0.36	15.57
B.11.	392.0	14.4	5.5	19.3			
U.11.	452.0				0.22	0.26	19.17

EXPERIMENT No. XXII

Name: S.M. Bantu Male 30. Convalescent gastrectomy for peptic ulcer.

Date: 20.10.55 Height: 5' 11" Weight: 160 lbs.

Calcium (1350 mg.) infused at a constant rate between t = 48.0 and t = 110.5

Urine collected by: Catheter and bladder washout.

Inulin primer given approx. 20 mins. before UD.

	Time (mins)	Ca. (mg.%)	P. (mg.%)	In. (mg.%)	Ca. (mg/min)	P. (mg/min)	In. (mg/min)	Na^+ mEq/min.
BB.		10.6	3.4					
UD.	00.0							
B.1.	11.5	10.6	3.4	14.7				
U.1.	21.0				0.30	0.38	16.14	0.19
B.2.	31.0	10.5	3.5	16.3				
U.2.	48.0				0.20	0.44	18.04	0.20
B.3.	58.5	14.0	3.7	17.3				
U.3.	68.0				0.71	0.93	26.15	0.97
B.4.	78.0	15.9	4.2	19.9				
U.4.	88.5				1.01	1.19	29.32	1.40
B.5.	98.5	16.7	4.6	19.9				
U.5.	110.5				1.21	1.40	25.82	1.60
B.6.	120.5	18.4	5.0	19.2				
U.6.	131.5				1.01	1.38	24.62	1.40
B.7.	141.5	18.4	5.2	21.1				
U.7.	151.5				1.14	1.54	21.10	1.60
B.8.	167.0	16.7	5.2	21.9				
U.8.	181.5				1.05	1.37	24.33	1.20
B.9.	197.5	17.3	5.3	22.4				
U.9.	212.0				0.82	1.12	24.39	0.91
B.10.	227.5	15.8	5.4	22.8				
U.10.	242.0				0.63	0.92	29.20	0.70
B.11.	302.5	15.8	5.3	22.4				
U.11.	362.0				0.35	0.43	24.08	0.26

EXPERIMENT No. XXIII

Name: O.L. Coloured Male 25 Normal

Date: 15.11.55 Height: 5' 8" Weight: 145 lbs.

Calcium (1350 mg.) infused at constant rate between t = 44.0 and t = 103.0

Urine collected by: Catheter and bladder washout.

Inulin primer given approx. ½-hour before UD.

	Time (mins)	Plasma			Urine			
		Ca. (mg.%)	P. (mg.%)	In. (mg.%)	Ca. (mg/min)	P. (mg/min)	In. (mg/min)	Na^+ mEq/min.
BB.		10.6	3.6					
UD.	00.0							
B.1.	10.0	10.6	3.6	12.1				
U.1.	20.5				0.04	0.10	13.9	0.07
B.2.	30.5	10.6	3.7	13.6				
U.2.	42.5				0.04	0.12	12.1	0.06
B.3.	54.5	13.9	4.0	12.1				
U.3.	62.5				0.12	0.19	14.0	0.10
B.4.	75.0	15.8	5.2	13.6				
U.4.	83.0				0.51	0.61	14.3	0.26
B.5.	94.0	17.1	5.9	12.8				
U.5.	103.0				0.75	0.76	10.6	0.33
B.6.	114.0	20.0	6.9	15.9				
U.6.	125.0				0.64	1.10	15.2	0.51
B.7.	140.0	18.4	7.3	15.9				
U.7.	157.5				1.85	1.66	13.2	1.44
B.8.	172.5	17.8	7.1	15.9				
U.8.	188.5				1.28	1.26	13.1	1.05
B.9.	203.5	18.2	6.6	18.1				
U.9.	220.0				0.62	0.71	14.4	0.49
B.10.	250.0	16.8	6.6	16.6				
U.10.	280.0				0.30	0.45	14.1	0.15
B.11.	320.0	-	6.9	15.9				
U.11.	340.0				0.15	0.34	13.0	0.04
B.12.	370.0	15.2	7.0	18.1				
U.12.	400.0				0.26	0.35	14.0	0.07

EXPERIMENT No. XXIV

Name: L.B. Coloured Male 32. Obsessional neurosis.

Date: 4.11.55 Height: 5' 10" Weight: 150 lbs.

Calcium (1350 mg.) infused at constant rate between t = 62.5 and t = 126.0

Urine collected by: Catheter and bladder washout.

Inulin primer given approx. ½-hour before UD.

	Time (mins)	Plasma			Urine			
		Ca. mg.%	P. (mg.%)	In. (mg.%)	Ca. (mg/min)	P. (mg/min)	In. (mg/min)	Na^+ mEq/min.
BB.		10.4	3.0					
UD.	00.0							
B.1.	9.5	10.6	3.1	19.6				
U.1.	20.5				0.11	0.11	23.6	0.25
B.2.	31.0	10.6	3.2	19.6				
U.2.	41.5				0.12	0.13	23.6	0.25
B.3.	51.5	10.5	3.3	17.8				
U.3.	62.5				0.12	0.21	24.0	0.27
B.4.	72.5	13.6	3.5	17.8				
U.4.	84.5				0.75	0.52	24.9	0.59
B.5.	94.5	17.3	4.8	19.6				
U.5.	104.5				1.58	1.07	28.3	0.85
B.6.	115.0	20.1	5.6	20.4				
U.6.	126.0				1.26	1.01	18.8	0.60
B.7.	135.0	19.7	7.0	22.2				
U.7.	147.0				0.67	1.56	23.7	0.03
B.8.	158.0	17.8	6.8	21.3				
U.8.	167.5				1.34	2.45	29.3	0.84
B.9.	182.5	16.9	6.6	26.7				
U.9.	198.0				1.58	2.18	38.7	1.27
B.10.	228.0	15.9	5.9	24.9				
U.10.	258.5				1.08	1.60	34.2	0.73
B.11.	289.5	15.0	5.9	24.9				
U.11.	309.0				0.62	1.32	31.7	0.28

EXPERIMENT No: XXV

Subject: U.G. **Coloured Female 18** **Normal Control.**

Date: 27.8.56. **Height: 4' 3"** **Weight: 83 lbs.**

Urine collected by: Catheter and bladder washout.

Parathormone 400 units administered intravenously at t = 61.5

Event	Time (mins)	Serum		Urine	
		P. (mg.%)	Inulin (mg.%)	P. (mg/min)	Inulin (mg/min)
Inulin primer	-56.0				
UD.	00.0				
B.1.	7.5	3.90	32.0		
U.1.	21.0			0.20	16.1
B.2.	29.0	3.71	32.4		
U.2.	41.0			0.21	17.4
B.3.	49.0	3.83	33.0		
U.3.	61.5			0.24	18.0
B.4.	69.5	3.59	32.8		
U.4.	81.5			0.45	16.3
B.5.	90.0	3.76	35.5		
U.5.	101.5			0.69	16.7
B.6.	109.0	3.76	31.4		
U.6.	122.0			0.64	16.6
B.7.	129.5	3.76	-		
U.7.	141.0			0.62	21.1
B.8.	149.0.	3.76	42.4		
U.8.	163.0			0.56	23.7
B.9.	173.0	3.76	41.9		
U.9.	182.0			0.58	25.5
B.10.	191.0	3.76	30.9		
U.10.	202.0			0.41	21.4
B.11.	209.0	3.76	41.0		
U.11.	222.0			0.39	21.6
B.12.	229.0	3.76	40.0		
U.12.	242.0			0.40	23.6

EXPERIMENT No: XXVI

Subject: A.F. Malay Male 22 Normal Control.

Date: 13.8.56. Height: 5' 0" Weight: 120 lbs.

Urine collected by: Catheter and bladder washout.

Parathormone 400 units administered intravenously at t = 72.

Event	Time (mins)	Serum			Urine		
		Ca. (mg.%)	P. (mg.%)	Inulin (mg.%)	Ca. (mg/min)	P. (mg/min)	Inulin (mg/min)
Inulin primer	-60.0						
UD.	00.0						
B.1.	7.5	8.64	3.26	43.0			
U.1.	49.0				0.06	0.22	49.6
B.2.	56.5	8.80	3.01	39.5			
U.2.	69.0				0.08	0.15	41.0
B.3.	76.5	9.45	3.14	41.5			
U.3.	90.0				0.13	0.35	44.8
B.4.	104.0	9.45	3.35	45.6			
U.4.	110.0				0.09	0.79	47.1
B.5.	117.5	9.60	3.38	43.8			
U.5.	132.0				0.07	0.70	36.8
B.6.	139.5	9.29	3.19	39.0			
U.6.	152.0				0.04	0.60	31.0
B.7.	159.5	9.76	3.76	33.0			
U.7.	172.0				0.02	0.64	39.6
B.8.	182.0	-	-	-			
U.8.	192.0				0.03	0.61	27.0
B.9.	200.0	-	-	30.0			
U.9.	212.0				0.03	0.83	37.2
B.10.	220.5	9.60	3.87	40.6			
U.10.	232.0				0.03	0.88	45.6
B.11.	239.5	9.60	3.82	40.6			
U.11.	252.0				0.06	0.91	47.6

EXPERIMENT No: XXVII

Subject: T.M. **Bantu Male 38.** **Sciatic Syndrome.**

Date: 27.4.56. **Height:** 5' 9" **Weight:** 143 lbs.

Urine collected by: Catheter and bladder washout.

Parathormone 400 units administered intravenously at t = 59.

Blood sampling at 5 min.intervals from indwelling intra-arterial needle, and specimens for each period pooled.

Sterile buffered phosphate solution infused at constant rate from t = -32.0 to t = 204.0

Event	Time (mins)	Serum			Urine		
		Ca. (mg.%)	P. (mg.%)	Inulin (mg.%)	Ca. (mg/min)	P. (mg/min)	Inulin (mg/min)
Primer	-32.0						
UD.	00.0						
B.1.		10.1	15.56	56.5			
U.1.	20.0				0.07	14.7	66.3
B.2.		8.9	14.56	52.6			
U.2.	40.0				0.07	13.8	60.4
B.3.		9.1	13.33	48.4			
U.3.	59.0				0.12	12.8	55.2
B.4.		9.1	12.48	46.7			
U.4.	80.0				0.24	18.6	89.1
B.5.		9.1	12.15	45.2			
U.5.	100.0				0.13	13.0	60.0
B.6.		9.3	11.36	41.3			
U.6.	127.0				0.09	10.9	45.6
B.7.		9.6	11.32	41.3			
U.7.	145.0				0.12	9.3	43.2
B.8.		9.6	10.64	40.6			
U.8.	166.0				0.06	10.4	47.4
B.9.		9.8	10.31	39.8			
U.9.	184.5				0.05	10.2	48.3
B.10.		10.4	10.03	36.9			
U.10.	204.0				0.05	9.7	44.9

EXPERIMENT No: XXVIII

Subject: J.J. Coloured Male 35 Normal control.

Date: 8. 6. 56. Height: 5'7" Weight: 157 lbs.

Urine collected by: Catheter and bladder washout.

Parathormone 400 units administered intravenously at t = 60.

	Time (mins)	Serum Ca. (mg.%)	Serum P. (mg.%)	Serum Inulin (mg.%)	Urine Ca. (mg/min)	Urine P. (mg/min)	Urine Inulin (mg/min)
Inulin primer	-60.0						
UD.	00.0						
B.1.	7.5	11.2	3.45	21.5			
U.1.	19.5				0.05	0.29	35.4
B.2.	27.5	11.2	3.50	24.1			
U.2.	40.0				0.05	0.31	37.5
B.3.	47.5	11.1	3.70	22.6			
U.3.	60.0				0.04	0.29	30.3
B.4.	67.5	11.4	3.56	23.7			
U.4.	80.0				0.05	0.51	36.6
B.5.	87.5	11.0	3.51	23.9			
U.5.	100.0				0.07	0.84	35.8
B.6.	107.5	11.0	3.66	22.4			
U.6.	120.0				0.06	0.92	36.8
B.7.	128.5	11.0	3.74	22.5			
U.7.	140.0				0.04	0.83	35.3
B.8.	147.5	11.2	3.55	22.1			
U.8.	160.0				0.01	0.77	35.3
B.9.	167.5	10.9	3.60	19.8			
U.9.	181.0				0.01	0.72	37.2
B.10.	188.5	10.6	3.63	21.1			
U.10.	202.0				0.04	0.70	35.7
B.11.	213.0	10.2	3.68	19.6			
U.11.	227.0				0.01	0.85	33.8
B.12.	234.5	10.2	3.90	19.8			
U.12.	247.0				0.04	0.71	33.6

EXPERIMENT No: XXIX

Subject: J.J. Coloured Male 35 Normal control.

Date: 11.6.56. Height: 5'7" Weight: 157 lbs.

Urine collected by: Catheter and bladder washout.

Parathormone 400 units administered intravenously at t = 60.

Sterile buffered phosphate solution infused at constant rate from t = -60.0 to t = 244.5

Event	Time (mins)	Serum Ca. (mg.%)	Serum P. (mg.%)	Serum Inulin (mg.%)	Urine Ca. (mg/min)	Urine P. (mg/min)	Urine Inulin (mg/min)
Primer	-60.0						
UD.	00.0						
B.1.	7.5	9.6	6.84	21.7			
U.1.	20.0				0.03	3.66	32.5
B.2.	27.5	9.6	7.38	25.0			
U.2.	40.0	-	-	-	0.02	4.06	37.9
B.3.	47.5	10.1	7.48	25.2			
U.3.	60.0				0.03	3.63	35.1
B.4.	67.5	9.6	6.98	26.3			
U.4.	80.0				0.14	5.23	42.5
B.5.	88.0	9.6	6.90	26.0			
U.5.	100.5				0.06	5.98	48.3
B.6.	108.0	9.6	6.96	25.8			
U.6.	120.5				0.05	4.89	41.2
B.7.	130.0	9.3	7.03	25.6			
U.7.	144.5				0.03	4.17	31.6
B.8.	152.0	10.1	6.96	23.5			
U.8.	164.5				0.03	3.87	30.7
B.9.	172.0	10.6	6.90	22.9			
U.9.	184.5				0.04	3.07	32.7
B.10.	192.0	10.7	7.45	26.9			
U.10.	204.5				0.02	3.70	30.2
B.11.	212.0	10.4	7.30	24.3			
U.11.	224.5				0.02	3.78	30.3
B.12.	232.0	9.8	7.14	25.0			
U.12.	244.5				0.01	4.28	32.0

EXPERIMENT No: XXX

Subject: H.R. European Male 53. Acromegaly.

Date: 27.6.56. Height: 5' 9" Weight: 197 lbs.

Urine collected by: Catheter and bladder washout.

Parathormone 400 units administered intravenously at t = 60.

Event	Time (mins)	Serum			Urine		
		Ca. (mg.%)	P. (mg.%)	Inulin (mg.%)	Ca. (mg/min)	P. (mg/min)	Inulin (mg/min)
Inulin primer							
UD.	00.0						
B.1.	7.5	8.72	4.50	27.2			
U.1.	20.0				0.13	1.11	37.3
B.2.	27.5	9.60	5.13	27.4			
U.2.	40.0				0.10	0.74	32.5
B.3.	47.5	8.80	5.03	28.2			
U.3.	60.0				0.11	0.80	32.6
B.4.	67.5	8.64	4.96	28.2			
U.4.	80.0				0.12	1.18	43.8
B.5.	87.5	8.80	5.27	27.3			
U.5.	100.0				0.11	1.51	51.5
B.6.	108.0	9.12	4.39	27.9			
U.6.	120.0				0.12	1.26	47.4
B.7.	127.5	9.12	4.78	28.4			
U.7.	140.0				0.08	1.39	52.0
B.8.	147.5	8.80	5.06	28.3			
U.8.	160.0				0.07	1.03	35.4
B.9.	167.5	8.80	4.78	28.1			
U.9.	180.0				0.06	0.79	27.0
B.10.	187.5	8.96	4.98	29.4			
U.10.	200.0				0.09	0.87	37.5
B.11.	207.5	8.80	4.85	30.1			
U.11.	220.5				0.08	0.91	43.4
B.12.	228.0	8.96	5.42	31.0			
U.12	240.0				0.08	0.79	39.0

EXPERIMENT No: XXXI

Subject: H.R. European Male 53. Acromegaly.

Date: 2.7.56. Height: 5' 9" Weight: 197 lbs.

Urine collected by: Catheter and bladder washout.

Parathormone 400 units administered intravenously at t = 60.

Sterile buffered phosphate solution infused at constant rate from t = -60 to t = 240.0

Event	Time (mins)	Ca. (mg.%)	P. (mg.%)	Inulin (mg.%)	Ca. (mg/min)	P. (mg/min)	Inulin (mg/min)
Primer	-60.0						
UD.	00.0						
B.1.	7.5	9.12	7.28	26.7			
U.1.	20.0				0.07	3.45	36.2
B.2.	27.5	8.16	7.45	27.2			
U.2.	40.0				0.05	3.13	34.5
B.3.	47.5	9.28	7.54	27.7			
U.3.	60.0				0.05	3.46	37.2
B.4.	67.5	9.12	7.34	27.1			
U.4.	80.0				0.07	4.42	42.8
B.5.	87.5	9.28	7.40	27.4			
U.5.	100.0				0.03	4.28	38.7
B.6.	107.5	8.48	7.29	28.1			
U.6.	120.0				0.03	3.23	29.5
B.7.	127.5	9.12	7.39	27.9			
U.7.	140.0				0.02	2.51	23.1
B.8.	147.5	9.12	7.30	27.9			
U.8.	160.0				-	6.38	52.4
B.9.	167.5	9.28	7.46	28.8			
U.9.	180.0				0.02	3.54	32.0
B.10.	187.5	9.12	7.40	28.1			
U.10.	200.0				0.04	4.35	39.9
B.11.	208.0	9.43	7.65	28.0			
U.11.	220.5				0.04	4.19	37.5
B.12.	228.0	8.96	7.55	26.1			
U.12.	240.0				0.03	3.92	35.4

EXPERIMENT No: XXXII

Subject: E.B. **Coloured Female 38** **Hypoparathyroidism.**

Date: 17.1.56 **Height:** 5' 4" **Weight:** 148 lbs.

Urine collected by: Catheter and bladder washout.

Calcium (1350 mg.) infused at constant rate from t = 43.5 to t = 90.0

Patient had been off all therapy for 4 weeks prior to the performance of the experiment.

Chvostek's and Trousseau's signs positive.

Inulin primer given 30 mins. before UD.

	Time (mins)	Serum			Urine		
		Ca (mg.%)	P (mg.%)	Inulin (mg.%)	Ca (mg./min.)	P (mg./min.)	Inulin (mg./min.)
UD.	00.0						
B.1.	13.5	7.2	5.4	10.8			
U.1.	23.5				0.72	23.3	0.17
B.2.	33.5	6.9	5.3	11.4			
U.2.	43.5				0.68	22.3	0.33
B.3.	54.5	8.6	5.4	11.3			
U.3.	63.5				0.86	25.9	-
B.4.	74.0	9.6	5.9	10.7			
U.4.	90.0				1.02	24.6	0.46
B.5.	10.15	15.7	7.1	10.9			
U.5.	113.0				1.92	21.5	1.27
B.6.	124.0	15.5	7.9	12.5			
U.6.	136.0				2.54	26.9	1.40
B.7.	146.0	18.4	8.7	13.9			
U.7.	166.5				2.85	29.3	1.01
B.8.	181.0	15.2	8.1	14.3			
U.8.	197.5				2.16	29.0	0.64
B.9.	228.0	13.4	7.9	14.2			
U.9.	257.5				1.43	25.3	0.23
B.10.	289.0	13.4	7.6	13.6			
U.10.	320.0				1.25	28.5	0.12

EXPERIMENT No: XXXIII

Subject: H.W. **European Female** **Hypoparathyroidism Malignant Exophthalmos**

Date: 22.1.57 **Height:** 5' 6" **Weight:** 145 lbs.

Urine collected by: Spontaneous voiding.

Calcium (1320 mg.) infused at progressively increasing speeds from t = 00.0 to t = 180.5

Patient on Calciferol and prednisone at time of experiment.

Inulin primer given approx. 1 hour prior to UD.

	Time (mins)	Serum			Urine		
		Ca (mg.%)	P (mg.%)	Inulin (mg.%)	Ca (mg./min.)	P (mg./min.)	Inulin (mg./min.)
UD.	00.0						
B.1.	10.0	9.52	3.73	34.8			
U.1.	21.5				0.08	0.11	6.32
B.2.	31.0	10.00	3.76	33.8			
U.2.	41.7				0.82	1.40	74.6
B.3.	50.7	9.76	3.50	39.0			
U.3.	62.5				0.81	1.17	55.7
B.4.	70.5	10.00	3.32	39.7			
U.4.	84.3				0.91	0.90	45.4
B.5.	92.8	10.00	3.58	33.5			
U.5.	103.6				1.43	1.12	45.7
B.6.	111.4	10.80	3.90	35.0			
U.6.	122.8				2.54	0.91	30.2
B.7.	130.8	11.04	4.33	30.1			
U.7.	142.8				2.95	1.65	46.0
B.8.	150.8	11.68	4.53	30.5			
U.8.	161.3				2.96	1.67	41.8
B.9.	168.8	12.24	4.57	31.9			
U.9.	180.5				3.17	1.86	37.5

EXPERIMENT No: XXXIV

Subject: N.N. **Bantu Male 31** **Sarcoidosis.**

Date: 23.1.57 **Height:** 5' 9" **Weight:** 168 lbs.

Urine collected by: Spontaneous voiding.

Calcium (2,000 mg.) administered intravenously at progressively increasing speeds from t = 0 to t = 180.6 mins.

Inulin primer given approx. 60 mins. before UD.

	Time	Serum			Urine				
	(mins)	Ca (mg.%)	P (mg.%)	Inulin (mg.%)	Ca (mg/min)	P (mg/min)	Inulin (mg/min)	Na^+ mEq/min.	K^+ mEq/min
UD.	00.0								
B.1.	7.5	10.08	3.13	40.0					
U.1.	20.5				0.68	0.32	37.1	0.280	0.066
B.2.	28.0	10.64	3.13	42.5					
U.2.	43.5				0.67	0.45	35.1	0.275	0.064
B.3.	51.0	10.80	3.17	43.4					
U.3.	62.5				1.14	0.47	36.6	0.594	0.127
B.4.	71.0	11.28	3.52	38.2					
U.4.	83.1				1.48	0.56	34.9	0.432	0.076
B.5.	91.1	11.52	3.55	37.7					
U.5.	103.6				2.03	0.86	33.6	0.700	0.095
B.6.	110.6	12.08	3.74	38.5					
U.6.	122.1				2.36	1.25	36.4	0.740	0.098
B.7.	130.1	12.48	3.87	39.1					
U.7.	142.8				2.90	1.45	38.1	0.886	0.107
B.8.	149.8	12.72	4.26	37.5					
U.8.	161.3				3.38	1.76	36.8	1.198	0.132
B.9.	168.3	13.04	4.74	34.9					
U.9.	180.6				3.61	1.85	36.1	1.633	0.158

EXPERIMENT No: XXXV

Subject: A.C. **Bantu Female 26** **Sarcoidosis.**

Date: 19.12.56. **Height:** 5' 3" **Weight:** 123 lbs.

Urine collected by: Indwelling catheter and bladder washout

Calcium (839 mg.) administered at progressively increasing speeds from t = 0 to t = 179.4

Inulin primer given 60 mins. before UD.

	Time (mins)	Serum			Urine				
		Ca (mg.%)	P (mg.%)	Inulin (mg.%)	Ca (mg/min)	P (mg/min)	Inulin (mg/min)	Na^+ mEq/min.	K^+ mEq/mi
UD.	00.0								
B.1.	8.5	12.09	5.20	88.4					
U.1.	20.5				0.35	0.66	23.8	0.138	0.051
B.2.	27.5	12.96	5.35	86.0					
U.2.	40.2				0.41	0.69	20.9	0.185	0.060
B.3.	47.7	13.52	5.55	89.0					
U.3.	60.5				0.47	0.79	20.5	0.276	0.067
B.4.	68.5	13.36	5.22	93.2					
U.4.	80.9				0.55	0.68	20.2	0.285	0.056
B.5.	89.9	13.76	5.26	90.8					
U.5.	100.9				0.69	0.76	20.2	0.336	0.066
B.6.	108.9	13.92	5.02	101.1					
U.6.	121.0				0.83	0.79	23.4	0.428	0.072
B.7.	128.5	14.88	4.88	101.1					
U.7.	140.0				0.93	0.81	24.4	0.465	0.079
B.8.	147.5	15.44	5.04	102.0					
U.8.	160.2				0.95	0.84	24.3	0.512	0.086
B.9.	168.7	15.92	5.15	126.4					
U.9.	179.4				1.04	0.86	25.0	0.551	0.089

CASE E.B. (No. 39859), a 38-year old Coloured female, was seen first at Groote Schuur Hospital in December of 1955 complaining of symptoms of tetany and hoarseness of voice.

These symptoms had been present for 8 years, following the last of three operations on the thyroid for a goitre that she had had since childhood.

Clinical examination revealed a generally well woman, with no evidence of hypothyroidism or systemic disease. She had signs of latent tetany with ridging of the finger nails. No other form of ectodermal dysplasia was present.

Initial laboratory examination revealed a serum calcium concentration of 5.9 mg %, serum inorganic phosphorus concentration of 6.2 mg % and a urinary calcium output of less than 25 mg/24 hours.

She remains symptom free and normocalcaemic on Calciferol 50,000 units daily and calcium gluconate 2 grammes t.i.d.

All therapy was withheld for 2 weeks before the experiment described on page 254 was performed.

CASE H.W. (No. 332532), a 37-year old European female, underwent a subtotal thyroidectomy in 1946 for symptoms of weight loss and mild proptosis following the birth of her second child.

Tetany supervened within three days of the operation and has remained since albeit relieved by calcium lactate and calciferol. Myxoedema appeared within three months of the operation, and exophthalmos and opthalmoplegia developed and progressed until 1948.

Examination at the time of the experiment described on page 255 revealed a well covered and generally healthy woman with moderate bilateral exophthalmos, lid retraction and paralysis of right upward gaze.

No signs of tetany were present as therapy was not withdrawn before the experiment.

The exophthalmos had improved considerably on prednisone 20 mg daily.

This case has been reported in greater detail by Jackson (138).

A P P E N D I X B.

General.

Infusion Techniques Employed.

Statistical Methods Used.

Analytical Methods.

Bibliography.

GENERAL

The glomerular filtration rate was estimated from the clearance of inulin by $$G.F.R. = \frac{UV_{in} \times 100}{P_{in}}$$

where UV_{in} = rate of excretion of inulin in mg./min.

P_{in} = plasma inulin concentration in mg.%

The filtered load was calculated as the product of the glomerular filtration rate and the plasma concentration in mg./ml.

The rate of tubular reabsorption was calculated as the difference between the filtered load and the rate of excretion.

The concentration of inulin and phosphate in the glomerular filtrate was presumed to be identical with that in the serum.

Urinary collection periods were timed by simultaneously pressing two stop watches, to start the one and to stop the other.

The urine specimens, after being passed, were diluted immediately with distilled water to give a minute volume of 30 ml./min.

10% pyrogen free inulin solution as supplied by Messrs. Kerfoot was used for intravenous infusion.

A solution of sterile buffered phosphate was prepared from the recipe given by Anderson[12] to contain 1 0% of phosphorus at pH 7.4. This solution, after preparation, was sterilised by filtration and autoclaving and the pH was checked with a Beckman model G pH meter before use.

10% calcium gluconate solution was used in the calcium experiments.

INFUSION TECHNIQUES EMPLOYED

When a constant rate of infusion was required, the simple and inexpensive apparatus of Josephs and Schnieden (Josephs, H., and Schnieden, H. 1955. Lancet 268 : 388) was used. This proved most effective in maintaining a constant rate of flow, and could be calibrated with reasonable accuracy to deliver any amount required in a given time.

A constant infusion machine was used for administering solutions at progressively increasing speeds. This machine uses the principle of a fixed syringe, the piston of which is driven in by a pushrod. The speed at which the pushrod is extruded, and hence the rate of flow from the syringe, can be regulated by means of a dial on the face plate of the machine. The machine was calibrated using a 50 ml. syringe, and a graph drawn from these readings in such a way that the dial setting could be obtained for any required rate of flow.

During the course of experiments using progressively increasing rates of infusion, the dial was set every five minutes.

The rates of flow required at the various times during the experiment were read off a graph drawn up as follows -

The abscissa was marked off in minutes from t = 0 to t = 180. The ordinate was marked off in mls./min. The total volume of fluid required for each individual was calculated so as to give 25 mg. of Phosphorus per Kg. body weight or 20 mg. of Calcium per Kg. body weight over the 3 hour period, depending upon which substance was being infused.

The graph of the equation $R = \frac{V}{180^2} t + \frac{V}{360}$ was then drawn where R = rate of flow in mls./min.

V = total volume of fluid required over the course of the experiment.

t = time in minutes from the start of the infusion.

STATISTICAL METHODS USED.

A. IN THE PHOSPHATE EXPERIMENTS. (Experiments I - XIII inclusive).

The regression equations were determined in the following manner.

Let x, X denote the variable plotted on the abscissa.

y, Y denote the variable plotted on the ordinate.

Let n = number of observations on each subject.

Let X = Σx - the total of all the values of x for each subject.

Let Y = Σy.

Let $Sxx = \Sigma x^2 - \frac{X^2}{n}$

$Sxy = xy - \frac{XY}{n}$

$Syy = \Sigma y^2 - \frac{Y^2}{n}$

Then the regression coefficient for each subject,

$a = \frac{Sxy}{Sxx}$.

The value of the negative intercept on the ordinate, b is given as $b = \bar{y} - a\bar{x}$

The level of probability at which the hypothesis that a = 1 for each patient could be rejected was determined from a table of t with n - 2 degrees of freedom (Table 14)

A value for t for each patient was obtained as

$$t_{,7} = \frac{1 - a}{\sqrt{\frac{s^2}{Sxx}}} \qquad \text{where } s^2 = \frac{Syy - a\,Sxy}{n - 2}$$

/B. IN THE ...

B. IN THE CALCIUM EXPERIMENTS. (Experiments XIV to XXIII inclusive).

(1) Here let x, X denote Plasma calcium concentration in mg°/o.

y, Y denote $\frac{UV_{ea} \times 100}{G.F.K.}$

Let k = number of patients (In this case k = 9)

Let n_i = number of observations on the ith patient.

Let $N = \sum_i n_i$, the total no. of observations. (Here N = 59)

Let (x_{ij}, y_{ij}) be the jth observation on the ith patient where j runs from 1 to n_i

Let $X_i = \sum_j x_{ij}$, the total of the x's for the ith patient.

Let $Y_i = \sum_j y_{ij}$, the total of the y's for the ith patient.

(2) Let $Sxx_i = \sum_j x^2_{ij} - \frac{X_i^2}{n_i}$

$Syy_i = \sum_j x^2_{ij} - \frac{Y_i^2}{n_i}$

$Sxy_i = \sum_j x_{ij} y_{ij} - \frac{X_i Y_i}{n_i}$

The slope $a_i = \frac{Sxy_i}{Sxx_i}$

$\therefore \quad b_i = \bar{y}_i - a_i \bar{x}_i$

(3) Let $Sxx_m = \sum_i Sxx_i$

Let $Syy_m = \sum_i Syy_i$

Let $Sxy_m = \sum_i Sxy_i$

/The ....

The "average" slope $a_m = \frac{Sxy_m}{Sxx_m}$

(4) The "error variance" σ_i^2 for each patient is estimated by

$$\sigma_i^2 = \frac{1}{n_i - 2} \left[Syy_i - a_i Sxy_i \right]$$

The pooled error variance is estimated by

$$S^2 = \frac{1}{N - 2k} \left[Syy_m - \sum_i a_i Sxy_i \right]$$

(5) The table of covariance to test the hypothesis that the a_i were equal (Table 26) was drawn up as follows.

	D.f.	Sums of Squares.
Difference between a_i	$k - 1$	$\sum_i a_i Sxy_i - a_m Sxy_m = S_1$
Deviations within patients from their own slopes a_i	$N - 2k$	$Syy_m - \sum_i a_i Sxy_i = S_2$
Deviations without patients from the average slope a_m	$N - k - 1$	$Syy_m - a_m Sxy_m$

Then $\frac{S_1 / k - 1}{S^2 / N - 2k}$ = F with k - 1, N - 2k degrees of freedom.

(6) The 95°/o confidence interval for a_i were calculated

as $a_i \pm t_{5°/o,\ N-2k} \sqrt{\frac{S^2}{Sxx_i}}$

(7) The level of probability at which the hypothesis that $a_i = 0{\cdot}5$ could be rejected (Table 27) was obtained from a table of t.

/A value ....

A value for t was obtained by

$$t_{5\%N-2k} = \frac{0.5 - a_i}{s^2/S_{xx_i}}$$

(8) It will be seen that in computing the 95% confidence interval and the levels of probability mentioned in (6) and (7), a pooled estimate of the error variance has been used rather than σ_i^2.

This assumes that the σ_i^2 are equal.

In order to test this assumption Bartletts' test was used.

This test says that B/C is distributed as χ^2 with $k - 1$ degrees of freedom.

Where $B = (\sum_i n_i - 2) \log_e s^2 - \sum_i [(n_i - 2)\log_e \sigma_i^2]$

and $C = 1 + \frac{1}{3(k-1)} \sum_i \left[\left(\frac{1}{n_i - 2}\right) - \frac{1}{\sum_i n_i - 2}\right]$

In the series of experiments under consideration

$B = 13.6452$

$C = 1.08728$

$\therefore \quad B/C = 12.55$

$\chi^2_{5\%,8} = 15.51$

The assumption that the σ_i^2 are equal is therefore permissible.

ANALYTICAL METHODS

1) Inulin in Serum and Urine. - Method of Behreiner[230]

(a) Serum: To one ml. of serum were added 5 ml. distilled water, 2 ml. of 5% Zinc sulphate solution and 2 ml. of 0.25N NaOH. The resulting protein flocculum was separated by centrifugation and the supernatant protein free fluid used for analysis.

(b) Urine: Where the urine contained protein, it was deproteinised in a similar manner to the serum. In none of the cases described in this thesis however was this found to be necessary. A suitable dilution of the urine was made (usually 1:200) and this was used for analysis.

To 2 ml. of protein free fluid or diluted urine were added 2 ml. of 0.1% resorcinol in 95% alcohol and 5 ml. of 30% HCl. This mixture was shaken well and placed in a water bath at 80°C. for 25 minutes. The red colour which developed was read against a similarly treated standard solution in a Klett Summerson colorimeter using a filter of 490 u.
An aqueous reagent blank was used for the zero setting of the colorimeter. The colour developed by this method was found to be stable for at least 45 mins.

2) Inorganic Phosphorus in Serum and Urine.

The method used was a modification of the method of Fiske and Subbarow[76] using ascorbic acid for reduction. The serum was deproteinised by adding to 0.5 ml. serum 2.5 ml. of 10% trichloracetic acid solution. The protein flocculum was separated by centrifugation.

To one ml. of protein free fluid, diluted urine or standard solution were added 4 ml. of distilled water, 1 ml. molybdate reagent and 0.5 ml. of ascorbic acid solution. The blue colour was allowed to develop for ten minutes and was read in a Klett Summerson colorimeter using a filter of 660 mu.

Reagents used: Molybdate reagent - 50 mg. ammonium molybdate dissolved in a mixture of 750 ml. of distilled water and 150 ml. of concentrated sulphuric acid and made up to final volume of 1,000 ml. with distilled water.

Ascorbic acid solution - 1 50 mg. tablet of ascorbic acid crushed and dissolved in 10 ml. of distilled water and filtered.

All estimations were done in duplicate.

Zero setting on the colorimeter was adjusted with an aqueous reagent blank.

3) Calcium in the Serum and Urine.

(a) Serum: The method used was that of Greenblatt, I.J., and Hartman, S. (Anal.Chem. 23: 1708).
To 1 ml. of serum were added 9 ml. H_2O, 1 ml.2N NaOH and a knife point of ammonium purpurate - sodium chloride mixture. This was titrated with EDTA solution from a 2 ml. burette to an end point marked by a colour change from pink to purple. The amount of EDTA solution used in the titration multiplied by 16 gave the concentration of calcium in mg.%

Reagents used: Ammonium purpurate - NaCl mixture
Ammon. purpurate 0.2 grammes
Sodium chloride 100.0 grammes
ground well together

EDTA solution:

Disodium - ethylene diamine tetraacetic acid 148.8 mg. in 100 ml. distilled water.

All estimations were done in duplicate.

The end point of this titration, while not easy to see at first, was quite readily seen when sufficient experience had been gained and the method was found to give accurate and reproducible results.

(b) Urine: The method used was that of Jackson and Irwin (139).

The Calcium from an accurately measured volume of urine (usually 10 ml.) was adsorbed onto a cation exchange column using "Dowex" 50 (Dow Chemicals), 50 - 100 mesh as the exchange medium. The column was approximately 12.5 cms. in length and 0.6 cms. in diameter with a rate of flow of approximately 1.0 ml./min.

The column was activated for use with 25 ml. of 6N HCl and washed with distilled water until neutral to litmus. After passage of the acidified urine, the column was washed again with distilled water and the washings tested qualitatively for calcium to ensure full adsorption of the cations.

Sodium and potassium were then eluted with N HCl by collecting an exact 20 ml. of eluate. Calcium was then obtained by elution with 3N HCl and collecting approximately 35 ml. of the eluate in a 100 ml. volumetric flask. The eluate was made up to volume and was analysed for calcium by flame photometry using standards for comparison.

The flame photometry was done on a Beckman Model D Spectrophotometer with flame and photomultiplier attachments. A hydrogen/oxygen flame was used with gas pressures of 10 lb. and 4½ lb. respectively, and readings were made at a wavelength of 554 u. with a slit width of 0.06 mm.

The column was cleaned between elutions with 10 ml. of 3N ammonia followed by a washing with distilled water.

The method was checked frequently by passing known amounts of calcium in solution through the columns and noting the recovery.

4) Sodium and Potassium in Urine.

These were estimated on suitably diluted urine using a Barclay internal standard flame photometer.

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