CALCIUM AND PHOSPHORUS METABOLISM IN NEPHRITIS

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Modern methods of biochemical research have revealed certain changes to be more or less constantly present in the blood of persons suffering from renal disease. Many of these variations are still imperfectly understood. Some of the earlier hypotheses as to their causation have had to be abandoned, and others have not as yet been definitely confirmed or disproved. The question of 'retention of phosphorus' falls into the latter group and it was primarily with a view to elucidating this question that the present work was undertaken. Since, however, retention of phosphorus is intimately associated with that of calcium, the metabolism of calcium was investigated at the same time.

In 1916 Marriott and Howland¹ showed that in nephritis with low urinary excretion, the inorganic phosphorus content of the serum was high. These results have been confirmed by other workers and the theory of phosphorus retention has been generally accepted. Likewise, the observations of Halverson, Mohler and Bergeim² in 1917, of low serum calcium and decreased urinary calcium have been frequently corroborated. These results were not questioned until 1926 when Boyd, Courtney and McLachlan³ supplied the first available information on the utilization of these substances in renal disease, and their studies, though inconclusive, seemed to throw doubt on the occurrence of any abnormal retention of phosphorus. Consequently the accepted explanation of the low serum calcium values on the basis of compensating over-excretion became unsatisfactory.

In 1927 Byrom and Kay⁴ cast still more doubt on the idea of excessive phosphorus retention by demonstrating that the total blood phosphorus showed no increase in nephritic subjects. They found merely a redistribution of the acid-soluble phosphorus, whereby the inorganic fraction increased at the expense of the non-hydrolyzable ester phosphorus of the red corpuscles, thereby supporting the conclusions of Boyd, Courtney and McLachlan.

In view of the conflicting evidence as to their utilization in renal disease it seemed worth while to investigate by balance studies the metabolism of calcium and phosphorus. While this was being done, observations were made on the serum values. The results, in agreement with those of other workers, showed subnormal values for calcium and high figures for inorganic phosphorus during the more acute stages of the disease.

The main part of the work is presented in three sections:—

- I. Utilization of calcium and phosphorus in nephritis, i.e., calcium and phosphorus balances.
- II. Absorption of calcium and phosphorus in nephritis.
- III. Excretion of calcium and phosphorus in nephritis.

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I. UTILIZATION OF CALCIUM AND PHOSPHORUS IN NEPHRITIS.

As has already been indicated, the only previous calcium and phosphorus balance studies in nephritis are those of Boyd, Courtney and McLachlan³. They found that nephritic children had a positive calcium balance unless the intake was very low, and that the amount of calcium retained could not be correlated with the blood calcium content. All the œdematous subjects showed a positive phosphorus balance except two who were on minimal intake, and the non-hydræmic patients in most instances had negative balances. They concluded that 'evidence of phosphate retention given by blood and balance studies does not always agree.'

These observations must be looked upon with caution as the duration of the balance period was in each case only two or three days. The fallacies of such short periods have been frequently demonstrated. Apart from this, however, they do not quote control observations of their own, but used the normal figures of Sherman and Hawley⁵ for comparison with figures for nephritic patients.

In the present work the calcium and phosphorus balances were determined in a number of nephritic children in each of whom clinical and biochemical data gave evidence of renal damage. The procedure followed was similar to that observed in other balance studies in this hospital. Diet was confined entirely to milk, approximately 100 c.cm. per kilogramme of body weight being allowed daily. Where the quantity, so calculated, was thought to be excessive, sugar was substituted for some of the milk, and by this means the caloric value of the diet was maintained. Each child was given the selected diet for at least three days before the collection of excreta was commenced.

Balance studies must ever be open to criticism until some method of marking the fæces is introduced, whereby collection of the appropriate specimens may be made with certainty. Bergeim's suggestion, that a carmine capsule be given with the first and last meals of the period, was tested in several normal children. The dye was found to be spread throughout the intestinal evacuations of 48 hours and the method accordingly was abandoned. In subjects where there was a tendency to constipation lavage was used daily. In all cases the bowel was washed out with sterile water at the beginning and at the end of each period.

Balance studies were carried out on eleven nephritic children, representing various phases of renal disease. Nine were classed as 'acute nephritis' since clinical recovery seemed complete within six months. The two remaining subjects suffered respectively from chronic interstitial nephritis accompanied by dwarfism (Case 6) and chronic parenchymatous nephritis (Case 4). In four of the acute cases a second balance period was observed after an interval varying from one to four months.

Control figures were obtained from three children who had recovered from acute ileo-colitis, bronchitis and rheumatism respectively. The details of the analyses are given in Table 1.

It has been shown by Telfer²⁶ that the retentions of CaO and P₂O₅ in healthy children are approximately equal and vary from 0.06 to 0.12 grm.

per kgrm. per day. Sherman and Hawley⁵ observed that in children between 3 and 12 years of age, on a mixed diet with 750 c.cm. of milk daily, the average retention of CaO was 0.01 grm. and of P_2O_5 0.008 grm. per kgrm. per day. They found that when the milk allowance was increased, the retentions of CaO and P_2O_5 rose. Therefore, since each child of the present series was having considerably more than 750 c.cm. of milk daily, the retentions might reasonably be expected to show values over 0.01 grm. per kgrm. per day.

MacRae⁶, working in this laboratory, found that in convalescent children between 3 and 12 years of age, on a diet consisting entirely of milk, the reten-

TABLE 1.

CALCIUM AND PHOSPHORUS INTAKE AND EXCRETION IN NEPHRITIC SUBJECTS (CASES 1-11) AND NORMAL CONTROLS (CASES 15-17).

Case No.	Age in years	Weight in kgrm.	Days of study	CaO in grm. per day			Balance grm. per kgrm. per day	P_2O_5 in grm. per day			Balance grm. per kgrm. per day
				Intake	Urine	Fæces		Intake	Urine	Fæces	
1	8	26	6	4.65	.044	2.61	+.077	6.54	2.45	1.575	+.096
2	6	16.4	6	3.234	.039	2.277	+.057	4.536	1.69	1.75	+.068
3	12½	26	7	4.8	.087	3.29	+.054	6.6	1.84	3.24	+.058
	12½	27.4	6	3.84	.136	2.41	+.048	5.67	1.92	2.31	+.053
4	13	25	4	2.7	.021	1.58	+.044	3.78	.32	2.11	+.054
5	8	26.4	6	2.293	.063	1.14	+.042	3.216	1.02	1.212	+.038
	8½	24.4	6	2.212	.038	1.62	+.023	2.884	.938	1.597	+.014
6	6	11.6	7	2.232	.088	1.74	+.035	3.08	.82	1.827	+.039
7	10	22.6	5	3.06	.041	2.46	+.025	4.36	1.87	2.038	+.02
8	6	18.8	5	2.1	.048	1.58	+.025	2.52	.764	1.44	+.017
	6½	17.5	6	2.15	.073	2.308	-.013	3.18	1.197	1.977	+.0003
9	5	15	6	1.95	.022	1.8778	+.0033	2.73	.914	1.803	+.00083
	5½	14.8	6	2.13	.0575	2.0965	-.0017	2.86	1.217	1.98	-.225
10	6	13.8	5	1.575	.046	1.53	-.0007	2.1	.81	1.49	-.014
11	8	20	7	2.79	.03	3.101	-.017	3.96	1.1	3.18	-.016
15	7	21.3	6	3.1	.2362	1.115	+.081	4.4	2.147	.717	+.071
16	6	20	6	2.87	.135	1.89	+.042	3.83	1.26	1.82	+.037
17	6	15	6	2.325	.166	1.603	+.037	3.3	1.408	1.205	+.046

tions of CaO and P_2O_5 were approximately equal and ranged between 0.016 and 0.052 grm. per kgrm. per day. The three control subjects of this series showed a similar state of affairs, the retentions varying between 0.037 and 0.081. (To avoid repetition hereafter all utilization figures will be merely stated in grammes on the understanding that they have been calculated in terms of body weight for one day.)

It is important to emphasize that Telfer found in young children retentions of calcium as high as 0.12 grm. and Holt, Courtney and Fales⁷, working with healthy infants, retentions averaging 0.09 grm. These being the highest figures recorded for healthy children, it might be expected that in the presence

of any 'retention' in nephritic subjects the balances would be at least as great. The utilization figures (Table 1) show that this expectation was not realized. The highest figure of the series was 0.096 grm., the remainder being less than 0.08 grm.

In these fifteen determinations the balances varied to a marked extent. In eleven the CaO balance was positive, in four negative; while the P_2O_5 balance was negative on three occasions. Holt, Courtney and Fales' state that an intake of less than 0.09 grm. of CaO per kgrm. per day will produce either a very low retention or a negative balance. The intake in this series

CHART I.
Ca. AND P. INTAKE CONTRASTED WITH Ca. AND P. RETENTION.

was only in one instance as low as 0.09 grm. and averaged 0.14 grm. CaO and 0.183 grm. P_2O_5 . It would therefore appear that the supply of both was adequate. From Chart I it will be seen that utilization bears no relationship to intake. The cases showing negative balances received a more ample supply than some of those with a positive retention. Similarly high retentions are shown to be quite unconnected with the intake.

Neither the age nor the weight of the children could be correlated with the utilization of either CaO or P_2O_5 .

In the four cases in which balance periods were observed on two occasions, the retentions during the second period were diminished (Table 1). In one of

these (Case 3) the difference in the balance figures might well be attributed to the slight decrease in intake. In each of the other three cases, however, this explanation is inadmissible, for two reasons: (1) the intake during each period was practically identical; (2) the decreased retention was very definite and of such magnitude that in two children a negative balance was found. These latter figures were obtained while the children were definitely convalescent, and it may be significant that the only other negative balances were found in two other convalescent children.

CHART II.
SERUM Ca CONTRASTED WITH CaO RETENTION AND URINARY CaO.

Attempts to correlate the retention figures for CaO and P_2O_5 with the urinary analysis and approximately contemporary blood observations were of no avail (Charts II and III). The levels of the serum calcium and phosphorus had no bearing on their retentions. These observations agree with those of Boyd, Courtney and McLachlan³ that 'the calcium balance is independent of the blood-calcium content,' and 'evidence of phosphate retention given by blood and balance studies does not always agree.' Case 4 illustrates this clearly. Similarly, the levels of blood non-protein nitrogen and chloride could not be associated with the CaO and P_2O_5 balances.

The remaining point shown by these balance studies in nephritic children is the remarkable manner in which the utilizations of CaO and P_2O_5 run parallel (Chart I). This has been demonstrated in healthy subjects and in other diseases, such as rickets and coeliac disease, and apparently there is no exception in nephritis. The graphs in this chart bring out the point clearly and taking this fact into consideration it is obvious that if excessive retention of phosphorus occurs in nephritis, an approximately equal extra retention of calcium accompanies it.

CHART III.
SERUM INORGANIC P. CONTRASTED WITH P_2O_5 RETENTION AND URINARY P_2O_5 .

Conclusions.—From the results so far noted it is permissible to draw the following conclusions regarding the nature of mineral metabolism in nephritis :

1. The retentions of CaO and P_2O_5 are as a rule approximately equal and within the normal range, except during convalescence when they are reduced.
2. There is no relationship between the serum levels of calcium and inorganic phosphorus on the one hand, and the retentions of CaO and P_2O_5 on the other.
3. There is no connection between the retention and the urinary excretion of either CaO or P_2O_5 .

II. ABSORPTION OF CALCIUM AND PHOSPHORUS IN NEPHRITIS.

(1) **Calcium.**—The balance investigations of this study showed low urinary excretion of calcium. This has been observed on many previous occasions and the decrease has been attributed to impairment of renal function without, as in the case of phosphorus, any accumulation in the blood. Boyd, Courtney and McLachlan³ concluded from their studies that the nephritic kidney was incapable of excreting calcium in normal fashion, citing as evidence of this the lowered urinary output and the abnormally small amount of calcium per 100 c.cm. of urine.

Before diminished excretion can be made responsible for the low urinary calcium, it must be shown that absorption is at least comparatively normal, but so far as can be ascertained from the literature this aspect of calcium metabolism in nephritis has received scant attention. Wills, Sanderson and Paterson⁸ state that even in presence of a negative balance, where there is increase in urinary calcium, it is fair to conclude that there is increased absorption. Since in this study the children in whom two balance periods were observed showed an increased urinary excretion of calcium in the second (convalescent) period it seemed important to study the efficiency of absorption both during the active and the convalescent stages of the disease.

In previous studies of the effect of ingested salts on the blood calcium two methods have been employed. The earlier results, obtained after prolonged administration of calcium salts, were extremely inconclusive and contradictory, but more recent observations, in which a series of estimations was carried out at intervals after a single dose, have shown much less variance.

The striking feature of the earlier reports on absorption of calcium is the stress laid on the difference in availability of different salts. Steenbock, Hart, Sell and Jones⁹ in 1923 decided that no difference in availability existed between the lactate, carbohydrate, phosphate, silicate or sulphate, so long as a sufficient quantity was supplied. Jansen¹⁰ in 1924 found that calcium lactate, given orally, had no effect on blood calcium but found increase with the chloride and bicarbonate, particularly well marked with the latter. Hjort¹¹ in 1925 was of opinion that the more soluble salts, the lactate, chloride and glycerophosphate, had a much more constant effect than the less soluble carbonate.

Attempts have been made to probe this suggestion that the availability of the calcium depends on the solubility of the salt exhibited. Wills, Sanderson and Paterson⁸ concluded that the gastric acidity did not seem to be a limiting factor in the supply of calcium to the body. This opinion was based on balance experiments, no observations on blood values being reported. Mason¹² in 1921 had already noted that solution of calcium in diluted HCl did not affect the rate of absorption. He also indicated that calcium lactate produced less effect on plasma calcium than did calcium chloride, and inferred that the latter was better absorbed.

Since different observers have favoured different salts, it would appear that there can be no marked difference in their suitability. The more recent workers have shown that calcium lactate is an adequate source of supply for

absorption studies, and this salt was accordingly selected as the preparation of choice in the present work. An additional point in its favour is that no change in the pH of the blood, such as occurs with calcium chloride, follows its administration.

As has been already mentioned, it is only recently that increase in blood calcium after ingestion of calcium salts has been recognized.

The earlier workers relied mainly on prolonged administration to effect an alteration in the blood level. Bergeim¹³, Blum and his co-workers¹⁴, Leicher¹⁵, Rockwood and Burrier¹⁶, working with nephritic subjects, and Salvesen, Hastings and McIntosh¹⁷ working with dogs, failed to obtain indisputable evidence of any rise in blood calcium after continued oral administration of calcium salts. They omitted, however, to allow for excretion and withdrew specimens of blood for examination at irregular intervals and often days apart.

Information obtained from serial observations at relatively short intervals after ingestion of a single dose of one or other of the calcium salts has proved much more constant and reliable. Mason¹² in 1921 found slight increase in plasma calcium with a single large dose of calcium lactate, but with calcium chloride obtained a more constant effect and higher rise. Jansen in 1923 and Hjort¹¹ in 1925 both demonstrated increase in blood calcium after ingestion of calcium salts, and a year later Kahn and Roe¹⁸ and Bauer and Ropes¹⁹ confirmed this occurrence in human subjects.

They differed considerably in their conclusions. The maximum increase reported varied between the wide limits of 14 and 81 per cent. of the fasting value, and its time of occurrence from 1 to 7 hours. It was also stated by Kahn and Roe¹⁸ that when food was given with the calcium the rate of absorption was decreased. Hjort had previously noted that administration of from 150 to 600 c.cm. of water produced in the serum calcium a decrease of 1 mgrm. which persisted for several hours.

Accordingly, before the absorption of calcium in nephritis could be assessed, it was necessary to obtain some control curves in normal subjects.

The procedure in all cases was as follows. Each subject fasted for three hours before the withdrawal of the first specimen of blood. Four grammes of calcium lactate in approximately 100 c.cm. of water were then given, and subsequent observations were made on the serum calcium at intervals of 2, 4 and 6 hours. No food or water was allowed during this period.

The seven control curves showed great similarity (Table 2). In five the maximum elevation occurred at 2 hours. In the remaining two there was a further slight increase at 4 hours. In all, the return to fasting level had been well advanced by the end of 6 hours. None of these curves showed a final reading which was below the original level.

In nephritic subjects 28 curves were observed in 16 children (Table 2). These showed variations from the normal and from one another. In 22 curves the increase 2 to 4 hours after the dose of lactate compared favourably with that in the controls, while in four of them it exceeded the rise shown in any of the normal curves. In the remaining six curves the increase was less than that shown in four hours by even the lowest of the control series. In all the nephritic curves, however, there was some evidence of absorption.

It seems, therefore, that as in normal subjects, so in children suffering from nephritis, administration of a single dose of calcium lactate orally leads to elevation of the serum calcium. As has been already indicated, in the large majority of nephritic curves the elevation corresponded to that in the control

TABLE 2.

SERUM CALCIUM OF NEPHRITIC AND CONTROL SUBJECTS AFTER INGESTION OF CALCIUM LACTATE.

Case No.	Age Years	Nephritis	Serum Ca in grm. per 100 c.cm.				Type of graph	Stage of disease
			Fasting	2 hr.	4 hr.	6 hr.		
1	11	Acute	9.0	10.04	9.7	9.24	I	Early convalescence.
3	12	Acute	10.34	11.7	10.73	9.9	II	Improving.
	+4 mth.		10.46	10.96	9.95	10.39	III	Almost well.
4	13	Chronic	5.82	6.04	5.91	5.42	II	Very ill; some œdema
5	9	Acute	7.87	8.76	7.34	7.87	III	Uræmia; some œdema
	+10 days		8.91	9.9	9.45	9.18	I	Albuminuria only.
	+2 mth.		9.24	10.31	—	9.76	I	Albuminuria persisting.
7	10	Acute	9.91	10.7	11.3	12.17	IV	Early convalescence.
	+1 mth.		9.41	10.49	9.86	10.22	III	Albuminuria only.
8	6	Acute	9.64	10.0	9.28	8.92	II	Early convalescence.
	+1 mth.		9.77	10.77	9.41	9.23	II	Late convalescence.
9	5	Acute	9.16	9.72	9.86	9.35	I	œdema.
	+1 mth.		9.74	10.26	9.74	9.58	II	Well.
11	8	Acute	11.6	14.1	12.3	10.3	II	Almost well.
12	11	Chronic	4.8	4.9	5.0	6.57	IV	III: Chvostek.
	+10 days		4.62	5.0	4.88	5.15	IV	I.S.Q.A.
	+2 mth.		9.24	9.86	9.9	9.52	I	Much improved.
18	1½	Chronic	10.96	13.07	11.14	10.52	II	œdema.
19	8	Acute	9.43	11.82	9.74	10.08	III	Almost well.
20	7	Acute	9.6	10.76	10.17	9.43	II	7 days after uræmic twitchings.
21	9	Acute	8.7	9.58	8.27	9.35	III	œdema.
22	5	Acute	6.97	7.6	7.29	8.88	IV	œdema.
23	9	Acute	8.54	8.54	8.77	8.77	IV	Uræmic convulsions.
	+1 mth.		9.86	10.45	9.4	9.68	III	Well.
24	8	Acute	7.91	8.91	8.7	8.26	I	œdema.
	+2 wk.		9.27	9.56	9.58	9.0	II	No œdema.
CONTROLS								
15	7	Colitis	9.71	10.1	10.2	10.0	C	Well.
16	6	Chorea.	10.38	11.41	11.5	10.72	C	Well.
17	6	Bronchitis	9.62	11.05	10.3	10.1	C	Convalescent.
25	9	Rheumatic heart Dis.	8.9	10	9.24	9.08	C	Convalescent.
26	10	Rheumatic heart dis.	9.62	10.95	10.1	10.05	C	Convalescent.
27	10	Rheumatic heart dis.	9.6	10.14	9.86	9.77	C	Convalescent.
28	9	Rheumatic heart dis.	9.76	10.7	9.86	9.86	C	Convalescent.

series but much wider variation in the increase was noted. This becomes more obvious if the control and nephritic figures are contrasted (Table 3).

Since each control curve reached its peak not later than 4 hours, this has been adopted as the maximum time during which comparisons of absorption may be drawn and the average increase in both cases is found to be exactly equal (0.97 mgrm. per 100 c.cm. of serum). In four of the nephritic curves, the maximum increase did not occur until 6 hours, and if these figures be included in the average, the increase in nephritis becomes slightly more than in normal, namely, 1.11 mgrm. per 100 c.cm. of serum.

Of the five nephritic children in whom a poor rise was found after 4 hours, two were the subjects of balance studies (Cases 4 and 8). Although the blood-calcium curves seemed to indicate some defect in absorption, in both a positive CaO balance was found, indicating that the balance cannot be predicted from the type of blood curve. If the rise in the calcium curve is contrasted with the retention of CaO and the percentage of CaO intake appearing in the urine, it will be found that no relationship exists between them. Three children

TABLE 3.

AVERAGE CALCIUM CONTENT OF BLOOD OF NORMAL AND NEPHRITIC SUBJECTS AFTER THE INGESTION OF CALCIUM LACTATE.

Subjects	No. of curves	Ser. Ca. mgrm. per 100 c.cm.			Increase mgrm. per 100 c.cm.		
		Fasting	4 hr.	Increase	% of fasting	Smallest	Greatest
Control	7	9.66	10.63	0.97	10.04	0.49	1.43
Nephritic	28	8.98	9.95	0.97	10.8	0.25	2.87

(Cases 11, 8 and 9) gave a negative balance, but the respective blood curves showed increases of 2.5, 1.0 and 0.52 mgrm. per cent. Nor did the highest retention figures correspond with the greatest elevation in the blood curve. Similarly with the urinary excretion, Case 9, while excreting by this route only 1.13 per cent. of CaO intake, showed a rise of 0.7 mgrm. in the blood-calcium curve. Some time later, when the urinary figure had risen to 2.68 per cent., i.e., more than double its previous amount, the calcium curve showed an increase of 0.52 mgrm. per cent. This lack of relationship is shown also by the control subjects in whom both the CaO balance and blood curve were observed.

Cases obtained during different stages of illness, however, show more definite indication of some upset in calcium metabolism during the active stage of the disease with a gradual return to normal as convalescence advances. In each of two children (Cases 5 and 12) three curves were obtained and in six others (Cases 3, 7, 8, 9, 23, 24) two curves. This procedure was suggested by the change in retention found in convalescent children, and also by the altered distribution of the calcium excretion. The increase in serum calcium had no relationship to either the fasting level or the stage of the disease.

Case 12, with fasting estimations, on two occasions, of 4.8 and 4.6 mgrm. per 100 c.cm., showed corresponding elevations of 1.77 and 0.53 mgrm. Similarly Case 5 with primary observations of 7.87 and 9.24 mgrm. per cent., showed on each occasion an increase of 0.89 and 1.07 mgrm. per cent., i.e., an almost identical increase in the height of the two curves. Case 24, with fasting levels of 7.91 and 9.27 mgrm. per cent., gave a greater rise on the first observation (1.0 mgrm.) than on the later one (0.31 mgrm.). In each case the later curves were observed after the onset of definite clinical improvement, and although the increase did not seem to be dependent on the fasting level, yet the type of curve obtained during different stages of the illness showed marked aberration from the normal. This is most easily appreciated when the figures are graphed.

CHART IV.
COMPOSITE GRAPHS OF DIFFERENT TYPES OF SERUM CALCIUM CURVE.

The graphs have been arranged in groups entirely from their general appearance, since no other method of classification could be found. Four categories have been adopted. The average curve of each group is graphically presented in Chart IV.

Type I. Curves very similar to the control curves. This includes two cases of acute nephritis with great oedema; two cases of acute nephritis during convalescence; and a convalescent chronic parenchymatous nephritis.

Type II. Curves characterized by the occurrence of a fall in serum calcium at 6 hours of such magnitude that the final reading had fallen below the fasting level. The subjects in whom this was found were one case of acute nephritis; six acute cases in various stages of convalescence; one case of renal

dwarfism ; one of functional albuminuria ; and a case of chronic parenchymatous nephritis showing much œdema ten days preceding the onset of a fatal pericarditis.

Type III. Curves in which a good increase was obtained at 2 hours, followed by a marked fall, attaining at 4 hours a level even lower than the fasting, with a subsequent terminal rise at 6 hours. This group also shows some variety in its subjects, namely, one case of acute nephritis with uræmia and some œdema ; two of acute nephritis, one of which had œdema ; two cases of acute nephritis during a rather slow convalescence ; and one late convalescent case.

Type IV. A final group in which a variable increase was found at 2 and 4 hours, with the maximum elevation shown by the 6 hours specimen. The cases falling into this category are :—one child immediately after cessation of uræmic convulsions ; one case of acute nephritis with generalized anasarca ; one of acute nephritis in early convalescence ; one of chronic nephritis which ultimately left hospital comparatively well.

Thus in each group are found examples of the various stages of the disease, with and without œdema, a factor which does not appear to have any constant effect on the behaviour of the calcium curve. If the curves observed in different stages of nephritis are followed, it is found that the one type of curve seldom occurs twice in the same child, suggesting that there is either defective absorption or alteration in the intermediate metabolism of calcium. Examination of the charted curves of other observers does not show variations to have been found in normal subjects similar to those just described in nephritis, and since the average increase in the serum calcium value has been shown to be quite as large in nephritic as in normal children, and also, as will be shown later, the urinary excretion of calcium is invariably diminished, it seems probable that the anomaly lies in the intermediate metabolism of calcium.

In view of these findings it looks as if the low urinary output of calcium in nephritis cannot be the result of an alteration in absorption, and the factors producing it must be sought elsewhere.

(2) **Phosphorus absorption.**—Wigglesworth and Woodrow²⁰ have shown that in healthy subjects an increase of 55 to 60 per cent. occurred in the inorganic blood phosphorus after ingestion of 25 gm. of acid or alkaline phosphate. They found that the inorganic phosphate was equally distributed between plasma and corpuscles, which means that an increase is present in both and therefore can be determined by estimation of the inorganic phosphorus in the serum alone.

As was the case with calcium two methods of investigating phosphorus have been tried by earlier workers : (1) prolonged administration of phosphorus salts with random blood observations, and (2) serial observations after one dose. The latter method, employed by Murdoch²¹ in her study of absorption in normal and rachitic children, seems preferable and was the method adopted in the present work. In each case food was withheld for a minimum period of 3 hours before the fasting specimen of blood was obtained. Four grammes of NaH_2PO_4 dissolved in approximately 100 c.cm. of water were then given, and

samples of blood withdrawn after 1, 2 and 4 hours. Estimation of serum inorganic phosphorus was carried out immediately after each specimen of blood was obtained. During the investigation food and water were temporarily withheld.

Twenty-two blood curves were thus obtained from fifteen nephritic children. Control curves were constructed in ten children convalescent from other diseases, mainly chorea and rheumatic arthritis (Table 4).

The control curves show considerable variation in detail, but closely resemble those obtained by Murdoch²¹ in normal children of the same ages, 6 to 12 years. The fasting level varied in different individuals from 4.6 to 6.3 mgrm. per 100 c.cm., i.e., within the normal range. In all, definite increase in the serum inorganic phosphorus was found one hour after administration of the phosphate. In six cases the maximum rise was shown at this time; in four it occurred at 2 hours, and in one, a case of diabetes, it occurred at 4 hours. At this time, in all but the case of diabetes, the return to fasting level was well advanced, but in none had that level been reached. The increase varied in amount as in the time of attaining a maximum, the range being 0.75 to 3.2 mrgm. per 100 c.cm., and there did not seem to be any relationship between the height of the curve and the fasting level.

In three of the subjects of these control tests balance studies were also carried out, and in these the rise in the curve gave no indication of the retention found. Nor did it indicate the percentage of intake which was excreted by the urine, except that in these three instances, the smaller the increase in the blood curve the higher the percentage excretion by the urine. This might be explained on the assumption that the lower curves indicate a more active renal excretion and occur as a direct consequence of the latter. Such an assumption, however, is not warranted on this small number of observations.

Failure of all these curves to regain fasting level in 4 hours aroused interest, and several curves were prolonged for periods varying up to 14 hours in an attempt to find how long a time would elapse before fasting level was again restored. As will be seen from Table 4, two cases had almost regained fasting level in 13 and 14 hours respectively. A third, falling at 7 hours, rose again, and at 10 hours was higher than at any part of its course. Of the remaining two cases, one was rapidly approaching normal at 7 hours, while the other showed considerable elevation persisting at this time.

Wigglesworth and Woodrow²⁰ found increase in blood inorganic phosphorus persisting 10 hours after a large dose (25 grm.) of phosphate, in the healthy adult. Not only did it persist, but having reached a maximum elevation in one hour, no decrease was found in frequent estimations made throughout the day. They therefore suggested that phosphate behaves as a threshold substance and is excreted by the kidney only when the blood concentration exceeds a certain undetermined value. This may explain the type of curve found by them, but it does not assist in explaining the slow, more or less steady fall extending over 10 or 12 hours found in the control cases of the present series. Wigglesworth and Woodrow²⁰, Brain and Kay²², and Salvesen²³ have all found marked increase in urinary excretion of phosphorus after elevation of the blood phosphorus has been induced either by oral or intravenous administration of phosphate.

The ability of the normal kidney to excrete much more than the customary amount cannot therefore be questioned and one is forced to conclude that the

TABLE 4.

SERUM INORGANIC PHOSPHORUS OF NEPHRITIC AND CONTROL SUBJECTS
AFTER INGESTION OF NaH_2PO_4

Case No.	Age in years	Nephritis	Serum inorg. P. in mgrm. per 100 c.cm.				Type of graph	Stage of disease
			Fasting	1 hr.	2 hr.	4 hr.		
1	11	Acute	6.66	9.23	8	6.85	I	Convalescent.
3	12	Acute	6	8	7.5	6.3	I	In acute stage.
	+5 mth.		5.21	7.5	7.06	6	I	Almost well.
4	13	Chronic	12	12.63	12.63	12.63	III	Very ill; œdema.
5	9	Acute	4.8	5.71	6.48	5.71	II	Convalescent.
7	10	Acute	6	7.06	7.06	6.66	III	Convalescent.
8	6	Acute	5	6.31	7.06	6.31	II	Early convalescence.
	+1 mth.		4	6.66	5.21	4.44	I	Late convalescence.
9	5	Acute	7.06	8.88	8	8.27	III	Just after uræmia.
	+1 mth.		4.8	6.48	6.48	5.6	I	Well.
11	8	Acute	4.6	4.7	5.3	5	II	Convalescent.
12	11	Chronic	12	13.33	13.33	13.33	III	Very ill.
	+2 wk.		9.23	10	10	9.6	III	I.S.Q.A.
	+2 mth.		6.15	7.27	7.27	7.27	III	Much improved.
19	8	Acute	4.28	5.45	5.58	5.1	II	Convalescent.
20	7	Acute	5.21	6.81	7.31	6.15	II	Early convalescence.
21	9	Acute	8.57	12	10.8	8.9	I	In acute stage.
22	5	Acute	4.9	6.15	5.71	6	III	œdema.
23	9	Acute	4.89	6	6.48	6.31	III	Just after uræmia.
	+1 mth.		5.1	6	6	5.6	III	Well.
24	8	Acute	5.66	6	6.31	5.85	II	œdema.
	+2 wk.		5.15	6.85	7.5	6.85	II	Improving.
CONTROLS								
15	7	Colitis	6.31	8.16	6.86	6.66	C.I	Well.
16	6	Chorea	4.8	8	7.7	6.6	C.I	Well.
17	6	Bronchitis	5.85	8	8.57	7.4	C.II	Convalescent.
25	9	Rh.arthritis	5.33	7.06	6.41	5.85	C.I	Convalescent.
26	10	Rh.arthritis	4.61	5.71	5.85	5.33	C.II	Convalescent.
29	12	Chorea	5	7.06	6.48	6	C.I	Convalescent.
27	10	Rh.arthritis	5	5.48	6.31	—	C.II	Convalescent 14 hr. 5.21 mgrm.
28	9	Rh.arthritis	5.71	7.06	6.31	—	C.I	Convalescent 13 hr. 5.77 mgrm.
30	7	Chorea	4.7	5.45	5.1	5.02	C.I	Convalescent 7 hr. 5.2 mgrm.
								10 hr. 5.6 mgrm.
31	8	Chorea	5	6.31	6.66	5.85	C.II	Convalescent 7 hr. 5.45 mgrm.
14	6	Diabetes	4.61	6.66	6.85	7.06	—	7 hr. 5 mgrm.

effect of high blood phosphorus content on the general metabolism is not such as to demand its urgent removal even in normal persons.

In fifteen nephritic subjects very similar results were obtained (Table 4, Chart V). The fasting level varied between 4 and 12 mgrm. per 100 c.cm., nine being over 6 mgrm. In 13 curves the maximum rise occurred at 1 hour, and in all it had been reached by 2 hours. Again, there was great variation in the amount of increase, the range corresponding to that of the control series, and as in the latter, no relationship could be found between the fasting level and the height of increase in the curve. This is well illustrated in Case 12, from whom three curves are available for study. The first, observed during an acute exacerbation of a chronic nephritis, showed a fasting level of 12 mgrm.

CHART V.
COMPOSITE GRAPHS OF DIFFERENT TYPES OF P. SERUM CURVE.

C.I.= Mean graph of 6 control curves
C.II.= " " 3 " "
I.= " " 6 nephritic "
II.= " " 7 " "
III.= " " 9 " "

and a rise of 1.33 mgrm. per 100 c.cm. The second, in early convalescence, showed the fasting level to be 9.2 and the rise 0.8; while the third, some time later, had a fasting level of 6.15 and a rise of 1.1 mgrm. per cent.

Nor could the phosphorus curves be correlated with the clinical condition. Case 12, on three occasions, showed the same type of curve; a maximum rise attained at 1 hour, and remaining steady at 4 hours. Improvement in his renal function, which had undoubtedly taken place, was not accompanied by a more rapid fall in the blood curve, and in view of the type of curve found by Wigglesworth and Woodrow²⁰ this cannot be labelled an abnormal curve.

Similarly presence of oedema was apparently of no significance so far as the phosphorus curve was concerned. Case 4 with generalized oedema showed a curve similar to those found in Cases 12 and 23 which had no oedema. Case 22

and Case 1 with marked œdema showed curves which were alike but different from the above, and also from the third type of curve shown by another œdematous subject, Case 24.

On the whole, the nephritic curves compared very favourably with normal curves from the point of view of absorption. Even when the fasting inorganic phosphorus was around 12 mgrm. per 100 c.cm., a state of affairs generally regarded as indicative of death within a few weeks, there was definite evidence of absorption. It will be seen from Table 5 that the average fasting level, highest level attained, and increase in the control subjects are in absolute agreement with Murdoch's normal curves. In the nephritic subjects the fasting levels, when averaged, are higher, as are the maximum elevations; but the increase is not quite so great, being about 25 per cent. lower. The range, however, is very similar in the control and nephritic cases of this series.

Defective absorption, as judged by the curves, cannot be held responsible for the lowered P_2O_5 balances observed in the repeated studies (Tables 1 and 4). Case 3 on each occasion showed an increase of 2 and 2.29; Case 8, 2.05 and

TABLE 5.

AVERAGE SERUM INORGANIC PHOSPHORUS CONTENT OF NORMAL AND NEPHRITIC SUBJECTS AFTER INGESTION OF NaH_2PO_4 .

Subjects	No. of curves	Ser. inorg. P. in mgrm. per 100 c.cm.			Smallest increase	Greatest increase
		Fasting	Highest	Increase		
Murdoch's	3	5.3	7.1	1.8	1.6	2.2
Control	9	5.25	7.05	1.8	0.75	3.2
Nephritic	22	6.22	7.58	1.36	0.65	3.43

2.65; and Case 9, 1.8 and 1.7 mgrm. per cent. In the other case it was not possible to determine an earlier phosphorus curve, but the one obtained during the second balance period showed an increase of 1.7 mgrm. per cent.

Comparison with the P_2O_5 retention figures and with the percentages of intake excreted by the urine shows that there is no correlation between these and the rise in phosphorus curve. Case 8, with a minimal retention of P_2O_5 , showed the highest rise in the curve (2.65 mgrm., i.e., well above the average normal rise of 1.8 mgrm.), and in Case 4 the rise in the curve was 0.65 mgrm. and the retention adequate. The urinary figures showed similar confusion, and the inverse relationship shown by the three control curves was not found in nephritis.

Summary of blood calcium and phosphorus findings.

1. The average rise of the calcium curve in nephritic subjects is equal to that found in control subjects, but the average rise of the phosphorus curve in nephritic subjects is lower than that of controls.

2. The height of the rise in both the calcium and phosphorus curves is independent of the fasting level both in nephritic and normal subjects,

3. The duration of the rise of both the calcium and phosphorus curves in the nephritic subjects is more variable than in the normal cases.

4. None of the types of calcium and phosphorus curves could be found to bear any relationship to the clinical condition of the cases.

5. The height of the calcium and phosphorus curves bore no relationship either to their urinary output or their retention.

III. EXCRETION OF CALCIUM AND PHOSPHORUS IN NEPHRITIS.

(1) **Urinary excretion.**—Reference has already been made to the fact that for many years diminution in urinary excretion of calcium and phosphorus in nephritis has been generally recognized.

This has been observed repeatedly, but the first significant work on excretion of calcium by the nephritic kidney seems to have been done by Hetenyi and Nogradi²⁴ in 1925. In order to avoid any difficulty arising from variations in absorption, they injected intravenously 10 per cent. solution of calcium chloride, and found that in uræmia the urinary calcium output increased very slightly in the two hours following injection. Even in patients convalescent from acute nephritis, the urinary excretion rose less than half the amount found in normal subjects, showing that, whatever the cause of the impairment of renal excretion, some time elapsed before the kidney recovered its excretory power. In common with others, they failed to find in nephritis, in marked contrast to the normal, any increase in urinary calcium excretion following increased intake.

Boyd, Courtney and McLachlan³ concluded that the nephritic kidney was incapable of excreting calcium in normal fashion as evidenced by the low total excretion and by the low concentration in the urine. With regard to phosphorus, they were of opinion that in nephritis urinary excretion compared favourably with normal.

Thus, while all previous workers are unanimous in the opinion that there is low renal output of calcium in nephritis, some doubt has been expressed as to whether the phosphorus output is also diminished. In the present investigation eleven cases of nephritis were examined, four of them on two occasions, and the figures for urinary excretion show that, without doubt, the quantities of calcium and phosphorus voided by this route were subnormal (Table 1). Before discussing these in detail, it is necessary to consider the normal urinary output of these elements.

Holt, Courtney and Fales⁷, while studying the calcium metabolism of normal children, indicated that the main source of recovery of the surplus calcium intake was the faeces. Findlay, Paton, and Sharpe²⁵, Telfer²⁶, Sherman and Hawley⁵ agree with this finding, placing the urinary calcium at from 1 to 12 per cent. of the intake. MacRae⁶, working in this laboratory with children under 12 years, has found that the urinary output of calcium represents on the average 6.74 per cent. of the intake, and the control subjects of this series showed a similar distribution, 4.7 to 7.6 per cent. of intake.

As regards urinary phosphorus, Sherman and Hawley's figures ranged between 44 and 64 per cent. and both Telfer's and MacRae's averaged around 50 per cent. of the intake in normal children. The control subjects of this investigation excreted from 33 to 49 per cent. of intake in the urine.

It appears, then, that there is considerable variation in the urinary excretion, even in normal children. The average percentage of intake recovered from the urine approximates to 6 per cent. for CaO, and 50 per cent. for P₂O₅.

The figures in the nephritic subjects show very definite variation from the

normal. The average amount of calcium in the urine, for all 15 balance experiments, whether in the acute stages, during convalescence, or in the chronic forms of the disease, was 2.02 per cent. of the intake. The maximum output for the series was that shown by a chronic case, 3.9 per cent. of intake, i.e., well below the average of 6 per cent. in health. In no instance of acute nephritis did the urinary calcium output exceed 3 per cent. of intake, while in one subject it was found to be only 0.94 per cent.

Of the four repeated balance observations, there was apparent in three definite improvement in renal excretion of calcium at the time of the second observation (Table 6). In the fourth case the first period was observed during a phase of extreme diuresis which followed an attack of acute uræmia complicating a bronchiectasis. No satisfactory explanation for the decreased excretion during the later balance period could be found.

The behaviour of the urinary phosphorus was found to be roughly parallel to that of the calcium. From the average urinary content expressed in terms of intake it seems that the decrease is not so marked as in the case of calcium. The average fraction of phosphorus intake appearing in the urine was 32 per cent. as compared with the normal average of 50 per cent. Thus while only one-third of the normal quantity of calcium appeared in the urine more than half the normal amount of phosphorus was excreted by this route. It was noted that the range of urinary phosphorus excretion was smaller than in healthy children, being with one exception between 26 and 43 per cent. of the intake. The lowest figure, 8.4 per cent. of intake, was shown by a case of chronic nephritis shortly before a fatal pericarditis. The maximum urinary excretion of phosphorus did not occur in the child who showed the maximum calcium excretion. In fact, in all forms of nephritis there was evident more defect in the excretion of calcium than of phosphorus although even the latter only approached the normal proportion in two subjects, one of whom was convalescent.

Here, as in the case of calcium, the four repeated balance observations showed that urinary excretion of phosphorus had improved in the second period (Table 6). It was found that Case 5, which showed a decreased output of calcium, had a slightly increased urinary phosphorus, demonstrating again that there is no absolute parallelism between the two.

As was indicated in the section on calcium and phosphorus utilization, no relationship could be traced between the retentions and the figures for urinary excretion (Charts III and IV). Likewise the urine volume was not found to have any bearing on the quantitative excretion of calcium and phosphorus.

With one exception, previous workers on the serum phosphorus in nephritis have accepted the theory of defective excretion by the kidney. That there is justification for this is shown when the serum and urinary phosphorus contents are compared (Chart III). The lowest urinary phosphorus of the present series occurred in Case 4, which showed the highest serum level. Serum values within normal limits, however, did not indicate a normal distribution of the phosphorus excretion, and one must therefore conclude that although a high

serum inorganic phosphorus may occur coincidentally with a low urinary output of phosphorus, yet they are not directly interdependent. Looking at the urinary calcium excretion from the same point of view it is evident that with the lowest serum values there is found the lowest excretion (Chart II). But again, normal serum values do not foretell a normal urinary excretion of calcium and thus it seems, as in the case of phosphorus, that the renal output is not directly dependent on the serum level.

(2) **Fæcal output.**—In healthy children the proportions of calcium and phosphorus intake excreted in the fæces are fairly constant. From Sherman and Hawley's⁵ figures it has been calculated that the fæcal portion of the total phosphorus excreted averaged 37·7 per cent. MacRae's⁶ figures for normal children showed an average of 40 per cent. in the fæces, of the total phosphorus excreted; while the control cases of the present series gave an average of 43·5 per cent. Thus in 22 normal children the fæcal phosphorus amounted to an average of 39·2 per cent. of the total output. If this be expressed in terms of intake it appears that 30·3 per cent., i.e., about one-third, of the ingested phosphorus is eliminated by the bowel.

When corresponding calculations are made for the cases of nephritis it is evident that 62·3 per cent. of the excreted phosphorus is present in the fæces. There is, therefore, a relative increase in the fæcal content. If this be expressed in terms of intake, it is found that an average of 54·2 per cent., i.e., more than half, of the ingested phosphorus appears in the fæces, showing that there is also an absolute increase in fæcal content in nephritis.

A similar aberration from normal is evident in the fæcal excretion of calcium. The average fæcal content in healthy children was found to be 91 per cent. of the total output, representing 64·7 per cent., or almost two-thirds of the intake. In nephritic subjects these figures were increased. A larger portion of the total output, averaging 97·4 per cent. appeared in the fæces, thereby accounting for 78 per cent., or more than three-quarters of the intake. Thus, an increase, relative and absolute, is found in both the calcium and phosphorus contents of the fæces, of which the increase in phosphorus is relatively the greater.

This inequality of increase is more easily appreciated if the CaO to P_2O_5 ratio be considered. In MacRae's⁶ normal children and in the control series of the present study considerably more CaO than P_2O_5 was present in the fæces. The ratio in these subjects averaged 1·34 : 1. When the nephritic figures are examined, it is found that the fæcal CaO and P_2O_5 contents were more nearly equal, the average ratio being 1·08 : 1.

The four repeated balance studies (Table 6) seem to show that these abnormalities have some significance since they approached normal as convalescence proceeded. In one patient (Case 3) after an interval of four months, analysis showed increased output of calcium and phosphorus in the urine, decrease in the fæcal content, and rise in the CaO to P_2O_5 ratio. Balance studies repeated in three other subjects at shorter intervals showed that the urinary phosphorus increased in three, the urinary calcium in two, and fæcal CaO to P_2O_5 ratio in all three. The intestinal output of both calcium and

phosphorus, however, had increased to such an extent that negative balances were found, and it is worthy of mention that the only other negative balances were found in children during early convalescence, along with abnormally high faecal output, low urinary excretion, and low CaO to P_2O_5 ratio in the faeces. From this it would seem that the upset in calcium and phosphorus metabolism in renal disease is not confined to the more acute stages of the disease, but persists for some weeks after convalescence has begun.

TABLE 6.

COMPARISON OF THE FÆCAL AND URINARY EXCRETIONS IN THE ACUTE AND IN THE CONVALESCENT STAGES OF NEPHRITIS.

	Fæcal CaO as % of intake	Urine CaO as % of intake	CaO reten- tion per kgm. per day	Fæcal P_2O_5 as % of intake	Urine P_2O_5 as % of intake	P_2O_5 reten- tion in gram. per kgm. per day	Ratio fæcal CaO to fæcal P_2O_5
Case 3.							
Acute stage	6.85	1.8	-.054	49.1	27.8	-.058	1.01
4 months later; late con- valescent	62.7	3.5	-.048	40.7	33.8	-.053	1.04
Case 5.							
Acute stage	49.6	2.7	-.042	37.7	31.7	-.038	0.94
1 month later; convales- cent	73.2	1.7	-.023	55.3	32.5	-.014	1.01
Case 8.							
Acute stage	75.2	2.3	-.025	57.1	30.3	-.017	1.10
5 weeks later; convalescent	107.3	3.25	-.013	62.1	37.6	-.0003	1.17
Case 9.							
Acute stage	96.3	1.13	-.033	66.0	33.6	-.0008	1.04
3 weeks later; convalescent	98.4	2.68	-.0017	69.2	42.5	-.0225	1.06

Summary of the results of experiments in urinary and intestinal excretion of calcium and phosphorus.—1. The excretion by the urine of both calcium and phosphorus is diminished in nephritis, the calcium excretion being diminished to a greater extent than the phosphorus.

2. The amounts of calcium and phosphorus excreted in the urine by nephritic patients bear no relationship to their respective retentions nor to urine volume.

3. The faecal output of calcium and phosphorus is increased in nephritis.

4. The increase in the phosphorus excretion by the bowel is greater than that of calcium, the normal CaO to P_2O_5 ratio being reduced almost to unity.

5. The process of restoration of the normal route of excretion is extremely slow and is delayed for some time after the onset of convalescence.

General conclusions.

Studies of the retention, absorption and excretion of calcium and phosphorus in nephritis indicate that :—

- (a) retention is not abnormally high ;
- (b) absorption, though more variable than normal, is adequate ;
- (c) there is a deviation of both calcium and phosphorus excretion from the urine to the faeces.

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