THE METABOLISM OF CALCIUM AND PHOSPHORUS IN HUMAN PARATHYROID DEFICIENCY

by

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A THESIS

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INTRODUCTORY

HISTORICAL

Shelling, in his book,[1] divides the historical development of the physiology of the parathyroids into five periods: First, is the half century from 1835 to 1885, during which time thyroidectomies were done without cognizance of the existence of the parathyroids as a distinct anatomical entity. The second period is marked by the discovery of the external parathyroids by Sandstrom in 1881, and their rediscovery by Gley in 1885. The description of the internal parathyroids by Kohn in 1895 represents the third period. The fourth period is marked by the discovery in 1908 by McCallum and Voegtlin[2] of the relationship of the nervous disorders following extirpation of the parathyroids to the level of blood calcium. Finally, the present epoch in the understanding of the physiology of the parathyroids began when an extract from these glands was prepared which would raise the blood calcium level, and in so doing relieve parathyroprivic tetany. This was finally achieved in 1924 when Berman, Hanson and Collip[3] were able to show that their extracts would elevate blood calcium levels in normal and in parathyroidectomized animals.

EMBRYOLOGY, ANATOMY AND PATHOLOGY OF PARATHYROIDS.

The parathyroid glands arise as paired structures from the endoderm of the third and fourth branchial clefts. There are two pairs of glands. The superior pair develop from the

fourth branchial cleft, and the inferior pair develop from the third.

The superior parathyroids are fairly constant in their position on the medial aspect of the dorsal surface of each lobe of the thyroid at the junction of the upper and middle thirds. In man they are usually imbedded in the thyroid substance and are for that reason often called 'internal'. The inferior pair are situated more caudally on the dorsal surface of each lobe of the thyroid, are less constant in their position, and are usually external in that they are well separated from the capsule of the thyroid gland. The parathyroid glands are small, yellow-brown, usually oval but occasionally pyriform bodies. In man there are usually four, and sometimes five, of these bodies. In the adult, each body on the average weighs 0.035 Gm. and measures 6 to 7 mm. by 3 to 4 mm. by 1.5 to 2 mm. In addition, aberrant parathyroids occur not infrequently in the thyroid or thymus, or in the connective tissue between them. In all normal cases, the tissue of the parathyroids is separated by a connective tissue capsule from the contigous tissues.

The parathyroid glands are composed of densely packed groups of cells which are sometimes arranged in cords. Between the cells is a framework of reticular fibers and a richly anastomosing network of sinusoidal capillaries. Two main types (4) of epithelial cells have been described in it, principal cells and oxyphil cells.

The most important of these are probably the principal cells. Their clear cytoplasm practically never contains granules; these cells have a relatively large vesicular nucleus. It issaid that these are the only cells found in human parathyroids until about the age of ten years. In some cases the principal cells form a continuous mass while in other cases they form trabeculae separated by sinuses. Occasionally they may give rise to acini with a colloidal material in their lumen. These glands are subject to considerable change in number, position and size and weight. Instances of inflammation, fibrosis, idiopathic degeneration,(5) amyloid infiltration, hemorrhage, cystic degeneration, hypoplasia, tumefaction, malignancy and other pathological changes have been recorded in the literature.

PHYSIOLOGY

Although it was early known that parathyroprivic tetany was accompanied by a decrease in the calcium content of the blood,(2) it was also believed that other conditions were necessarily associated. Paton(6) and his colleagues thought that guanidine appeared in the blood following loss of the parathyroids and tetany was caused by the toxic effect of guanidine. Underhill(7) attributed the tetany following parathyroidectomy to hypoglycemia. Wilson, Thurlow, and Stearns(8) found an increase in the excretion of ammonia during tetany and considered that, after parathyroidectomy, the first stage was an alkalosis.

The development of the most recent concepts of normal and pathologic physiology of the parathyroid glands has followed the work of Albright,[5], [8], [9], [10], [11] Thompson and Collip[12] - and others.[13]

Albright[14] writes, "The parathyroid hormone has a marked influence on the metabolism of calcium and phosphorus. Its primary action, in my opinion, is on phosphorus metabolism and the changes in the metabolism of calcium are dependent on the preceding changes in the metabolism of phosphorus."

CALCIUM METABOLISM

The metabolism of calcium is considerably simpler than that of phosphorus. Over 99 per cent of the body's calcium is in the form of a calcium phosphate-calcium carbonate compound which is deposited in the organic matrix of bone and teeth. In addition, there are small amounts of calcium in body fluids. The calcium in the blood except for a negligible part is in two forms, calcium ions and calcium proteinate.[14] The parathyroid hormone apparently regulates the level of calcium ions. There is good evidence that the stimulus for the parathyroid glands to produce more hormone is a serum calcium ion level below normal. Even when calcium is absent from the diet, a considerable amount is excreted in the urine, since the normal serum calcium value (10.5 mg. per hundred cubic centimeters) is above the threshold (approx. 8.5 mg. per cent) for calcium excretion.

Calcium is also excreted in the feces. There is evidence that fecal calcium represents not only calcium which has not been absorbed by the gastro-intestinal tract but calcium which has been excreted into the intestinal tract. (15) The latter portion, however, is of small quantitative significance except in hyperthyroidism. (15) Two other routes of calcium loss from the body are the lactating breast and the placenta. Since the amount of calcium which can be held in the body fluids is small and quite constant, it follows that if the calcium intake is greater than the calcium output the balance must for the most part be represented by the calcium deposited in bones or possibly in teeth. The converse must be true if a negative calcium balance exists.

Under normal conditions both calcium deposition and calcium absorption from bone are going on at all times. One process, to be sure, may be more active than the other, depending on the calcium balance as a whole. There is no such metaplasia of tissue in the teeth.

PHOSPHORUS METABOLISM

Phosphorus, likewise, is found in large amounts in bones and teeth (calcium to phosphorus ratio, approx. 2 to 1), and in smaller amounts in body fluids. The normal blood level for adults is 3.5 mg. per 100 cc. of serum, plus or minus 0.7 mg. It is higher in children.

There are, in addition, in the body many organic phosphate compounds, such as phosphoprotein, phospholipids and various phosphate esters, which liberate phosphate ions on hydrolysis. Thus, a postive phosphorus balance does not necessarily mean that phosphates are entering the bones.

RELATION OF CALCIUM METABOLISM TO PHOSPHORUS METABOLISM IN DISEASE OF THE PARATHYROIDS.

If one stops substitution therapy with parathyroid extract in a parathyroidectomized patient, four cardinal metabolic changes occur. There is first an immediate decrease in the phosphorus excreted in the urine; second, the serum phosphorus level rises; almost simultaneously the serum calcium level falls; finally, with the fall in serum calcium there is diminished excretion of calcium in the urine. If one administers parathyroid extract to a normal person these same four metabolic functions are altered in the opposite directions; i.e., one obtains hyperphosphaturia, hypophosphatemia, hypercalcemia and hypercalciuria. There are two schools of thought as to the mechanism by which these four cardinal metabolic effects are mediated. One school holds that the hormone acts on bone tissue (osteoclasts) and tends to dissolve or otherwise remove calcium phosphate deposits from the bones (12). Such a theory might explain the hypercalcemia, hypercalciuria, and hyperphosphaturia; it does not explain the hypophosphatemia unless one hypothesizes some secondary adjustment.

The other theory is based on the hypothesis that the hormone affects phosphates in the circulating body fluids in such a way that their excretion in the urine is increased. This would explain the immediate hyperphosphaturia on administration of parathyroid extract and the resulting hypophosphatemia. Furthermore, because of the lowered level of serum phosphorus, it is contended that the serum would be less saturated with respect to calcium phosphate and there would be an increased tendency for calcium phosphate to enter the serum from the gastro-intestinal tract or from the bone. This would lead to hypercalcemia, and finally the hypercalcemia would lead to hypercalciuria.

Collip recently (18) contributed considerable evidence in favor of the theory that the action of the parathyroid hormone is primarily upon phosphate excretion by the kidney. He was able to show that parathyroid extracts produced no effects on the serum calcium in rats, cats, or dogs immediately following bilateral nephrectomy or ligation of the renal vessels or ligation of both ureters. Reestablishment of urine flow in one cat by cutting the ligated ureters was followed by a normal parathyroid hypercalcemia. During anuria induced by posterior pituitary extract no parathyroid hypercalcemia was noted. Injection of parathyroid extract, followed by repeated intravenous administrations of sodium acid phosphate to maintain a constant serum phosphate level, prevented the customary rise in serum calcium.

In rickets of the variety associated with spasmophilia there is a constant stimulus for increased parathyroid activity (hypocalcemia) with the result that the parathyroid glands become hypertrophied. It is believed that the hypertrophy occuring in rickets, osteomalacia, multiple myelomata, carcinomatous metastases to bone, experimental rickets and osteoporosis, is due to a secondary compensatory change in the glands resulting from disturbed calcium metabolism. This must be differentiated from osteitis fibrosa cystica, (Von Recklinghausen's disease of bone) in which the parathyroid tumor is primary and the disordered calcium metabolism secondary thereto.

In diseases wherein bone formation is faulty because of disorders in the mechanisms responsible for maintaining optimal levels of calcium and phosphorus, phosphatase levels are elevated. Normally the concentration of calcium ions and phosphate ions is such that the solubility product of calcium phosphate is approached but not attained. If the values given as normal are considered to represent the ion concentrations, (Calcium 10.5 milligrams per cent; phosphorus 4.0 milligrams per cent) the product of these values will normally be somewhat in excess of 40, numerically. Arbitrarily it is taken as a rule that if this product is less than 30, clinical rickets exists, between 30 and 40 rickets may exist, and, above 40 healing rickets may exist.

In the region of the osteoblasts the solubility product of calcium phosphate may be locally exceeded by the liberation of phosphate ion as a result of the action of phosphatase on serum organic phosphates. Normally, this mechanism is local, and since a condition of saturation of calcium phosphate is approached, no excess amount of phosphate needs be released to effect a precipitation of insoluble salts in the bone. In conditions wherein the product of the ion concentrations does not so closely approach the solubility product of the salt, this mechanism may compensate by increased phosphatase activity. Blood levels of phosphatase may be greatly increased due to the dissemination of this enzyme into the blood stream. In active rickets, characterized by failure of the bones to grow at the epiphyseal line, this mechanism has failed to compensate. It is significant that healing of rickets may take place at the epiphyseal line simultaneously with loss of mineral from the bones generally, due to a combination of antirachitic treatment and parathyroid activity.

REVIEW OF THE WORK OF ALBRIGHT

The basis for the evolution of these concepts follows in part from a series of metabolic studies by Albright and coworkers carried out on patients with hypoparathyroidism, rickets, etc., the essentials of the results of which will be reviewed here:

GRAPH 1 shows the results (8) of a typical study on a case of idiopathic hypoparathyroidism in a male. (14 year old Italian boy).

The effects upon the urinary excretion and serum levels of calcium and phosphorus following, first, the administration of parathormone and, second, changes in the phosphorus intake are noted. The following comments seem indicated:

1. The hypoparathyroid state is characterized by an essentially normal phosphate excretion, phosphatemia, hypocalcemia, and a very low urinary calcium excretion.

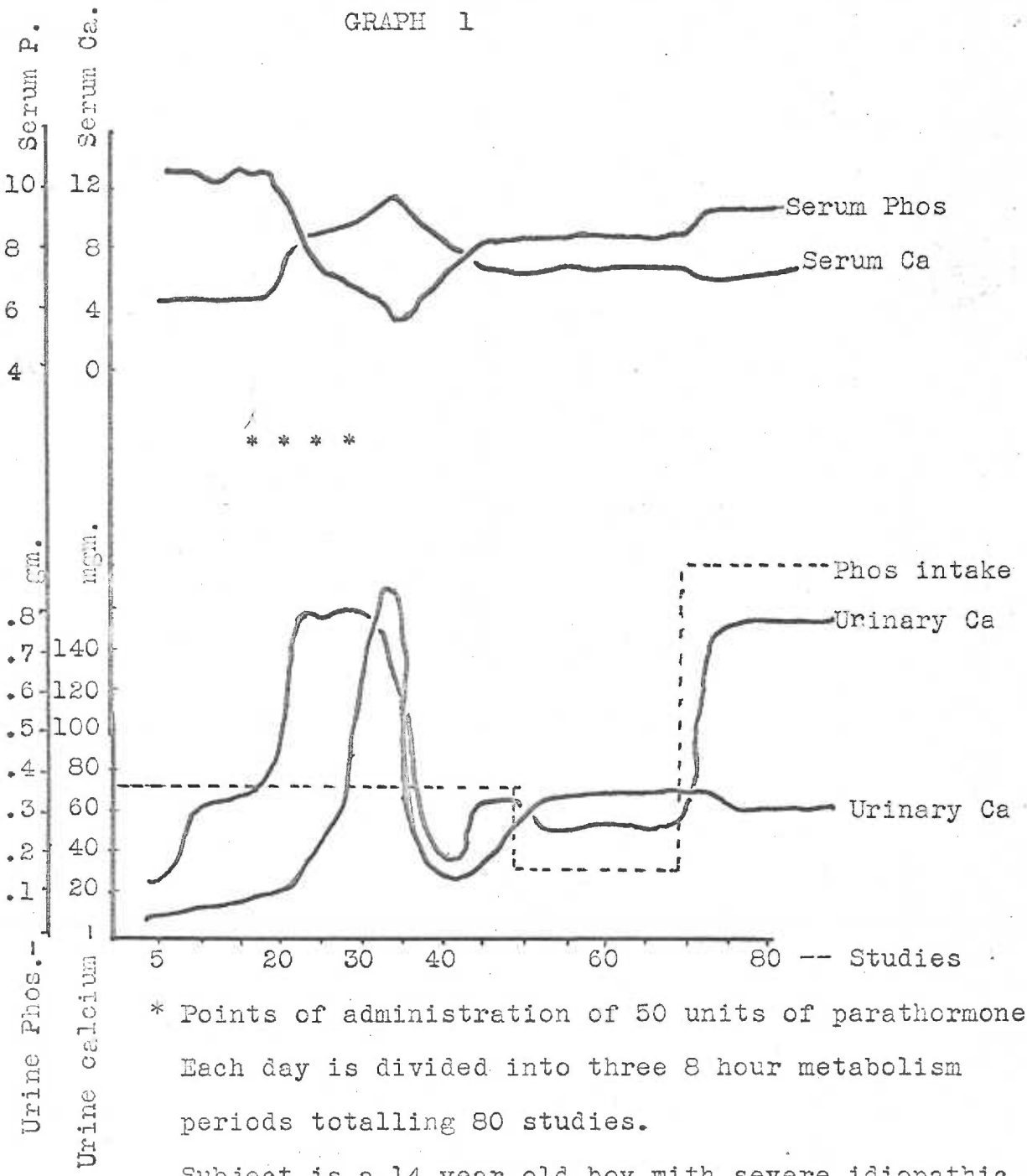
2. Administration of parathormone results in increased phosphorus excretion, lowered serum phosphorus, elevated serum calcium, and increased urinary calcium.

3. Cessation of parathormone is followed by a fall in phosphorus excretion, a rise in serum phosphorus, a fall in serum calcium, and a fall in calcium excretion. The latter is less precipitous than the fall of phosphorus excretion.

4. Decrease in phosphorus intake was followed by a decrease in phosphorus excretion, and no significant change in the serum level of either phosphorus or calcium.

5. Great increase in phosphorus (primary sodium phosphate) intake was followed by a marked increase in phosphorus excretion, and a slight tendency for serum phosphorus to increase and serum calcium to fall.

GRAPH 1



* Points of administration of 50 units of parathormone.

Each day is divided into three 8 hour metabolism periods totalling 80 studies.

Subject is a 14 year old boy with severe idiopathic hypoparathyroidism.

Serum levels are expressed in milligrams per 100 cc. of serum.

GRAPH II (10) shows the results of a metabolic study on a boy of 14 years who was suffering from "vitamin D resistant rickets". The six consecutive studies indicated were as follows: Study 1, low calcium diet; 11, identical conditions except that 5 grams of calcium lactate was given daily by mouth; 111, unchanged except that 0.575 gms. of phosphate was given daily by intravenous route; IV, same except that 600,000 units daily of vitamin D were added; V, intravenous administration of phosphate changed to oral administration; VI, vitamin D discontinued.

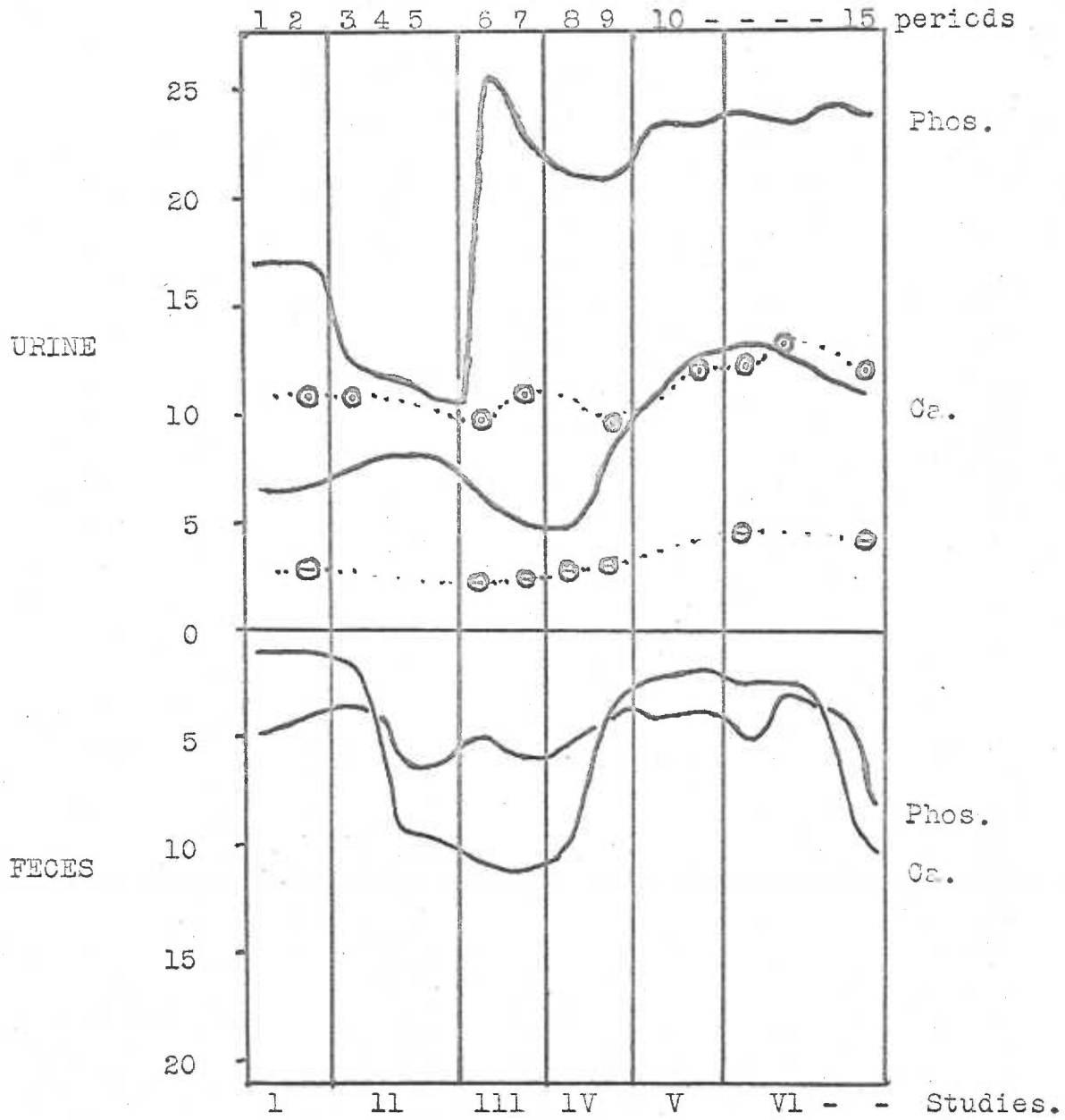
The remarkable result here is the fall in urinary phosphorus excretion on administration of large doses of vitamin D, and the subsequent rise when vitamin D was discontinued.

GRAPH III shows results on a patient with an extreme degree of hypoparathyroidism. The five studies indicated were as follows: I, low calcium, moderately low phosphate diet; II, same except that 1.63 grams of calcium (as gluconate) was given intravenously each day; III, same regime as study number 1; IV, 1.63 grams of calcium (as gluconate) by mouth; V, no change from previous regime except that 600,000 units of vitamin D (Viosterol) were given daily.

Note here that urinary phosphorus excretion fell about 300 mg. per cent following intravenous calcium,

GRAPH 11

METABOLIC STUDIES ON A PATIENT WITH RICKETS RESISTANT TO VITAMIN D THERAPY

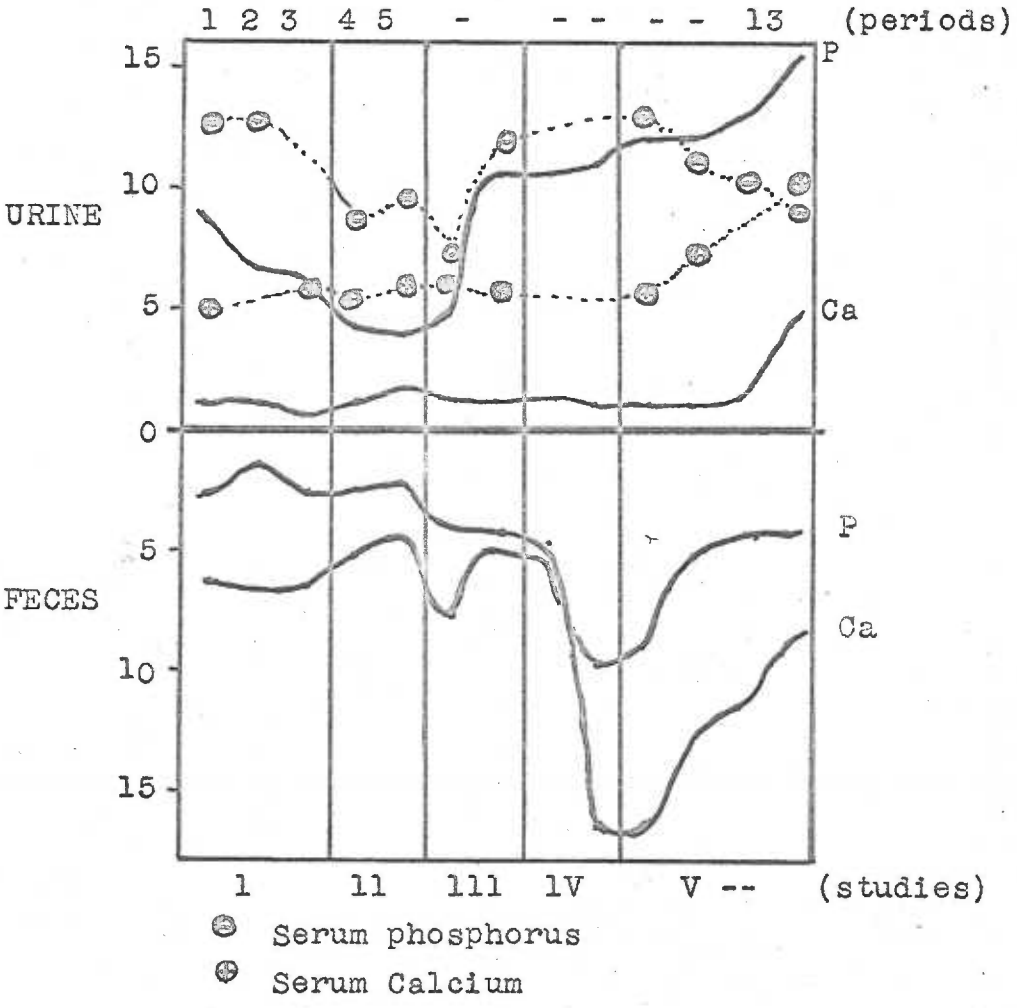


Distribution of calcium and phosphorus in the blood, urine, and feces in 15 consecutive 3-day periods. (see text)

Excretion is expressed in grams per 3-day period.

GRAPH 111

METABOLIC STUDIES ON A PATIENT WITH SEVERE IDIOPATHIC HYPOPARATHYROIDISM.



Distribution of calcium and phosphorus in the blood, urine, and feces in 13 consecutive 3 - day periods. (see text)

Excretion is expressed in grams per 3 - day period.

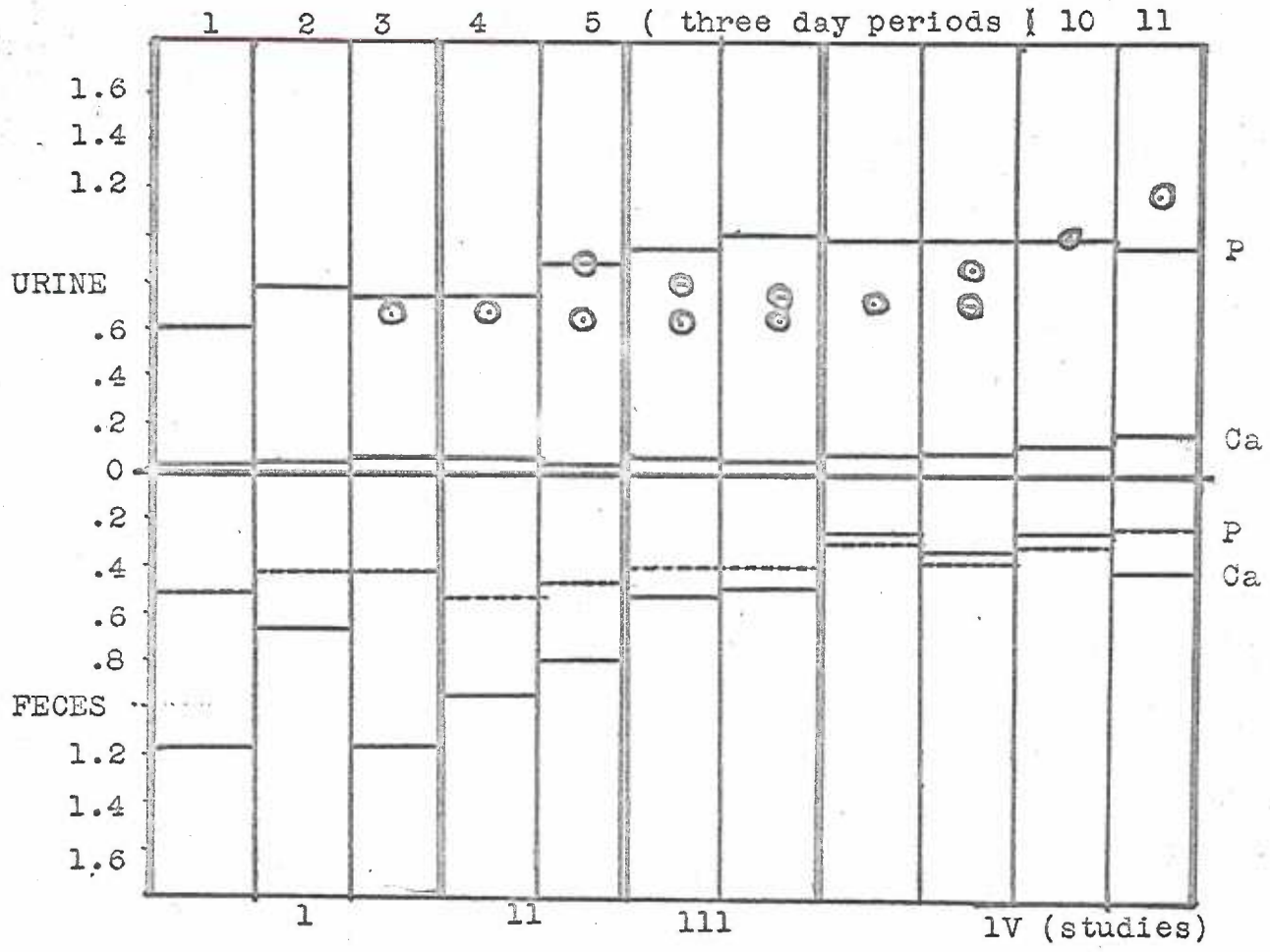
and rose following administration of vitamin D.

GRAPH IV shows results of study on another patient with severe hypoparathyroidism. This was done more clearly to evaluate the effect of vitamin D. The four studies indicated were as follows: I, control period with low calcium and moderately low phosphorus intake; II, 200,000 units of vitamin D daily; III, 400,000 units of vitamin D daily; IV, vitamin D discontinued. During this entire study the patient received 5 grams of calcium gluconate daily by mouth. Since there is no parathyroid activity in this case, the increase of urinary phosphate excretion following administration of vitamin D is intrepreted as an additional function of vitamin D.

GRAPH V shows results of studies (21) on a boy with severe hypoparathyroidism. A.T.10 tended to stimulate absorption of calcium from the gut, and stimulated excretion of phosphates in the urine. Vitamin D is the more potent in the former and A.T. 10 more potent in the latter effect. Obviously the effect of the vitamin D lasts much longer than that of the A.T. 10.

GRAPH VI shows subsequent studies, in which vitamin D, A.T.10, and parathormone were compared. (11) The subject here was not a hypoparathyroid but rather a case of chronic, "Vitamin D resistant" rickets. It will be noted that urinary phosphorus excretion was slightly depressed by vitamin D. From these metabolic studies and

GRAPH 1V



Serum phosphorus level.

Serum calcium level.

Subject is a patient with severe pypoparathyroidism.

Study includes 11 periods of three days grouped into four (1V) studies:

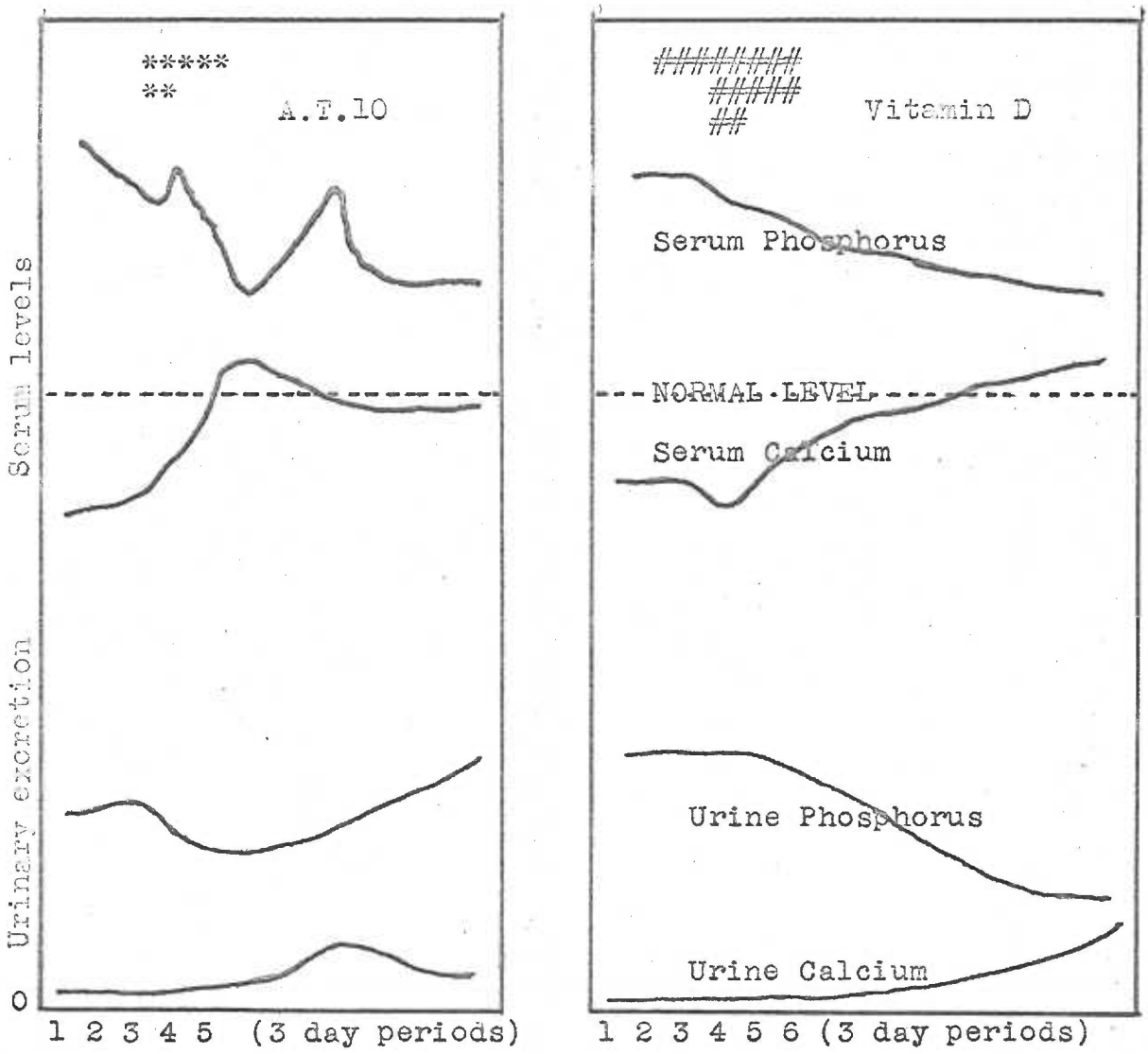
1. Control of period with 5 grams Calcium gluconate daily p.o.

11. Same except that 200,000 units of vitamin D is added.

111. Same except that 400,000 units are given.

1V. Vitamin D discontinued.

GRAPH V



** Administration of A.T. 10..

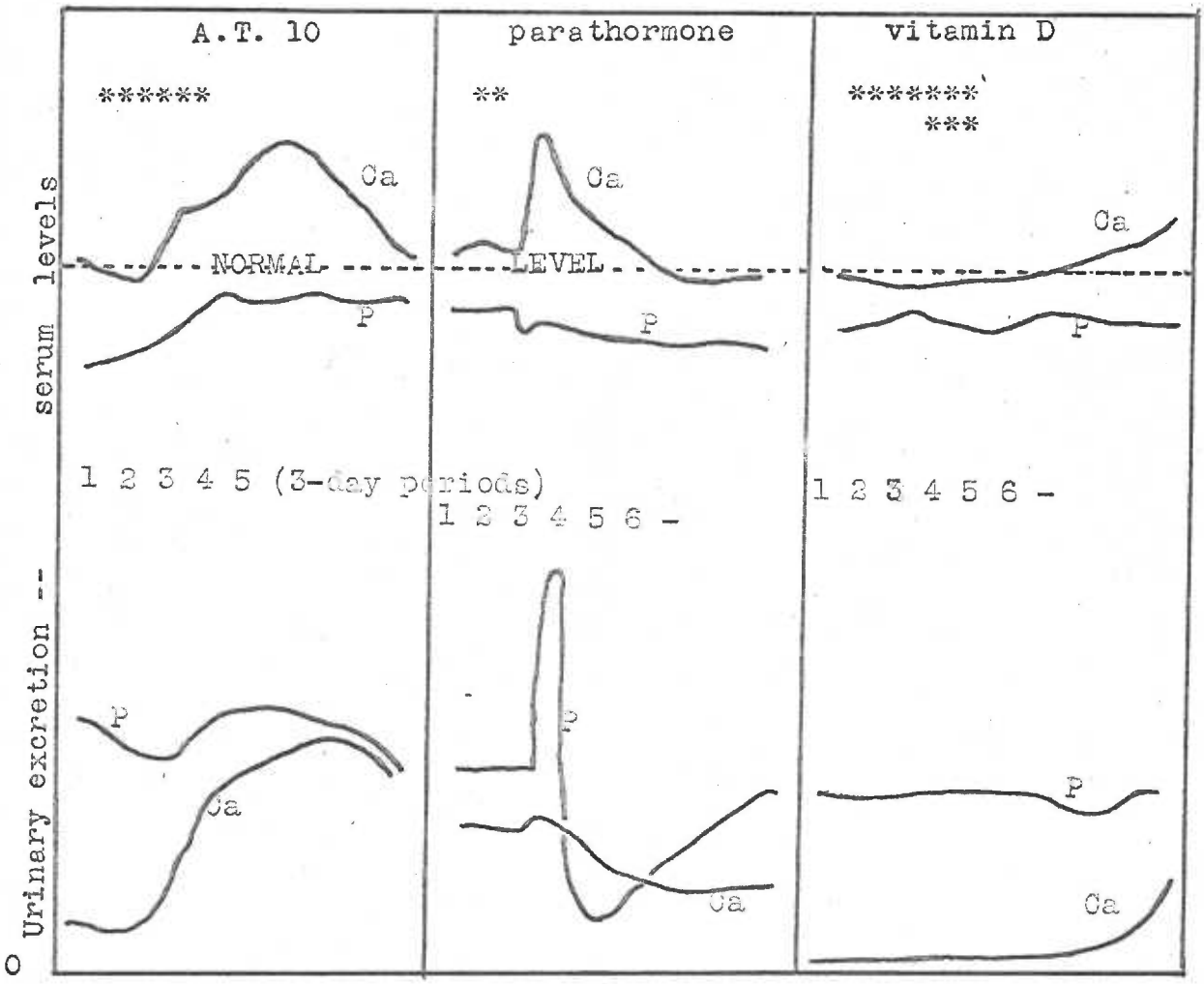
Administration of Vitamin D..

Comparison of the effects of Vitamin D and A.T. 10 on the metabolism of calcium and phosphorus in a patient with severe hypoparathyroidism.

Normal level in graph is arbitrarily taken as 10 mg. per cent for calcium, and 4 mg. per cent for phosphorus.

The curves showing urine phosphorus and urine calcium are entirely relative.

GRAPH VI



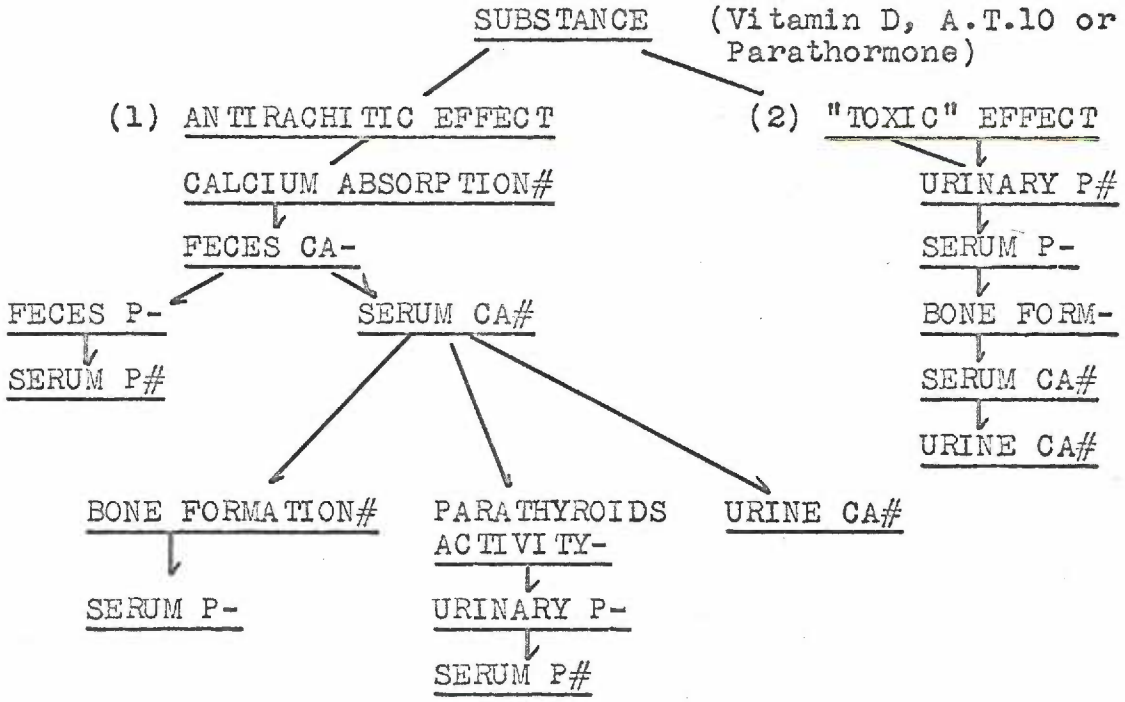
** Points of administration of substance.

Comparison of the effects of A.T. 10, parathormone, and vitamin D. The subject is a patient with vitamin D resistant rickets. Graphical representation here is the same as in graph V..

other similar studies the following concepts of the mode of action of these three substances upon the metabolism of calcium and of phosphorus may be formulated:

	(Antirachitic) Calcium absorption	(Toxic) Phosphorus excretion
Vitamin D	XXXX	X
A. T. 10	X	XXX
Parathormone	0	XXXXX

The inter-relations of the resulting alterations may be shown schematically as follows:



The figure '#' indicates increase and the sign '-' indicates decrease.

From this it may be seen that vitamin D and A.T.10 have at least two actions. The antirachitic effect following increased calcium absorption results in increased bone formation, while the so called toxic effect resulting from increased phosphorus excretion in the urine results in lessened bone formation or loss of mineral from bone. Whether these substances cause one or the other of these results depends upon which one of these two effects dominates. The healing of rickets with vitamin D administration represents the antirachitic or 'Calciuric' (17) effect, while the osteoporosis of hyperparathyroidism represents the other extreme.

THERAPY IN HYPOPARATHYROIDISM

Therapy in cases of hypoparathyroidism has in the past been limited to preventing tetany, or to maintaining the patient on a regime that would keep the blood calcium levels within a normal range. (20) The condition of the patient at any time may be evaluated by several clinical and laboratory tests:

(1) Laboratory determinations of serum calcium and phosphorus.

(2) The Chvostek sign.

(3) Erb's sign.

(4) The Sulkowitch test.

The patient is assumed to be bordering on tetany if the Chvostek sign is positive, in which case tapping the

cheek over the facial nerve causes rapid contractions of the muscles supplied by it.

Erb's sign is elicited by the use of galvanic currents, which reveals signs of hyperexcitability in the peripheral nerves. If these two signs are negative the patient is assumed to be safely out of the range of hypocalcemia associated with tetany.

The Sulkowitch test shows the presence of significant amounts of calcium in the urine and so indicates that the blood level is above the kidney threshold (8.5 milligrams per 100 cc.) and that the patient is accordingly not bordering on tetany (6 mg. per cent.). Conversely, absence of calcium in the urine as shown by this test is a strong indication that the patient is bordering on tetany.

The medications used (19) are as follows: Calcium salts, dihydrotachysterol (A.T.10), vitamin D, parathyroid extracts, acidifying preparations, and various combinations of these. In severe tetany intravenous administration of 5 to 20 cc. of 5% calcium chloride or 5 to 20 cc. of 10% calcium gluconate may be given. Also in 40 to 60 units of parathormone may be given intravenously. For the maintenance of patients with hypoparathyroidism the use of parathyroid extracts has been almost entirely abandoned in favor of A.T.10 or vitamin D preparations, both of which are used with calcium preparations by mouth. Repeated administration of parathormone results in the development

of an increasing tolerance for the drug. Since the hormone is itself a protein in nature, this is undoubtedly an immune body reaction involving the development of antibodies. A.T.10 is a derivative of cholesterol and is chemically related to vitamin D and, like vitamin D, is not antigenic and may be given over long periods of time without the development of tolerance.

EXPERIMENTAL

METABOLIC STUDIES IN THREE CASES OF HYPOPARATHYROIDISM

OBJECTIVES:

The objectives of the study may be listed as follows: (1) To repeat the work of Albright on the distribution of calcium and phosphorus in patients with hypoparathyroidism. (2) To study the alterations in excretion of calcium and phosphorus under various dietary and therapeutic regimes. (3) To determine the balance of calcium and phosphorus under various dietary and therapeutic regimes. (4) To establish, if possible, a therapeutic test to indicate the presence of active parathyroid tissue. (5) To arrive at an optimum therapeutic regime for the patient in question.

In view of the actions of the drugs used in patients with hypoparathyroidism, it is well within the realm of possibility for a patient to show normal blood levels of calcium and of phosphorus and be free of tetany, but be in a constant state of negative balance so far as calcium and phosphorus is concerned. Thus, after years of otherwise successful treatment a patient may develop osteoporosis, spontaneous fractures, and other symptoms of generalized bone decalcification. Therefore, it seems indicated that in any study of methods for handling these patients one should keep in mind the long time balance of

calcium and phosphorus as an essential part of the treatment. Furthermore, since each patient will show individual differences in absorption, amount of parathyroid tissue remaining, kidney function etc., it is suggested that every patient may require individual metabolic balance studies to arrive at an optimum regime of treatment that will not only prevent tetany and spasmophilia, but also maintain a positive balance of these bone building elements.

PROCEDURES AND METHODS:

Eighteen studies were made. All, except two, were five day studies, in which all the feces and all the urine were collected for five consecutive days. In each study the patient was brought into equilibrium by controlling and establishing a constant dietary and therapeutic regime several days before collections were started. In some cases large amounts of vitamin D were given to the patients. Since the effect of vitamin D is known to persist for about 30 days, at least a month was allowed to elapse following administration of vitamin D before subsequent studies were started. Determinations of both calcium and phosphorus were made on each 24 hour sample of urine and feces. Blood levels were not followed daily, but during each study at least one blood sample was taken, and calcium and phosphorus levels in the serum determined.

Analytical procedures were in most cases modified to better adapt them to the studies. In each case the method

was checked against known preparations to assure accuracy.

The steps in the analytical procedures used are as follows:

I. Urinary Phosphorus.

1. Pipette 25 cc. sample into digestion flask.
2. Boil out toluol. (2 minutes).
3. Add 4 cc. conc. nitric acid; boil to red fumes.
4. Cool.
5. Add 2 cc. conc. sulphuric acid; boil until red fumes are gone.
6. Cool.
7. Add 1 cc. perchloric acid (60%); boil 5 minutes.
8. Make up to 100 cc. in volumetric flask; shake.
9. Pipette 10 cc. of this into 100 cc. volumetric flask.
10. Add 40 to 50 cc. water; adjust PH to neutral.
11. Pipette 5 cc. of standard solution (containing 0.50 mg. phosphorus per 5 cc. solution) into another 100 cc. volumetric flask.
12. Proceed as in feces phosphorus determination.

II. Urinary calcium.

1. Shake up urine sample thoroughly.
2. Pipette 10 cc. of urine into a 15 cc. centrifuge tube.
3. Adjust pH acid to congo red.
4. Add 2 cc. of 4% ammonium oxalate. Adjust pH to 5.5.
5. Proceed as in procedure for feces calcium.

III. Preliminary preparation of feces:

1. Dilute sample to 1000 cc. with 10% sulfuric acid.

2. Take 50 cc. sample of this; neutralize with 40% NaOH.

3. Evaporate to dryness; powder well with pestle.

4. Mix with 4 grams of well powdered sodium nitrate.

5. Heat (hot) over Fischer burner until bleached.

6. Take up with about 30 cc. of HCl. (Use HCl made from one part of conc. HCl and two parts of water).

7. Dilute to 100 cc. in volumetric flask.

8. Filter. Filtrate is used for calcium and phosphorus determinations.

IV. Feces phosphorus.

1. Pipette 10 cc. of above filtrate into 100 cc. volumetric.

2. Adjust pH to neutral (pH 6.5 to 7.0).

3. Pipette 5 cc. of standard solution (containing 0.5 mgm. of phosphorus per 5 cc. solution) into another 100 cc. volumetric flask.

4. Add 40 to 50 cc. of water to each flask.

5. Add 10 cc. molybdate solution to each flask.

6. Add 4 cc. reducing solution to each flask.

7. Dilute to mark; compare in colorimeter after 10 min.

V. Feces calcium.

1. Pipette 10 cc. of above filtrate into 15 cc. centrifuge tube.

2. Add 2 cc. of 4% ammonium oxalate.

3. Adjust pH to 5.5 to 6.0.

4. After 2 hours centrifuge, invert, drain.

5. Wash down with dilute ammonia; stir.

6. Centrifuge, invert, drain.

7. Add 10 cc. 1.N sulphuric acid; place in boiling water bath.

8. Titrate with 0.01 N. Potassium permanganate while hot.

VI. Serum calcium. Total.

1. To 2 cc. of clear serum add 2 cc. of water and 1 cc. of 4% ammonium oxalate.

2. After two hours, centrifuge, until sediment is packed.

3. Decant off the supernatant liquid.

4. Invert tube on clean filter paper and allow to drain for five minutes.

5. Add 3 cc. of dilute ammonia, stir up with a glass rod.

6. Centrifuge, decant and drain as before.

7. Add 2 cc. of N. sulphuric acid. Stir.

8. Titrate with 0.01 N. Potassium permanganate.

VII. Serum calcium. Diffusible.

1. Place 6 cc. of clear serum in a high pressure filter. (200 lbs. per square inch pressure for 25 minutes).

2. Collect approx. 2.8 cc. of ultrafiltrate.

3. To 2 cc. of this filtrate add 2 cc. of water and 2 cc. of 4% ammonium oxalate solution.

4. Proceed as in total serum calcium determination.

VIII. Serum phosphorus.

1. Use 2 cc. of serum.

2. Add 8 cc. of 10% trichloracetic acid. Shake well.

3. Filter.

4. Place 5 cc. of filtrate in 15 cc. test tube.

5. Add 1.5 cc. of 2.5% ammonium molybdate in 3 N. sulphuric acid.

6. Add 0.6 cc. of reducing agent. Wait 10 minutes.

7. Compare with standard, which is made as follows:

Standard solution:

(a) Use 5 cc. of standard containing 0.5 mg. phosphorus per 5 cc.

(b) Add 10 cc. of 2.5% ammonium molybdate in 5. N. sulphuric acid.

(c) Add 4 cc. of reducing agent.

(d) Dilute to 100 cc. in volumetric flask.

The early phosphorus determinations were made by comparison with a standard in a comparison colorimeter. Later a Klett-Sumerson photo-electric colorimeter was used.

DESCRIPTION OF TABLE I

Three patients with hypoparathyroidism were studied.*

Subject "K" (J. K. #104209) is a 53 year old man with a moderate degree of idiopathic hypoparathyroidism. Eight studies were done and are referred to as K-1, K-2 etc.

Subject "S" (G. S. #38790) is a 36 year old woman with a very severe case of post-operative (thyroidectomy) hypoparathyroidism. This patient suffered tetany unless active treatment was maintained, and therefore was not suitable for many studies that were carried out on the other two subjects. Two studies were done and are referred to as S-1 and S-2.

Subject "C" (Z. C. #106403) is a 55 year old woman with a moderately severe hypoparathyroidism. She was the subject of eight studies, which are referred to as C-1, C-2.

* Multnomah County Hospital, Portland, Oregon.

The studies carried out are described in the chart (page 26) in which the following headings are listed:

Medication: The medication used.

Calcium diet: The daily intake of calcium (in grams)in the diet.

Calcium Lact.: The daily intake of calcium (grams) as lactate.

Calcium total: The sum of the above.

Calcium serum: The serum calcium level in milligrams per 100 cc. of serum. Sample taken some time during study.

Calcium bal.: The balance of calcium in milligrams. "-" indicates negative balance. No sign indicates positive balance.

Phosphorus diet: Phosphorus intake in the diet (grams).

Phosphorus serum: The serum level of inorganic phosphorus in milligrams per 100 cc. of serum.

Phosphorus balance: The balance of phosphorus in milligrams.

Date begun: The month and day of year 1940 on which the study was started.

COMMENTS

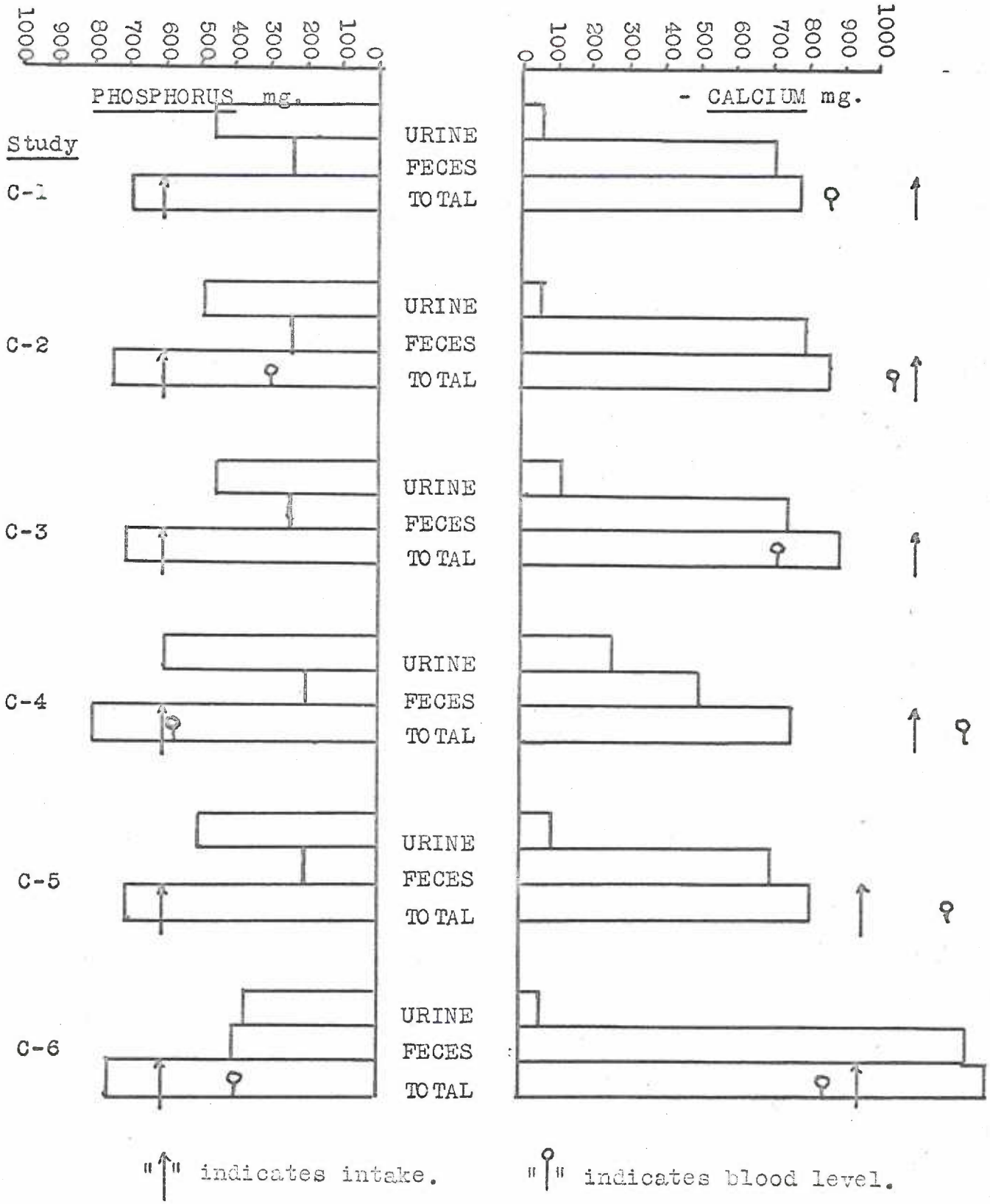
The ideal clinical treatment of a case of hypoparathyroidism should be reflected in the levels of calcium and phosphorus in the serum, their excretion in the urine and feces, and the balances as determined by comparing the total intake with total output. It has been pointed out in previous discussion that the characteristic thing about hypoparathyroidism is that calcium and phosphorus is predominantly excreted by the feces, and that the opposite condition of hyperparathyroidism is characterized by an

TABLE "I".

EXP.	MEDICATION	CALCIUM DIET	CALCIUM LACT.	CALCIUM TOTAL	CALCIUM SERUM	CALCIUM BAL.	PHOSPHORUS DIET	PHOSPHORUS SERUM	PHOSPHORUS BAL.	DATE BEGUN
C-1	Control	.45	.65	1.10	8.5	.335	.60	-	-.080	4/17
C-2	Parathormone 1cc. daily	.45	.65	1.10	10.0	.230	.60	3.0	-.150	4/26
C-3	A.T.10,1cc. daily	.45	.65	1.10	7.0	.220	.60	-	-.100	5/18
C-4	A.T.10,2cc. Daily	.45	.65	1.10	12.5	.340	.60	6.0	-.200	6/28
C-5	Control	.30	.65	.95	12.0	.140	.60	-	-.090	7/24
C-6	Low fat 200/80/20	.30	.65	.95	8.5	-.350	.60	4.0	-.150	8/1
C-7	A.T.10,1cc twice weekly	.30	.65	.95	10.5	-.160	.60	6.0*	-.320	8/27
C-8	Vitamin D, 100,000 daily	.30	.65	.95	12.0	.480	.60	-	-.175	10/19
K-1	Vitamin D, 100,000 daily	.15	.65	.80	10.8	.080	.30	4.5	-.150	3/25
K-2	High fat, 100/60/200	.30	.65	.95	-	.080	.60	-	.050	5/21
K-3	Low fat, 150/80/20	.30	.00	.30	-	.015	.60	-	.020	7/1**
K-4	A.T.10 2cc. daily	.30	.00	.30	9.0	-.050	.60	-	-.100	7/17
K-5	A.T.10 discontinued at start of study	.30	.00	.30	8.0	-.050	.60	3.8	-.020	8/26
K-6	Low fat, 150/80/20	.30	.00	.30	10.0	-.150	.60	4.0	-.120	8/31
K-7	Low fat, 150/80/20	.30	.00	.30	-	-.025	.60	-	-.070	9/11
K-8	Low fat, 150/80/20	.30	.00	.30	-	(See text)				9/16
S-1	A.T.10, 5cc daily	.15	.69	.84	8.5	.230	.30	2.5	-.100	2/20
S-2	Vitamin D, 400,000 daily	.15	.69	.84	8.5	.100	.30	-	-.180	3/25

* Checked. **Tetany on 3rd. day; Patient was given an indefinite, large amount of calcium gluconate and parathormone intravenously to relieve tetany.

FIGURE I



"↑" indicates intake. "♀" indicates blood level.

scale for blood level is above divided by 100.

FIGURE II

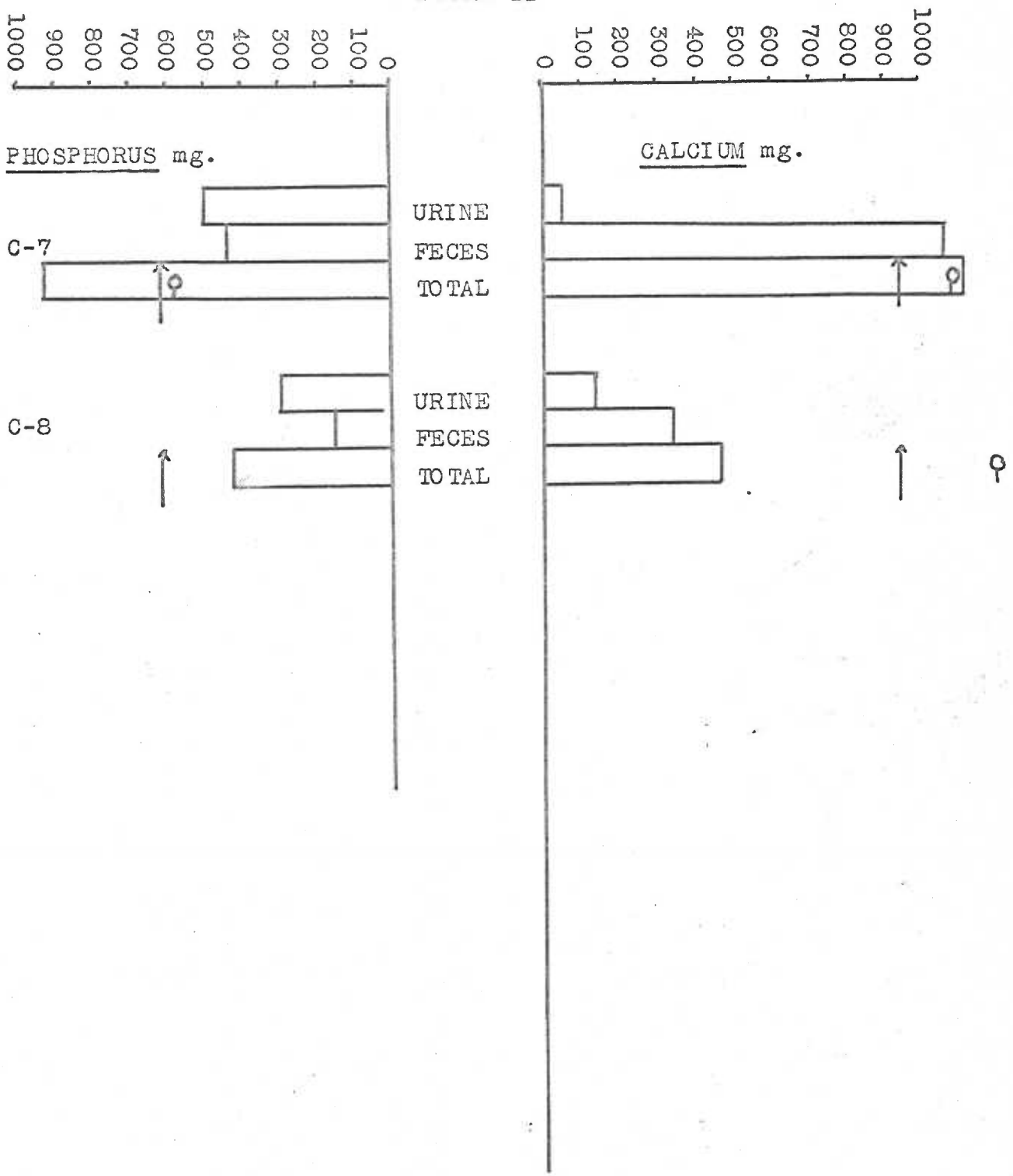

1000 900 800 700 600 500 400 300 200 100 0
PHOSPHORUS mg.
0 100 200 300 400 500 600 700 800 900 1000
CALCIUM mg.
C-7
URINE
FECES
TOTAL
C-8
URINE
FECES
TOTAL

FIGURE III

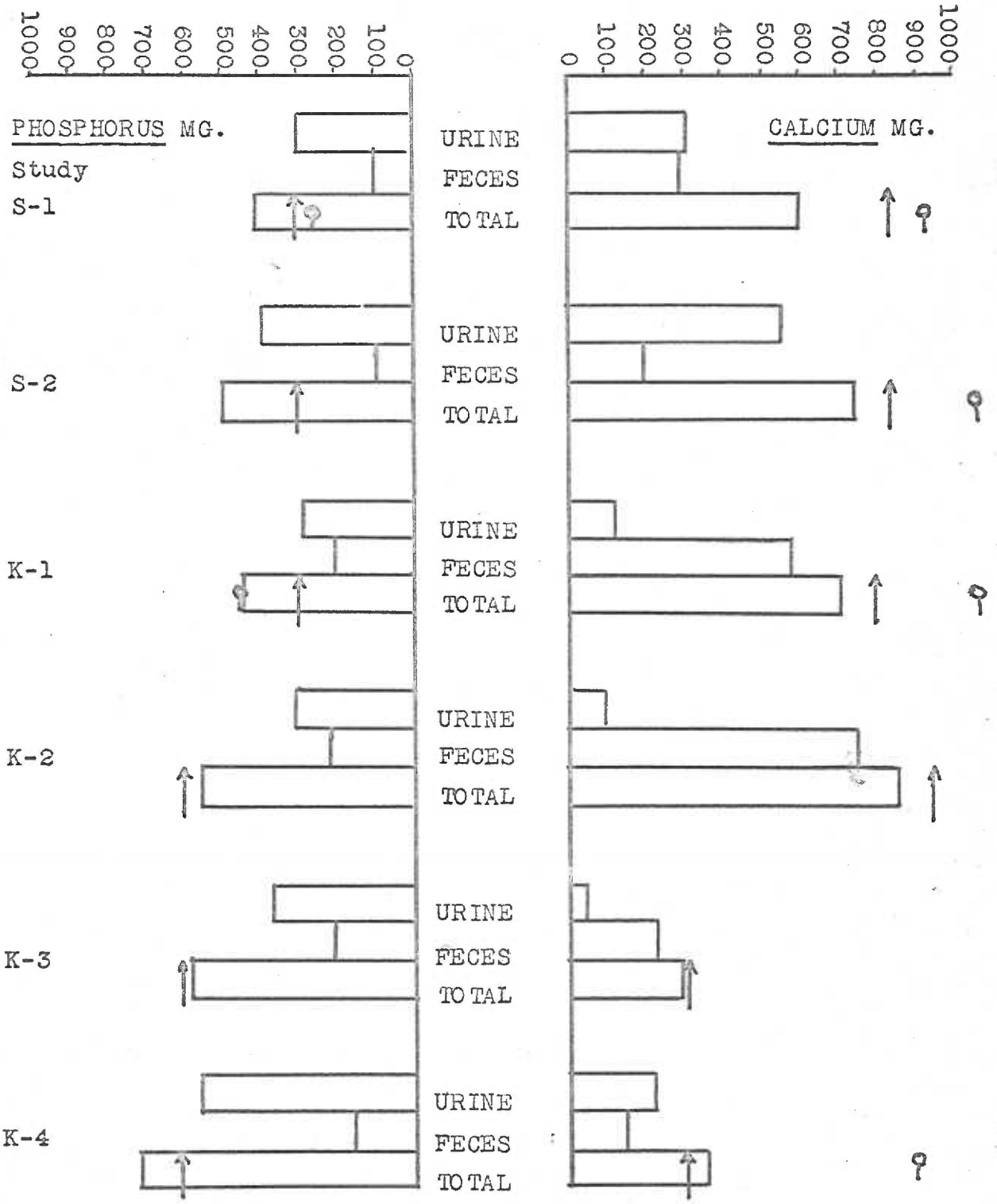

1000
900
800
700
600
500
400
300
200
100
0
PHOSPHORUS MG.
Study
S-1
S-2
K-1
K-2
K-3
K-4
URINE
FECES
TOTAL
0
100
200
300
400
500
600
700
800
900
1000
CALCIUM MG.

FIGURE IV

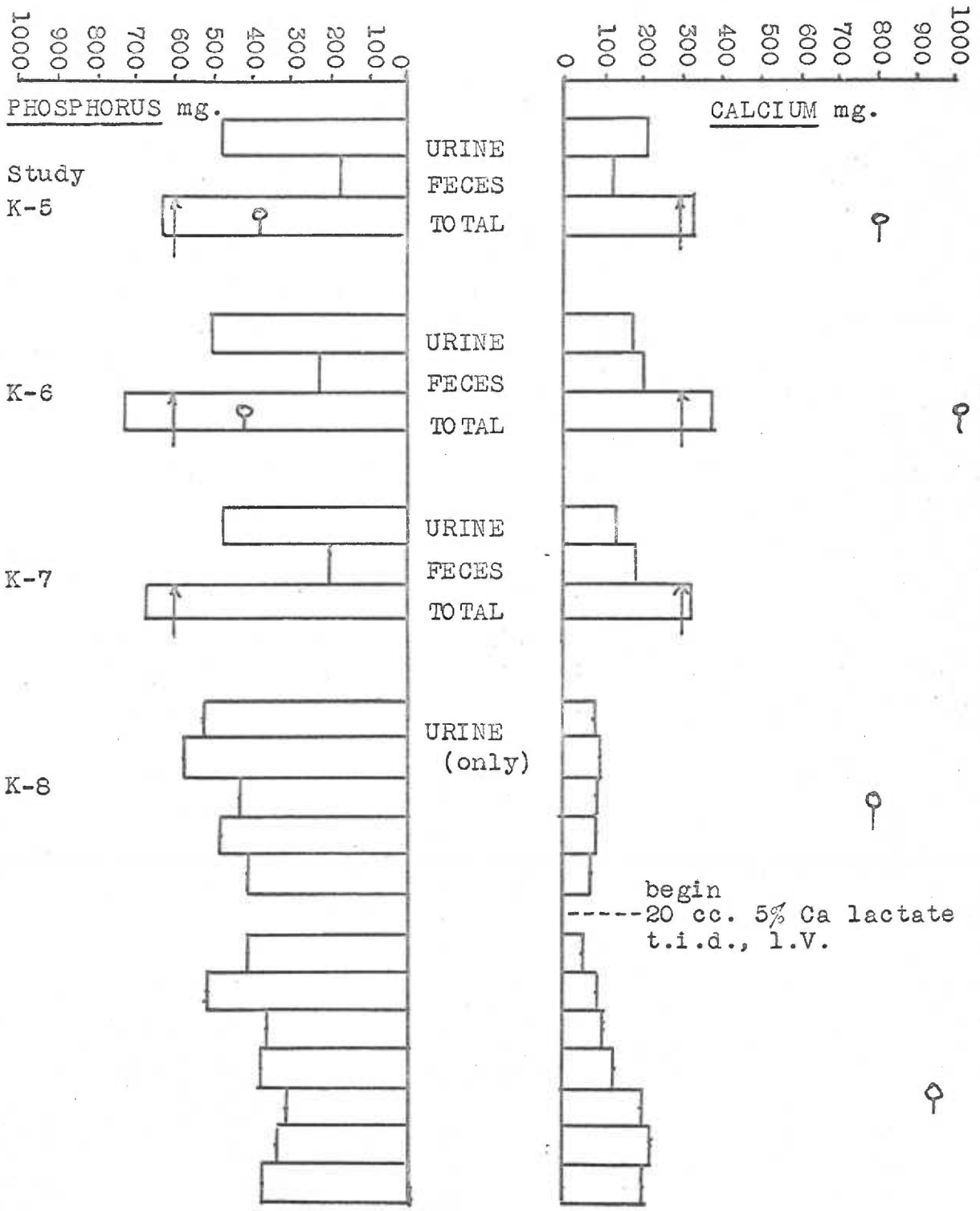

1000
900
800
700
600
500
400
300
200
100
0
PHOSPHORUS mg.
0
100
200
300
400
500
600
700
800
900
1000
CALCIUM mg.
Study
K-5
URINE
FECES
TOTAL
K-6
URINE
FECES
TOTAL
K-7
URINE
FECES
TOTAL
K-8
URINE
(only)
begin
-----20 cc. 5% Ca lactate
t.i.d., I.V.

TABLE II

		CALCIUM			PHOSPHOROUS		
S-1	Days	URINE	FECES	TOTAL	URINE	FECES	TOTAL
	1	306.	122.	428.	256.	51.3	307.3
	2	318.	260.	578.	326.	95.0	421.0
	3	323.	200.	523.	268.	78.5	346.5
	4	305.	328.	633.	318.	120.0	438.0
	5	305.	588.	893.	318.	165.0	483.0
	Total	1557.	1498.	3055.	1486.	509.8	1995.8
	Average	311.	299.	611.	297.	101.9	399.1
S-2	1	538.	108.	646.	393.	43.8	436.8
	2	579.	120.	699.	364.	74.0	438.
	3	594.	344.	938.	466.	119.0	585.
	4	520.	334.	854.	314.	114.5	428.5
	5	495.	154.	649.	355.	52.5	407.5
	Total	2726.	1060.	3786.	1892.	403.8	2295.8
	Average	545.	212.	757.	378.	80.7	459.2
K-1	1	153.	602.	755.	360.	178.	538.
	2	141.	502.	643.	465.	157.	622.
	3	101.	144.	245.	206.	93.	299.
	4	120.	693.	813.	186.	200.	386.
	5	89.	896.	985.	136.	262.	398.
	Total	604.	2837.	3441.	1352.	890.	2243.
	Average	121.	567.	688.	270.	178.	448.
K-2	1	99.6	196.	295.6	377.	68.5	445.5
	2	63.3	880.	943.3	257.	187.0	444.0
	3	57.3	368.	425.3	268.	135.0	403.0
	4	82.0	1650.	1732.0	388.	488.0	876.0
	5	68.1	668.	736.1	300.	232.0	532.0
	Total	370.3	3762.	4132.3	1590	1110.5	2700.5
	Average	74.0	752.	826.	318.	222.	540.
K-3	1	101.0	304.	405.	520.	283.	803.
	2	41.4	172.	213.4	276.	140.	416.
	3	43.5	196.	239.5	341.	180.	521.
	Total	185.9	672	857.9	1137.	603.	1740.
	Average	61.9	224	285.9	379.	201	580.
K-4	1	281.	161.	442.	658.	180	838.
	2	271.	132.	403.	675.	144.	819.
	3	185.	178.	363.	442.	198.	640.
	4	159.	112.	271.	410.	114.	524.
	5	187.	170.	357.	474.	123.	597.
	Total	1083.	753.	1736.	2659.	759.	3418.
	Average	216.6	150.6	367.2	531.	152.	683.

Each daily figure represents excretion in milligrams per 24 hour samples.

TABLE III

		CALCIUM			PHOSPHORUS			
K-5	Days	URINE	FECES	TOTAL	URINE	FECES	TOTAL	
	1	192	120	312	402	122	524	
	2	229	183	412	494	317	811	
	3	191	139	330	355	187	542	
	4	236	118	354	591	147	738	
	5	175	103	278	342	118	460	
	Total	1023	663	1686	2184	891	3075	
	Average	204	132	337	437	178	615	
K-6	1	126	208	334	407	226	633	
	2	212	178	390	525	252	777	
	3	283	195	478	535	351	866	
	4	158	182	340	504	158	662	
	5	107	223	330	515	172	687	
	Total	886	986	1872	2486	1139	3625	
	Average	177	197	374	497	228	725	
K-7	1	135	178	313	520	230	750	
	2	140	195	335	411	170	581	
	3	137	152	289	475	185	660	
	Total	412	525	937	1406	585	1991	
	Average	137	175	312	469	195	664	
K-8	1	79			527			
	2	105			570			
	3	85			430			
	4	80			472			
	5	78			415			
	6	60			410			
	7	89			520--began 20cc of 5%			
	8	111			360 Ca lactate, thrice			
	9	138			365 daily I.V.			
	10	183			315			
	11	211			340			
	12	205			395			
C-1	1	67	522	589	560	143	703	
	2	44	986	1030	456	341	797	
	3	48	580	628	435	169	604	
	4	29.6	886	915.6	376	300	676	
	5	39.2	650	689.2	500	210	710	
	Total	227.8	3624	3851.8	2327	1163	3490	
	Average	45.4	725	770.3	466	232	698	
C-2	1	58	1120	1178	587	334	921	
	2	87	600	687	500	181	681	
	3	41	642	683	420	182	602	
	4	48	600	648	600	193	793	
	5	27.2	1030	1057.2	402	286	688	
	Total	261.2	3992	4253.2	2509	1176	3685	
	Average	52.2	798	850.6	502	235	737	

TABLE IV

		CALCIUM			PHOSPHORUS		
C-3	Days	URINE	FECES	TOTAL	URINE	FECES	TOTAL
	1	91	1010	1101	372	268	640
	2	110	628	738	386	185	571
	3	97	440	537	476	146	622
	4	105	816	921	462	276	738
	5	139	912	1051	567	332	899
	Total	542	3806	4548	2263	1207	3470
	Average	108	761	869	452	242	694
C-4	1	274	756	1030	585	244	829
	2	234	570	804	622	171	793
	3	255	470	725	463	156	619
	4	258	356	614	553	152	705
	5	309	596	905	684	225	909
	Total	1330	2748	4078	2907	948	3855
	Average	266	549	815	581	189	771
C-5	1	92.5	1010	1102.5	555	220	775
	2	71.5	820	893.5	442	191	633
	3	80.3	296	376.3	430	214	644
	4	50.4	750	800.4	400	222	622
	5	93.2	575	668.2	514	200	714
	Total	387.9	3451	3840.9	2341	1047	3388
	Average	77.6	690	768.1	468	209	677
C-6	1	59.0	2780	2839.0	430	890	1320
	2	53.0	575	628.0	349	154	503
	3	42.0	376	418.0	184	130	314
	4	17.1	1065	1082.1	411	432	843
	5	12.6	988	1000.6	412	364	776
	Total	183.7	5784	5967.7	1786	1970	3756
	Average	36.7	1157	1193.5	357	394	751
C-7	1	44	815	859	558	362	920
	2	47	1344	1391	517	486	1003
	3	59	1605	1664	450	652	1102
	4	41	541	582	515	222	737
	5	52	1060	1112	451	453	884
	Total	243	5365	5608	2471	2175	4646
	Average	48	1073	1121	494	435	929
C-8	1	125	280	405	265	105	370
	2	130	525	655	280	210	490
	3	111	380	491	220	140	360
	4	155	295	450	310	147	457
	5	130	205	335	333	95	428
	Total	651	1685	2336	1408	697	2105
	Average	130	337	467	282	139	421

abnormally abundant excretion of these elements in the urine. A normal condition, and one to be attained in treatment, is then one of moderate excretion of phosphates and of calcium in the urine, a normal level of serum calcium and of serum phosphorus, and a condition of balance of these two elements, since this is incompatable with persistent loss of mineral from the bones. The blood level of calcium is reflected in the urinary calcium output, and the absorption from the gut is reflected in the distribution of urine and fecal excretion.

Study C-1, which is a control study with a moderately low calcium and phosphorus intake, shows a scanty urine calcium, a lowered serum calcium, and a poor absorption of calcium. The calcium, because of the greatly lowered excretion, remains in positive balance, but the phosphorus balance is negative. This fits the expected picture of hypoparathyroidism, and is clearly one in need of correction.

Study C-2, in which 1 cc. of parathormone was given daily shows only a slight increase in urine phosphorus, a slight increase in urine calcium, and a return to normal of serum calcium. The calcium balance remains positive, and the phosphorus balance remains negative. This treatment may be interpreted as one giving symptomatic relief only, since all that has been accomplished is the establishment of normal levels of calcium and phosphorus in the blood.

Study C-3, in which A.T.10, (1 cc. daily) was given shows a substantial increase in urinary calcium and a fall in feces calcium. The fall in serum calcium level may be disregarded, since it is probably an error. No improvement in balance of calcium or of phosphorus is noted. Probably this amount of A.T.10 is inadequate.

Study C-4, shows the marked improvement in giving larger amounts of A.T.10. Excretion of calcium in the urine is now well within the normal range. Absorption from the gut is efficient, and there is a substantial positive balance of calcium. Probably this would constitute good treatment except for the negative balance of phosphorus. Later it will be pointed out that this negative balance could have been corrected by administration of larger amounts of phosphorus in the diet.

Study C-5, a second control period without medication, shows a return to the picture seen in the first control period in study C-1. However, the blood level of calcium is not low, and the excretion of calcium in the urine is not as low as in the first control study. This is explained by the fact that A.T.10 was administered prior to the second control study, while parathormone was administered prior to the first control study, and that the action of A.T.10 is more persistant than that of parathormone and may well be exerting its effect during this study.

Study C-6 was done to determine the effect of a low fat diet. No medication was given. Calcium excretion in the urine and the serum calcium level are the same as in the first control study, which indicates that the subject has merely relapsed into the same metabolic picture seen in the first control study as far as the danger of tetany is concerned. Excretion of calcium and of phosphorus is substantially increased in the feces indicating poor absorption. This effect may be due to the lack of the cholegogue effect of fats and the consequent lack of bile acids in the gut. The negative balance of both calcium and phosphorus has increased by approximately the amount that the fecal excretion of them has increased. In other words the positive balance has decreased with the decrease in absorption from the gut.

Study C-7 shows the effect of very small amounts of A.T.10, which was given at the rate of one cc. twice weekly. The results are in all respects intermediate between that in the controls and in the studies, in which larger amounts of A.T.10 were given..

Study C-8 shows the effect of 100,000 units of vitamin D with no increase in the intake of either calcium or phosphorus. It should be pointed out that the calcium intake during this study was lower than occurs in most diets. All the criteria of good treatment are met in this study. The calcium excreted in the feces is at the lowest level seen in this series ofstudies. The serum calcium level is high as evidenced both by the determination, and by the increased excretion of calcium in the urine.

Phosphorus is efficiently absorbed and the excretion of phosphorus by the kidney is not excessive nor scanty. For the first time in the "C" series of studies the balance of both calcium and phosphorus is definitely positive.

Study K-1 corresponds to study C-8 in that 100,000 units of vitamin D was administered per day. The calcium intake was low and the phosphorus intake even lower. The serum calcium is in the normal range and urinary excretion of calcium is in the lower range of normal. The calcium balance is positive, but the phosphorus balance is negative, which fact may be due to the unusually low phosphorus intake. Apparently this subject requires more vitamin D than subject "C" to effect efficient absorption of calcium from the gut. It should be noted that this subject excretes approximately the same amount of phosphorus in the feces in all the "K" studies regardless of medication, phosphorus intake or phosphorus balance. Probably if the phosphorus intake was at a reasonable level in this study the balance would be positive and the patient could be maintained indefinitely under such a regime.

Study K-2 was done to determine the effect of a high fat diet. Unfortunately, since the intakes of both calcium and of phosphorus were increased, vitamin D discontinued, and no blood levels determined, very little can be learned from the changes that took place. Obviously, absorption is less complete, and in spite of this a positive balance of calcium and of phosphorus is maintained. This could have resulted only from the increased intake of these elements.

Study K-3 was done to determine the difference in the effect of a high fat diet and the effect of a low fat diet. This experiment also met with misfortune in that the standing orders for 5 grams of calcium lactate daily were discontinued by mistake with the result that the daily intake of calcium was reduced from 0.95 grams to 0.30 grams. No medication was given with the diet. The subject developed severe tetany on the third day of the study, and was given calcium gluconate and parathormone intravenously. No conclusions can be drawn as to the effect of the fat intake under the circumstances, but it is remarkable that this substantial decrease in the intake of calcium did not result in the development of a negative balance of that element.

Study K-4, in which A.T.10 was administered gave results exactly as in subject "C" in study C-4. Excretion of calcium and phosphorus was increased in the urine and decreased in the feces. Apparently, due to increased urinary excretion, the balance of both elements is slightly negative.

Study K-5 was preceded by over six weeks of therapy with A.T.10 which was stopped on the day that the study was started. The daily urine excretion of calcium and of phosphorus shows no tendency to diminish during the subsequent four days of the study.

Study K-6 is a continuation of the previous study with no change in the regime except that low fat diet used in K-3 was resumed on the first day. The object of the regime

was to subject the patient to the same conditions in which he developed tetany (study K-3), watch the urine excretion of phosphorus and of calcium closely with the Sulkowitch test as well as with urine analyses, in order to prevent the reoccurence of tetany, and note the changes which take place. It was not until the last days of this study, approximately a week after the A.T.10 was discontinued, that a significant fall in urine calcium could be detected. Study K-7 was started sixteen days after the A.T.10 was discontinued, and is a continuation of the preceding study with no interruption of above conditions. An additional small increment of decrease in urinary excretion of calcium and of phosphorus is seen, but no other change is significant. It may be seen from the last three studies, in which conditions were identical to those in K-3, that the subject did not develop tetany as in K-3 but that the urinary excretion of phosphates and of calcium tended to remain at a reasonably high level, and that the subject showed a very slow return to the picture of tetany seen in K-3. The regime of treatment leading up to K-3, in which tetany was precipitated, was characterized by a high fat diet plus five grams of calcium lactate per day over a period of about sixty days. In comparison, the regime leading up to the study extending through K-5, K-6, and K-7 was preceded by about six weeks of therapy with A.T.10. Apparently the action of A.T.10 persists for weeks after its use is discontinued. On the other hand

when a patient is maintained by administration of calcium lactate orally, without medication with A.T.10, sudden cessation of the calcium lactate will rather suddenly precipitate the syndrome of tetany. Probably in clinical management of these cases it would be wise to administer some A.T.10 along with the calcium lactate whether the salt alone is sufficient or not, in order to protect the patient by buffering the calcemia through periods during which the medication may for some reason be neglected.

Study K-8 was carried on immediately following K-7 and begins with a condition characterized by low urine calcium excretion. In this condition it is assumed that any existing parathyroid tissue will be stimulated to activity, cause increased urinary excretion of phosphates and a corresponding fall in serum phosphorus. It was attempted to demonstrate the existance of active parathyroid tissue in subject "K" at this time by giving large intravenous injections of calcium lactate. This should, by elevation of the serum calcium level, diminish the stimulus for parathyroid activity and result in decreased excretion of phosphorus in the urine. These changes should take place rapidly if the action occurs on this basis. The serum calcium was raised from 8.0 to 9.5, the urinary excretion of calcium was increased and the urinary excretion of phosphorus was decreased. However, it can not be contended that these changes are due to alterations in parathyroid activity.

The changes here developed slowly in a cumulative manner over a period of days. It is more likely that this represents merely a building up in the serum of a higher calcium level and the secondary changes resulting therefrom. Certainly, this can not be intrepreted as a test for the presence of parathyroid tissue.

Study S-1 was done to evaluate the action of A.T.10 (5 cc. daily) in a severe case of post-operative parathyroidism.

Study S-2 shows the effect of vitamin D (400,000 units daily). It is readily seen that vitamin D effects more efficient absorption of calcium from the gut and establishes a higher blood level of calcium than does A.T.10. This patient developed tetany rapidly when therapy was not vigorously carried out, and for this reason was not studied extensively. She was later discharged from the hospital and maintained on 200,000 units of vitamin D daily (Ertron caps q.i.d.) and five to ten grams of calcium lactate daily by mouth.

INTERPRETATION

Studies S-1 and S-2 indicate that vitamin D is more effective than A.T.10 in promoting absorption of calcium and of phosphorus. Also the blood level of calcium is maintained at a higher level with vitamin D therapy. In studies D-7 and C-8 these same changes are even more apparent. It will be noticed that with subject "S" large amounts of A.T.10 and especially of vitamin D were used

and that the balance of calcium and of phosphorus was better with A.T.10. On the other hand studies with subject "C", who has a much milder degree of hypoparathyroidism, much smaller amounts of medication were used, and the balance of these minerals was greatly improved by changing from A.T.10 to vitamin D. This is in accord with the accepted theories of the actions of these two medications. It may be suggested here that in severe cases of hypoparathyroidism the administration of excessive amounts of vitamin D in an attempt to control tetany may lead to a negative balance of one or both of these elements.

In studies S-1 and S-2 it will be seen that the phosphorus balance was negative and that the intake of phosphorus was low. In study K-1, likewise, the balance of phosphorus was negative and the intake low. When the phosphorus intake was doubled (K-2) the balance was restored to a positive level. It may be inferred from this that the phosphorus balance hinges definitely on the phosphorus intake. (It will be seen later that this does not follow in the case of calcium). It should be pointed out that throughout the entire "C" series with low phosphorus intake, the phosphorus balance remained negative until the last study, in which vitamin D was administered.

In study C-4 the administration of A.T.10 was doubled. As anticipated the urinary excretion of calcium and of phosphorus was increased and the feces excretion decreased. However, the already negative balance of phosphorus became

more severe while the balance of calcium improved. Apparently in the treatment of these cases with preparations which have the property of promoting excretion of phosphates in the urine, an adequate intake of phosphorus must be provided to insure continued positive balance of that element.

When the "K" series is compared with the "C" series, it will be seen that subject "C", while under reasonably adequate treatment, maintained a substantial positive balance of calcium, and that subject "K", regardless of treatment, failed to establish a satisfactory positive balance. When (K-3) the total calcium intake was changed from about 0.95 grams to about 0.30 grams by discontinuing the daily intake of 5 grams of calcium lactate, the amount in the feces decreased by almost this amount, and although tetany developed, a slight positive balance of calcium and of phosphorus was maintained. It is apparent, in this study, that the balance of calcium is not materially altered by the intake. When, in K-4, A. T. 10 was administered urinary excretion of calcium and of phosphorus increased, feces calcium and phosphorus decreased, serum calcium returned to normal, but the balances of these minerals became negative. This indicates that in the hypoparathyroid the ability to mobilize calcium and phosphorus from the bones, in the absence of an exogenous supply, is limited; that the

development of a negative balance follows increased urinary excretion, and is prevented, in the case of phosphorus, by adequate intakes. When C-6 and C-8 are compared it is seen that, in subject "C", absorption of calcium from the gut was greatly promoted by vitamin D. On the other hand, comparison of K-6 and K-1 reveals no such marked effect. Studies K-6 and C-6 were "low fat diet" studies in which no medication was given, and may be regarded as controls. It may be inferred from these observations that, first, a positive calcium balance depends on adequate absorption of calcium from the gut, and, second, that the dosage of vitamin D for one subject may be inadequate for another.

CONCLUSIONS

(1) The concepts of the physiology of the parathyroids as described by Albright and others are compatible with and are repeatedly confirmed by the results of these studies.

(2) Adequate intakes of calcium and of phosphorus are indicated as an adjunct to therapy with A.T.10, in order to avoid long continued negative balances of these elements.

(3) Vitamin D, unless given in extremely large doses, is more effective than A.T.10 in promoting positive balances of calcium and of phosphorus.

(4) Dietary alterations of fat intake (from 20 to 200 grams of fat per day) did not disturb the absorption of calcium nor of phosphorus to any marked degree.

(5) Substantial positive balances throughout this study are consistently associated with efficient absorption of these elements from the gut, whether this be effected by vitamin D or not.

(6) Probably prolonged treatment of a given case of hypoparathyroidism should be checked by studies of the nature described here to avoid negative balances, especially if large amounts of A.T.10 are used.

Esther O. Wells.

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