CALCIUM AND PHOSPHORUS METABOLISM IN CERTAIN EXPERIMENTAL AND DISEASED CONDITIONS.

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Contents.

T H E O R Y .

The elements calcium and phosphorus are among those that are essential to the life of mammals, and the study of their metabolism is a problem which presents many interesting aspects to the biochemist. The role of the parathyroid glands in the regulation of calcium and phosphorus metabolism, and the possibility of a relationship between the functions of parathormone and of vitamin D form the subject matter of this paper.

Normal Calcium and Phosphorus Metabolism.

1. Absorption and Excretion.

Adults normally exist in a state of calcium equilibrium, for the maintenance of which about 0.45 gm. of calcium per day is required.[1] This element is absorbed from the intestine, but owing to its lessened solubility, its absorption never approaches the completeness of that of sodium. Calcium is excreted in both urine and faeces, the proportion being about one-third in the urine to two-thirds in the faeces.[2] In infants, 90% to 95% is excreted in the faeces. Faecal calcium is a mixture of insoluble calcium soaps and $Ca_3(PO_4)_2$.

Phosphorus is excreted normally in the proportion of two-thirds in the urine to one-third in the faeces.[3] Urinary phosphorus is almost entirely inorganic.

Acidic conditions in the intestine favor the absorption of both calcium and phosphorus, by increasing the solu-

bility of calcium phosphate. The increased absorption is usually mirrored by an increased urinary output, in order to maintain equilibrium. Diets high in either element cause increased excretion of the other in the faeces through the formation of $Ca_3(PO_4)_2$.

In infants and growing children a positive balance of both calcium and phosphorus is normally maintained, in order to supply the needs of growing bone and tissues. Telfer[5] gives figures for the normal retention of infants, and shows that in rickets, which is characterised by faulty bone growth, the retention is lessened. No negative figures for retentions are found, even in marked rickets.

2. Blood and Bone Calcium and Phosphorus.

Serum normally contains from 10 to 11 mg. of calcium per 100 cc., while the red cells contain little or no calcium.[1] The serum calcium is present in three different forms:

(a) Non-diffusible, representing between 40% and 60% of the total calcium. This fraction is considered to be loosely bound by protein.

(b) Diffusible. This portion is inorganic.

(c) Ionic. So far no reliable method for estimating the amount of ionised calcium in serum has been devised.

Phosphorus is present in the blood in both organic and inorganic form. Normally plasma contains 4 to 5 mg. of inorganic phosphorus per 100 cc.[4]

It has been determined repeatedly, both theoretically and experimentally,[7,8,9,10] that blood contains in solution more calcium and inorganic phosphate than can be accounted for by the known solubilities of the calcium phosphates and of calcium carbonate, and by the union of part of the calcium with protein. The mechanism by which the extra calcium and phosphate is held in solution in the blood is not known; it may be through some property of the internal secretion of the parathyroid glands. Thus, although the actual calcium compounds present have not been determined, it has become convenient to speak of blood as being "physiologically" saturated with calcium phosphate.

Bone consists essentially of a deposition of some compound of calcium and phosphorus (together with smaller amounts of other elements) upon an organic matrix. Analysis of bone ash indicates a remarkable constancy of composition, and although a great deal of work has been done upon this problem, the exact nature of the inorganic part of bone has not yet been determined definitely. Sheer and Kramer's contention that it consists of a mixture of $CaHPO_4$, $Ca(OH)_2$ and $CaCO_3$, receives no support from the physical evidence of Taylor and Sheard[11] that no $CaHPO_4$ is present. Klement and Morgulis, seperately, propose two different complexes of $Ca_3(PO_4)_2$ and $Ca(OH)_2$, while physical evidence points to a structure similar to that of the apatite minerals. Thus, Roseberry, Hastings and Morse[12] represent the inorganic part of bone by the formula $Ca_3(PO_4)_2.n\ CaCO_3$ where n is between 2 and 3.

Like the actual structure of bone, the mechanism whereby the inorganic material is laid down upon the organic matrix is very much in dispute. The work of Robison and his associates[10] has led to a very reasonable theory of ossification. Robison has demonstrated the presence of an enzyme, phosphatase, in cartilage. This enzyme hydrolyzes organic phosphates, liberating inorganic phosphate. There has accumulated a good deal of evidence tending to connect bone phosphatase with calcification, and Robison has proposed the following theory of its function. At the site of calcification, the bone phosphatase hydrolyzes some of the organic phosphate esters of the blood, liberating inorganic phosphate. The phosphate concentration in the blood is thus increased, and the effective solubility product of calcium phosphate in the blood is exceeded. The result is deposition of solid calcium phosphate in the bone. There is a considerable amount of evidence supporting this theory.

It is interesting to note that the phosphatase theory of calcification emphasizes the primary importance of phosphorus, rather than calcium, in bone formation. It will be apparent, as other phases of calcium and phosphorus metabolism are examined, that this tendency is increasing in almost every branch of the subject.

The Parathyroids and Calcium and Phosphorus Metabolism.

The parathyroid glands have been shown by Collip to elaborate an internal secretion, which he has named "parathormone". The effects of active extracts of the

glands have been studied by several investigators, and the relation of parathormone to calcium and phosphorus metabolism has been ably reviewed by Thomson and Collip.[14]

1. Effect of Extract on Normal Animals.

Large doses of parathormone produce in normal animals an extreme hypercalcaemic reaction of the serum, with a characteristic series of symptoms leading to death.[15] There is a striking increase in the viscosity of the blood; anorexia, vomiting, apathy and even coma may be present, and in the premortal stages there is a marked rise in serum phosphate, blood urea and non-protein nitrogen, while chloride and bicarbonate fall.

Smaller doses have a different effect, which has been analysed by Albright and his associates.[16] The first action is to sweep out phosphorus in the urine; later serum phosphate falls, probably due to urinary loss. Serum calcium then rises in response to the fall in phosphate, and urinary calcium increases due to the hypercalcaemia. The total result is a loss from the body of calcium and phosphorus, which is found to be in the same ratio as these elements are present in $Ca_3(PO_4)_2$. Thus the chemical evidence indicates that the bones have been indirectly decalcified, as a result of the series of events following the initial phosphaturia. Since, however, large doses of parathormone cause a rise in both calcium and phosphate of the blood, as mentioned above, parathormone must have the power to decalcify the bones directly.

Albright[16] has shown further that if parathormone is administered over a long period of time to a normal animal, it loses its effect, and normal excretion of calcium and phosphorus is resumed. Since in an animal thus "immunized" to the action of the hormone, administration of acidic substances will still cause a marked increase in the excretion of both calcium and phosphorus in the urine, it must be concluded that parathormone is capable of mobilising only a certain fraction of the lime salts of bone.

In view of the chemical evidence pointing to the decalcification of bone by parathormone, histological studies of such bones have been made by several investigators. Johnson and Wilder[17] found that repeated injections of parathormone produced, in puppies and in young rats, the typical picture of osteitis fibrosa cystica (von Recklinghausen). Lacunar resorption of trabeculae and corticalis of bones was noted, with replacement of marrow and cortex with fibrous connective tissue and osteoid tissue. Multinucleated giant cells (osteoclasts) appeared and cysts were formed.

Jaffe, Kodensky and Blair[18] produced a similar picture, without the osteoid tissue, in young guinea pigs, by means of a single large dose (20 units per 100 gm. wt.) of parathormone. The changes did not appear until 12 to 24 hours after the dose was given. In another experiment they found that osteoid tissue could be obtained by means of smaller doses at repeated intervals.[19]

Selye[20] injected 10-day rats, intraperitoneally,

with 5 units of parathormone daily for three days, and then changed to 10 units daily. He found their growth retarded. About the sixth day, bowing of the legs appeared. Only sections that had not been decalcified were examined at autopsy. These showed no calcium in the zone of provisional calcification, and marked hyperaemia in the zone of primary marrow formation. Below this, nearly all the bone consisted of eosinophilic osteoid tissue, the cause of the bending. At the point of bending the periosteum invaded the marrow cavity, and there a fibrous marrow developed. This he considers to corroborate the theory that osteitis fibrosa is a secondary effect, due to the bending of the bones, and not a primary effect of parathormone itself.

Selye[54] has shown, further, that continued administration of sub-lethal doses of parathormone produces first, osteitis fibrosa cystica, and later, a stimulation of new bone growth and even of new calcium deposits, so that a condition very similar to marble bone disease is the final result. He used 5-day rats, giving them 5 units daily, the osteitis fibrosa developing in 5 days. He has demonstrated also that very small doses of parathormone stimulate osteoblast formation and bone apposition. This action of small doses is very probably the normal action of the hormone.

2. Parathyroidectomy and Parathormone.

It has been shown by Hastings[21] and others that, after removal of the parathyroid glands, serum calcium falls and inorganic phosphate rises, while the excretion of both elements in the urine is diminished. When the serum calcium falls to a concentration of 7 mg. per 100 cc., or less, a

condition of excessive irritability of the motor nervous system, known as tetany, sets in. The chemical changes can all be reversed or prevented, and tetany relieved, by the administration of an active extract of the parathyroids. Evidently a sufficient supply of the parathyroid secretion is necessary to maintain blood calcium at a normal level. The fall in urinary excretion of calcium and phosphorus may also be taken to be due to the insufficiency of parathormone resultant upon the removal of the parathyroid glands.

The tetany accompanying parathyroidectomy may be relieved temporarily, and the serum calcium raised, by the administration of calcium salts. Thus it appears that this type of tetany is a direct result of the low serum calcium.

3. Clinical Parathyroid Disorders.

Hypoparathyroidism may occur clinically as the accidental result of thyroidectomy, or it may occur as an idiopathic disease. The symptoms, as might be expected, are hypocalcaemia, hyperphosphataemia, diminished output of calcium and phosphorus in the urine, and active or potential tetany. The condition may be relieved by the administration of parathormone.

Hyperparathyroidism occurs most frequently as the result of a tumor of the parathyroids. It was first described accurately by von Recklinghausen, and is generally termed "von Recklinghausen's disease", or "generalised osteitis fibrosa". The chemical symptoms are hypercalcaemia, hypophosphataemia, and increased urinary output of calcium and phosphorus. Muscular weakness and hypotonia are accompanied by pain in

the joints. There is present a progressive and uniform skeletal decalcification, which in long-standing cases may lead to severe deformities. Operation and removal of the tumor generally results in a return to normal, although the sudden drop in serum calcium sometimes gives rise to symptoms of tetany, which may be controlled by parathormone until equilibrium at the new calcium level is established.

Some cases of hyperparathyroidism have been reported, due to hyperplasia of the parathyroid glands, in which removal of part of the glandular tissue has corrected the condition.

The recognition of the true cause of von Recklinghausen's disease has been the result mainly of the experimental work on parathormone. The symptoms of this disease, as described above, are the same as those produced in animals by repeated dosage of parathormone. This has been emphasized particularly by Johnson and Wilder[17], who have shown that the uniform skeletal abnormalities of von Recklinghausen's disease may be reproduced in puppies and in young rats by means of daily injections of parathormone. Since simultaneous administration of vitamin D did not modify significantly the end result, they concluded that von Recklinghausen's disease in man is due to a hyperfunction of the parathyroid glands, and has no relation etiologically either to osteomalacia or to rickets. They have shown further, that a dosage of 50 units of parathormone per day, given a normal human subject, produced an increased urinary output of calcium and phosphorus, an increase in serum calcium and decrease in phosphate, weakness, and pain in the joints. This

is considered as sufficient evidence that the effect of parathormone upon the human subject is the same as its effect upon animals, and confirms the authors' contention as to the cause of von Recklinghausen's disease. The number of cases which have been treated successfully by surgery constitutes the final proof.

Rickets.

1. Calcium and Phosphorus Metabolism.

Rickets is a disease, occurring usually in infants or in very young children, which is characterized mainly by deficient calcification of bone. This leads to softening and bending of the bones, and to enlargement and deformity of their growing ends. Two types of rickets are known:

(a) The type in which the serum calcium is normal but the inorganic phosphate low. This type is the more common.

(b) The type in which the serum calcium is low and the phosphate normal. In this group, tetany may accompany the rickets.

Rickets may be produced experimentally in animals by diets deficient in either calcium or phosphorus, especially if the diet contains a deficit of one and an excess of the other. This has been shown to be due to the fact that, under such conditions, the element in which the diet is deficient is largely removed in the faeces as solid $Ca_3(PO_4)_2$, thus depriving the animal of its necessary supply for bone building. Telfer[5] has shown that, in rickets in children, there is an increased faecal excretion of calcium and phosphorus, and hence a diminished retention of these elements by the

body.

Recent experimental work upon "beryllium rickets" in rats is interesting in this connection. It has been shown by Kay and his co-workers[23,24] that if a small amount of beryllium carbonate is added to the adequate diet of a growing rat, the animal develops severe rickets, in which the plasma phosphate may fall as low as 0.3 mg. of phosphorus per 100 cc. Beryllium phosphate has no effect whatever. The results are explained by the fact that, in acid solutions, beryllium forms an insoluble phosphate; thus, beryllium carbonate, dissolved in the acid contents of the stomach, precipitates available phosphorus as the insoluble phosphate, which is removed in the faeces. Rickets then results from the phosphate deficiency. This work illustrates once again the tendency of present-time research to give to phosphorus the place of primary importance in calcium and phosphorus metabolism .

2. Vitamin D.

It has been shown that most children with rickets are receiving diets entirely adequate in calcium and phosphorus, and that the faulty bone formation is the result of an insufficiency of vitamin D. Administration of the vitamin will increase the retention of calcium and phosphorus, restore the blood levels of these elements, and cure the bone lesions. Thus, the action of vitamin D is to promote the retention of calcium and phosphorus by the body; this is generally believed to be accomplished by an increase in the abs-

orption of these elements from the intestine. Limited improvement in rickets may be obtained by other measures that promote calcium absorption, such as the administration of acid or of acid-forming substances.

The exact mechanism by means of which the vitamin acts upon calcium and phosphorus absorption is not known.

Hypervitaminosis D.

Moss and Lewis[25] have shown that extremely large doses of vitamin D in the form of irradiated ergosterol cause hypercalcemia, hypercalcification of the skeleton, and pathological metastatic calcification in the soft tissues. With even larger doses, the shafts of the long bones are thinned, and pathological calcium deposits appear at the epiphyses. There is a negative calcium balance, with increased urinary excretion of calcium. Large enough doses are toxic, animals dying apparently from the hypercalcemia.

Jones and Nepoport[26] have shown that the hypercalcemic effect of large doses of the vitamin is exaggerated by the simultaneous administration of soluble calcium salts by stomach tube. This is what would naturally be expected to occur.

It has been observed[25] that metastatic calcification due to large doses of vitamin D takes place only when the serum calcium is falling, after the initial rise. This the authors consider to indicate that the state of serum calcium is different during the fall. This assumption seems to me to be unnecessary; if calcium were deposited during

the rise, such a deposition would remove calcium from the serum and so prevent any increase in its concentration. Since the increase in concentration does occur, calcium is deposited only when a certain blood level is reached; deposition then causes the fall in serum calcium.

Tetany.

1. Types of Tetany.

Reference has already been made to the tetany of parathyroidectomy and of hypoparathyroidism, which is due to the low serum calcium resultant upon the insufficiency of the parathyroid hormone. The tetany which sometimes accompanies low calcium rickets may be considered also to be the result of the level of serum calcium.

It has been shown, in addition, that typical tetany may be produced by hyperventilation[27], by continuous removal of hydrochloric acid from the stomach[28], or by administration of excessive bicarbonate, all of which measures increase the pH of the blood plasma. The administration of acidic substances will lower the pH and relieve the tetany. Thus the cause of this type of tetany is undoubtedly an alkalosis.

Acidic substances have also been found to relieve temporarily the symptoms of parathyroid tetany, although there is no change in the level of serum calcium. Nevertheless, attempts to demonstrate an alkalosis in this type of tetany have not been successful.

The tetany of rickets responds to the same type of treatment as other types of tetany. Obviously such measures can only be temporary, and an attempt should be made to

correct the underlying cause, the rickets itself.

2. Tetany and Calcium.

Attempts have been made by various investigators to associate all types of tetany with the level of serum calcium, by means of equations connecting calcium ions with pH. Nothing very convincing has been evolved.

Since in nephritis the level of serum calcium may fall below 7 mg., due to protein deficiency, without the appearance of tetany, it seems probable that parathormone affects the inorganic calcium. Interesting attempts to settle this question have been made by several investigators in studies upon cerebrospinal fluid.

3. Tetany and Cerebrospinal Fluid.

Cerebrospinal fluid normally contains about half as much calcium and 38% as much phosphorus as blood serum. It has been assumed, generally, that the calcium of the fluid is the inorganic portion of the serum which reaches the fluid by diffusion. Thus, if parathormone governs inorganic calcium of serum, one would expect it to influence spinal fluid calcium as well. Cameron and Moorehouse[29] found the spinal fluid calcium of dogs only very slightly reduced after parathyroidectomy; similarly, Rourke, Smith and Hartman[30] found little change in spinal fluid calcium in infants treated for tetany, even when serum calcium rose considerably. These results would seem to indicate that parathormone does not control diffusible calcium, or else, perhaps, that the calcium of cerebrospinal fluid is not the diffusible fraction of serum calcium. Critchley and O'Flynn[31], on the other hand,

found a definite fall in cerebrospinal fluid calcium in tetany, and an even more striking rise during recovery. These contradictory results have not yet been explained.

It is interesting, in this connection, to note that there is a certain amount of evidence appearing to indicate that cerebrospinal fluid calcium is not the inorganic, diffusible fraction of serum calcium. Fluid calcium has been shown to vary with the level of serum protein.[32] Injection of calcium salts increases serum calcium more than fluid calcium. Finally, in nephritis, the fluid calcium agrees with the filtrable serum calcium only when the total serum calcium is normal.

Evidently more evidence is required in order to settle definitely the state of cerebrospinal fluid calcium.

Relation Between Parathormone and Vitamin D.

It may be seen from the material so far presented that vitamin D, the parathyroid glands, and the ratio and amounts of calcium and phosphorus in the diet all play a part in the regulation of calcium and phosphorus metabolism. The question arises as to whether or not there exists an interrelationship between the actions of the vitamin and of the hormone. The available evidence on this point is frequently conflicting, although many of the apparent contradictions are undoubtedly due to insufficient care in the regulation of experimental conditions, such as the calcium and phosphorus content of diets.

1. Parathyroid Stimulation by Vitamin D.

Large doses of parathormone or of vitamin D have been shown to raise the serum calcium and to produce pathological calcium deposits in the soft tissues. On the basis of the similarity in the action of these two substances, it has been proposed that vitamin D produces its effect by means of stimulation of the parathyroid glands to elaborate more of their secretion. At the present time, there is evidence both for and against this hypothesis.

Taylor and his associates[33] found that the objective symptoms, the blood chemistry changes, and the postmortem findings in toxic overdoses of irradiated ergosterol were indistinguishable from those produced by toxic overdoses of parathormone. They suggest, therefore, that toxic doses of vitamin D act by stimulation of the parathyroid glands. They found, in addition, that administration of irradiated ergosterol was active in relieving tetany after ordinary parathyroidectomy, but inactive if aberrant neck tissues had been excised at the operation. This they explain by assuming that excision of aberrant neck tissues removes accessory rests not touched by the ordinary parathyroidectomy; the effectiveness of irradiated ergosterol in ordinary parathyroidectomy is taken to be a result of hyperplasia of this accessory parathyroid tissue due to the stimulus of the vitamin. In their experiments, relief from tetany by ergosterol was obtained within one hour after parathyroidectomy.

Johnson[34] found that when irradiated ergosterol in

therapeutic doses was administered to rats, together with parathormone in such dosage as he had shown formerly would produce typical osteitis fibrosa and metastatic calcification, the bone lesions were more marked than with parathormone alone. He showed also, by means of roentgenographic studies, that the metastatic calcium deposits were much more intense; kidney deposits were obtained of sufficient density to make the whole kidney distinctly visible in X-ray photographs, an effect impossible to achieve by the use of either parathormone or vitamin D singly. He suggests that vitamin D may act either by stimulation of the parathyroid glands to secrete more of their hormone, or by intensification of the action of the hormone already present.

It appears to me that the similarity of action of large doses of parathormone and of vitamin D observed by Taylor need not be explained by an interaction of the two substances. All the effects observed may be traced to the hypercalcaemia, thus accounting for the similarity, and it seems unnecessary to assume that the hypercalcaemia is produced by the same mechanism in each case. Similarly, in Johnson's work, vitamin D and parathormone have similar actions, and it is natural that their effects should be additive; it does not follow, however, that they are brought about by the same mechanism. The relief of tetany by vitamin D, is, of course, another matter, and will be discussed further in connection with the work of Shelling.

Gruner[35] has produced in 500 gram guinea pigs, by means of daily administration of irradiated ergosterol, bone

lesions which he interprets as being identical with those of osteitis fibrosa cystica. He concludes that vitamin D, in this respect, is similar in its action to parathormone. His experiments might, of course, be considered as evidence in favor of the theory that vitamin D acts by stimulation of the parathyroid glands.

There is, perhaps, a greater volume of evidence against, than for, this hypothesis.

If the effects of large doses of parathormone and of vitamin D are similar, the effects of therapeutic doses are not. Hugaley[36] has shown that parathormone in dosage of from 5 to 20 units daily produces in rats an increase in serum calcium, in faecal calcium, and in urine phosphorus, and a striking increase in urine calcium. Irradiated ergosterol increases urine calcium, but decreases faecal calcium and phosphorus. This seems to indicate a different mode of action. Further, he found that rats treated with parathormone until "immunity" developed, i.e., until further dosage no longer produced the former hypercalcaemic response, could still be made to excrete large quantities of calcium and phosphorus in the urine by means of administration of irradiated ergosterol. This, too, would seem to show that response to vitamin D could not have been through the medium of the action of parathormone.

If the vitamin acts by stimulation of the parathyroid glands, then complete extirpation of parathyroid tissue should prevent the action. Several investigators[35,37,38,39,40] have shown that large doses of ergosterol are effective in

relieving tetany after ordinary parathyroidectomy, at the same time restoring the serum calcium to its normal level. In these experiments, of course, there is always the question as to whether the removal of parathyroid tissue has been complete. If this could be considered a certainty, these experiments would constitute definite proof of the independence of action of vitamin D and of parathormone.

Rappenheimer[41] found that cod liver oil and viosterol in therapeutic doses were effective in preventing rickets in rats on a rachitogenic diet, even after parathyroidectomy. He concluded that the action of vitamin D is not dependent upon the parathyroids.

Selye[30] produced, in young rats, a condition of typical osteitis fibrosa which has been described before. He states that this condition is not the same as the bone lesions of hypervitaminosis D, and therefore it cannot be considered that vitamin D acts through the medium of the parathyroids. His work, therefore, contradicts the claims of Gruner.

Shelling[40] believes that many apparent contradictions in this field might be cleared up by attention to dosage and to the calcium and phosphorus content of diets. He finds that large doses of viosterol will raise the serum calcium and alleviate tetany in parathyroidectomized animals on low calcium diets, and is effective even if no calcium or phosphorus at all is present in the food. He does not think that Taylor's explanation of his failure to obtain beneficial action upon tetany after parathyroidectomy and excision of neck tissue, is correct. Hypertrophy of parathyroid rests in the space of one hour after operation, he says, is contrary to all clin-

ical experience. Besides, ordinarily the response of serum calcium to viosterol is not seen until 24 to 48 hours after its administration. He thinks it possible that the ultimate explanation may lie in the difference in the effects of small, therapeutic, and large doses.

Shelling, Asher and Jackson[42] have pointed out some further differences in the actions of vitamin D and of parathormone. They found that small doses of parathormone did not prevent rickets in animals on rachitogenic diets, while it is well known that small doses of vitamin D do have this effect. Analyses of bone in which calcification had been promoted by vitamin D showed that there is an increase in the ratio of ash to organic matter over the value for normal bone; this they interpreted to mean that vitamin D produces calcification in the existing matrix. On the other hand, bones pathologically calcified by parathormone showed no increase in the ratio; parathormone first stimulates trabeculation, so that increase in organic material keeps pace with the inorganic calcium deposits.

It may be seen, therefore, that the evidence upon this question is so contradictory as to be indecisive. It appears to me that, although there may be some relationship between the parathyroids and vitamin D, it very probably is not the relationship which has been discussed above.

2. <u>Parathyroid Hypercalcemia in Rickets</u>.

A rather different theory of relationship between vitamin D and parathormone has been proposed by several investigators. It was first demonstrated by Mellheim that in

rickets in rats there is an hypertrophy of the parathyroid glands, due, he stated, to an increase in the size of the individual cells. Hitter demonstrated hypertrophy in human rickets, as did Pappenheimer and Minor[43]. These latter investigators found that the hypertrophy was due to an increase in the size of the whole gland, due to a multiplication of cells.

Shelling[45] notes that Walther has shown that injection of parathormone into rats increases their susceptibility to rickets, and Pappenheimer that removal of the parathyroids increases the resistance to rickets; both of these experiments suggest that there may be an hyperfunction of the parathyroids in rickets.

Johnson[34] suggests that the hypertrophy of the parathyroids observed by Erdheim may be compensatory in nature, i.e., that in rickets there is an extra demand for parathormone to maintain normal serum calcium level.

Wilder, Higgins and Sheard[46] have shown that a slight deficiency of vitamin D produces in chicks hypertrophy of the parathyroid glands, which may be prevented by the administration of parathormone. Individual cells of the glands were enlarged, and there was also a multiplication of cells. A deficiency of the vitamin great enough to cause rickets, however, results in an hypertrophy of the parathyroids which is not preventable by the administration of parathormone. They suggest that parathormone sensitizes the tissues to the action of vitamin D, so that less of the vitamin is necessary to guard against rickets. They consider, also, that the deficiency of the vitamin, acting directly upon the parathyroids, constit-

utes the stimulus to hyperplasia. As confirmatory evidence they cite the experiments upon relief of tetany by vitamin D: large doses are necessary because of the insensitiveness of tissues to its action when there is a parathormone deficiency. Johnson's experiments on simultaneous administration of D and parathormone they explain by assuming that the parathormone sensitizes the tissues, so that therapeutic doses of the vitamin produce more than therapeutic effects. Altogether the hypothesis of these investigators sounds very attractive.

Hamilton and Schwartz[45] found that administration of calcium chloride or calcium gluconate by mouth to rachitic rabbits (100 gm. per animal) produced an extreme hypercalcemic reaction of the blood from which many of the animals died; the same treatment produced, of course, only a slight increase in serum calcium in normal animals. Hypercalcemia indicates the ability of the serum to hold in solution more calcium than normally, and they thought that this property of the serum of rachitic animals might be explained by assuming an abnormally high parathormone content. If this were the case, it would show that the hyperplasia of the parathyroids in rickets is accompanied by a definite increase in the secretion of parathormone.

Accordingly, they devised a method which they claim will detect the presence of small quantities of parathormone, and applied this method to rachitic and to normal serum. Their method is as follows:

If calcium chloride is given at intervals to normal rabbits, plotting serum calcium against time yields a curve

with a maximum near the beginning followed by a fairly rapid fall. If, however, parathormone is injected into the animals before the commencement of the experiment, the curve falls off much more gradually after the maximum. They found that doses as small as 1.5 units of parathormone per kg. weight of the animal produced a recognizably pathological calcium curve.

Using this method, they injected rachitic serum into one group of normal animals, normal serum into another group, kept a third as controls, and followed the calcium curves of each group. They found that the injections of normal serum did not produce any change in the curve in comparison with the curve of the control animals, while the effect of rachitic serum was to delay the fall of the curve in a manner very similar to parathormone injections, as described above. They conclude from this work that rachitic blood is rich in parathormone, or in some other substance having an identical effect upon serum calcium. They suggest that the hyperparathyroidism in rickets may be secondary to a low calcium absorption, which, in the absence of the hyperparathyroidism, would lead to a low serum calcium and tetany.

The existing evidence, therefore, seems to indicate that the established hyperplasia of the parathyroid glands in rickets is accompanied by an increase in secretion of parathormone, thus producing an increased concentration of this chemical agent in the blood.

If one could obtain, by means of repeated injections of rachitic blood, the typical bone lesions of osteitis fibrosa cystica, this would be confirmatory evidence of the presence of

an abnormal amount of parathormone in rachitic blood. On the other hand, negative results in such an experiment would not necessarily indicate a normal parathormone content in rachitic serum; it might merely mean that the increase in parathormone is not great enough to produce a pathological effect upon bone.

In this paper, experiments are reported investigating this point. The results, with one possible exception, are all negative; this work, therefore, does not add any evidence to either side of the question.

Chemistry of Parathormone.

The active principle of the parathyroids has not yet been isolated in a chemically pure condition, and attempts have been made to make chemical studies upon the available concentrated extracts.

Collip[46] prepared an active extract from fresh ox parathyroid glands, by a process of acid hydrolysis, followed by purification by fractional precipitation. The final active material obtained was an amorphous powder, exhibiting protein characteristics.[47] It is precipitated from aqueous solution at an iso-electric point of pH 4.8 to 4.9. With salts present in minimal amounts, its precipitation range is from pH 4.6 to 5.2. It gives the common protein tests, and is precipitated from acid solution by half-saturation with ammonium sulphate or by saturation with sodium chloride. It is soluble in 90% alcohol, and insoluble in ether, acetone and pyridine. It is destroyed by pepsin and trypsin, and does not dialyze through collodion membrane. It is apparently similar in many respects to insulin.

Tweedy[48] has modified Collip's method of preparation of the hormone by removing inert material with acetone, and precipitating the active fraction with trichloracetic acid. If the material obtained by this method is not entirely water-soluble he suspends it in five times its volume of acid alcohol, and precipitates the soluble fraction with ether. The final material is dissolved in normal saline for physiological use.

Tweedy and Torigoe[49], using the active material prepared by the above method to study the chemistry of the hormone, found among other things that it is absorbed from acid solution by permutit, and released by 5% ammonia at 0 to 2 degrees C. Sodium chloride, carbolic acid, pyridine or ammonium chloride do not release it. This, together with other experiments, suggested the importance of amino or imino groups in its constitution.

In this paper, an attempt to concentrate parathormone from normal serum is described. Permutit absorption, release by ammonia, and trichloracetic acid precipitation are employed.

E X P E R I M E N T A L .

The experimental work here described concerns the relation of the parathyroids to calcium and phosphorus metabolism in conditions of various kinds. It may be conveniently divided into three parts, as follows:

1. Calcium and phosphorus metabolic balances upon several patients with obscure bone diseases, in which it was suspected that the parathyroids might play a part.

2. Comparisons of the effects of injections of rachitic serum and of parathormone, into young rats, with a view to determining whether or not there exists an hyperparathyroid state in rickets.

3. An attempt to concentrate parathormone from normal serum, with tests upon rats of the material obtained.

I. CALCIUM AND PHOSPHORUS BALANCES.

Patient # 1. Jean Lewis.

This patient was a baby who at birth had deformities of the bones resembling those of very marked rickets. At ten days she was admitted to hospital with convulsions. She was found to have a raised blood calcium, varying at different times from 14.3 to 18.0 mg. per 100 cc., and a normal or slightly lowered blood phosphorus, varying from 3.3 to 4.8 mg. per 100 cc. The condition was, therefore, at first very suggestive of hyperparathyroidism, and it was decided to conduct studies upon her daily calcium and phosphorus output in the urine, and

subsequently upon her daily balance with respect to these elements.

Urinary Calcium and Phosphorus.

In all the chemical studies upon urine recorded in this paper, calcium was determined by the method of Shohl and Pedley[50]. Phosphorus was determined by the method of uranium acetate titration.[51]

In Table 1 is given a record of Jean Lewis' daily urinary output of calcium and phosphorus, determined over several days, each determination being done upon a 12-hour specimen. Table 2 gives a similar record for a normal infant, Belle Wexler. Jean was two months old at the time of these determinations, while Belle's age was three months.

Table 1. Jean Lewis' Urine Ca. and P.

Date	Specimen	Ca/100cc. gm.	Total Ca. gm.	P/100cc. gm.	Total P. gm.
Oct. 12.	night	0.0155	0.0184	0.0355	0.0426
"	Day	0.0160	0.0220	0.0357	0.0493
Oct. 13.	night	0.0161	0.0112	-	-
Oct. 14.	day	0.0187	0.0302	-	-
"	night	0.0173	0.0224	0.0395	0.0500
Oct. 15.	day	0.0093	0.0208	0.0342	0.0763
Oct. 17	night	0.0170	0.0233	0.0414	0.0547
"	day	0.0188	0.0261	0.0401	0.0554
Oct. 19	night	0.0184	0.0319	-	-
"	day	0.0191	0.0382	-	-
Mean	day	0.0164	0.0291	0.0367	0.0603
Mean	night	0.0165	0.0214	0.0385	0.0491
Mean	12-hr.	0.0165	0.0253	0.0376	0.0547

Table 2. Normal Control.

Date	Specimen	Ca/100cc.	Total Ca	P/100cc.	Total P
		gm.	gm.	gm.	gm.
Sept.27	day	0.0005	0.0010	0.0105	0.0019
"	night	0.0014	0.0026	0.0366	0.0634
Oct. 2	night	0.0003	0.0005	0.0236	0.0341
"	day	0.0002	0.0003	0.0204	0.0325
Oct. 5	night	0.0002	0.0003	0.0171	0.0306
Oct. 6	day	0.0001	0.0001	0.0253	0.0354
Oct. 8	night	0.0003	0.0004	0.0236	0.0377
Oct. 8	day	0.0004	0.0005	0.0237	0.0330
Oct. 9	day	0.0002	0.0004	0.0223	0.0429
Oct. 10	day	0.0003	0.0004	0.0258	0.0374
"	night	0.0002	0.0003	0.0190	0.0256
Oct. 11	day	0.0003	0 -	0.0255	-
"	night	0.0002	0.0002	0.0290	0.0290
Mean	day	0.0003	0.0005	0.0225	0.0326
Mean	night	0.0004	0.0007	0.0245	0.0374
Mean	12 hr.	0.0004	0.0006	0.0235	0.0255

Calcium and Phosphorus Balance

The calcium and phosphorus balance upon Jean Lewis was carried out as follows. Urine was collected from 6 P.M. Nov. 25 till 6 P.M. Nov. 27, and analysed in 12-hour specimens. One-half gm. carmine was given with the 6 P.M. feeding on Nov. 25, and again with the 6 P.M. feeding on Nov. 27. The first stool showing carmine was collected, and subsequent stools also collected, up till the first specimen to show the second carmine. This and subsequent specimens were discarded. The stools were dried, mixed, sampled, and the samples ashed, dissolved in 10% HCl, and analysed for calcium or phosphorus. The amounts of food taken by the baby at each feeding were recorded for the 48 hour period. Samples of her formula were obtained later, and 10cc. samples ashed and analysed. The mean of these analyses was taken as the true calcium and phosphorus content of the formula.

The methods of analysis for calcium and phosphorus in the acid solutions of stools and of feedings were as follows:

Calcium. To the HCl solution were added 5cc. of concentrated sulphuric acid, and the solution heated to boiling. 5cc. of 2.5% oxalic acid were added, the solution cooled to room temperature, and neutralised with concentrated ammonium hydroxide, using methyl red as an indicator. It was allowed to stand over night, and precipitate filtered and estimated by potassium permanganate titration, as in the method used for urine.

Phosphorus. The HCl solution was made just acid to litmus, using NaOH and dilute acetic acid. 5cc. of the acetate

buffer solution, used in the method for urine, were added, the solution heated to boiling and titrated with uranium acetate in exactly the same manner as for phosphates in urine.

The results of this experiment are given in Tables 3 to 6, inclusive.

Table 3. Jean Lewis' Urine Analyses.

Date	Specimen	Ca/100cc.	Total Ca	P/100cc.	Total P.
		gm.	gm.	gm.	gm.
Nov. 25	night	0.0101	0.0311	0.0349	0.1075
Nov. 26	day	0.0093	0.183	0.0399	0.0762
"	night	0.0133	0.0307	0.0385	0.0885
Nov. 27	day	0.0085	0.0184	0.0368	0.0794
Totals			0.0985		0.3516

Table 4. Jean Lewis' Stool Analyses.

No.	Sample Wt. gm.	Total P gm.	No.	Sample Wt. gm.	Ca content of sample gm.
1		0.3799	1	1.0272	0.0703
2		0.3457	2	1.3809	0.0731
33		0.3471	3	0.5622	0.0684
Mem		0.3369	4	0.7772	0.0722
			5	0.6136	0.0663
			Total	4.3707	0.3503
Total weight of stools was 8.5 gm. Remaining Ca by proportion:					
			Rest	4.1293	0.3335
			Total Stool Ca		0.6838

Table 5. Jean Lewis' Food Analyses.

Date	No.	KMnO4 used	Total Ca	No.	Uranium Acetate used	Total P
		cc.	gm.		cc.	gm.
Feb.7	1	11.00		3	4.70	
	2	10.85		4	4.65	
	Mean	10.90	1.4508	5	4.78	
				Mean	4.64	1.2275
Feb.19	1	10.7		3	4.40	
	2	10.7		4	4.35	
	Mean	10.7	1.4010	Mean	4.38	1.1340
Mean of two dates			1.4309			1.1807

Table 6. Jean Lewis' Balance.
(48 hours)

Material	Ca Intake	P Intake	Ca output	P Output
	gm.	gm.	gm.	gm.
Food	1.4309	1.1807		
Urine			0.0985	0.3516
Stools			0.6988	0.3569
Total	1.4309	1.1807	0.7973	0.7085
Calcium balance..... + 0.6336 gm.				
Phosphorus balance..... + 0.4722 gm.				

Patient #8. James McSheiron.

This patient was a normal baby as far as bone metabolism was concerned, and the 48-hour calcium and phosphorus balance carried out in his case was done in order to provide normal figures to compare with Jean Lewis. The baby's age at the time of the experiment was 5 months.

The experiment was carried out from 8 P.M. Mar. 23 to 8 P.M. Mar. 25. The routine of stool and urine collections was the same as in the case of Jean Lewis. Samples of each separate feeding lot were obtained and analysed.

The total 48-hour specimen of urine was combined and analysed by the usual methods. The stools were ashed in toto, dissolved in dilute HCl, made up to a definite volume, and 10cc. samples analysed, using the methods before described. 10cc. samples of each feeding lot were analysed in the same manner as for Jean Lewis. The record of the amounts of each feeding was then used to determine the total calcium and phosphorus intake.

The results of this experiment are recorded in tables 7, 8, and 9.

Table 7. James McPherson's Feeding Analyses.

Feeding Lot	Sample	Amount Taken	$KMnO_4$	Total Ca	Uranium Acetate	Total P
		Oz.	cc.	gm.	cc.	gm.
#1	1	42	6.75		2.35	
"	2		6.30		2.15	
"	3		6.45			
Mean			6.50	0.7621	2.25	0.5477
#2	1	43	7.20		2.70	
"	2		7.50		2.45	
"	3		7.05		2.55	
Mean			7.35	0.9798	2.57	0.7076
Totals				1.7419		1.2553

Table 8. James McPhedran's Stool Analyses.

No.	$KMnO_4$	Total Ca	No.	Uranium Acetate	Total P
	cc.	gm.		cc.	gm.
1	38.95		1	3.80	
2	39.00		2	3.65	
Mean	39.28	0.9824	Mean	3.73	0.1552

Table 9. James McPhedran's Urine Analyses.

No.	$KMnO_4$	Total Ca	No.	Uranium Acetate	Total P
	cc.	gm.		cc.	gm.
1	7.05		1	16.65	
2	8.00		2	16.35	
3	8.30		3	16.35	
Mean	7.77	0.0952	Mean	16.45	0.7192

Table 10. James McPherson's Balance.
(48-hour)

Material	Ca. Intake	P. Intake	Ca. Output	P. Output
	gm.	gm.	gm.	gm.
Feedings	1.7419	1.2553		
Urine			0.0652	0.7192
Stools			0.9824	0.1852
Totals	1.7419	1.2553	1.0476	0.9044

Calcium Balance..... + 0.6943 gm.

Phosphorus Balance..... + 0.3509 gm.

Patient #3. Peter Wilson.

This patient was a normal infant, to serve as an additional control for Jean Lewis. The experiment was carried out from 6 P.M. Apr. 29 to 6 P.M. May 1. Urine was collected over this period, and the specimens combined and analysed. Carmine was given to mark the stools in the same manner as before. The stools were combined, dried, ashed, dissolved in dilute HCl, made up to a definite volume, and 10cc. samples analysed. Samples of each separate feeding lot were obtained, and 10cc. portions analysed in the same manner as before. Peter's age was 6 weeks at the time of the experiment.

The results of this experiment are recorded in Tables 11, 12, 13 and 14.

Table 14A gives a comparison of the metabolism of Jean Lewis with her controls. Table 14B shows the relative calcium and phosphorus retentions of Jean Lewis, her normal controls and normal and rachitic subjects studied by Telfer.[55]

Table 11. Peter Wilson's Feeding Analyses.

Feeding Lot	Sample No.	Amount Taken	$KMnO_4$	Total Cu	Uranium Acetate	Total P
		cc.	cc.	gm.	cc.	gm.
1.	1.	24 3/8	10.05		3.90	
"	2.		10.30		3.75	
"	3.		9.30			
Mean			9.88	0.6502	3.82	0.5340
2.	1.	23	9.25		4.00	
"	2.		9.35		3.90	
"	3.		9.00			
Mean			9.20	0.5917	3.95	0.5230
Totals of Feeding lots 1. and 2.				1.2409		1.0570

Table 12. Peter Wilson's Urine Analyses.

Sample No.	$KMnO_4$	Total Ca.	Sample No.	Uranium Acetate.	Total P.
	cc.	gm.		cc.	gm.
1.	0.20		1.	15.10	
2.	0.30		2.	15.45	
3.	0.20		Mean		0.0400
Mean	0.23	0.0021			

Table 13. Peter Wilson's Stool Analyses.

Sample No.	$KMnO_4$	Total Ca.	Sample No.	Uranium Acetate	Total P.
	cc.	gm.		cc.	gm.
1.	43.10		1.	4.30	
2.	43.20		2.	4.90	
3.	43.70		3.	4.20	
Mean	43.33	1.0500	Mean	4.53	0.2450

Table 14. Peter Wilson's Balance.
(43-hour)

Material	Ca. Intake	P. Intake	Ca. Output	P. Output
	gm.	gm.	gm.	gm.
Feedings	1.2049	1.0570		
Urine			0.0021	0.6400
Stools			1.0500	0.2450
Totals	1.2049	1.0570	1.0521	0.8850

Calcium balance..... + 0.1528 gm.

Phosphorus balance..... + 0.1720 gm.

Table 14A. Comparison of Metabolism of Jean Lewis with Controls.

Name	Ca. Output		P. Output		Ca. Balance (48-hour)	P. Balance (48-hour)
	Urine	Stools	Urine	Stools		
	gm.	gm.	gm.	gm.	gm.	gm.
Jean Lewis	0.0985	0.6988	0.3516	0.3569	0.6336	0.4722
James McPherran	0.0052	0.9624	0.7192	0.1052	0.6943	0.3509
Peter Wilson	0.0021	1.0500	0.6400	0.2450	0.1382	0.1720

Table 143. Comparison with Telfer's Figures
for
Normal and Rachitic Calcium and Phosphorus Metabolism.

Name	Age	Percentage Retention of Intake	
		Calcium	Phosphorus
Jean Lewis	8 months	44.3	40.0
James McPhedran	5 months	39.8	28.0
Peter Wilson	6 weeks	12.7	16.2
Telfer's Normals	7 to 8 months	21.0 to 34.0	15.0 to 17.0
Telfer's Rachitics	6 to 7 months	6.0 to 17.0	5.0 to 6.1

Remarks:

Comparison of Table 1 with Table 2 indicates that Jean Lewis had a markedly increased excretion of calcium in the urine, compared with the amount for a normal infant. The urine phosphorus is also apparently increased in amount, although not so greatly.

Table 14A gives a comparison of the calcium and phosphorus output and balance for Jean Lewis with those of her two normal controls. This table shows a comparatively large positive balance for both calcium and phosphorus for Jean Lewis, a fact which is rather surprising in view of the marked state of decalcification of the patient's bones, as shown by X-ray photographs. It agrees, however, with the fact that no progressive decalcification could be discerned by means of X-rays taken at different times. The table also shows that Jean Lewis' positive calcium balance lies between the amounts obtained for the two normal controls. Her positive phosphorus balance, however, is slightly greater than that of either of the normal controls.

The table indicates normal distributions of calcium between urine and stool in ratios of approximately 1 to 12, and 1 to 500. Evidently in an infant, the normal amount of calcium excreted in the urine is very small. For Jean Lewis, the ratio is approximately 1 to 7, showing relative increase in urinary calcium.

The distribution of phosphorus between urine and stool is shown by the table to be normally in the ratio of about 1 to

S. Jean Lewis' ratio is about 1 to 1. Thus the phosphorus metabolism of this patient is definitely abnormal.

Table 14B gives a comparison of the percentage retention of calcium and phosphorus for Jean Lewis, her normal controls, and for normal and rachitic subjects studied by Telfer.[58] These figures do not yield much information, except to show that Jean Lewis' calcium and phosphorus metabolism is not that of an ordinary rachitic child.

There are, as far as I know, no available metabolic studies of hyperparathyroidism in infants. It is therefore rather difficult to interpret these results. By analogy with the reports on older children and adults, one would expect an initial phosphaturia followed by calcuria. There is no marked phosphaturia, in Jean Lewis' case; in fact, phosphate is diverted from urine to stool. There is, however, a marked calcuria. The metabolic disturbance is thus apparently not a simple hyperparathyroidism.

Dr. Aub[52] reports calcium and phosphorus studies on a patient of Cushing's, a case diagnosed as an adenoma of the basophil cells of the anterior pituitary. This girl showed herself to be in nitrogen and phosphorus equilibrium, but her calcium output in both urine and stools was far in excess of the normal figures for the test diet she was given. Her bones showed decalcification, which was especially marked in the skull. Her blood calcium was normal. She had, in addition, very definite symptoms of pituitary basophilism.

It seems to be possible that the disturbance in Jean

Lewis' case might be of pituitary origin, analogous to the case noted above, with which it presents a similarity with respect to calcium output. This is, however, the only similarity in the cases. Thus the chemical findings, alone, do not point to any definite diagnosis.

The view of the case as the result of a pituitary malfunction is considerably strengthened by the autopsy findings.

Jean Lewis died at the age of six months, apparently from increased intracranial pressure. Measurements taken at intervals during her life revealed a very much retarded rate of growth, and at death it was apparent that her brain had been growing more quickly than her skull, the brain expanding against a broad band of osteoid tissue at the edge of the bones of the cranial vault. Autopsy further revealed marked lesions of the bones, calcium deposits in the kidneys, and hemi-agenesis of the posterior lobe of the pituitary.

It seems very probable that the pituitary lesion was the primary one; a lesion of the posterior pituitary might conceivably upset the functioning of the anterior pituitary. Disturbance of the growth factor of the anterior pituitary would produce dwarfing, and the upset in calcium and phosphorus metabolism might result from a malfunctioning of the parathyrotropic principle of the pituitary. The calcium deposits in the kidney are probably the result of the continued calcuria.

Patient # 4. Mrs. Lewis.

Mrs. Lewis, the mother of Jean Lewis, was apparently a normal, healthy woman. Her blood calcium was normal, but her blood phosphorus consistently low. Mrs. Lewis had had, two years previously, another child, Gwen, with the same congenital condition as that observed in Jean. Gwen died at the age of three months. It was first thought (before Jean's death), therefore, that a state of asymptomatic hyperparathyroidism - if such a condition can exist - might be the cause of the congenital condition in the two children. A calcium and phosphorus balance was conducted, as follows:

The experiment was carried out from 8 A.M. Oct. 27 to 8 A.M. Oct. 31, i.e., a period of four days. Urine was collected over this period, and analyzed in several specimens. Charcoal was given before breakfast on Oct. 27, and again before breakfast on Oct. 31. The first specimens of stool to show charcoal were preserved, and also all subsequent specimens up to, but not including, the first specimen to show the second charcoal. The stools were combined, dried, powdered, mixed, weighed, and weighed samples ashed, dissolved in dilute hydrochloric acid and analyzed as before described.

A duplicate of each meal eaten by Mrs. Lewis during the metabolism period was weighed out, subtracting from the duplicate any food left by the patient. These duplicate meals were combined, dried, ground finely, mixed, and the total weighed. It was then analyzed by sampling, as were the stools. Mrs. Lewis' water intake over the four-day period was measured.

A sample of water was analysed for calcium, and the calcium content of the water consumed by the patient calculated from this analysis.

The results of the experiment are shown in Tables 15 to 18, inclusive.

Table 15. Mrs. Lewis' Food Analyses.

Sample No.	Percentage of Ca.	Total Ca.	Sample No.	Total P.
		gm.		gm.
1	0.1539	2.1312	1	3.3088
2	0.1415	1.9601	2	3.2167
3	0.1472	2.0390	Mean	3.2914
4	0.1562	2.1642		
Mean		2.0736		

Table 16. Mrs. Lewis' Urine Analyses

Date	Specimen	Total Ca.	Total P.
		gm.	gm.
Oct. 27	24-hr.	0.0316	0.7363
Oct. 28	24-hr.	0.0777	0.3356
Oct. 29	12-hr.	0.0620	0.2798
Oct. 30	36-hr.	0.1161	0.6641
Totals		0.3473	2.2158

Table 17. Mrs. Lewis' Stool Analyses

Sample No.	Percentage of Ca.	Total Ca.	Sample No.	Total P.
		gm.		gm.
1	0.1332	1.2773	1	1.0915
2	0.1342	1.2365	2	1.0729
3	0.1375	1.3183	3	1.0778
Mean	0.1350	1.2940	Mean	1.0807

Table 18. Mrs. Lewis' Balance.

Material	Ca. Intake	P. Intake	Ca. Output	P. Output
	gm.	gm.	gm.	gm.
Food	2.0736	3.2914		
Water	0.0220	"		
Stools			1.3340	1.0807
Urine			0.3473	2.2158
Totals	2.0956	3.2914	1.6813	3.2965

Balances:

Calcium..... + 0.4143 gm.

Phosphorus..... + 0.0050 gm.

Remarks:

Table 18 shows a calcium distribution between urine and stool in the ratio of about 1 to 4, and a phosphorus distribution in a ratio of about 2 to 1, both of which are normal for an adult. There is a positive calcium balance over the four day metabolism period, of about 0.45 gm., i.e., 0.11 gm. per day, which may be considered normal for a woman after child-birth. Phosphorus equilibrium is evidently being maintained.

The results of this experiment, of blood studies, and of X-ray examinations showed that there was no condition of hyperparathyroidism present in Mrs. Lewis.

The findings of the post-mortem upon Jean, together with a very abnormal history of pregnancies in the case of Mrs. Lewis, strongly suggest some pituitary malfunction in the mother, as well as in the children, and which might be the cause of the congenital condition in the children.

Patient # 5. Edward Lavoque.

This boy had been in the Children's Hospital at the age of six, with a diagnosis of late rickets. He was brought back several years later (at the age of 11), when it was found that his rickets had healed, leaving severe bone deformities. It was thought that perhaps there might be some endocrine disturbance causing the rickets to appear as late as the age of six. Therefore a calcium and phosphorus balance was carried out.

The metabolism period was from 8 A.M. Apr. 11 to 8 A.M. Apr. 14. Urine was collected over this period, and analysed in two portions. Carmine was given with breakfast in Apr. 11, and again with breakfast on Apr. 14. Stools showing the first carmine were collected, and also those up to,but not including the first specimen to show the second carmine. The stools were combined, dried, ashed, dissolved in dilute HCl, made up to 250 cc., and 10 cc. samples analysed as before.

The patient was given a weighed, low calcium diet. Duplicates of every meal were weighed out, combined and dried. The food was then ground, and extracted twice with ether, by decantation. The fatty portion, after evaporation of the solvent, was ashed, dissolved in HCl, and analysed. The dry residue from the original extraction was weighed, finely ground, mixed, and samples ashed and analysed. The water intake of the patient was measured, and the calcium content calculated from previous analyses of tap water. The results of the experiment are shown in Tables 19 to 22, inclusive.

Table 19. Edward Laroque's Food Analyses.

Material	Sample	$KMnO_4$	Total Ca	Sample	Uranium Acetate	Total P
		cc.	gm.		cc.	gm.
Fatty Portion		2.35		1	4.60	
	2	2.40		2	4.25	
	Mean	2.38	0.0590	3	4.65	
				Mean	4.50	0.2230
Extracted Residue	1	1.70	0.2641	1	6.00	0.9365
	2	2.75	0.2345	2	6.60	0.9951
	3	2.40	0.2148	Mean	6.30	0.9658
	4	2.80	0.2270			
	5	3.20	0.2165			
	Mean	2.57	0.2314			

Table 20. Edward Laroque's Urine Analyses.

Specimen	No.	$KMnO_4$	Total Cc.	No.	Uranium Acetate	Total P.
		cc.	gm.		cc.	gm.
Apr. 11. to 12.	1	0.30		1	6.05	
	2	0.28		2	5.65	
	3	0.35		Mean	5.85	0.4457
	Mean	0.30	0.0054			
Apr. 13.	1	0.40		1	7.00	
	2	0.35		2	6.55	
	3	0.60		Mean	6.78	0.6825
	Mean	0.45	0.0109			
Totals			0.0163			1.1282

Table 21. Edward Lavegne's Stool Analyses.

Sample No.	$KMnO_4$	Total Ca.	Sample No.	Uranium Acetate	Total P.
	cc.	gm.		cc.	gm.
1	17.00		1	6.85	
2	16.75		2	6.90	
3	16.60		3	6.90	
Mean	16.78	0.4070	Mean	6.88	0.4575

Table 22. Edward Lacoque's Balance.

Material	Ca. Intake gm.	P. Intake gm.	Ca. Output gm.	P. Output gm.
Extracted Food	0.2314	0.0960		
Fatty Food	0.0590	0.2230		
Water	0.0303	-		
Stools			0.4070	0.3675
Urine			0.0163	1.1282
Totals	0.3207	1.2190	0.4233	1.4957

Ca. Balance..... = 0.1026 gm.

P. Balance..... = 0.2769 gm.

Remarks:

Table 22 shows a distribution of calcium between urine and stools in the ratio of approximately 1 to 20, and a phosphorus distribution in the ratio of about 3 to 1. These may be considered normal for a boy of eleven. The slight negative balances over the three day period might be expected on a low calcium diet. Evidently there is no calciuria and no phosphaturia.

Blood studies showed a normal calcium and phosphorus, and these, together with the metabolic balance, rule out the possibility of hyperparathyroidism.

Patient # 6. Annie Slechynsky.

This child, a girl of thirteen, had developed very thin and fragile bones, that tended to fracture easily. She had a condition of progressive bone atrophy, the bones becoming not more rarefied but of smaller and smaller diameter. Her legs were very markedly deformed. It seemed advisable, therefore, in such a condition whose cause was unknown, to determine by chemical studies whether or not there was any endocrine disturbance involved.

Her blood calcium was found to be 10.0 mg. per 100 cc., and her blood phosphorus 3.9 mg. per 100 cc.

A calcium and phosphorus balance was conducted from 7 A.M. June 13 to 7 A.M. June 16. For the purposes of this experiment she was placed upon the same low calcium diet that was given Edward Laroque. Beyond weighing the food, then, there was no necessity for further analysis. The water intake was recorded, and the calcium content calculated. The stools were marked off with carmine in the same manner as for Edward Laroque, and dried and analysed in the same manner. Urine was analysed as usual.

The results of this experiment are given in Tables 23 to 25, inclusive.

Table 23. Annie Slochynsky's Urine Analyses.

Sample No.	$KMnO_4$	Total Ca.	Sample No.	Uranium Acetate	Total P.
	cc.	gm.		cc.	gm.
1	1.15		1	7.10	
2	1.40		2	7.10	
Mean	1.28	0.0496	Mean	7.10	1.1989

Table 24. Annie Slochynsky's Stool Analyses.

Sample No.	$KMnO_4$	Total Ca.	Sample No.	Uranium Acetate	Total P.
	cc.	gm.		cc.	gm.
1	42.95		1	15.45	
2	42.75		2	15.25	
3	42.85				
Mean	42.85	0.9730	Mean	15.35	0.3174

Table 25. Annie Glochynsky's Balance.

Material	Ca. Intake	P. Intake	Ca. Output	P. Output
	gm.	gm.	gm.	gm.
Food	0.2904	1.2168		
Water	0.0354	-		
Urine			0.0496	1.1989
Stools			0.9790	0.3174
Totals	0.3258	1.2168	1.0286	2.0163

Calcium Balance..... = 0.7028 gm.

Phosphorus Balance..... = 0.7975 gm.

Remarks:

The calcium distribution between urine and stools, in the ratio of approximately 1 to 20, is normal. The phosphorus distribution ratio of about 1.5 to 1 shows that phosphorus is diverted from urine to faeces. The patient's condition is therefore not related to hyperparathyroidism.

The marked negative balances found fit in well with the clinical picture of progressive bone atrophy. The normal blood calcium and phosphorus figures in this case illustrate very strikingly that the appearance of normality obtained from blood studies may not always be a true indication of existing conditions. It is evidently possible to obtain an increased calcium and phosphorus output without an elevated value for either element in the blood.

This case appears to be a failure to utilize the available material, the fault presumably lying in the substrate in which the calcium and phosphorus normally would be deposited.

II. INJECTIONS OF RACHITIC SERUM AND OF PARATHORMONE.

1. Injections of Rachitic Serum into Young Rats.

If there is a state of hyperparathyroidism in rickets, it should be possible, by injection of large quantities of rachitic serum, to produce in the experimental animal the same effects as are produced by parathormone injections. Thus, repeated injections should produce osteitis fibrosa cystica.

The following experiments, in which an attempt was made to duplicate the effects of parathormone injections by injections of rachitic serum into young rats, were necessarily limited by the supply of rachitic serum available. Only blood from children with marked clinical rickets, and who were not being treated therapeutically for the condition, was used.

All the injections were subcutaneous. It was found advisable to paint the affected part with collodion, in order to prevent leakage. The bone sections were prepared according to the routine laboratory method, as follows:

The bones were fixed in formalin, decalcified in 5% nitric acid, and washed in running water for 24 hours. They were then passed through the following fixative:

70% alcohol, overnight.

Aniline, 30 minutes.

Aniline till clear.

Chloroform, 15 minutes.

Paraffin in chloroform in oven, 30 minutes.

Paraffin, as long as required to clear in aniline.

They were blocked in paraffin, sectioned, and stained with hematoxylin and eosin. In some cases rib sections were made in addition to femur sections.

Experiment 1.

1 cc. of rachitic serum was injected into each of four 13-day rats, daily, for five days. The rats were killed the following day, and sections of the femurs made.

Microscopics: Normal bone pictures.

Experiment 2.

Two 3-day rats were given the following injections of serum from a child with rickets:

May 13...0.2 cc. each.
May 14.. 0.25 cc. each, twice during the day.
May 15.. 0.5 cc. each, twice during the day.
May 16.. 0.5 cc. each, twice during the day.
May 17.. 0.5 cc. each, once, and 1.0 cc. each, later.
May 18.. 1.0 cc. each, twice during the day.
May 19.. 1.0 cc. each, twice during the day.
May 20.. Rat # 1 was killed, and rib and femur sections made.
.. Rat # 2 was given 1.0 cc. twice during the day.
May 21.. 1.0 cc.twice during the day.
May 22.. 0.25 cc. Later in the day rat # 2 was killed, along with a normal control, rat # 3. Sections were made.

Microscopics:

Rat # 1. There may be a few more osteoclasts near the costo-chrondral junction in the growing zone.

Rat # 2. There may be a few more osteoclasts on the exterior surface of the rib.

On the whole, the bones of both these rats are to be taken as normal.

Rat # 3. Normal bone picture.

Experiment 3.

On May 20, a sample of serum was obtained from Alva Lambrecht, a child with marked clinical rickets. Her blood calcium was 10.2 mg. per 100 cc., and phosphorus 2.6 mg. One 3-day rat was given injections of this serum, as follows:

June 7. 12 noon..... 0.5 cc. of serum.

5 P.M..... 0.75 cc. of serum.

June 8. 10 A.M. Rat appeared healthy. Given 1.0 cc. of serum

2 P.M. Rat seemed oedematous. Given 1.0 cc. of serum.

5 P.M. Rat apparently sick. Given 1.0 cc. of serum.

June 9. Rat found dead with very marked general oedema. The whole rat was preserved in formalin, and sections of the femur made.

Microscopic:

Rat # 11. Marked increase in osteoclasts on the shaft side of the growing zone, and to a less degree in the cortex.

Experiment 4.

Using the same specimen of serum, one 3-day rat was injected as follows:

June 14. 12 Noon. Given 0.5 cc. of serum.

5 P.M. Rat looked oedematous and sick. It was decided not to give any more injections.

June 20. Rat had apparently fully recovered.

Remarks:

From the above experiments it is seen that only when a very young rat was given large doses of rachitic serum at frequent intervals was any evidence of changes in the bones produced. The only change observed was an increase in osteoclasts, and it will be discussed further in connection with the experiments upon parathormone injections.

The oedema produced by the specimen of rachitic serum which produced the bone changes, is very interesting.

Harrop and his associates[56] have recently shown that sodium is the determining factor in the regulation of proper tissue hydration. Sodium metabolism is thus related to oedema.

It has also been shown that the excretion of calcium and phosphorus, due to parathormone administration, is influenced by sodium chloride. Thus, sodium metabolism is in some way related to calcium metabolism. Therefore, there may be a connecting link between calcium metabolism and hydration through the medium of sodium.

Oedema and fits occur in the new-born, without known cause. Fits may also occur alone; these latter are controlled by calcium and are in some way related to tetany. It may be questioned, therefore, whether there is not present in the serum of new-born, tetanic infants, some substance which influences both calcium metabolism and tissue hydration (possibly through the medium of sodium) so that tetany and oedema result. In this connection, the oedema in rat # 11 is very interesting.

The results of experiment 4, above, suggest that the origin of the oedema is non-bacterial; it is very unlikely that the rat would have recovered after one injection, if there had been a bacterial infection present. A culture of this sample of rachitic serum was found to be sterile, a further indication that an infection was not present.

2. Injections of Parathormone into Young Rats.

The experiments in this section have been divided into two series.

First Series of Experiments.

The first series of experiments is an attempt to determine the minimum dosage of parathormone which will produce a recognisably pathological lesion in the bones of young rats. Both 5- and 3-day rats were utilised. Even if a state of hyperparathyroidism does exist in rickets, the concentration of parathormone in the blood must be very small. Therefore the minimum parathormone effects were sought, in order that these might be compared with any effects obtained from the injections of serum from rickety children.

Injections were performed in the same manner as for rachitic serum. With 5-day rats, Selye's work was repeated and confirmed, and dosage was reduced to discover the minimum. Some of the later bone sections were decalcified in a mixed ferric and aluminum nitrate solution, which gives a clearer picture than the nitric acid. With the small bones of the young rats, decalcification is complete in one week.

In the later experiments, kidney sections were made, and stained with silver nitrate, haematoxylin and eosin. An attempt was made to follow the developing lesion in bone and kidney.

Experiment 1.

Two 3-day rats were injected with 1/5 unit and 2/5 unit, respectively, of Parathormone Lilly, daily, for 9 days. On the tenth day, the rats were killed, together with a normal control from the same litter. Bone sections were made.

Microscopics:

Rat # 5. (1/5 unit) Normal bone picture.

Rat # 4. (2/5 unit) Normal bone picture.

Rat # 6. (Control) Normal bone picture.

Experiment 2.

Two 3-day rats were injected with 2/5 unit, and one with 1/5 unit of Parathormone Lilly, daily for 12 days. On the 13th day, the rats were killed, together with a normal control. Femur sections were made.

Microscopics:

Rats # 7 and # 8. (2/5 unit) Normal bone picture.

Rat # 9. (1/5 unit) Normal bone picture.

Rat # 10. (Control) Normal bone picture.

Experiment 3.

Three 5-day rats were injected with 5 units of Parathormone Lilly per rat, daily, for 5 days. On the following day they were killed, together with three normal controls. Femur

sections were made.

It was observed that the growth of the injected rats was retarded; they were all smaller than the controls. Their stomachs were found to be flat and empty, as though they did not nurse. The weights of the rats at death are given below:

Test Rats.		Control Rats.	
# 1 T.....	12.6 gm.	# 1 N.....	20.0 gm.
# 2 T.....	12.5 gm.	# 2 N.....	18.6 gm.
# 3 T.....	7.2 gm.	# 3 N.....	14.6 gm.

Microscopics:

Rat # 1 T. The marrow cavity is shut off from the metaphysis by thickly-felted osteoblast tissue. There are a few capillaries running in the long axis of the marrow and piercing the felt-work. The line of the proliferating cartilage is very irregular; osteoclasts are active in it, but do not appear to be attacking the bone. There is hemorrhage in the metaphysis.

Rat # 2 T. (1) Section decalcified in aluminum nitrate.

There is an increase in bone. There are very few osteoclasts near the lower epiphysis. The marrow cavity is now shut off by bone which is heavy, quite vascular, and the vessels are overlaid by fat osteoblasts.

(2) Section decalcified in ferric nitrate.

There are more osteoblasts between the marrow and the bone. The appearance suggests bone formation in the metaphysis without cartilage as an intermediary.

Rat # 3 T. The metaphysis is essentially granulation tissue, with irregular peninsulas of cartilage and no bone. No osteoclasts.

Rats # 1 N, 2 N, and 3 N. Normal bone pictures.

Comment:

Experiment 3 confirms Selye's results.[54] However, there is obviously a good deal of variation in response from the same apparent dosage. The result may depend upon the actual dose per gram weight, the sex factor and the appetite.

Experiment 4.

One 5-day rat was injected with 4 units, and two others with 3 units each, of Parathormone Lilly, daily, for 5 days. On the sixth day they were killed, together with a normal control from the same litter. Femur and kidney sections were made. The test rats were smaller than the control; their weights before being killed ranged from 16.4 gm. to 19.2 gm., while the control weighed 21.3 gm.

Microscopics:

Rat # 29. (4 units).

Bone: Moderate increase in osteoclasts with beginning fibrosis at the end of the marrow cavity. The zone is about three times as deep as the zone in the normal control.

Kidney: Small amount of calcium in the lymphatics of the cortex and medulla.

Rat # 30. (3 units)

Bone: This section shows the same type of lesion as in rat # 29, but a little less marked. The zone is between two and three times as deep as in the normal control.

Kidney: Calcium in the lymphatics and in the tubules. The presence of calcium in the tubules appears to be a secondary

process, as there are cells about some of the "pseudo-casts" as if the calcium had broken through from the lymphatic into the tubule. There is pus in the tubules, and minute areas of acute interstitial nephritis.

Rat # 31.(3 units).

Bone: This section shows a lesion similar to rat # 30, perhaps even more marked. The zone is between two and three times as deep as in the control.

Kidney: Marked calcium deposits, especially in the interstitial tissue of the tip of the papilla. The collecting tubules are dilated and filled with pus. The calcium in the lymphatics distorts the walls of the tubules higher up, and there is some pus here also.

Rat # 32. (Control)

Bone: Normal.

Kidney: Normal.

Experiment 5.

Two 5-day rats were injected with 2 units, each, of Parathormone Lilly, daily, for 5 days. They were killed on the following day, and femur sections made.

Microscopics:

Rat # 14. (2 units) An increase in osteoclasts in the cortex.

Rat # 15. (2 units) Apparently normal.

Experiment 6.

Three 5-day rats were injected with 1 unit, each, of Parathormone Lilly, daily, for 5 days. They were killed on the

sixth day, and bone sections made.

Microscopics:

Rat # 16. (1 unit) Narrow zone. Osteoclasts prominent.

Rat # 17. (1 unit) Normal picture.

Rat # 17A. (1 unit) Normal picture.

Experiment 7.

Three 5-day rats were injected with ½ unit, each, of Parathormone Lilly, daily, for 5 days. They were killed, together with a normal control, on the following day. Bone sections were made.

Microscopics:

Rat # 18. (½ unit) There is an increase in the width of the trabeculae in the growing zone. Otherwise, a normal picture.

Rat # 19A. (½ unit) Increase in width of the trabeculae in the growing zone. Definitely pathologically wide zone. Heaping up of osteoblasts; increase in osteoclasts; haemorrhages.

Rat # 19. (½ unit) Similar to rat # 19A, but less in degree.

Rat # 20. (Control) Narrow zone; a few small osteoclasts; well-developed osteoblasts.

Remarks:

From the results of experiments 1 to 7, above, it is seen that definite pathological bone lesions may be produced in the 5-day rat by doses of parathormone of less than 5 units per day. It was found that doses of 4 and of 3 units both produced definite lesions; doses of 2, 1 or ½ unit produced slight lesions in some cases, but not consistently in all rats treated. Doses of 1/5 and of 2/5 unit had no effect upon the bones. The first

noticeable change appears to be an increase in osteoclasts, as is shown in experiments 5 and 6. Again, a considerable variation in response to the same dosage was observed.

In the following experiment, the course of the developing lesion produced by 5-unit doses daily, was followed. Parallel bone and kidney sections enable direct comparisons to be made.

Experiment 8.

Five 5-day rats were injected with 5 units of Parathormone daily, per rat. One rat was killed 24 hours after every injection, thus giving a series of rats that had been given 5 units for 1, 2, 3, 4, and 5 days, respectively. Femur and kidney sections were made.

Microscopics:

Rat # 38. (One injection).

Bone: There is a dotted line of osteoclasts at the junction of the marrow cavity and the metaphyseal trabecular bone, also a spotty lining of these cells to the cortex.

Kidney: Small calcium deposits in the cortical lymphatics and tubules, and also to a less degree in the medullary lymphatics. Large calcium casts in the collecting tubules.

Rat # 39. (Two injections).

Bone: The multinucleated giant cells (osteoclasts) at the junction of the marrow and trabecular bone have to some extent coalesced, and there are also fibroblasts filling in the interstices. Similarly there is a loose feltwork of fibroblasts (osteoblasts ?) beneath the cortex.

Kidney: Many small calcium deposits in the cortical lymphatics and larger ones in the medullary lymphatics at the base of the pyramid. There are a few calcium casts in the collecting tubules, one accompanied by cells.

Rat # 40. (Three injections).

Bone:

(a) Cartilage.

(1) Resting cartilage. This takes one blue colour throughout, in place of the normal band on the joint side of the proliferating cartilage.

(2) Proliferating cartilage. Normal.

(3) Metaphyseal cartilage. Heavier and more irregular than normal.

(b) Bone.

(1) Metaphyseal. Essentially no matrix laid down on the cartilaginous ribs, but instead there is a filling in with fat fibroblast cell types.

(2) Metaphyseal-marrow junction. Band of mixed osteoblasts and osteoclasts. Cortex has telescoped at this point.

(3) Cortex. Thin. Along one side are many osteoclasts and osteoblasts, on the other side osteoblasts only.

Kidney: A remarkable picture of calcium deposition in all the lymphatics of the kidney, with no casts.

Rat # 41. (Four injections).

Bone: Very few osteoclasts, but many osteoblasts which

now enclose the marrow cavity and fill in the interstices of the cancellous bone.

Kidney: There are many minute calcium deposits in the cortical lymphatics, but in the pyramid all is concentrated in the lymphatics of the tip, where there is also an acute interstitial inflammation. The collecting tubules are dilated. One group of cortical tubules is dilated.

Rat # 42. (Five injections)

Bone: The osteoclasts are now concentrated in the metaphysis. The shaft is collapsed, and the whole cortex appears wavy, as though it had lost its strength. There does not appear to be any increase in osteoblast formation.

Kidney: There is some calcium scattered throughout the lymphatics of the medulla, and two large patches in the lymphatics, one on each side of the pyramid. Apparently there are no deposits in the cortex, and no glomerular involvement. A few of the collecting tubules, near the calcium deposits, are dilated.

Remarks:

From the bone sections, it is evident that parathormone stimulates the formation both of osteoclasts and of osteoblasts. At first the former increase more rapidly than the latter; later the osteoblasts also increase rapidly, until they outnumber the osteoclasts.

The kidney sections show calcium deposits from the beginning. At first there is a small amount in the lymphatics and large calcium casts in the tubules. With further injections

the deposits in the lymphatics increase and the casts in the tubules soon disappear; finally all the calcium is in the lymphatics.

It appears from comparison of bone and kidney sections that calcium casts in the tubules roughly parallel the osteoclastic activity in the bones.

Second Series of Experiments.

The second series of parathormone injections was designed to give sections to compare directly with those of rat # 11, which died with marked oedema after injections of rachitic serum. Three-day rats were used, therefore, and injections given for two days. Again, it was endeavoured to find the minimum dosage needed to produce any bone change.

Experiment 1.

Two 3-day rats were each injected with 2 units of Parathormone Lilly, twice daily, for 2 days, i.e., 4 units per day. One rat died on the second day, and was discarded. The other was killed on the third day, and femur and kidney sections made.

Microscopic:

Rat # 22. (2 units, twice daily)

Bone: Apparently complete disappearance of the trabeculae into the growing zone, with fibrous tissue and osteoclasts present.

Kidney: There are calcium deposits present, probably both in the lymphatics and in the tubules.

Experiment 2.

Two 3-day rats were each injected with 1 unit of Parathormone Lilly, twice daily, for 2 days, i.e., 2 units per day. On the third day they were killed, together with a normal control, and bone and kidney sections made.

Microscopic:

Rat # 23. (1 unit twice daily)

Bone: A condition similar to that of rat # 22, but not quite so far advanced. There is still some trabeculation.

Kidney: Extensive calcium deposits.

Rat # 24. (1 unit twice daily)

Bone: Similar to rat # 23.

Kidney: Similar to rat # 23.

Rat # 25. (Control)

Bone: Normal.

Kidney: Normal.

Note: The test rats in both these experiments showed marked retardation of growth. For example, at death the weights of rats # 23 and # 24 were 7.5 and 6.9 gm., respectively, while the weight of the control (# 25) was 9.3 gm.

Experiment 3.

Two 3-day rats were each injected with 1 unit of Parathormone Lilly, daily, for 2 days. On the third day they were killed, and bone and kidney sections made.

Microscopica:

Rat # 27. (1 unit)

Bone: No fibrosis. No increase in osteoclasts. The new bone zone is narrow, probably narrower than normal.

Kidney: No calcium deposits.

Rat # 28. (1 unit)

Bone: Narrow and ill-developed growing zone. Increase in osteoclasts in the growing zone and in the cortex near it.

Rat # 20. (1 unit).

Kidney: No calcium deposits.

Experiment 4.

One 3-day rat was injected with ½ unit of Parathormone Lilly, twice daily, for 2 days. On the third day, the rat was killed, together with a normal control. Femur and kidney sections were made.

Microscopics:

Rat # 52. (½ unit twice daily)

Bone: There is an increase in osteoclasts and a slight increase in fibroblasts.

Kidney: There is a little finely granular blackish material at the tip of the pyramid, and again in and under the pelvic mucosa. The section is not very good, and this material may be calcium, or it may be an artefact of staining.

Remarks:

It may be seen from the results of the above experiments that, with 3-day rats, doses of 4 units per day (2 units twice daily) and of 2 units per day (1 unit twice daily) produce very definite bone lesions, and, in addition, extensive calcium deposits in the kidneys. A dose of 1 unit per day is evidently insufficient to produce calcium deposits in the kidneys, while it may or may not produce a slight bone lesion. There is some indication that a greater effect is produced by giving the daily dose in two portions.

Again, the first noticeable effect is an increase in

osteoclasts. This is the same change as was observed in rat # 11, which died with oedema following injections of rachitic serum.

From the data available, then, it is impossible to state whether or not there exists a state of hyperparathyroidism in rickets. In the one case in which bone changes were found to result following rachitic serum injections, these changes are apparently similar to the early lesions produced by minimum dosage of parathormone. This suggests the possibility of an abnormally large concentration of parathormone in rachitic serum. The case is, however, complicated by an unexplained oedema.

Three-day rats are so very tiny that injection of 1 cc. of serum at a time was difficult, and injection of larger amounts would be practically impossible. In order to obtain a more extensive lesion by the use of rachitic serum, therefore, it would be necessary to employ some method of concentrating the active principle from the serum.

The results by the injection of rachitic serum being inconclusive as to the presence of a state of hyperparathyroidism in rickets, it was decided to attempt to concentrate parathormone from serum. If this could be accomplished successfully by the use of large quantities of normal serum, it might be possible to demonstrate by the same method whether or not there is an excess of the principle in rachitic serum.

III. CONCENTRATION OF PARATHORMONE FROM NORMAL SERUM.

Experimental Procedure.

About 3 litres of blood were taken from two cows, under sterile conditions. The blood was allowed to clot for two hours at room temperature, and then for 24 hours in the refrigerator. The serum was decanted off, filtered from small clots, centrifuged to remove cells, and decanted. Throughout this and subsequent processes, the material was kept in the refrigerator as much as possible. About 1300 cc. of serum were obtained altogether.

Permutit was prepared as for urinary ammonia,[57] being set up in a calcium chloride tube, with a plug of cotton wool in the end. It was washed three times with 2% acetic acid, and three times with distilled water, by suction. The serum was passed through the prepared permutit, by the aid of suction, at the rate of about one drop per second. The permutit was renewed after each 300 cc. of serum. Last traces of serum were washed out of each lot of permutit with distilled water. Each new lot was treated with acetic acid and water before using.

The permutit was combined and washed four times, by decantation, with a saturated solution of sodium chloride, and four times with distilled water. This procedure is stated not to remove parathormone absorbed on permutit.[49] Whitehorn has shown that potassium chloride and sodium chloride are good general reagents for removing substances absorbed on permutit;[50] it was thought, therefore, that this treatment might remove some unwanted substances which must necessarily have been absorbed from the serum. Whitehorn[50] has also shown that only the rel-

atively strong bases are capable of being absorbed upon permutit; thus most of the ordinary constituents of serum would have passed through unabsorbed. The saline extract appeared cloudy, very like some protein solutions.

The permutit was next extracted three times with a 5% ammonium hydroxide solution in the refrigerator at 5 degrees Centigrade. The extract appeared fairly clear and of a slight brownish tint. It was concentrated in vacuo at about 50 degrees (water-bath temperature) to a volume of about 200 cc., using caprylic alcohol to prevent foaming. The protein content of this solution was precipitated by the addition of excess 20% trichloracetic acid. The precipitate, which was a dirty white in colour, was filtered and dried in vacuo over concentrated sulphuric acid. During the process of drying, it became hard, shiny, and a very deep brown colour. It was preserved in the refrigerator over calcium chloride. The total amount of dried material obtained was approximately 0.03 gm.

The dry product was found to be insoluble in water and in HCl at a pH of 3. It was also insoluble in normal saline. Parathormone has been stated to be soluble in all these three reagents. The material dissolved slowly in a moderately strong solution of ammonium hydroxide, from which solution it could be precipitated by the addition of concentrated HCl, drop by drop. The precipitate thus obtained was a dirty greyish-white, similar to the original trichloracetic precipitate. It seems, therefore, that the change in colour noted on drying the material very likely did not represent an irreversible chemical

alteration.

Unlike the dried material, the precipitate obtained from alkaline solution by the addition of acid was found to be readily soluble in HCl at a pH of 3, and soluble, though in smaller amounts, in distilled water and in normal saline. The latter two solutions were rather cloudy, while the HCl solution was clear and slightly brownish.

Biological Tests.

The material obtained was tested upon young rats, using solutions in ammonia, HCl at pH 3, and in normal saline. The solutions were not sterilized in any way, although a sterile syringe was used for the injections.

Experiment 1.

A solution of the dry material was made by dissolving a small amount in as little ammonium hydroxide as possible, and diluting the resulting solution with an equal volume of 1.7% saline. Two 3-day rats were given injections of this solution, as follows:

July 23. 10:00 A.M. Each rat given 0.25 cc.

10:15 A.M. Rats had developed hematoma around the point of injection.

4:45 P.M. One rat killed, and bone and kidney sections made.

Microscopics:

Rat # 33.

Bone: Normal.

Kidney: No calcium deposits.

July 24. The other rat was apparently recovering. The region of haematoma appeared as if it were becoming necrotic.
July 25. Rat was killed, and femur and kidney sections made.

Microscopics:

Rat # 34.

Bone: Normal.

Kidney: No calcium deposits.

Experiment 2.

A small amount of the dried material was dissolved in as little ammonium hydroxide as possible. Concentrated HCl was added, drop by drop, until a maximum precipitate resulted. This was centrifuged, the supernatant fluid decanted off, and the residue dissolved in a small amount of HCl of pH 3.

One 5-day rat was injected with this solution, as follows:

July 24. Rat given 0.5 cc.
July 26. Slight haematoma present. Rat was given 0.5 cc.
July 28. Rat given 0.75 cc.
July 29. The rat was killed, and bone and kidney sections made.

Microscopics:

Rat # 35.

Bone: Normal.

Kidney: No calcium deposits.

Experiment 3.

Some of the dry material was dissolved in ammonia, and precipitated by HCl, as before. The precipitated material was dissolved in normal saline, in which it formed a slightly cloudy

solution. One 5-day rat was injected with this solution, as follows:

July 24. Rat given 0.5 cc. of solution.

July 25. Rat given 0.75 cc. of solution.

July 26. Rat given 0.9 cc. of solution.

July 27. Rat given 1.0 cc. of solution.

July 28. Rat given 1.0 cc. of solution.

July 29. The rat was killed, together with a normal control, and femur and kidney sections made.

Microscopics:

Rat # 36. (Test rat)

Bone: Normal.

Kidney: No calcium deposits .

Rat # 37. (Control)

Bone: Normal.

Kidney: Normal.

Experiment 4.

One 5-day rat was injected, as follows, with the saline solution prepared in experiment 3.

July 31. The rat was given 0.75 cc. of the solution.

Aug. 1. Rat given 1.0 cc. of the solution.

Aug. 2. The rat was killed, together with a normal control. Bone and kidney sections were made.

Microscopics:

Rat # 45. (Test rat)

Bone: Normal.

Kidney: Normal.

Rat # 44. (Control)

Bone: Normal.

Kidney: Normal.

Experiment 5.

The precipitated material, obtained as before, was suspended in five times its volume of acid alcohol (35 cc. of 95% alcohol to 15 cc. of 10% HCl), allowed to stand for awhile, and centrifuged. Only a small portion of the material dissolved in the alcohol. The alcoholic solution was decanted off, and to it was added an excess of ether. A slight precipitate formed, which was centrifuged. The supernatant liquid was removed, the precipitate washed with ether and dissolved in normal saline.

This process has been stated to purify parathormone.[40]

The solution obtained as above was injected into a 3-day rat, as follows:

Aug. 12. Rat given 1.0 cc. of the solution.

Aug. 13. There was present a slight hematoma from the last injection. The rat was given 1.0 cc. of the solution.

Aug. 14. The rat was killed, along with a normal control. Bone and kidney sections were made.

Microscopics:

Rat # 50. (Test rat).

Bone: Normal.

Kidney: Very congested; no calcium.

Rat # 51. (Control)

Bone and Kidney: Both normal.

Remarks:

Since the results of all the biological tests upon the material isolated from serum are negative, it follows that this material cannot contain an appreciable quantity of the active principle of the parathyroid glands. Two explanations are possible:

(1) Normal serum does not contain a sufficient amount of parathormone to enable its isolation in the manner described, or

(2) The parathormone was lost at some point in the chemical procedures.

If the work of Collip and of Tweedy upon the chemistry of parathormone is correct, the second possibility is very unlikely: the method of isolation was based upon the chemical properties of the hormone.

There are, however, two stages at which loss of the active material might have occurred. Firstly, removal of the absorbed material from permutit, by means of ammonia, was done in the refrigerator at 5 degrees C., while Tweedy states that parathormone is removed at 0 to 2 degrees C. There is thus the possibility of incomplete removal. Secondly, when the trichloracetic acid precipitate was drying in vacuo over concentrated sulphuric acid, oxidation may have occurred, inactivating the material.

With the information available, it is impossible to say definitely where the fault lies.

S U M M A R Y .

1. Calcium and phosphorus balances upon several patients with pathological bone conditions are presented.

2. A marked generalized oedema was produced in a young rat by the injection of a specimen of serum from a rachitic child.

3. An increase in osteoclasts was produced in the femur of a young rat by frequent injections of relatively large amounts of rachitic serum.

4. An increase in osteoclasts in the bones was found to be one of the first noticeable effects of injections of very small doses of parathormone into young rats. The possibility of a state of hyperparathyroidism in rickets is suggested.

5. It was found that doses of parathormone large enough to produce extensive bone lesions in young rats, also produced calcium deposits in the kidney substance. Calcium casts were observed in the tubules, at first, but upon continued administration of parathormone these disappeared, leaving deposits of calcium in the interstitial tissue only.

6. An attempt to concentrate parathormone from normal cow's serum is described.

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