THE RICE INSTITUTE

STUDIES ON THE INFLUENCE OF THE
PARATHYROID ON THE RENAL EXCRETION
OF PHOSPHATE IN DOGS

by

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A THESIS
SUBMITTED TO THE FACULTY
IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
MASTER OF ARTS

Houston, Texas
May, 1954
ACKNOWLEDGMENTS

I wish to express my appreciation to Doctor Roy V. Talmage who has directed this research, for his generous aid and most helpful advice.

I wish also to thank my fellow graduate students for much indispensable assistance in administering intravenous anesthesia to the experimental animals.

Finally, to my wife, Suzanne, for typing this thesis from my illegible notes, my heartfelt thanks.
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INTRODUCTION

The parathyroid glands may be considered the "youngest" of the endocrine glands both from the standpoint of knowledge about their existence and function and from an evolutionary standpoint as well.

Parathyroid tissue has been demonstrated only in mammals, birds, reptiles and the terrestrial forms of the amphibia. The other endocrine organs can be traced back at least to some of the fishes and in many cases can be shown to occur throughout the Vertebrata.

Some seventy years ago the parathyroids were recognized and described, but it was not until the end of the first decade of this century that their function began to be perceived. The major progress toward understanding their physiology came in the decade between 1925 and 1935 following the preparation of a potent extract of the glands by several workers. Since that time, progress in parathyroid research has been slow. This has been due partly to the lack of a pure preparation of the hormone, or hormones, from the glands, and partly to the fact that parathyroid function though of major import to the normal economy of an animal, may be replaced effectively by proper calcium therapy.

Of late there has been an increased interest in parathyroid research with the result that the older theories have been examined, revised, occasionally discarded, and new ideas have been put forth in attempts to explain the mode of action of these glands.

The work described below is one phase of a larger work concerned with parathyroid function which is being carried out in these laboratories.
These experiments have been concerned with certain aspects of the factor in the parathyroid extract which leads to an immediate and sharp rise in urinary phosphate following the injection of the extract into normal or hypoparathyroid dogs.
HISTORICAL BACKGROUND

Early knowledge of the function of most of the endocrine glands came from the clinical observation of the problems attendant to their loss, or malfunction. With the parathyroids, however, their functional significance was first noted in deliberate physiological studies and only later was it possible to relate certain clinical syndromes to disturbed function of these glands.

Remak (1855) probably was the first to describe the parathyroids, but his reference to them was vague. Other workers (Owen, 1862; Virchow, 1863) seem to have noticed the parathyroids but they thought these "yellow glandules" were thyroid tissue. The first lucid description of the parathyroids came from Sandström (1880) and Baber (1881). Their descriptions of the glands received little notice and not until 1891, when Gley redescribed the external parathyroids, did serious consideration of the role of these glands come into being.

Actually the symptoms of parathyroid insufficiency had been seen clinically and experimentally for some time without any realization of their significance. Clarke (1815), Dance (1831), and Corvisart (1852) had observed hypothyroid tetany. Parathyroprivic symptoms following thyroidectomy were observed and described by Raynard (1836), and Schiff (1859) in laboratory animals and by Kocher (1882) and Schiff (1884) in humans.

Although Gley knew that total parathyroidectomy was fatal and ascribed the survival of the rabbit to accessory parathyroid tissue (1892), he...
thought the glands were thyroid tissue and disclaimed Moussu's suggestion (1892) that the glands might have a separate function. Kohn (1895) described the internal parathyroids and later, on the basis of what embryological and pathological evidence was available, declared that the parathyroids were distinct organs of internal secretion. Vassale and Generali (1896, 1900) demonstrated that removal of the parathyroids only, caused tetany and said that post-thyroidectomy tetany was due to inadvertent removal of the parathyroids.

Although the mode of action of the parathyroids was as yet unknown, Jeandelise (1902) suggested that infantile, maternal and idiopathic tetany might be of parathyroidal origin. At this same period, Sabbatini (1901) and Loeb (1901) were demonstrating the relationship between low serum calcium and muscle hyperirritability. MacCallum and Voegtlin (1909) closed this chapter of the study of parathyroid function by demonstrating that hypoparathyroid tetany was a consequence of the low serum calcium accompanying the loss of the glands.

Greenwald (1911) was the first to note that urinary phosphorus was strikingly decreased following parathyroidectomy. Subsequently, this work was amply confirmed and it was shown that when parathyroid extract is administered, an increased urinary elimination of phosphate follows.

The first clue to the relationship of the parathyroids to bone disease came from Askanazy's work (190h) in which he described a case of osteitis fibrosa (von Recklinghausen's disease) with an associated parathyroid adenoma. Mandl (1926) obtained remarkable improvement of a case of osteitis fibrosa by removing a parathyroid tumor. Conclusive proof of an
effect of the parathyroids on bone was provided by the experimental pro-
duction of bone lesions in animals by Jaffe and Bodansky (1930).

Koch in 1912 demonstrated the presence of methyl guanidine in the urine
of parathyroidectomized dogs and thus began an unfortunate episode in the
story of parathyroid function. Some of the guanidines when injected intra-
venously produce symptoms resembling tetany, and several workers, (Paton
and Findlay, 1917; Sutherland Simpson, 1922), proposed that the parathy-
roids served to detoxify some toxin in the body, probably a guanidine.

The preparation of a potent extract of the parathyroid glands by Ber-
man (1924), Hanson (1925), and Collip (1925) gave workers the tool they had
needed to demonstrate conclusively the role of the parathyroid glands in
the economy of the animal body.

In the thirty years since the first preparation of an extract contain-
ing the active principle of the parathyroid glands, much progress has been
made concerning the physiology of the parathyroid hormone. There is no un-
certainty regarding the effects of the parathyroid hormone, but as yet the
mode of action of the hormone has not been determined. The principal
stumbling block is the lack of a pure preparation of the hormone of the
parathyroid glands. The best preparations today contain about half a
dozens components, any one of which could be the active principle. As a
matter of fact, there is increasing evidence that there may be more than
one type of activity in parathyroid extract. It is fortunate for the clin-
ician, but not so for the physiologist, that substances other than parathy-
roid extract, i.e. calcium therapy and dihydrotachysterol administration,
may serve successfully to replace parathyroid hormone in the body's economy.
There is thus no pressing need for the further purification of the extract and as a result very little headway has been made in this field for some time. Actually the problems concerning the mode of action and purification of the parathyroid hormone are interrelated and it may be that the most profitable line of future investigation will be one which embraces both problems at once.
METABOLISM

The historical review given above makes one thing clear, the hormonal elaborations of the parathyroid glands are involved in the metabolism of calcium, phosphorus and bone. It is appropriate, therefore, to examine in some detail the salient aspects of the metabolism of these minerals and this tissue before proceeding with a discussion of the physiology of the parathyroid glands.

Calcium Metabolism

It is important to remember that calcium, phosphorus and bone metabolism are intimately interrelated and cannot be discussed completely separately. This section will discuss some aspects of metabolism peculiar to calcium, and a correlation between this and the succeeding section on phosphorus will be made in the section on bone physiology.

A discussion of calcium metabolism must include several points: (1) the introduction of calcium into the body by intestinal absorption, (2) transport via the bloodstream, (3) participation in various metabolic schema, notably bone growth, and (4) loss of calcium through the urine and/or the feces.

The physiology of calcium metabolism has been reviewed recently by Nicolaysen et al. (1953). The Transactions of the conferences on Metabolic Interrelations of the Josiah Macy, Jr. Foundation contain excellent discussions of calcium and bone metabolism in general.

The net amount of calcium absorption is the sum of absorbed calcium, minus secreted calcium, plus reabsorbed calcium. The second term, secreted
calcium, has been in controversy for some time. If one places an animal on a calcium-free diet, calcium can be recovered in the feces for some time. It must be remembered that at the time the animal commences a calcium-free regimen, there may be a considerable quantity of calcium remaining in the gut, which is eliminated over a considerable period of time. Well conducted experiments involving the parental injection of calcium salts, (to produce a hypercalcemic state) (Nicholls and Nimalasuriya, 1939; McCance and Widdowson, 1939; Greenberg, 1945) indicate that no regulated secretion of calcium occurs. Colectomized animals excrete just as much calcium as control animals and an isolated colon secretes no calcium into the lumen according to Nicolaysen (1934).

The foregoing reports demonstrate convincingly that there is no active secretion of calcium by the gut; however, the fact remains that even during long periods on a calcium free diet, some calcium is eliminated in the feces. It is now known that the source of this calcium is the digestive juices, particularly bile (Nicolaysen et al, 1953).

**Calcium Absorption**

Several factors influence the absorption of calcium by the gut, the chief one being vitamin D (Nicolaysen and Eeg-Larsen, 1953). The effect of cod liver oil on the clinical disease, rickets, has been known for centuries. Actually rickets may be caused by a deficiency of several factors besides calcium, such as phosphorus, magnesium, deficient bone protein, etc. However, rickets, as commonly understood, is caused by a deficiency of calcium ordinarily occasioned by either a low calcium diet or defective absorption of calcium due to avitaminosis D. While vitamin D is known to be
essential for the absorption of calcium in most species, the mechanism of
calcium absorption is not known. The recent review of Nicolaysen and Eeg-
Larsen (1953) indicates that the effect on calcium and an effect on citric
acid in the bone are the only direct actions of vitamin D.

Steatorrhea may lead to low calcium absorption due to both the removal
of vitamin D, which is fat soluble, and the formation of calcium soaps in
the gut.

Phytic acid depresses calcium absorption by forming an insoluble cal-
cium compound (Mellanby, 1921; Bruce and Callow, 1934). Oxalic acid has
an effect on calcium absorption similar to that of phytic acid (Sherman,

A high phosphate diet may depress calcium absorption by forming in-
soluble calcium phosphates. A diarrhea of any magnitude also leads to a
negative calcium balance by denying sufficient opportunity for absorption.

Transport of Calcium

Calcium is moved in the body by the blood, lymph, and extracellular
fluid. Normally the serum calcium ranges near 10 mg%, but in pathologi-
cal conditions it may be twice this figure or as low as one-third normal.
The fact that serum calcium remains normal for some time in spite of de-
ranged calcium metabolism, points to the fact that the bones contain a
large reserve of excess calcium which is easily mobilized. About one-half
of the serum calcium is bound to protein. Most of the rest is ionized
(McLean and Hastings, 1935) but a small fraction is in the form of a com-
plex, probably calcium citrate, which is diffusible but not electrically
charged.
Utilization of Calcium

Over 99% of the calcium in the body occurs in bone and as a result the most important function of calcium is its participation in skeleton formation. The non-osseous calcium is, however, very important to the animal body. The best known but not, perhaps, most important function of this small amount of calcium is its participation in the clotting of blood.

One of the chief values of calcium to the organism is the role it plays in holding cells together by controlling their viscosity. Calcium participates in the buffer system of the body, and by the formation of soaps plays an important part in the passage of cell membranes by the fatty acids. Calcium is concerned with nervous stimulation of muscle, probably at the synapse and/or end plate of the nerves. A deficiency of ionized calcium causes hyperirritability of muscle and may lead to tetany. Just how calcium ions serve to inhibit contraction of the muscle is not understood. Paradoxically some calcium must be present in muscle for it to contract at all. Heilbrunn's *General Physiology* is recommended for a further discussion of the physiological role of calcium. The role of calcium in bone physiology will be discussed under that heading.

Excretion of Calcium

Calcium is lost from the body via the urine or the digestive juices. The latter route though tangible is not an important avenue of calcium loss. With adequate calcium diets the loss of calcium into the lumen of the gut is unimportant, and when an animal is on a calcium deficient diet, there is an increased efficiency of absorption which would be expected to reduce fecal loss (Nicolaysen *et al*, 1953).
Little is known of the specific mechanism whereby calcium is excreted in the urine. Urinary calcium fluctuates in response to changes in calcium absorption, phosphate absorption, blood pH, and certain hormonal influences, notably parathyroid hormone. Nicolaysen et al. (1953) have calculated that on a dietary intake of 10 mg./kilo, normal humans excrete an average of 25% of the ingested calcium. If intake is halved, the urinary calcium remains nearly unchanged in short term studies. Thus the urinary calcium excretion for an individual at any given time is relatively constant. The authors report that individual fluctuations do occur over a long period of time (more than two weeks). Day to day fluctuations do occur and may be correlated with alterations in calcium intake, but these changes do not approach the magnitude of the dietary changes which they reflect. Apparently this tendency toward a "constant" calcium excretion is conditioned by the hormonal status of the individual.

Aside from the marked effect of parathyroid hormone on calcium excretion, to be discussed presently, several other hormones may be involved. Estrogen is known to depress calcium excretion probably by increasing calcium storage in the bones (Albright and Reifenstein, 1948). ACTH may or may not effect calcium excretion (Albright and Bartter, 1950).

**Phosphate Metabolism**

As with calcium, most of the body phosphate (80-90%) is found in the bones. Thus a deranged phosphate metabolism could easily lead to an altered calcium metabolism by effecting the physiology of the bone.

**Phosphate Absorption**

Normally the absorption of phosphate from the gut is complete and ra-
pid. It has been shown definitely that vitamin D plays no direct part in phosphate absorption (Nicolaysen and Eeg-Larsen, 1953). Calcium can bring about a reduction in phosphate absorption in the gut by the same mechanism discussed above for the reduction of calcium absorption, namely the formation of insoluble calcium phosphates. Other metals such as iron and aluminum can operate in the same manner.

Transport of Phosphate

Serum phosphate levels are more variable than calcium levels both as regards different species and different individuals. The normal serum phosphate value for dogs is 5 mg,% (Albright and Reifenstein, 1948). The value in young animals is higher. Practically all of the serum phosphorus is unbound and freely diffusible (Peters and Van Slyke, 1931). Recent work (Jacobs and Verbanck, 1953; Jacobs et al, 1953) indicates that the non-diffusible fraction is on the order of 7-8% of total serum phosphate. Jacobs and his workers note the possibility that the demonstration of a non-diffusible fraction may be artefactual.

Utilization of Phosphate

Quantitatively, the principal function of phosphate in the body is participation in the skeleton, but the appreciable fraction of non-bone phosphate is of profound import to the body. An excellent survey of the role of phosphorus in the body is contained in Glass' summary of the First Symposium on Phosphorus Metabolism of the McCollum-Pratt Institute (1951). Phosphorus, as phosphate, is incorporated in the structure of the phospholipids, which by their participation in cell membrane structure contribute to the regulation of cell permeability. Phosphates also participate in
the structure of ribose and deoxyribose nucleic acids. Phosphate forms one of the major buffer constituents in the body fluids. The major role of phosphate, however, is concerned with intermediary metabolism. An incredible amount of knowledge has been gathered in this field in the last half century. The elucidation of the part played by phosphorus in carbohydrate metabolism, oxidation-reduction reactions, and the discovery of the value and role of the high-energy phosphate bond in the body economy form the foundation of present day biochemistry. Pertinent aspects of the role of phosphate in bone metabolism are discussed in the succeeding section.

**Excretion of Phosphate**

Phosphate excretion has been admirably dealt with by Smith (1951) and will be treated here only to point out those aspects which seem to have some bearing on the problem.

The possibility exists that phosphate is secreted into the tubules (Kleeman and Cooke, 1951) but there is no convincing evidence that this is so (Hogben and Bollman, 1951a). Presumably phosphate reabsorption in mammals occurs in the proximal tubules as is known to be the case in *Necturus* (Walker and Hudson, 1937).

Several workers (Harrison and Harrison, 1941a, 1941b; Pitts and Alexander, 1944; Schies. et al., 1948) have shown that there exists a maximal phosphate reabsorption rate (designated $T_{m}$ in the succeeding discussion) in the kidney of the dog. Reabsorption of phosphate is essentially total when the ratio filtered phosphate is less than $0.75$. Excretion of phosphate begins when the above value is exceeded and tubular
saturation occurs when the ratio equals about 1.5. Additional phosphate presented to the kidney at this point is unavailable for reabsorption and is all excreted.

The $T_{mp}$ for an individual animal (dog) has been shown to be relatively constant (Pitts and Alexander, 1944; Ayer et al., 1947), indicating the presence of a specific mechanism for the handling of this ion. The exact scheme of this mechanism has not yet been determined, however several factors are known which modify phosphate reabsorption.

$T_{mp}$ may be lowered by several procedures including cortisone administration (Roberts and Pitts, 1953), general anesthesia (Bartter, 1954), glucosuria (Hogben and Bollman, 1949), and prolonged perfusion of phosphate (Hogben and Bollman, 1949, 1951). In the last case it is possible that the lower $T_{mp}$ is a result of adrenal cortical stimulation. Hydrochloric acidosis and bicarbonate alkalosis fail to influence $T_{mp}$. The depression of $T_{mp}$ caused by glucose may be either a competition for available "space" for reabsorption or else a competition for available energy. Smith (1951) indicates that the specific interference by glucose may be an artefact.

The reabsorption of phosphate is related to the reabsorption of alanine and glycine (Ayer et al., 1947) since increased reabsorption of these substances leads to a marked decrease in phosphate reabsorption. Glucose can lower phosphate excretion by causing a decrease in plasma phosphate.

Osmotic and water diureses fail to alter phosphate excretion, but acidosis leads to an increased excretion. This latter effect is referable to the role of phosphate as the principle buffer in the urine and not to an alteration in the mechanics of reabsorption.
As yet there is no proven, direct effect of parathyroid hormone on phosphate reabsorption although certainly this hormone greatly alters the excretion of phosphate. This aspect of phosphate excretion will be discussed below.

The Physiology of Bone

We are not concerned here with bone growth from a morphological or gross point of view, but rather with the composition of bony tissue and the biochemical processes whereby bone is formed. The best references to present concepts of bone formation and structure is to be found in the Transactions of the conferences on Metabolic Interrelations, mentioned previously, of which four volumes have been published.

Composition of Bone

It is necessary to think of bone as being composed of two aggregates of material, an organic matrix and an inorganic crystalline compound composed principally of calcium, phosphate, and bicarbonate, with variable amounts of several other substances such as magnesium, sodium, and citric acid. It now appears that the inorganic portion of bone is basically a hydroxyapatite crystal with the various other substances trapped and/or adsorbed on the crystal surface. The following quotation from Hendricks and Hill (1951) summarizes the current concept of bone salt structure.

"Apparently, what happens is the following: The crystal starts growing and, as it grows as hydroxy apatite, it has got a trashpile of stuff that it does not want in the hydroxy lattice, and it starts excluding these. Among these ions is carbonate, magnesium, and sodium. As the crystal grows these materials apparently completely cover the surface and stop the growth. So one characteristic of bone salt is that it is prevented by the mere adventitious materials that get upon the surface from growing beyond a certain size. A new surface can be started
"by covering up this old one, and this is done in the case of enamel. A new surface can also be started just by growing as an absolutely independent crystal, and that apparently is done in the case of bone."

The organic substance of bone is composed of an amorphous ground substance in which is found a fibrillar structure. The exact chemical status of these components of bone matrix has not been worked out, but for cartilage matrix which is presumably similar, the fibrillar substance seems to be collagen and the amorphous ground substance chiefly a muco-polysaccharide, chondroitin sulfuric acid (Partridge, 1948). Meyer (1952) believes that the collagen and the chondroitin sulfate are polar-linked in cartilage.

Formation of Bone

If we assume that the foregoing statements fairly characterize the substances which compose bone, then the problem is how are these components assembled into proper form to yield the tissue we call bone?

Certainly part, if not all, of the process of bone elaboration is the result of cellular activity. There is found in bony tissue two principal types of cells, mononuclear osteoblasts and polynuclear osteoclasts. It is well established that the former are characteristically associated with bone deposition and the latter with bone resorption. There is some question as to whether these cells are different types, or are different phases of one basic cell. It is also possible that each may in part serve both to form and destroy bone.

One theory of bone formation, championed notably by Albright and Reifenstein (1948), holds that the osteoblasts elaborate the bone matrix and that the inorganic salts "precipitate" within the matrix. This same
theory would explain resorption by saying that bone salts are removed from the matrix in accordance with the physico-chemical laws governing solid-fluid phase equilibria, and that the osteoclasts function as foreign body giant cells and remove the "decalcified" matrix. Such an hypothesis, however, must undertake to explain how the precipitation of bone salt occurs and why it occurs only at the site of the bone matrix. The Albright-Reifenstein school proposes that there is some solubility product for calcium and phosphate which is exceeded at the site of calcification, leading to precipitation of a calcium-phosphate compound, probably hydroxyapatite. How, then, is this solubility product exceeded? It is known that there is phosphatase present at the site of calcification (Robison, 1923). Phosphorylase has also been demonstrated (Gutman and Gutman, 1941). It is claimed that at the site of calcification, a local elevation of phosphate ions occurs due to organic dephosphorylation. The increased titer of phosphate ions exceeds the solubility product, and bone salts are precipitated. This theory has two major difficulties. First, according to the calculations of Albright and Reifenstein themselves, blood appears normally to be super-saturated with calcium and phosphate ions, which is obviously not probable. Second, there has been no demonstration of an organic phosphate to serve as a substrate in the dephosphorylation scheme outlined above.

The observation that glycogen accumulates in hypertrophic cartilage and disappears just prior to, or during, calcification, was thought to provide substrate for dephosphorylation by phosphatase or phosphorylase. Indeed Gutman and Yu (1949) and Marks and Shorr (1950) were able to show that blockage of the glycogenolytic cycle stopped calcification,
although blocking the Krebs cycle did not effect calcification. None- 
theless, these authors were unable to conclude that it was phosphate which 
was supplied by glycogenolysis. It might as easily supply energy or (instead 
of excess phosphate ions) some special form of phosphate.

Recent years have seen the presentation of some impressive data indica-
tion that early research designed to determine the critical factor in cal-
cification of osseous tissue, may have been directed at the "cart and not 
the horse." Utilizing Lison's metachromatic staining technique with tolui-
dine blue and Hotchkiss' periodic-leucofuchsins stain, Levine et al (1949) 
and Rubin and Howard (1950) made some observations which led them to con-
clude that the acid muco-polysaccharide, chondroitin sulfate, plays a de-
finite role in determining the calcifiability of osseous tissue. Toluid-
dine blue attached to highly polymerized compounds gives a metachromasia 
(purple) color. A red color following treatment with leucofuchsins indi-
cates the presence of ketone radicals formed from adjacent hydroxyl groups 
or adjacent hydroxyl and amino groups. The authors cited above conclude 
that when both of these tests are positive in a tissue, the presence of 
chondroitin sulfate is demonstrated. It was already known that uncalci-
fied cartilage or osteoid (uncalcified bone) gave positive responses to 
both tests. It was claimed, however, that the acid muco-polysaccharide 
disappeared when calcification occurred (Sylven, 1947). It was shown by 
Rubin and Howard that this was not the case, but rather, the metachromatic 
material was obscured by the calcium salts. These authors observed that 
in cartilage immediately prior to calcification there occurred a sharpening 
of the two staining reactions in the area adjacent to the hypertrophic
cartilage cells. This is the same area where calcification first appears.

Their interpretation of this phenomenon is best expressed in their own words:

"...we believe there is a cause and effect relationship between the change in state of acid muco-polysaccharides of the organic matrix, and calcification of that matrix. The same change which is responsible for a change in their dye-binding capacity is probably also responsible for an increased calcium affinity which thereby established calcifiability."

**Destruction of Bone**

As far as the breakdown of osseous tissue is concerned, the bone of contention seems to be whether this is merely the reverse of bone formation or if the osteoclasts phagocytize osseous tissue, and if so is the ingested tissue first decalcified. Actually it seems that little will be learned in this respect until more is known of the process of bone formation.
PHYSIOLOGY OF PARATHYROID HORMONE

The determination of the effects of a hormone on the body may proceed in two directions; the effects of removal of the hormone from the body economy and the effect produced by administering the hormone to both normal and deficient animals. In order to limit the discussion of parathyroid physiology, only studies of the hormone in the dog will be discussed unless work in other species seems pertinent.

Effects of Parathyroprivy

Removal of the parathyroid glands instigates a characteristic chain of events which if unaltered leads to the death of the animal. However, to set off this sequence of bodily malfunctions, it is necessary to remove all or practically all of the parathyroid tissue, for in spite of their minute size the parathyroids normally represent more than twice as much tissue as is necessary for normal existence. Several workers (Mackallum and Voegtlin, 1909; Shelling, 1935) have described in detail the symptoms of parathyroprivy in dogs. About half a day following parathyroidectomy the animal refuses to eat and becomes restless. Muscular twitching develops which becomes progressively worse. As the tremors become more general, the animal becomes apprehensive, the body temperature is elevated and hyperpnea may develop. Finally clonic and tonic spasms develop and the animal may go into general convulsions. Death, if it occurs, usually is the result of laryngeal spasm or exhaustion. If the first attack is not fatal, spontaneous remission occurs, but a new attack follows some hours later.

It is now known that the frank symptoms of parathyroprivy are the results of the lowered serum calcium, and that any means which will serve
to elevate the serum calcium level will produce remission of the symptoms. Since the alterations of body function described above are results of the altered composition of the body fluids, it is to them that one must turn to seek an explanation of the function of the parathyroid hormone.

Changes in Calcium Following Parathyroidectomy

It was first shown by MacCallum and Voegtlin (1909) and since amply confirmed by other workers, that there is a prompt and steady decline in serum calcium following loss of the parathyroids. Some calcium is lost via the kidneys; however, even if there were no urinary calcium lost, the amount of loss which would lower blood calcium to tetanic levels is negligible. As mentioned in the section on calcium metabolism, about half of the serum calcium is ionized and it is this fraction which is concerned with nervous irritability. There is no precise level at which tetany occurs, since one occasionally sees tetany with a serum calcium of 8 mg. % and fails to find tetany with a serum calcium below 5 mg. % (Turner, 1943).

Changes in Phosphorus Following Parathyroidectomy

As previously mentioned, Greenwald (1911) was the first to demonstrate the abrupt hypophosphaturia which follows parathyroidectomy. His observations were substantiated in his later work and have been amply confirmed by many other workers. He was unable to demonstrate any increase in serum phosphate and concluded that the excess phosphate was stored in the tissue (1924). Subsequently, several workers (Salvesen, 1923; Albright and Ellsworth, 1929; Shelling, 1932) have shown that a definite rise in serum phosphate accompanies the hypophosphaturia.
Effect of Parathyroidectomy on Bone

Little change in bone structure is seen following parathyroidectomy, but the metabolism (i.e., turnover) of the osseous tissue is reduced.

Effects of Parathyroid Extract Administration

The events following parathyroid extract administration are generally the same whether the animal be hypo- or iso-parathyroid, except that they are exaggerated in the hypo-parathyroid animal.

If the dosage of parathyroid extract is too high or administration too frequent, a syndrome is seen which may culminate in the death of the animal. The changes noted above in the body fluids are forced to extremes, especially in the blood. The serum calcium may reach 20 mg. % or more. The serum phosphorus drops to a very low level but later begins to rise. This pre-terminal rise in serum phosphorus begins while the excretion rate is still considerably above normal (Talmage et al., 1953b). The cause of the rise is a calcinosis of the tubules, which makes the kidney unable to handle the large phosphate load from the bone which is being resorbed. In this extreme hyperparathyroid state, the animal becomes first ill at ease with vomiting and diarrhea and later apathetic and finally comatose. Death usually results from circulatory failure. These symptoms are caused by a combination of the high serum calcium and renal failure.

Changes in Calcium Following Parathyroid Extract Administration

If parathyroid extract is given intravenously, there is a rise in serum calcium which reaches a peak in about four to eight hours and then subsides. By the subcutaneous or intramuscular route, the peak rise in serum calcium does not occur until the fifteenth to twenty-fourth hour. There
is also an eventual rise in urinary calcium, but this change seems to depend upon the serum calcium rising above a threshold for excretion.

**Changes in Phosphorus Following Parathyroid Extract Administration**

It has long been known that the first effect of administration of parathyroid extract is an immediate rise in phosphate excretion (Greenwald, 1911; Greenwald and Gross, 1925; Ellsworth, 1932; Logan, 1939; Handler *et al.*, 1951). This augmented phosphaturia is followed by a depression of the serum phosphorus (Cantarow *et al.*, 1938).

The speed with which the rise in urinary phosphate occurs was noted by Logan (1939) who found a definite rise in one hour; and Tweedy and Campbell (1944) who detected the rise in the first hour with $^{32}P$ and were of the opinion that it started immediately after injection. Handler *et al.*, (1951) detected the increased phosphaturia within 30 minutes to one hour following intravenous injection of parathyroid extract.

**Effects of Parathyroid Extract on Renal Hemodynamics**

The abrupt and marked changes in phosphorus excretion following parathyroid extract administration have suggested that the parathyroid hormone may exert a direct influence on the way phosphate is handled by the kidney. Harrison and Harrison (1941b) report decreased reabsorption of phosphate by the kidney following subcutaneous injection of parathyroid extract. Fay *et al.*, (1942) found no change in phosphate/creatinine clearance in either parathyroidectomized or parathyroid extract treated animals which they studied. Handler *et al.*, (1951) have examined the immediate effects of parathyroid extract upon renal hemodynamics as related to phosphate excretion. They found that following intravenous administration of the extract,
there were marked increases in GFR and RPF. There was an increase in reabsorption of phosphate which was, however, less than the increased filtration, so that a phosphuria resulted. Following subcutaneous injection, the renal changes were less marked and the phosphuria resulted chiefly from a decreased reabsorption. They found no change in plasma calcium, but found a rise in plasma phosphate following intravenous injection. In contrast, animals injected subcutaneously showed a decline in phosphate and an elevated plasma calcium.

In a later paper, these authors reported that the increased plasma phosphate resulted from the conditions of the experiment and not the administration of parathyroid extract. They did confirm the rest of their previous work although the alterations in renal hemodynamics were not as great as previously reported. They cite evidence to suggest that the several results achieved by them might be due to the presence of more than one factor in the parathyroid extract (Handler and Cohn, 1952).

**Bone Changes Following Parathyroid Extract Administration**

It was observed by Jaffe et al., (1932) that following administration of parathyroid extract to dogs, there was a marked resorption of bony tissue, especially at the epiphysis. This finding has also been noted in other species. Conversely, Selye (1932) found that if very small doses of parathyroid extract were given, there was produced not bone resorption, but bone deposition. This effect was also seen when larger doses were given to animals previously made hyperparathyroid by hormone administration. Selye thought that the so-called immunity to repeated injections of parathyroid extract was due to a reversal of effect of the hormone and not to an immune
Thus it is seen that in hyperparathyroidism there occur alterations in calcium and phosphorus just as in hypoparathyroid conditions only the direction of the alterations are reversed. The symptoms of acute overdosage, too, are the reverse of those obtaining in parathyroprivy and again are due to serum calcium changes.

**Theories of Parathyroid Function**

Out of the above facts concerning various states of parathyroid function, there have evolved two divergent views as to the exact mode of action of the parathyroid hormone. One idea holds that the primary effect of parathyroid hormone is on the bone, specifically the osteoblasts. This theory is well summed up by one of its chief protagonists, Selye (1947).

The other theory of parathyroid function is commonly called the Albright-Reifenstein theory and is a part of their theory of bone metabolism discussed previously. These workers and their adherents feel that the primary function of parathyroid hormone is to control phosphate excretion at the kidney. They explain the other metabolic alterations as secondary to the renal phosphate change, being mediated through the hypothetical solubility product of ionized calcium and phosphate in the blood. Albright and Reifenstein summarize their views as follows:

"...the parathyroid hormone in some way affects the phosphate dissolved in body fluids in such a way as to make it more readily excreted by the kidney with a resulting decrease in the serum phosphorus level; this tends to make the body fluids less saturated in regard to whatever equilibrium constant governs the serum calcium and phosphorus values ... resorption of the calcium-phosphate salt from the bone-resorbing surfaces is thereby increased; there results an elevated serum calcium level together with the depressed serum phosphorus level. Once this new state of
"equilibrium has been reached there would be no further changes if it were not for the fact that the higher serum calcium level leads to an increased calcium excretion in the urine; this loss of calcium in the urine is a factor tending to cause undersaturation of the body fluids again so that unless there is a supply of calcium from the gastro-intestinal tract, the bones will have to supply the deficit; there will result, therefore, a decrease in the total amount of bone tissue and the bones will become weak."

When the same set of observations lead to such divergent opinions, it is likely that the truth of the matter lies somewhere between. Indeed there is now quite a body of evidence to show that neither theory is sufficient in itself and there is considerable speculation that the parathyroid hormone acts at both bone site and kidney or that there are, perhaps, two hormonal substances each acting at one site.

The chief evidence advanced by Albright and Reifenstein in support of their theory is the fact that the changes in phosphate metabolism generally precede the other changes when parathyroid function is altered. The evidence against their theory is much more impressive. First, their basic premise regarding solubility products is invalid, since it has been observed that calcium and phosphorus can vary independently of each other (Dent, 1953; Hopkins et al, 1952; Talmage et al, 1953a). Further, Munson et al (1952) and Talmage and Kraintz (1954) have been able to demonstrate a serum calcium fall in parathyroidectomized rats which precedes the rise of phosphorus. Conclusive proof of an action of parathyroid hormone independent of the kidneys has been provided by several works which demonstrate the typical bone changes in nephrectomized animals (Ingalls et al, 1943; Selye, 1942; Heller et al, 1950). Other workers have shown the serum calcium may still be controlled by parathyroid extract in nephrectomized and nephrectomized-para-
thyroidectomized animals (Ellsworth and Fuchter, 1935; Stoerk, 1943; Mohahan and Freeman, 1944; Talmage et al., 1953a).

The above experiments, while offering evidence against the Albright-Reifenstein theory, also offer evidence to support the contention that there is a primary action on the bone. They do not, however, exclude the possibility that there might also be an effect directly on the kidney. Several observations, in fact, lead one to the conclusion that there must be a direct effect of parathyroid hormone on the kidney. Presumptive evidence for a direct renal effect is available in the fact that phosphate excretion changes occur within the first hour after either parathyroidectomy (Talmage and Kraintz, 1954) or parathyroid extract administration (see Effects of Parathyroid Extract Administration above). Furthermore, the fact that, following parathyroidectomy, there is a state where a high serum phosphorus and low (practically none) urinary phosphorus coexist, makes it obvious that there must be a change in the kidney's handling of phosphate. That the curtailment of urinary phosphate can occur in the face of an already high serum phosphate is shown by the work of Talmage and Kraintz, (1954). Rats given 2 mg. disodium phosphate at parathyroidectomy and each hour thereafter showed a diminution of phosphate excretion on the same order as animals not receiving phosphate.

**Evidence for the Presence of More than one Active Component in Parathyroid Extract**

It is well known that commercial parathyroid extract is an inhomogeneous substance. This has been one of the major difficulties in evaluating the effects of parathyroid extract administration. The most potent
preparations known are those of Ross and Wood (1942) and L'Heureux et al. (1947). These latter workers, using electrophoresis and the ultracentrifuge, examined preparations made by their own methods as well as preparations made by the methods of Ross and Wood and Collip and Clark (1925), and found Ross and Wood's and their own preparation to contain two major components. Collip and Clark's extract contained five separate components, the activity of which appeared to be higher in the lighter components.

Handler and Cohn (1952) reported the removal, by dialysis, of a factor in parathyroid extract which raises the blood pressure following injection. They found that the capacity of the extract to raise both GFR and RPF is associated with the calcium raising factor since enzymatic digestion destroys both. However, if the extract is given subcutaneously, renal hemodynamic alterations are not seen although the ability of the extract to raise serum calcium is unimpaired. These authors suggest that the capacity to raise blood pressure, the capacity to increase GFR and RPF, and the capacity to raise serum calcium are mediated by separate factors.

In 1952, Stewart and Bowen reported that destruction, by formalin inactivation, of the calcium raising factor in parathyroid extract did not destroy the ability of the extract to produce increased phosphate excretion following intravenous injection. They feel that the phosphate factor is an artefact, produced by the extraction procedure. Kenny et al. (1954) report different ratios of calcium activity to phosphorus activity in commercial parathyroid extract and extract prepared by them.

In view of the above evidence, the presence of two pharmacologically active components in parathyroid extract is a distinct possibility.
STATEMENT OF THE PROBLEM

From the discussion above it is apparent that the chief problem in
the field of parathyroid research, aside from the problem of procuring a
pure extract, concerns the site of action of the hormone. There is ample
evidence to show effects upon both bony tissue and the kidney. Since the
bone changes can be seen in the absence of the kidney, it follows that
these changes cannot be secondary to the alterations in kidney metabolism.
It does not follow, however, that renal effects are secondary to the bone
effects for they (renal changes) can be and usually are, seen in advance
of any alterations in the bone. It is entirely possible that there are
two sites of primary action of parathyroid extract.

The simultaneous occurrence of high serum phosphate and low urinary
phosphate seen in early hypoparathyroidism, means that the loss of the
hormone from the body must cause a change in either the state of the serum
phosphorus, making it unavailable for excretion, or in the conditions of
filtration and reabsorption in the kidney. The available evidence points
to the probability that the latter condition obtains.

The technique described below was designed to provide a very sensitive
technique to demonstrate the immediate renal phosphate changes which take
place following the administration of parathyroid extract to experimental
animals. A number of experiments have been performed utilizing this tech-
nique in an attempt to determine whether the immediate renal alterations
seen are mediated by the active principle of the parathyroid extract. The
response to the intravenous and subcutaneous administration of parathyroid
extract has been investigated in normal and parathyroprivic dogs.

In addition, the effect of formalin inactivated parathyroid extract upon the urinary excretion of phosphorus was investigated in an attempt to confirm the findings of Stewart and Bowen (1952) that inactivation did not destroy the phosphorus factor of the extract.

Another series of experiments was performed using parathyroid extract which had been dialysed against, or diluted with acetate buffer to determine whether this extract acted in the same manner as intact extract.

Finally, five long-term experiments, involving a total of 26 animals, were performed. These experiments were designed to check the change in phosphate excretion produced by injections of intact and "acetate treated" parathyroid extract over a period of days, rather than hours as in the short term experiments.
MATERIALS AND METHODS

The experiments described below are of two general types: short term experiments involving major surgical techniques, and long term experiments in which animals are maintained in metabolism cages for several days.

Experimental Animals

One hundred and fifty nine adult mongrel bitches were utilized in the experiments. All animals were obtained from the local pound and used within twenty four hours of procurement. The criteria for the selection of the experimental animals were: apparent good health, weight of approximately ten kilograms, range 7-14 kilos, and sexual maturity. The genital tract of a few animals appeared to be immature but these animals could as easily have been nulliparous adults.

In the short term experiments, water (40 ml./kilo) was given by stomach tube to insure an adequate urine flow. Sodium pentobarbital was delivered into the saphenous vein one hour later. (See the section below on anesthesia for dosages.)

Operative Technique in Short Term Experiments

The abdominal cavity was entered through a mid-ventral incision which extended forward about four inches from a point some 3 inches above the symphysis pubis.

It was usually necessary to empty the bladder by manual expression or with a large syringes. The omentum and small intestine were retracted anteriorly and the horns of the uterus were deflected laterally to expose the ureters. A section of the ureter was freed from the dorsal mesentery
and partially severed. A polyethylene catheter with an outside diameter of 1.57 mm. was introduced into the cut, inserted into the ureter for a distance of about 3 cm. toward the kidney, and secured by tying the ureter just above the cut. The catheters were brought through the incision and bent to drip into weighed cupped planchets or, in some experiments, into graduated centrifuge tubes.

When a flow of urine was established, carrier free $^{32}$P was injected into the femoral or jugular vein.

In an ideal experiment, test substances were administered one and one half hours after the injection of the radio-phosphorus. It was observed, however, that the administration of additional sodium pentobarbital gave rise to certain alterations in phosphate excretion which could mask the changes produced by the test substances. These effects are discussed in some detail below. If the animal required additional anesthesia within 45 minutes of the proposed time of injection of the test substance, the injection of the test substance was delayed so that an adequate time interval separated it from the injection of the pentobarbital.

Early experiments were designed to run for two hours after the injection of the test substances but were terminated sooner if the depth of anesthesia became too shallow. Other experiments, involving changes in phosphate excretion following thyroparathyroidectomy and subcutaneous injection of test substances, were run for two to six hours longer. In these cases it was necessary to administer additional quantities of anesthetic agent.
Collection and Measurement of Samples

Twenty minutes after the injection of P$^{32}$, collection of samples was begun. Samples were taken at 5, 10, or 15 minute intervals. Where test substances were administered intravenously, ten minute intervals were employed except for the first half hour following the injection, when five minute intervals were used. In other experiments ten or fifteen minute intervals were employed throughout. The planchets were reweighed at the end of the collection period and the urine output obtained in grams. The planchets were dried under an infra-red lamp for counting. When urine was collected in graduated tubes, as was done in certain experiments involving longer time intervals, an aliquot, by volume, was placed in a planchet and dried as above.

Dried samples were counted on a Tracerlab SC-1B Autoscaler, with automatic sample changer, using a thin window Geiger-Muller tube. A sufficient number of counts was recorded to make a counting error of less than 2%.

Calculation of Data

The data were calculated as total radioactivity excreted per minute in the collection intervals and as average radioactivity per gram (or per milliliter) for the collection intervals.

It was observed that if, during the control period, the urine volume fluctuated significantly; there was a corresponding change in total radioactivity excreted. However, there was practically no change in the amount of radioactivity per gram of urine. For this reason it is felt that changes in radioactivity/gram, or /milliliter, of urine provide the most reliable index of changes in phosphate excretion by the kidney.
volume of urine is used in all tables below, but the graphs show in addition total radioactivity and actual urine volumes.

Urine weight as a quantitative measure of output was employed because the urine volumes, encountered under antidiuretic conditions and/or short time intervals, were such that errors in pipetting such samples would be very large. The validity of this technique was checked by specific gravity determinations on urine samples. It was determined that the change in specific gravity of urine was very slight and in all cases was well within the limits of experimental error. As a result it is felt that changes in weight of urine samples fairly reflect changes in urine volume.

The utilization of such short time intervals and the correspondingly small quantities of urine was made possible by the use of $^{32}P$ as an indicator of urinary phosphate excretion. That this method is accurate has been demonstrated by Handler and Cohn (1951), who showed that the specific activity of $^{32}P$ in the urine paralleled that of the serum if the time lag for the passage of urine through the ureters was considered. Of course the specific activity of the serum, and the urine, declines steadily due to the progressive dilution of the isotope, but changes in excretion of the radiophosphorus adequately reflect changes in the excretion of the stable isotope.

Actual radioactivity values are meaningless due to variations in dosage of $^{32}P$, size of test animals, and in time lapse between $^{32}P$ injection and test substance injection. To allow for comparison between animals, the value for the last pre-injection sample is arbitrarily set at 100 and the other values adjusted to this standard. Therefore, a value of 200 following
injection of a test substance indicates a two-fold increase in excretion rate, a value of 300, a three-fold increase, etc.

Blood samples for serum calcium and phosphate determinations were taken before test substance administration and at the conclusion of the experiment. When appropriate (as following thyroparathyroidectomy and in long term experiments) intermediate samples were also taken. Serum calcium was determined by the method of Kramer and Tisdall as modified by Clark and Collip (1925). Determination of urinary and serum inorganic phosphate was made by the method of Fiske and Subbarow (1925).

**Long Term Experiments**

For the long term experiments, animals were placed in metabolism cages 24 hours prior to the beginning of the experiment and maintained on a diet of raw chopped horse meat and water *ad libitum* for the entire period.

At the beginning of the experiment, approximately 10 μc. of P₃² was administered subcutaneously and urine collections were made twice a day thereafter. Twenty-four hours later thyroparathyroidectomies were performed on part of the animals. Following a 48-hour post-operative period, administration of test substances was begun. All of the thyroparathyroidectomized dogs and half of the normal dogs received test substance injections. All test substances were injected subcutaneously, and were given twice or three times a day throughout the period.

Blood samples were taken at appropriate intervals, and serum calcium and inorganic phosphorus were determined as outlined above. An aliquot of urine was placed in a planchet and the radioactivity determined as previously described. In addition, urinary phosphate values were determined
chemically.

The data in these experiments are calculated as total radioactivity excreted per day.

**Dialysis of Parathyroid Extract**

In a number of experiments, commercial parathyroid extract was dialysed against acetate buffer (pH 3.6) or against distilled water. The extract was placed in a three-fourths inch diameter cellophane tube and suspended in the dialysing solution in the cold (ca. 5°C). The dialysing solution was agitated slowly with a magnetic stirrer or a motor driven glass stirring rod.
INFLUENCE OF ANESTHETIC AGENTS UPON EXCRETION

Early in this experimental work it was observed that following the administration of supplemental doses of sodium pentobarbital, there sometimes occurred a rise in the urinary phosphate excretion which resembled, but was not quantitatively similar to, that seen following parathyroid extract administration. It was further noted that the dosage employed, 30 mg./kilo, maintained the animals in a satisfactory state of anesthesia for only about one and one half to two hours, although this dosage should keep a dog in deep anesthesia for at least 4-6 hours.

There was also the thought that perhaps the immediate rise in phosphate excretion which we obtained from the administration of parathyroid extract was in some way connected to the anesthetic agent. It was conceivable that injection of parathyroid extract into the anesthetized dog produced the same sort of effect as an additional dose of sodium pentobarbital, only more marked. Consequently, a series of experiments were performed to test the effect of various anesthetic agents on our experimental results, and to seek a method of prolonging the anesthetic state.

Alpha Chloralose

The results obtained with dogs anesthetized by an intravenous dose of alpha chloralose (50 mg./kilo) were like those obtained under sodium pentobarbital anesthesia. Since the effects of sodium pentobarbital and alpha chloralose on the metabolism are essentially the same (Topete, 1951, for rats; Alvarez-Buylla, 1951, for dogs) no advantage accrued from using this anesthetic.
Sodium Barbital

With this anesthetic agent (in dosages of 200 mg./kilo) the response to parathyroid extract administration was still seen but was reduced in magnitude. Sodium barbital made the animals somewhat diuretic. This diuresis was augmented considerably when the test substance was injected. It was thought that this diuresis and its accompanying increase in total phosphate excretion, in part, masked the effect of the test substance.

Ether

Ether was administered by cannulation of the trachea following tracheotomy. It was necessary to use a minimum of 300 U.S.P. units of parathyroid extract to secure an effect under ether anesthesia.

Sodium Pentobarbital

Sodium pentobarbital was given in doses of 40 mg./kilo in an attempt to secure a better anesthetic state than that produced by 30 mg./kilo. This procedure met with some success. However, this dosage is near the lethal dose which in our work was determined to be between 45-50 mg./kilo.

Combinations of subcutaneous and intravenous doses of sodium pentobarbital were given. This technique often lead to profound changes in the animal's respiration and circulation and for that reason was discarded.

It was noted that if following surgical procedures the animals were not kept restrained, a considerable prolongation of a satisfactory anesthetic state was obtained. Therefore, all subsequent experiments were performed by anesthetizing the animals with 40 mg./kilo of sodium pentobarbital, and allowing them to lie unrestrained on the operating table following surgery.
TECHNIQUE OF THYROPARATHYROIDECTOMY

One parathyroid gland lies on the lateral surface of the thyroid and can be removed easily, but the other is imbedded in the thyroid. Therefore, to insure complete parathyroidectomy, it is necessary to thyroidectomize the animal.

The dogs were anesthetized by ether or intravenous injection of sodium pentobarbital. Ether was used only if no experiment was to follow within 24 hours of surgery.

The animal is fastened to the table on its back with the head drawn well forward. A mid-ventral incision about two inches long is made posteriorly from a point just below the larynx. The skin is spread, the fascia is removed, and the sternohyoideus muscles are separated in the mid-ventral line and spread apart to reveal the trachea. The thyroids are found lateral to the trachea from which they are separated by connective tissue. The sternothyroideus muscle, located just lateral to the thyroid is grasped and pulled upward to reveal the thyroid closely applied to its medial surface. The thyroid is grasped with hemostatic forceps near its center and separated from the sternothyroideus by blunt dissection beginning at the posterior end.

The thyroid may be pulled upward now and out through the incision. Arteries and veins enter the gland at either end. In addition, a vein from the posterior end of the gland courses forward adjacent to the gland to about its mid-section where it turns inward in the fold of connective tissue which invests the gland. The connective tissue is "fanned out" to locate the blood vessels and all those at the posterior end of the gland are tied...
off with a single suture. Before severing these vessels, it is important to apply a hemostat distal to the suture as the superior branch of the thyroid artery is not yet constricted and considerable bleeding can result when the inferior thyroid vein is cut.

The small vein, previously mentioned, which leaves the thyroid at its mid-section must be tied off separately. The connective tissue is cut up to the anterior end of the gland, a suture is made in the manner described for the posterior end, and the gland is removed from the body.

As no muscles are cut in this operation, it is only necessary to release them and to close the skin with wound clips at the conclusion of the operation, following which the wound is painted with tincture of merthiolate.

The surgical instruments are scalded prior to the operation but aseptic technique is not employed, due to high resistance to infection of female dogs and due also to the short term nature of the experiments. Although animals have been kept for as long as two weeks after surgery, post operative infection was encountered only once and this in a preliminary experiment in which sterile operative technique was employed.
EXPERIMENTAL RESULTS

Changes in Phosphate Excretion in Short Term Experiments

Response of Normal Dogs to Intravenous and Subcutaneous Injections of Parathyroid Extract

Table I summarizes the results of several experiments in which normal dogs received parathyroid extract either intravenously or subcutaneously. Figures 1 and 2, respectively, illustrate typical experiments of each type.

The increase in phosphate excretion following intravenous injection was always observed to commence within five or ten minutes of the injection. The peak excretion rate was obtained twenty to thirty minutes following the administration of small doses (50-150 units) of the extract, and was somewhat postponed when larger doses were used (see Table I). The height of the peak response was independent of the dose; however, the post-maximal curve was roughly inversely proportional to the dosage. It was found that the minimum dose giving a response in these experiments was 50 units. The apparent difference in peak response to small and large doses of extract (Table I) is caused by an abnormally high response (ca. 100) of one of the animals receiving 100 units of the parathyroid extract.

As shown in Figure 2, the responses to the subcutaneous injection of 200 units parathyroid extract (administered in multiple injections of 25 units per site) is essentially like the response to intravenous injection. As noted in Table I, half of the dogs receiving subcutaneous doses of parathyroid extract failed to show any change in phosphate excretion. This is felt to be due to the fact that there is considerable variation in the rate
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<th>time peak attained minutes</th>
<th>change in serum calcium -mg.%</th>
<th>change in serum phosphate -mg.%</th>
<th>no. of diuretic animals</th>
<th>no. of antidiuretic animals</th>
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<td>5</td>
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<td>2</td>
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<tr>
<td>SQ responsive</td>
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<td>243.8</td>
<td>56.3</td>
<td>+0.6</td>
<td>+2.0</td>
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Table I: Results of Intravenous and Subcutaneous Injections of Commercial Parathyroid Extract into Normal Dogs.

Note: Changes in serum phosphorus and calcium not significant, due to large variations.
Figure 1. Results of a typical experiment involving the intravenous administration of parathyroid extract to a normal dog.

Time zero set at middle of last pre-injection period.

Actual counts, volumes, etc. shown on scale at right.
Figure 2. Results of a typical experiment involving the subcutaneous administration of parathyroid extract to a normal dog.

Time zero set at middle of last pre-injection period.

Actual counts, volumes, etc., shown on scale at right.
at which a subcutaneous injection is absorbed by the bloodstream. It is suggested that higher doses would yield a more uniform result. Of those animals which responded to the subcutaneous administration of the extract, the response was of the same order as that obtained following intravenous injection. Table I indicates that the peak response following subcutaneous injection occurs nearly one hour after administration. This figure is somewhat misleading since one animal failed to attain the peak until two hours after injection. It is felt that the correct time for the peak should be on the order of 30 minutes.

In the experiments above, no significant changes in serum calcium or phosphorus occurred within the experimental period. In general, however, serum calcium remained unchanged or slightly elevated while serum phosphorus was variable, showing both increases and decreases.

Of the eleven dogs in Table I which received intravenous injections, seven showed a marked antidiuresis following injection. The remainder showed a moderate diuresis. All but one of the dogs receiving subcutaneous injections showed a marked diuresis.

In a few experiments, animals received no test substance injections. The hyperbolic curve obtained (shown in Figure 3) is a typical dilution curve resulting from the progressive fall in specific activity of the injected isotope. All experiments yielding a curve of this nature indicate a negative response.

Intravenous injections of 2 ml. fresh beef serum, 100 mg. beef albumin in 15 ml. water (pH 3.5), and 2.5 ml. fresh rat plasma gave no response, indicating that the response to parathyroid extract was not due to the
Figure 3. Results of a control experiment in which no test substance was administered.

Time zero set arbitrarily at 1.5 hours after start of experiment.

Actual counts, volumes, etc. shown on scale at right.
volume, acidity, or protein nature of the injected substance.

**Response of Normal Dogs to Intravenous and Subcutaneous Injections of Acetate treated" Parathyroid Extract**

The parathyroid extract used in this group of experiments was treated in one of three ways. Initially quantities of extract were dialysed against 100 times their own volumes of distilled water. The pH change caused precipitation and the extract was resuspended in acetate buffer at pH 3.6. To avoid possible alteration of the extract due to the precipitation, subsequent dialyses were made against the acetate buffer. In a number of cases parathyroid extract and acetate buffer were mixed in various proportions.

The results of experiments on eight dogs which received either acetate-dialysed, or water-dialysed extract are shown in Table II. No change in urinary phosphate excretion was seen following injection of either extract. Serum calcium was unchanged but there was a consistent drop in serum phosphate. All of these animals showed a moderate to marked diuresis following injection of the extract. Dosages of dialysed parathyroid extract range from 100 to 250 units.

Six dogs receiving 1:1 mixtures of acetate buffer and parathyroid extract in doses of 50-150 units showed a small increase in phosphate excretion, indicating an insufficiency of acetate (Table II). The blood picture with respect to calcium and phosphorus is like that seen with dialysed extract. All but one of these dogs showed a diuresis following injection of the extract.

Injection of 100 units of 1:2 or 1:4 mixtures of acetate buffer and
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Table II: Results of Intravenous and Subcutaneous Injection of "Acetate Treated" Parathyroid Extract into Normal Dogs.

Note: Serum phosphate changes barely significant.
parathyroid extract gave the same result as did dialysed extract (see Table II).

Subcutaneous injections 200 units of 1:2 mixtures of extract and buffer were given to two animals with no results. Serum phosphate values were obtained for only one of these animals and showed a drop of 3 mg.%. Owing to the failure to obtain consistent results with subcutaneous injections of the same dosage of intact extract in normal dogs, no conclusions can be drawn from these two experiments.

Intravenous administration of 2 or 3 ml. of acetate buffer only, to normal dogs produced an average drop in serum phosphate of 1.3 mg. % and a slight to moderate diuresis.

Response of Thyroparathyroidectomised Dogs to Intravenous and Subcutaneous Injections of Parathyroid Extract

The results of experiments on nine dogs, thyroparathyroidectomised 24 hours previously, receiving doses of from 50 to 150 units of parathyroid extract intravenously are shown in Table III. The average rise in phosphate excretion was from a value of 100 to near 8500, however the variation was considerable. One dog responded in essentially a normal manner implying the presence of accessory parathyroids. The largest change in excretion was from 100 to 51,300. In about half of these animals, the amount of radioactivity in the urine prior to injection of the extract was very small, indicating a considerable phosphorus retention. The rest of the data in this group of animals is similar to that obtained with normal animals. It is to be noted that only in this group of animals, and the group of normal animals receiving intact extract, was an antidiuretic condition encountered.
<table>
<thead>
<tr>
<th>treatment</th>
<th>no. of animals</th>
<th>peak rise phosphate excr. from base 100</th>
<th>time in minutes</th>
<th>change calcium %</th>
<th>change phosphate %</th>
<th>no. of animals diuretic</th>
<th>no. of animals antidiuretic</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV 50-150 units</td>
<td>9</td>
<td>8639</td>
<td>41.3</td>
<td>+0.6</td>
<td>+0.3</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>SQ 100-300 units</td>
<td>3</td>
<td>3382</td>
<td>100</td>
<td>+1.3</td>
<td>+0.6</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

Table III: Results of Intravenous Injections and Subcutaneous Injections of Commercial Parathyroid Extract into Thyroparathyroidectomized Dogs.

Note: Serum calcium changes following subcutaneous injections are barely significant.
Data from three thyroparathyroidectomized dogs which received parathyroid extract subcutaneously in doses of 100, 250, and 300 units are shown in Table III. The extract was given in multiple injections, 25 units per site. The response is similar to that obtained with intravenous injections above, however, the time of the peak response was uniformly lengthened in these animals being 90, 90, and 120 minutes respectively.

Blood changes were similar to those obtained in normal animals receiving the extract subcutaneously, and there was a moderate diuresis in all cases. Beef serum, given to two control thyroparathyroidectomized dogs, failed to alter the phosphate excretion during the experimental period.

Response of Thyroparathyroidectomized Dogs to Intravenous and Subcutaneous Injections of "Acetate treated" Parathyroid Extract

Table IV shows the results of the intravenous administration of 150-200 units of water and acetate dialysed extract to three thyroparathyroidectomized dogs. The peak responses of these dogs were 266, 530, and 2190. The dog showing the response of 2190 did not reach the peak figure until 110 minutes after the injection. It is felt that the response of the other two dogs more fairly represents the response of parathyroprivic dogs to acetate treated extract. As indicated in Table IV, the blood changes were minor and there was a uniform diuretic response as was the case in all other animals receiving acetate treated extract.

One animal, shown in Table IV, received acetate treated extract subcutaneously. There was a definite response which reached a peak one hour after the injection of the extract.

Acetate buffer only, given intravenously to thyroparathyroidectomized
<table>
<thead>
<tr>
<th>treatment</th>
<th>no. of animals</th>
<th>peak rise phosphate excreted from base 100</th>
<th>time peak attained in minutes</th>
<th>change in serum calcium mg.%</th>
<th>change in serum phosphate mg.%</th>
<th>no. of diuretic animals</th>
<th>no. of antidiuretic animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV 150-200 units</td>
<td>3</td>
<td>1095</td>
<td>60</td>
<td>+1.1</td>
<td>+0.2</td>
<td>3</td>
<td>0</td>
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<tr>
<td>SQ 200 units</td>
<td>1</td>
<td>556</td>
<td>60</td>
<td>+4.5</td>
<td>-1.2</td>
<td>1</td>
<td>0</td>
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Table IV: Results of Intravenous and Subcutaneous Injections of "Acetate Treated" Parathyroid Extract into Thyro-parathyroidectomized Dogs.

Note: Note changes in serum phosphorus and calcium not significant, due to small number of animals.
dogs did not affect phosphate excretion, although a slight to moderate diuresis was seen.

Intravenous Administration of Formalin Inactivated Parathyroid Extract to Normal and Thyroparathyroidectomized Dogs

This group of experiments utilized parathyroid extract inactivated with formalin as outlined by Stewart and Bowen (1952). That the resultant material was altered, was shown by the fact that it was soluble in distilled water. Table V shows the response of two animals which received 50 and 100 units of the inactivated extract on the day following the completion of the inactivation process. There was a rise in phosphate excretion from the base figure of 100, to 150. The shape of the curve of phosphate excretion is similar to Figure 1, except for the low value of the peak. Four dogs receiving the extract one week or more following inactivation showed no response (see Table V). One animal received 50 units of the extract, the others received 100 units. One animal in each group showed a slight diuresis, the others showed an unaltered urine output.

Three dogs were given extracts of beef spleen prepared after the method of L'Heureux et al (1947) for parathyroid extraction. These animals showed no response to injections of 50 mg. in 13.5 ml. acid water (pH 3.6), 20 mg. in 15 ml. acid water, and 93 mg. in 25 ml. acid water (see Table V).

Two thyroparathyroidectomized animals showed peak responses averaging 1385 as indicated in Table V.

Changes in Phosphate Excretion in Long Term Experiments

These experiments were performed as outlined in Materials and Methods
<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Animals</th>
<th>Change in Serum Phosphate Peak from Base</th>
<th>Time Peak Attained</th>
<th>Change in Serum Calcium</th>
<th>% Increase Ca Excreted from Base</th>
<th>% Increase Phosphate Excreted from Base</th>
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<tr>
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<td>160</td>
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<td>none</td>
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<tr>
<td>IV inact. 1 week</td>
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<td>160</td>
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<td>IV spleen extract</td>
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<td>1</td>
</tr>
<tr>
<td>IV inact. 1 week</td>
<td>2</td>
<td>1350</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

*These animals thyroparathyroidectomized.*

Table V: Results of Intravenous Injections of Inactivated Parathyroid Extract into Normal and Thyroparathyroidectomized Dogs and Intravenous Injections of Spleen Extract into Normal Dogs.
except that experiments I and II were run only three days. In these first experiments, no test substance was given to the thyroparathyroidectomized dogs and injections to the animals receiving intact and acetate treated extract were begun on the second day. There was a considerable variation in the volume of urine excreted by different animals, and more importantly there was considerable variation in the number of times the animals urinated. This latter factor makes it impossible to calculate phosphate excretion on any sort of an activity per unit volume basis. Consequently the data are presented as total radioactivity excreted per 24 hours. Parallel chemical determinations of urinary phosphate gave the same results as radioactivity measurements. Table VI shows the results of five experimental groups totalling 26 animals. It is necessary to retain the identity of the experimental groups, since group changes were observed. For example, in experiment III, two of the three thyroparathyroidectomized dogs (dogs 114 and 115) showed a considerable rise in phosphate output on the third day. At this time injection of extract had not begun. This result was both surprising and interesting, however, as can be seen, two control animals responded in the same fashion. In another instance (experiment V), four of six animals failed to urinate at all on the second day of the experiment. No adequate explanation can be offered for these occurrences. It is certain that no samples were lost in experiment V, as happened unfortunately in experiment IV. It is suggested that a change in the temperature of the room in which the animals were kept might be part of the answer, especially in experiment III.

If the animals in columns 2 and 3 of table VI (normals, receiving para-
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<th>AcPTH</th>
<th>PTX</th>
<th>PTX+</th>
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<td>139</td>
<td>136</td>
<td>134</td>
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</table>

Table VI: Results of Five Long Term Experiments.

Note: PTH=parathyroid extract (commercial)
AcPTH=acetate treated parathyroid extract
PTX=thyroparathyroidectomized
--- = thyroparathyroidectomy
----- = test substance administration
thyroid extract and acetate treated extract, respectively) are compared
with their own controls, it will be seen that there was no daily change
in phosphate excretion as a result of the injection of the extracts. Com-
paring the data on dogs in column h, and data for the first three days on
dogs in columns 5 and 6 with the appropriate control periods, reveals that
while there is no demonstratable change in phosphate excretion in the first
twenty four hours after loss of the parathyroid glands, there is a diminu-
tion in total phosphate excretion in the second twenty four hours. This
decreased phosphate output may be due to failure to eat. No valid com-
parisons can be made between columns 5 and 6 (thyroparathyroidectomized
animals receiving intact and acetate treated extracts, respectively). The
apparent higher excretion of the animal receiving intact extract in experi-
ment III may be an artefact, since this animal failed to show the increase
in excretion on the third day which was seen in the other animals. A com-
parison of the survival of the animals in columns h, 5, and 6 demonstrates
that acetate treated extract is adequate replacement therapy in parathy-
roprivic dogs. In one case (animal 122, experiment IV), the animal was
found in profound tetany late at night, about the middle of the third
twenty four hour period. Fifty units of acetate treated extract were given
subcutaneously and 100 units were given intravenously. Respiration failed
as the injections were being given, and artificial respiration was applied
for about five minutes. In an attempt to reduce the high fever which caused
a marked hyperpnea (leading to an alkalosis, contributing to tetany) the
animal was placed in a cold room for about an hour and a half. A marked
improvement was noted and the animal was replaced in the metabolism cage.
Although the hyperpnea returned, no further treatment was instituted.

Eight hours later the animal appeared normal.
DISCUSSION AND CONCLUSION

A technique has been developed which makes it possible to characterize the phosphuria which follows the administration of parathyroid extract to dogs. Less than ten minutes after the extract is given by either the intravenous or subcutaneous route, there is a sharp increase in the concentration of phosphate in the urine. This increased phosphate output (which is not a function of urine volume) reaches a maximum approximately 30 minutes after the injection and subsides quickly, being essentially nil by the end of the second hour following the injection. In consideration of the speed of this change in phosphaturia and in the absence of any significant blood alteration, it appears that the phosphuria is caused by the direct action of some component of parathyroid extract on the kidney. The alterations in phosphate excretion are of the same nature in either normal or parathyroidectomized animals, except that dogs parathyroidectomized 24 hours previously show a much larger phosphuria. This is felt to be due to the fact that parathyroprivic animals have elevated serum phosphorus levels which makes more phosphorus available for immediate excretion.

By treating commercial parathyroid extract with acetate buffer at a pH of 3.6, it is possible to so alter the extract that it fails to produce an immediate increase in phosphate excretion in normal dogs, and gives a reduced effect in parathyroprivic dogs. No explanation of the manner in which acetate operates can be offered at this time. The removal of, or diminution of, this immediate phosphuric factor does not alter the ability of the extract to prevent or reverse the symptoms of parathyroid insufficiency.
as is shown by the failure of thyroparathyroidectomized dogs given acetate treated extract to develop tetanic conditions.

The fact that thyroparathyroidectomized dogs show a characteristic, albeit reduced phosphuria immediately following injection of acetate treated extract, coupled with the observation that thyroparathyroidectomized dogs receiving intact or acetate treated extract, in the five day experiments, showed the same ability to maintain normal serum and urinary phosphate levels, make it appear that the acetate extract still has the ability to influence phosphate excretion. This conclusion was further substantiated by Talmage and Kraintz on rats. They employed the technique outlined in their recent paper (Talmage and Kraintz, 1954) administering acetate treated extract to the parathyroidectomized rats immediately following the operation. Just as in the case of intact extract, the precipitous fall in urinary phosphorus and the rise in serum phosphorus which normally follow parathyroidectomy were prevented by the acetate treated extract.

The foregoing data all point out the nonessential nature of the factor in parathyroid extract which causes an immediate phosphuria. In addition, in the five day experiments, normal animals receiving intact extract failed to show any increased phosphate excretion over a 48 hour period, indicating that the immediate phosphate effect is neither prolonged nor significant.

In view of the above data, the following conclusions seem justified:

1) The factor in commercial parathyroid extract which causes an immediate and marked phosphuria following injection, is labile in the presence of acetate buffer at pH 3.6.

2) This factor is not essential to the therapeutic action of the
parathyroid extract.

3) Although able to increase phosphate excretion several fold for a short period of time, this factor produces no demonstrable change in phosphate excretion over a 24 hour period.

4) The unimpaired ability of acetate treated parathyroid extract to maintain both normal phosphate excretion and normal serum phosphate in parathyroidectomized dogs and rats indicates the presence of a second phosphate excretion factor in parathyroid extract.

5) The conclusions above indicate that commercial parathyroid extract contains two distinct activities. One of these is able to produce an immediate phosphuria; the other is able to maintain normal serum phosphate levels, serum calcium levels, and normal urinary excretion of these ions.

The suggestion that the phosphuric factor in parathyroid extract is an artefact was advanced by Stewart and Bowen two years ago (1952). These workers claimed to be able to demonstrate the presence of the phosphate factor in extract which had been inactivated with respect to its ability to elevate serum calcium of dogs. Attempts to repeat their work in this laboratory have been unsuccessful. Immediately following the incubation of parathyroid extract with formalin, slight increases in urinary phosphate can be produced by intravenous injection of the extract. If however, the extract is allowed to sit in the cold for a few days, no change in phosphate excretion results from its injection into normal animals, although a phosphuria can be produced in thyroparathyroidectomized animals. It appears
that formalin treatment only partially inactivates parathyroid extract, but that inactivation proceeds if the extract, dissolved in distilled water, is allowed to sit for an additional period following formalin treatment. While we are in agreement with Stewart and Bowen as to the possible artefactual nature of the phosphuric factor in parathyroid extract, we are in some disagreement with them as to the demonstration of its nature.

In summary, then, the studies contained in this thesis have shown the presence in parathyroid extract, of two factors which influence urinary phosphate excretion. One factor produces a transient but immediate increase in phosphate excretion without affecting serum phosphate values. The other factor is capable of maintaining normal phosphate levels in both urine and blood of parathyroidectomized animals. The nonessential and labile character of the former factor suggest that it may be artefactual, whereas the second factor appears to be the mechanism whereby the parathyroid hormone influences renal and, consequently, blood phosphate levels.

It is suggested that chemical studies of acetate treated parathyroid extract might yield information to better characterize, and perhaps purify the hormone of the parathyroid glands. In addition reexamination, with this altered extract, of earlier works concerned with alterations in renal hemodynamics and excretion produced by parathyroid extracts might allow a better concept of the real function of this hormone in the economy of the animal body.
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