THE RICE INSTITUTE

STUDIES OF THE RELATIONSHIP OF
DIHYDROTACHYSTEROL AND CALCIFEROL
TO PARATHYROID FUNCTION

by

Bernard F. Dodds

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

Houston, Texas
May, 1955
ACKNOWLEDGMENTS

I wish to express my appreciation to Doctor Roy V. Talmage for his efforts in directing and aiding me in these studies.

I also wish to thank my wife, Bonnie, for her help and support in making this work possible.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>CHEMISTRY OF VITAMIN D</td>
<td>2</td>
</tr>
<tr>
<td>CLINICAL USES OF DIHYDROTACHYSTEROL</td>
<td>6</td>
</tr>
<tr>
<td>In Hypoparathyroidism</td>
<td>6</td>
</tr>
<tr>
<td>In Pseudohypoparathyroidism</td>
<td>8</td>
</tr>
<tr>
<td>OTHER EFFECTS OF DIHYDROTACHYSTEROL</td>
<td>10</td>
</tr>
<tr>
<td>Antirachitic Effects</td>
<td>10</td>
</tr>
<tr>
<td>Effect on Bone</td>
<td>11</td>
</tr>
<tr>
<td>EFFECTS OF PARATHYROIDECTOMY</td>
<td>12</td>
</tr>
<tr>
<td>THEORIES OF PARATHYROID FUNCTION</td>
<td>13</td>
</tr>
<tr>
<td>Albright-Reifenstein Theory</td>
<td>13</td>
</tr>
<tr>
<td>Munson-Kenny Theory</td>
<td>14</td>
</tr>
<tr>
<td>Talmage-Kraintz Theory</td>
<td>14</td>
</tr>
<tr>
<td>Other Theories</td>
<td>14</td>
</tr>
<tr>
<td>MODE OF ACTION OF DIHYDROTACHYSTEROL</td>
<td>15</td>
</tr>
<tr>
<td>STATEMENT OF THE PROBLEM</td>
<td>16</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>17</td>
</tr>
<tr>
<td>EXPERIMENTAL RESULTS</td>
<td>20</td>
</tr>
<tr>
<td>Effect of Route of Administration of Dihydrotachysterol</td>
<td>20</td>
</tr>
<tr>
<td>Effect of Dihydrotachysterol on Normal Rats</td>
<td>23</td>
</tr>
<tr>
<td>I. Effects of Dose Level of A.T.-10 on Normal Rats</td>
<td>23</td>
</tr>
<tr>
<td>II. Cumulative Effects of A.T.-10 on Normal Rats</td>
<td>26</td>
</tr>
<tr>
<td>Effect of A.T.-10 on Parathyroidectomized Rats</td>
<td>28</td>
</tr>
<tr>
<td>Comparison of the Effects of A.T.-10 and Calciferol on Normal and Parathyroidectomized Rats</td>
<td>30</td>
</tr>
</tbody>
</table>
INTRODUCTION

In 1933 Holtz, a German worker, developed dihydrotachysterol (Antitetanic preparation 10), an irradiation product of ergosterol, as a specific drug for the treatment of hypoparathyroidism. It is used extensively for this condition at the present time as, in contrast to parathyroid extract, it produces no refractoriness when administered, even after long periods. The drug is used because it is able to maintain normal serum calcium levels without accompanying toxic effects. This action is not specific to dihydrotachysterol but is also found in varying degree in several related compounds, especially calciferol, another irradiation product of ergosterol. In fact, calciferol is often used in place of dihydrotachysterol because of its lower cost and the fact that, in humans, it appears to be as effective (McLean, 1953; Martin and Rilliet, 1953).
CHEMISTRY OF VITAMIN D

The similarity in action of calciferol and dihydrotachysterol, although in different ratios, is not surprising when one considers the chemical makeup of the two. For this reason a discussion of the chemistry of vitamin D and related compounds seems advisable at this time.

The term Vitamin D covers a number of compounds all possessing antirachitic activity. In recent years several chemically distinct forms of the "vitamin" have been reported. Custom now limits the application of the term to antirachitic sterol derivatives and to the unidentified antirachitic components of fish oils and other foods which are supposed to belong to this group (Bills, 1939).

The two forms of vitamin D which have the most widespread use and are the most important are activated ergosterol and activated 7-dehydrocholesterol. For the purpose of this study we will consider only ergosterol since both calciferol and dihydrotachysterol are derivatives of this compound.

Ergosterol, the pro-vitamin of calciferol and tachysterol, is the characteristic sterol of fungi. It is prepared from yeasts and molds and may make up to 2% of the dry weight of these organisms. When ergosterol is exposed to ultraviolet light a transformation to calciferol begins immediately. However, even under the best controlled conditions the transformation is only about 50% to calciferol. This is due to the fact that the transformation is not directly to calciferol but takes place in a series of overlapping steps of which calciferol is not the last. The following steps are involved when ergosterol is irradiated.
At no point in the series of reactions is any one of the substances present alone. Calciferol may be separated partially from the rest due to the fact that it requires more energy to convert it to toxisterol than is needed to produce it from tachysterol. Thus, if the irradiation is not overly prolonged, calciferol is the chief product (Bills, 1939).

These above reactions are purely photochemical. Nothing enters or leaves the molecule during the transformations. The only difference in the compounds formed is in the arrangement of the molecules. The activation can be induced by any wave length which is absorbed by the ergosterol molecule, although those between 305 and 230 nm are the most effective. The reactions are not noticeably changed by differences in temperature. They have been carried out with the same results in boiling solutions, at room temperature, and at the temperature of liquid air. The activation is more rapid in solution than if done while in the solid state, and is even more rapid if the solution is agitated while being irradiated.

If the irradiation of calciferol is carried too far toxisterol is formed. This substance, as the name implies, is toxic. One of the early preparations of calciferol (Vigantol) was actually a mixture of Calciferol and toxisterol. It was prepared commercially under the
mistaken idea that the irradiation of ergosterol should proceed until the absorption was at a maximum. Actually the most active form of the vitamin, calciferol, is formed before this. From this impure preparation of calciferol stems much of its reputation of being toxic. The preparations of calciferol on the market today are free from toxisterol (Reed, et al., 1939).

Dihydrotachysterol, or A. T.-10, is also a photochemical derivative of ergosterol. It is the reduced form of tachysterol which precedes calciferol in the series following irradiation of ergosterol. When calciferol is produced from ergosterol only about 50% of the final product is calciferol. The remaining 50% is made up of a mixture of lumisterol and tachysterol. The development of dihydrotachysterol was done in a deliberate effort to produce a compound which would be effective in the treatment of hypoparathyroidism. Its development was based on the idea that the toxic and antirachitic effects of vitamin D were due to two different factors. The earlier concept had been that the toxic effects of vitamin D was due to an overabundance of the antirachitic factor and, therefore, was due to hypervitaminosis. The idea was to produce a compound which would contain a large amount of the "Calcinosefaktor" (toxic factor) which would be effective in relieving the low serum calcium found in hypoparathyroidism. In 1933 Holtz developed dihydrotachysterol from tachysterol and this compound was shown to fulfill the expectations in the treatment of hypoparathyroidism (Albright and Reifenstein, 1948).

A look at the structural formulas of ergosterol, calciferol, and dihydrotachysterol will further emphasize the similarity between these compounds. It can be seen that the only difference between these com-
pounds is a spatial rearrangement of the molecule and the addition of two hydrogen atoms between C-22 and C-23 in the side chain of dihydrotachysterol.

Ergosterol

Calciferol

Dihydrotachysterol
CLINICAL USES OF DIHYDROTACHYSTEROL

Dihydrotachysterol (A.T.-10) is most commonly used in the treatment of hypoparathyroidism although it has been used in the treatment of pemphigus (Lever and Talbott, 1941), scleroderma (Bernstein and Goldberger, 1946), otosclerosis (Gunten, 1943), and pseudohypoparathyroidism (Alexander and Tucker, 1949).

In Hypoparathyroidism: McLean (1941) reviewed the use of dihydrotachysterol (and calciferol) in parathyroid insufficiency. The following is quoted from his article:

"It is now well established that certain products derived from activated ergosterol are highly effective in increasing the concentration of calcium in the blood and in relieving the symptoms of parathyroid insufficiency. It appears ....... that these products may be administered over a considerable time with reasonable safety ....... and that their prolonged use is attended neither by injury to the patient nor by the development of tolerance."

Among the advantages in the use of dihydrotachysterol (and calciferol) in the treatment of parathyroid insufficiency is that no tolerance is built up against this drug as is the case with parathyroid extract. It possesses the additional advantage of being effective when given by mouth while the extract must be given by injection. The activated sterols, because of their relatively slow action, cannot be used in emergencies and in this case the extract must be used. However, because of their slow and prolonged action the activated sterols do not need to be given as frequently as the extract. Dihydrotachysterol possesses the disadvantage of being expensive so that from a financial point of view calciferol is often the drug of choice.
One of the earliest reports of the use of dihydrotachysterol in clinical use is that of MacBryde (1938). He reports on seven cases; six of post-operative tetany following accidental removal of the parathyroid glands during thyroidectomy and one of the idiopathic type. All were successfully treated with dihydrotachysterol. Previously their symptoms had been uncontrolled for periods ranging from three and one half to seventeen years, except for temporary periods when calcium salts and parathyroid extract had been effective. Three had developed resistance to the extract. All symptoms were completely relieved with dihydrotachysterol and at the time of his report none of the patients had any toxic symptoms or had developed resistance to the drug. This, and most of the other clinical reports, emphasize the importance of periodic checks of urine calcium as an indication of overdosage. This is done easily by means of the Sulkowitch Test, which can be performed by the patient, himself, without any expensive equipment, and can be used as a gauge of the level of serum calcium. Using this simple test the patient is able to adjust his own dosage of dihydrotachysterol.

Many other similar reports of the successful control of parathyroid tetany with dihydrotachysterol are available in the literature (Franco, 1940; Harding, 1942; Berk, 1939; Albright, 1939; Margolis and Krause, 1939; and others).

It should be mentioned at this point that the use of calciferol in the treatment of human hypoparathyroidism appears to duplicate the action of dihydrotachysterol. The dosage required for calciferol is about twice as much as that of dihydrotachysterol on a milligram basis.
(McLean, 1941). Much of the use of calciferol in this syndrome has been confused by the fact that at two different dose levels calciferol exhibits two different and distinct actions. At a dosage of 700 to 1,000 international units per day it is effective in curing and/or preventing rickets (Park, 1940). However, at a dosage of 60,000 to 200,000 international units per day calciferol is effective in elevating and maintaining serum calcium levels (McLean, 1941). This dual action of calciferol at two separate and distinct dose levels has caused much of the confusion in its usage in parathyroid insufficiency. Dihydrotachysterol, on the other hand, has its dosages based on the effect on calcium in the blood. Because of the confusion about calciferol, and the mistaken impression that dihydrotachysterol's action on serum calcium is specific to that drug, dihydrotachysterol has been considered by some to be overemphasized as treatment. McLean (1941) claims the calcemic factor of the two compounds to be identical if the proper dosages are used.

In Pseudohypoparathyroidism: In 1942 Albright et al described a syndrome which they chose to call pseudohypoparathyroidism. The outward signs and symptoms are those of hypoparathyroidism, but the cause is not a lack of the parathyroid hormone but an apparent inability to respond to it. They report that the discovery of this condition was a result of a lucky coincidence. A patient with apparent hypoparathyroidism was treated with parathyroid extract, even though the authors had given up this treatment of hypoparathyroidism in favor of dihydrotachysterol. Upon finding no response to the extract a biopsy was performed and the parathyroid gland appeared to be normal. The patient was successfully treated with dihydrotachysterol. Up to 1948 Albright
and Reifenstein reported a total of seven cases of this unusual condition. Here then is a condition where the extract would not be effective even if no refractoriness were built up against it. In this case dihydrotachysterol is able to relieve the symptoms and return the serum calcium levels to normal where even large doses of parathyroid extract (7,400 units) had absolutely no effect.
OTHER EFFECTS OF DIHYDROTACHYSTEROL

Antirachitic Effects: Much of the early work with dihydrotachysterol reported that this substance had no antirachitic effects. Harnapp (1935), however, did report an antirachitic effect of the drug, and in 1941 Shohl and Farber confirmed his work on the rat. The latter investigators reported that dihydrotachysterol was only one four-hundredth as effective as vitamin D\textsubscript{2} (calciferol) and was five times as toxic.

Correll and Wise (1942) assayed dihydrotachysterol using both the rat and the chick. They found that dihydrotachysterol had an antirachitic potency of 30,000 international units per gram using the U.S.P. XI rat technique as compared to calciferol's 40,000,000 international units per gram. They also concluded that on a weight basis dihydrotachysterol was one eighth as potent as calciferol as an antirachitic for the chick, but, in contrast, on a rat unit basis it was about 400 times as potent as calciferol. Another surprising result of their experiments was the finding that dihydrotachysterol was four times as effective as vitamin D from cod liver oil, rat unit for rat unit, as an antirachitic for the chick. This was the first time an antirachitic substance from vegetable source (see section on Chemistry of Vitamin D) was found to be superior to that of vitamin D from cod liver oil. In a later paper (1943) Correll and Wise emphasize the individuality of the antirachitic action of dihydrotachysterol by pointing out that only 0.5 international units per day were required to produce normal mineral metabolism as compared to 100 international units per day for calciferol.

McChesney (1943a) confirmed this work and in addition contrasted
the effectiveness of dihydrotachysterol, calciferol, and vitamin D₃ (a derivative of 7-dehydro-cholesterol) when given orally or intramuscularly. He found the oral effectiveness of these to be 35:1 vitamin D₃ to calciferol and 4.5:1 dihydrotachysterol to vitamin D₃, rat unit for rat unit. However, when given intramuscularly dihydrotachysterol was not as effective as when given orally. In a second paper (McChesney, 1943b) he found that dihydrotachysterol was less effective when given intraperitoneally than orally.

Harrison and Harrison (1942) demonstrated that while dihydrotachysterol has the same physiological effects as calciferol in the normal dog it could not be used as a substitute for calciferol in the rachitic animal.

Effect on Bone: Latta and Tristan (1950) reported in a histological study, that dihydrotachysterol, if given in large enough doses, was able to cause a marked dissolution of the long bones of young rats. They consider that the hypercalcemia and renal tubular calculi seen in the late stages of their experiments were a direct result of the removal of calcium from the bones. Their report also stated that dihydrotachysterol was effective more rapidly when given orally than when given subcutaneously, although the subcutaneous route gave equal results if a longer period of time was used.
EFFECTS OF PARATHYROIDECTOMY

When an animal is parathyroidectomized, or the parathyroids cease to function for some reason, a characteristic series of effects can be observed. In the rat, phosphate excretion drops immediately and remains low for about 16 hours, at which time it returns toward normal. Calcium excretion, on the other hand, increases following parathyroidectomy becoming highest one to two hours following removal of these glands. It gradually decreases until approximately 16 hours when it, like phosphorous, returns to near normal levels. The serum phosphorous levels gradually increase following the operation. Serum calcium levels drop following the operation and if allowed to remain too low may lead to tetany and death of the animal. The return of urine phosphorous excretion to normal levels takes place when the serum phosphorous reaches a level of about 12 to 14 mg %. That of calcium excretion returns to normal values when the serum calcium level drops to between 6 and 7 mg %. These changes have recently been described from this laboratory (Talmage et al, 1955).
THEORIES OF PARATHYROID FUNCTION

Since this study deals partially with the influence of dihydro-tachysterol on parathyroidectomized animals it seems advisable at this time to present some of the current theories of parathyroid function in order to determine how the results obtained fit into the concepts advanced.

Albright-Reifenstein Theory: The theory which has received the most widespread acceptance, up to the present, was originally proposed by Albright, and since developed by Albright and Reifenstein (1948). They believe that the chief influence of the parathyroid glands is on the renal excretion of phosphate. The other effects of the hormone (and parathyroidectomy) they consider to be secondary to this effect. The best way to sum up their concept is to quote directly from their description.

"...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 phosphate 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 phosphorous 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 gastrointestinal tract the bones will have to supply the deficit; ...." (Albright and Reifenstein, 1948)

For the main evidence in favor of the Albright-Reifenstein theory the reader is referred to their book *Parathyroid Glands and Metabolic Bone Disease.*
Munson-Kenny Theory: Briefly stated these workers believe that the calcium-mobilizing activity and phosphaturic activity of the parathyroids are due to two separable hormonal activities. However, their work is done using parathyroid gland extracts, both with commercial forms, and with extracts which they prepare from bovine parathyroids. It is possible that these extracts do not behave in the same way as the hormone in vivo, as has recently been suggested by Buchanan (1954).

Talmage-Kraintz Theory: Recent work in this laboratory has challenged the Albright-Reifenstein school of thought. Talmage and Kraintz (1954) have demonstrated that it is possible to show a serum calcium fall before any change is seen in the serum phosphorous. It is also possible to show that the calcium and phosphorous in blood can vary without any apparent solubility product being involved (Talmage et al., 1953). The theory proposed from this laboratory is that the primary action of the parathyroid hormone is to remove calcium and phosphorous from the bone. In addition to the action on bone the hormone also is able to affect the kidney thresholds for calcium and phosphorous (Talmage and Kraintz, 1954).

Other Theories: Stewart and Bowen (1951) demonstrated that the parathyroids had an effect on serum calcium even in nephrectomized animals. Thus, they conclude that the action of the hormone on calcium is not mediated through the kidney. In a later work (1952) these same workers raised the question as to whether the effect of commercial extracts on renal excretion of phosphate was real or an antefact. Grollman (1954) concluded from studies on nephrectomized animals that the primary effect of the hormone on serum calcium is through direct action on the bone rather than through any action via the kidney.
As to the mode of action of dihydrotachysterol there is also some disagreement. Albright and Reifenstein state that the action of this drug is more similar to the parathyroid hormone than it is to vitamin D. Their idea is that the drug affects the kidney causing an increase in urine excretion of phosphate, followed by a decrease in serum phosphate and an increase in serum calcium. The other action of dihydrotachysterol, similar to vitamin D, in increasing the absorption of calcium from the intestine is believed by these authors to be of lesser importance. They do not make any statements to the effects, if any, of dihydrotachysterol on the bone.
STATEMENT OF THE PROBLEM

In view of the work being done in this laboratory on the function and mode of action of the parathyroid glands many of the earlier concepts of parathyroid action have been questioned. It was with these questions in mind and in view of the fact of dihydrotachysterol's wide acceptance as a drug in the treatment of hypoparathyroidism that this study was undertaken.

When one surveys the literature in regards to the mode of action of dihydrotachysterol one is immediately impressed with the lack of work done using this material on experimental animals, other than humans, in an effort to understand its actions. There is much clinical data using dihydrotachysterol but most of the reasons for its use appears to be empirical. Most of the reports on animals, other than humans, are concerned with the antirachitic effects of the drug.

This study was made, therefore, in an effort to try to elucidate the action of dihydrotachysterol on parathyroidectomized and normal rats. Also, since calciferol is often used in place of dihydrotachysterol in the treatment of parathyroid insufficiency some comparative studies of the two compounds were done.

These experiments were all performed under the same conditions and using the same methods as those done in this laboratory involving parathyroid extract in order that some idea of the comparative effects of the different materials commonly used in treatment of parathyroid insufficiency could be made.
MATERIALS AND METHODS

In preliminary experiments it was found that a dose of 200 μg per day gave the best desired responses; therefore, unless otherwise specified, this dosage of dihydrotachysterol (referred to hereafter as A.T.-10) was used in all subsequent experiments. It was also determined that dihydrotachysterol was relatively slow in action so, in most cases, the animals used in the experiments were pre-dosed. That is, the experimental animals were given A.T.-10 for two days prior to the experiment and a third dose on the day of the experiment, unless otherwise noted. Preliminary work also showed that the Sesame oil used as a vehicle for the A.T.-10 had no effect on serum calcium or phosphorous levels. The excretion of radiophosphorus was, likewise, not affected by this oil.

A total of 190 male Spargue-Dawley rats were used in these experiments, having a weight range of 175 to 250 grams. For most of the experiments the animals were maintained on a standard purina diet throughout the duration of the experimental period. However, in some cases as noted, the animals were kept on a calcium free diet for up to ten days before the start of the experiment in order to rule out any effect of A.T.-10 or calciferol on the intestinal absorption of calcium. When parathyroidectomies were performed, the glands were removed individually under ether anesthesia, using the method of Richter and Birmingham (1941).

The radioisotope, P^{32}, was injected intraperitoneally in doses ranging from 5 to 10 μc., the dose being kept constant for any one ex-
periment. At least four normal controls were run in each experiment. The time of administration of the radioisotope varied according to the experimental procedure and is noted in the results. The dried urine was "counted" using standard procedures on a Tracerlab SC-1B Autoscaler with an automatic sample changer and a Geiger-Muller tube. In order to correlate the various experiments to each other the radioactivity of the normal controls were set at 1000 and all other counts adjusted accordingly to this standard. By this method variations in dosage, in radioactive decay and in types of measurement equipment used were negated and the radiophosphorous excretion of all the experiments could be compared. Radiophosphorous excretion was used as an indication of phosphate excretion, a procedure which has previously been shown to be reliable (Talmage and Kraintz, 1953).

In a preliminary experiment A.T.-10 was given both orally and subcutaneously in equal doses in order to determine the most effective route of administration. As a result of this experiment the oral route was chosen and all following experiments used this method. Both Hytakerol (Winthrop-Stearns Inc. brand of dihydrotachysterol in Sesame oil) and Drisdol (Winthrop-Stearns Inc. brand of crystalline vitamin D₂ (calciferol) from ergosterol in propylene glycol), when used, were administered by stomach tube. For the collection of urine the animals were placed on glass metabolism cages and the urine collected in graduated cylinders. For those experiments running over eight hours on metabolism cages the animals were given food (calcium-free to prevent excess calcium contamination of the urine) and distilled water ad lib. When re-
peated short urine collections were used; the animals were given 5 ml. of distilled water by stomach tube at the beginning of each period in addition to the dose of 5% body weight given each animal at the beginning of the experiment.

At the termination of each experiment the animals were bled by heart puncture in order to obtain serum for calcium and phosphorous determinations. For both serum and urine determinations, where done, total phosphorous values were determined by the method of LePage (1949) and total calcium values by the Clark and Collip modification of the method of Kramer and Tisdall (1925).
EXPERIMENTAL RESULTS

Effect of Route of Administration of Dihydrotachysterol: This study was the first undertaken and was in the nature of a preliminary experiment. Two methods of administration, oral and subcutaneous, were tested in order to determine which one was the preferable route to be used in later experiments. Two groups of rats received a total of 600 \( \mu g \) of A.T.-10 (200 \( \mu g \) / day) over a three day period; one group was given subcutaneous injections and the other received A.T.-10 by stomach tube. In addition, parathyroidectomized animals receiving no treatment were run simultaneously as controls. The results are summarized in Table I.

Examination of these data shows that while A.T.-10 when given orally partially prevented the drop in \( P^{32} \) excretion normally seen following parathyroidectomy, the same drug at the same dose level was ineffective in preventing this drop when administered subcutaneously. Since this experiment covered three days from the time of the first dose of A.T.-10, the subcutaneous dose had ample time to be absorbed and the explanation for the differences in action when the drug is given other than orally must be looked for in some other factor. Just as striking as the effect on \( P^{32} \) excretion was the effect of the drug on serum calcium. Note again that if given orally the drug almost entirely prevented the hypocalcemia normally seen following the operation, while this drop was not prevented by subcutaneous administration of the same dose. The effects on serum phosphorous are just as graphic; the effect being greatest when A.T.-10 was given orally.
As a result of this experiment in all subsequent experiments using A.T.-10 the oral route of administration was employed. The results of this experiment are in agreement with those of Latta and Tristan (1950) and McChesney (1943a).
### TABLE I

**ORAL VERSUS SUBCUTANEOUS ADMINISTRATION OF A.T.-10**

<table>
<thead>
<tr>
<th></th>
<th>Renal P$^{32}$ Excretion (Total)</th>
<th>Terminal Serum Calcium (mg%)</th>
<th>Terminal Serum Phosphate (mg % P)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normal Controls</strong></td>
<td>1000 (660-1355)</td>
<td>9.7 (9.0-10.2)</td>
<td>10.1 (9.4-11.1)</td>
</tr>
<tr>
<td><strong>Parathyroidectomized Controls</strong></td>
<td>11 (9-13)</td>
<td>6.9 (6.4-7.8)</td>
<td>11.1 (9.7-12.0)</td>
</tr>
<tr>
<td><strong>Parathyroidectomized A.T.-10 by Stomach Tube</strong></td>
<td>563 (440-757)</td>
<td>9.0 (8.4-10.2)</td>
<td>12.7 (11.5-13.9)</td>
</tr>
<tr>
<td><strong>Parathyroidectomized A.T.-10 Subcutaneously</strong></td>
<td>10 (9-10)</td>
<td>7.4 (7.0-7.8)</td>
<td>10.9 (9.9-12.4)</td>
</tr>
</tbody>
</table>

**Notes:**

1. Dosage: 200 μg daily. Initial dose 48 hours prior to parathyroidectomy.
2. Urine collection period: 1-4 hours after parathyroidectomy; animals bled at end of collection period.
3. Each value given = average of three animals; ranges are indicated.
4. P$^{32}$ given 1 hour after parathyroidectomy. Radioactivity excreted by normal animals set at 1000; experimental values adjusted to this standard.
Effect of Dihydrotachysterol on Normal Rats: This part of the study of A.T.-10 is divided into two general sections. The first is concerned with the effects of various dose levels on normal rats. The second is concerned with the cumulative effect of one constant dosage.

I. Effects of Dose Level of A.T.-10 on Normal Rats: For this study four groups of animals were used. All were maintained on a standard purina diet and distilled water ad lib. In this particular experiment no radioactivity was given. The purpose of this study was to determine at what dosage of A.T.-10 the best responses could be obtained. The doses of A.T.-10 were as follows:

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose per day (total dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>25 µg per day (75 µg)</td>
</tr>
<tr>
<td>Group II</td>
<td>50 µg per day (150 µg)</td>
</tr>
<tr>
<td>Group III</td>
<td>100 µg per day (300 µg)</td>
</tr>
<tr>
<td>Group IV</td>
<td>200 µg per day (600 µg)</td>
</tr>
</tbody>
</table>

The results are given in Table II. For comparative purposes the normal phosphate excretion in normal rats is 300-400 mg/hr. Normal serum calcium values in rats are between 10.0 and 11.0 mg%, while normal serum phosphate levels are from 9.0 to 10.0 mg%. It might be noted that the serum phosphate levels of all the groups given in the table are high. This is not a result of A.T.-10 administration but a result of the procedure of determining this level. When the experimenter ran normal animals from another group with some from this experiment, it was determined that all the readings were too high. For this reason those figures given in Table II are not absolute but are for comparative purposes.

Examination of these data reveals that A.T.-10 had no effect on increasing urine phosphate excretion until a dose of approximately...
<table>
<thead>
<tr>
<th>Urine Collection Period (Hrs. after initial dose)</th>
<th>Terminal Urine Excretion Results (mg P/hr)</th>
<th>Terminal Serum Calcium (mg %)</th>
<th>Terminal Serum Phosphate (mg % P)</th>
<th>Urine Calcium Excretion (mg/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I 25 µg A.T.-10 daily</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-48</td>
<td>0.577</td>
<td>10.7</td>
<td>12.3</td>
<td>0.054</td>
</tr>
<tr>
<td>(total dose = 75 µg.)</td>
<td>(0.482-0.783)</td>
<td>(10.4-10.9)</td>
<td>(12.0-12.9)</td>
<td></td>
</tr>
<tr>
<td>Group II 50 µg A.T.-10 daily</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-48</td>
<td>0.530</td>
<td>12.0</td>
<td>13.5</td>
<td>0.052</td>
</tr>
<tr>
<td>(total dose = 150 µg.)</td>
<td>(0.460-0.705)</td>
<td>(11.2-13.0)</td>
<td>(13.1-13.8)</td>
<td></td>
</tr>
<tr>
<td>Group III 100 µg A.T.-10 daily</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-48</td>
<td>0.566</td>
<td>13.2</td>
<td>12.6</td>
<td>0.104</td>
</tr>
<tr>
<td>(total dose = 300 µg.)</td>
<td>(0.431-0.650)</td>
<td>(12.7-13.5)</td>
<td>(12.3-13.1)</td>
<td></td>
</tr>
<tr>
<td>Group IV 200 µg A.T.-10 daily</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-48</td>
<td>0.991</td>
<td>14.8</td>
<td>12.1</td>
<td>0.131</td>
</tr>
<tr>
<td>(total dose = 600 µg.)</td>
<td>(0.903-1.031)</td>
<td>(11.1-12.1)</td>
<td>(1.08-1.169)</td>
<td></td>
</tr>
</tbody>
</table>

Notes:
1. A.T.-10 given by stomach tube
2. Values given are averages of each group; ranges are indicated
3. Animals bled 72 hours after initial dose of A.T.-10.
4. Animals maintained on stock diet and distilled water.
5. Normal Urine Calcium Excretion: approximately 0.035 mg/hr.
300 µg (total accumulated dose) was reached. The excretion rate was approximately doubled by a total dose of 600 µg. The effects on serum calcium levels could be seen at much lower dosages. A total dose of 150 µg had the effect of raising these levels slightly, while larger doses increased the serum calcium levels to a greater extent. Except for an unexplained increase in Group II no effect on serum phosphate was shown under the conditions of this experiment. It should also be noted that there was some individual variation among the animals as to the dose at which the various responses to the treatment were manifested.

From this experiment it can be seen that a dose of 200 µg per day was the one that gave the greatest responses. This dosage is used in all experiments following unless otherwise noted.
II. Cumulative Effects of A.T.-10 on Normal Rats: In order to study some of the effects of A.T.-10 on calcium and phosphate metabolism in normal animals the drug was administered at a daily dose of 200 µg to two groups of rats. The first group was maintained on normal laboratory rat chow preceding and throughout the experimental study. The second group was placed on a specially prepared calcium-free regime ten days prior to the actual experimentation period. Urine collections were made starting 18-24 hours after the initial dose of A.T.-10. A second collection was made from 24-48 hours after initial dosage and in some instances 48-72 hours after the initial dose. In this way the cumulative effects of A.T.-10 on normal rats could be studied. The results are summarized in Table III.

Examination of these data reveals that the drug produced a gradual increase in serum calcium levels in both groups of animals while producing little, if any, effect on serum phosphate. In regard to the latter, there appeared to be some increase in serum phosphate by 48 hours after the initial treatment with A.T.-10, but since by 72 hours phosphate values were normal, the significance of the increase at 48 hours is questionable. It is of interest to note that Hoff (1935) reported an increase in serum phosphate following A.T.-10 administration. This result is in direct contrast to Albright and Reifenstein's theory as to the mode of action of dihydrotachysterol.

Renal excretion of these two ions appeared to follow the serum values. As the serum calcium level rose, calcium excretion increased markedly and in some cases rose to 15 times the normal value. This
### TABLE III

**EFFECT OF AT-10 ON NORMAL RATS**

<table>
<thead>
<tr>
<th>Urine Collection Periods - in Hours after initial dose of AT-10</th>
<th>18-24 Hours</th>
<th>24-48 Hours</th>
<th>48-72 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium Excretion (mg/hr)</td>
<td>Normal</td>
<td>AT-10</td>
<td>Normal</td>
</tr>
<tr>
<td>Normal Diet</td>
<td>0.019± (.001)</td>
<td>0.035± (.003)</td>
<td>0.039± (.005)</td>
</tr>
<tr>
<td>Calcium Excretion (mg/hr)</td>
<td>Calcium-free Diet</td>
<td>0.020± (.003)</td>
<td>0.036± (.005)</td>
</tr>
<tr>
<td>P32 Excretion (mg/hr)</td>
<td>Normal</td>
<td>AT-10</td>
<td>Normal</td>
</tr>
<tr>
<td>1000± 200 310 130 281 343</td>
<td>1130± 5 310 130 281 343</td>
<td>2048± 9 310 130 281 343</td>
<td>1000± 9 310 130 281 343</td>
</tr>
<tr>
<td>Serum Phosphate (mg % P)</td>
<td>Normal</td>
<td>AT-10</td>
<td>Normal</td>
</tr>
<tr>
<td>9.6± .28 310 130 281 343</td>
<td>11.3± .31 310 130 281 343</td>
<td>10.2± .19 310 130 281 343</td>
<td>10.2± .35 310 130 281 343</td>
</tr>
<tr>
<td>Serum Calcium (mg %)</td>
<td>Normal</td>
<td>AT-10</td>
<td>Normal</td>
</tr>
<tr>
<td>10.0± .35 51 18 .36</td>
<td>11.3± .51 51 18 .36</td>
<td>11.2± .18 51 18 .36</td>
<td>13.1± .36 51 18 .36</td>
</tr>
</tbody>
</table>

**Notes:**
1. Dosage = 200 µG AT-10 daily
2. Numbers in parentheses = number of animals
3. Values given with S.E.
4. Animals bled at conclusion of collection period.
5. P-32 given simultaneously with initial injection of AT-10. Radioactivity excreted by normal animals in each collection period set at 1000, and experimental values adjusted to this standard.
phenomenon occurred also in the animals maintained on a calcium-free
diet which would indicate that the effect of A.T.-10 was not limited
to a vitamin D-like activity of increasing the intestinal absorption
of calcium. Renal phosphate excretion increased slightly following
A.T.-10 administration, doubling its rate by 48 hours. However, as
in the case of serum phosphate levels, this rate was not maintained.

Effect of A.T.-10 on Parathyroidectomized Rats: Since dihydro-
tachysterol is used clinically for the treatment of hypoparathyroidism,
this series of experiments was undertaken to study the effects of the
drug on parathyroidectomized rats. Also, since the drug is known to
be relatively slow acting, in most cases the animals were pre-dosed
up to 48 hours prior to the operations. Some animals, however, were
given the initial dose of A.T.-10 from 12 to 18 hours prior to parathyroid-
ectomy and a third group received the drug at the time of the operation.
These last two groups of animals were used in an effort to determine
just how quickly the effects of A.T.-10 could be detected. For a sum-
mary of the progressive changes in the rat following parathyroidectomy,
the reader is referred to the section on the effects of parathyroidecto-
cy or to Talmage et al (1955). The results of the above experiments
are summarized in Table IV.

One the the most striking effects produced by A.T.-10 on the
progressive changes which follow parathyroidectomy was its influence on
phosphate excretion. Not only was the drop in phosphate excretion pre-
vented for the most part, but even the small drop noted was reversed
within a few hours. The serum phosphate values, however, were not
markedly effected, and rose steadily as did the parathyroidectomized
controls run simultaneously. In no case was a reduction of the high
serum phosphate following parathyroidectomy noted after treatment
### TABLE IV

**EFFECT OF AT-10 ON PARATHYROIDECTOMIZED RATS**

<table>
<thead>
<tr>
<th>Time of Initial Urine Collection Period</th>
<th>p 32 Excretion (mg/hr)</th>
<th>Calcium Excretion (mg/hr)</th>
<th>Terminal Serum Calcium (mg%)</th>
<th>Terminal Serum Phosphate (mg% P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Controls</td>
<td>1000 ± 62</td>
<td>.034 ± 0.03</td>
<td>10.5 ± 0.19</td>
<td>9.3 ± 0.35</td>
</tr>
<tr>
<td></td>
<td>(34)</td>
<td>(18)</td>
<td>(36)</td>
<td>(19)</td>
</tr>
<tr>
<td>PTX Controls</td>
<td>24 ± 6</td>
<td>.228 ± 0.036</td>
<td>7.6 ± 0.21</td>
<td>11.0 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>(30)</td>
<td>(9)</td>
<td>(24)</td>
<td>(17)</td>
</tr>
<tr>
<td>AT-10 Given Simultaneously with PTX</td>
<td>17 ± 3</td>
<td>.300 ± 0.037</td>
<td>8.5 ± 0.11</td>
<td>12.1 ± 0.63</td>
</tr>
<tr>
<td></td>
<td>(17)</td>
<td>(7)</td>
<td>(11)</td>
<td>(11)</td>
</tr>
<tr>
<td>AT-10 Given 4-6 HOURS Prior to PTX</td>
<td>898 ± 335</td>
<td>.231 ± 0.043</td>
<td>11.1 ± 0.35</td>
<td>12.8 ± 0.32</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
</tr>
<tr>
<td>AT-10 GIVEN 24-48 HOURS Prior to PTX</td>
<td>553 ± 90</td>
<td>.600 ± 0.062</td>
<td>11.1 ± 0.38</td>
<td>10.9 ± 0.38</td>
</tr>
<tr>
<td></td>
<td>(1173-2583)</td>
<td>(12)</td>
<td>(13)</td>
<td>(13)</td>
</tr>
</tbody>
</table>

\(^a\) PTX = Parathyroidectomized

**Notes:**
1. Dose = 200 mg AT-10 daily.
2. Number in parentheses = number of animals
3. Values given with Standard Error (Range given when less than 4 animals)
4. Animals bled at end of urine collection period.
5. P-32 given 1 hour following PTX, Radioactivity excreted by normals set at 1000; experimental values adjusted to this standard.
with A.T.-10. This again is in contrast with the effect which was noted by Albright and Reifenstein.

The other striking effect of A.T.-10 treatment of parathyroidectomized rats was the prevention of the fall in serum calcium which normally follows this operation. When the initial dose of A.T.-10 preceded parathyroidectomy by 12 hours or more, serum calcium was reduced by parathyroidectomy to normal levels, indicating only a slight drop from the elevated serum calcium produced by the previously administered A.T.-10. When the first dose was given at the time of parathyroidectomy there was a rise in serum calcium by the 7th hour and an even greater rise by the 24th hour. The urine calcium excretion rise following parathyroidectomy was even more marked when parathyroidectomy was preceded by A.T.-10 administration, but this is probably explained by the higher excretory rate existing at the time of operation.

Comparison of the Effects of A.T.-10 and Calciferol on Normal and Parathyroidectomized Rats: In view of the chemical similarity between A.T.-10 and various forms of vitamin D (see section on Chemistry of Vitamin D) and the fact that Vitamin D₂ (calciferol) is also often used in the clinical treatment of parathyroid insufficiency, the entire series of experiments was repeated. An additional group of animals in each instance was treated with calciferol in an amount double by weight that of A.T.-10 administered in the previous experiments. This increase was based on McLean's (1941) ratio of 2:1 for the effectiveness of dihydrotachysterol to calciferol. In order to rule out the effect of calciferol on intestinal absorption of calcium all the animals in this series of experiments were maintained on a calcium-free diet for ten
days prior to the experiment. These data are summarized in Table V.

In normal animals calciferol and A.T.-10 had about the same effect on urinary excretion of radiophosphorous. However, in parathyroidectomized animals calciferol afforded no protection against the drop seen following the operation while A.T.-10 prevented this drop. Calciferol had some slight effect of increasing urinary calcium excretion both in normal and operated animals but this effect was not nearly of the magnitude seen in the A.T.-10 treated animals. The major effect seen with calciferol was on the serum calcium levels of parathyroidectomized animals where it partially protected against the drop normally seen following the operation. But here again the protection was not nearly as great as that given by A.T.-10 which also raised the serum calcium levels in normal animals while calciferol had no effect on this group. Neither A.T.-10 nor calciferol had any pronounced effect on the level of serum phosphate.

From these data it may be seen that, for the phenomena described, calciferol will not replace A.T.-10 in the rat. For the most part calciferol could not be shown to influence any of the physiological functions studied in these experiments. The only possible effects seen for calciferol were minor increases in renal calcium excretion and a slight retardation in the fall in serum calcium following parathyroidectomy.
TABLE V

COMPARISON OF VITAMIN D₂ AND AT-10 IN NORMAL AND PARATHYROIDECTOMIZED RATS

<table>
<thead>
<tr>
<th></th>
<th>Renal P³₂ Excretion (Total)</th>
<th>Renal Calcium Excretion (mg/hr)</th>
<th>Terminal Serum Calcium (mg %)</th>
<th>Terminal Serum Phosphate (mg % P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Controls</td>
<td>1000 ± 220</td>
<td>.020 ± .003</td>
<td>10.6 ± .35</td>
<td>9.8 ± .28</td>
</tr>
<tr>
<td>Normals + Vitamin D₂</td>
<td>1246 ± 303</td>
<td>.073 ± .016</td>
<td>10.1 (9.5-11.4)</td>
<td>9.0 (8.6-9.5)</td>
</tr>
<tr>
<td>Normals + AT-10</td>
<td>1540 ± 104</td>
<td>.390 ± .044</td>
<td>13.1 ± .36</td>
<td>10.2 ± .35</td>
</tr>
<tr>
<td>Parathyroidectomized</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>4 (.043 (.042-.045)</td>
<td>7.6 ± .21</td>
<td>11.0 ± .21</td>
<td></td>
</tr>
<tr>
<td>Parathyroidectomized</td>
<td>100 (33-151)</td>
<td>.076 ± .022</td>
<td>9.1 ± .22</td>
<td>11.9 ± .37</td>
</tr>
<tr>
<td>+ Vitamin D₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parathyroidectomized</td>
<td>1738±230</td>
<td>.214 ± .022</td>
<td>12.2 ± 1.04</td>
<td>10.9 ± .38</td>
</tr>
<tr>
<td>+ AT-10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes: 1. All animals on Calcium-free diet 10 days prior to experimental use.
2. Dosage of drugs: 400 μg daily. Initial dose 48 hours prior to parathyroidectomy.
3. Urine collection periods: 4-7 hours after parathyroidectomy; animals bled at end of collection period.
4. Each value given = average of 4-7 animals with standard error. In the 5 cases were only three animals were used, range rather than S.E. is given.
5. P-32 given 1 hour following parathyroidectomy. Radioactivity excreted by normal animals set at 1000; experimental values adjusted to this standard.
DISCUSSION

It appears from the data presented in this study that the action of dihydrotachysterol in rats is restricted almost entirely to its ability to remove calcium and phosphate from bone. This is borne out by the evidence that increases or decreases in the renal excretion of both calcium and phosphate appear to follow similar increases or decreases in the serum levels of these two ions and that the drug was unable to lower serum phosphate levels even in the presence of slightly increased renal phosphate excretion. In addition to this, an effect, similar to vitamin D, on intestinal absorption of calcium would appear to be partially responsible for increased renal excretion of this ion. However, when animals were maintained on special calcium-free diets, an increase in renal excretion of calcium could still be demonstrated indicating that the intestinal effect was not totally responsible. In regard to the effects of dihydrotachysterol, other than intestinal absorption, the action of this drug in the rat appears to be different from the action of parathyroid hormone as suggested by recent works from this laboratory. This laboratory has postulated that the parathyroids are able, in the rat, to control the renal thresholds for both calcium and phosphate while simultaneously influencing the removal of calcium and phosphate from bone. Albright and Reifenstein believe that dihydrotachysterol has its primary action on the renal excretion of phosphate; causing an increase in the excretion of this ion. As a result of this action, they state that serum phosphate levels are lowered. In no experiment were we able to demonstrate such an action
of the drug. This indicates, to us, that the drug's primary action is not via the kidney but, rather, on bone. Whether the actions on bone by dihydrotachysterol and the hormone are similar has not as yet been determined.

Attempts are now being made to demonstrate the action of A.T.-10 on bone. Autoradiographs have been made using both radiocalcium and radiophosphorous. The results of these have been indicative but not conclusive. Radiocalcium appears to have been removed from the bone, but, at the present, no clear-cut evidence has been obtained upon which to make decisive conclusions. The difficulties of showing small changes in the quantity of material present in bone are numerous; and, at this writing, have not been overcome. Perhaps, if large amounts of the drug were administered over an extended period of time changes could be demonstrated. Latta and Tristan (1950) were able to show changes in bone induced by dihydrotachysterol using histological methods. This may be the method of choice in dealing with the phenomenon, but it is beyond the scope of this study.

These studies also bear out the evidence of Latta and Tristan (1950) and McChesney (1943) that dihydrotachysterol is less effective when injected than when administered orally. At the dose levels used in these experiments, no "calcemic effect" could be shown following subcutaneous injection. Since the above authors state that effects are seen following subcutaneous injections of A. T. -10, it is assumed that either the dosage in these experiments was not high enough or not prolonged enough for the effects to become evident. No explanation of the differences between oral and subcutaneous administration of the drug can be attempted.
at this time.

These studies also indicate, fairly conclusively, that in the rat the action of dihydrotachysterol and calciferol in regard to calcium and phosphate metabolism are not comparable. This appears to be in marked contrast to the similar effects produced by both drugs in humans as reported by McLean (1941, 1953) and Martin and Rilliet (1953). This could well be a species difference since dihydrotachysterol and calciferol are known to act differently in the rat and chick with regards to their antirachitic effect (Correll and Wise, 1942). Except for the effect on intestinal absorption of calcium, which was ruled out for the most part by maintaining the animals on calcium-free diets, calciferol proved virtually ineffective in regard to the studied physiological functions when administered to the rats in a dose approximating 2 mg. of crystalline calciferol per kilogram of body weight per day. One-half of this dose, by weight, of dihydrotachysterol was shown to be effective, when administered orally, in raising serum calcium levels in both normal and parathyroidectomized rats. Since this is the desired result in the treatment of hypoparathyroidism, it follows that, if the rat and human are comparable in this phenomenon, dihydrotachysterol would be the drug of choice.

Note: Part of the material presented above will soon appear in Endocrinology under the title Comparative Study of Some Effects of Administration of Dihydrotachysterol and Calciferol in Rats by R. V. Talmage and B. F. Dodds.
CONCLUSIONS

Based on the results of the above studies, the following conclusions regarding dihydrotachysterol and calciferol can be reached.

1. At the dose levels used, dihydrotachysterol is effective only if given orally.

2. While a minimum dose of 300 µg over a three day period may elicit a response, a dose of 200 µg per day is regarded as preferable for the best results.

3. In normal rats dihydrotachysterol is able to raise serum calcium levels and renal calcium excretion markedly, while only minor increases in phosphate excretion can be demonstrated.

4. When given prior to parathyroidectomy, dihydrotachysterol prevents the fall in serum calcium but does not prevent the temporary increase in renal calcium excretion which follows the loss of circulating hormone. Conversely, while the drug is able to prevent the fall in renal phosphate excretion, it is unable to prevent the rise in serum phosphate levels which follows removal of these glands.

5. When given at the time or following parathyroidectomy, dihydrotachysterol is able to raise serum calcium levels without any marked effect on serum phosphate levels or renal excretion rates of these two ions.

6. In the rat, equal or double amounts, by weight, of calciferol is unable to replace dihydrotachysterol in its effects on the above mentioned physiological functions.

7. Based on the results of these studies, it is concluded that dihydrotachysterol functions in the rat by removing calcium and phosphate from the bone.
BIBLIOGRAPHY


