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The effect of parathyroidectomy and thyroidectomy upon the subsequent levels of calcium and phosphorus in the blood of swine

Barry Reit

To the Graduate Council:

I am submitting herewith a thesis written by Barry Reit entitled "The effect of parathyroidectomy and thyroidectomy upon the subsequent levels of calcium and phosphorus in the blood of swine." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

R. L. Murphree, Major Professor

We have read this thesis and recommend its acceptance:

G. M. Merriman, D. O. Richardson

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

January 15, 1968

To the Graduate Council:

I am submitting herewith a thesis written by Barry Reit entitled "The Effect of Parathyroidectomy Upon the Subsequent Levels of Calcium and Phosphorus in the Blood of Swine." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

R. L. Murphree
Major Professor

We have read this thesis and recommend its acceptance:

Don O. Richardson

G. M. Merriman

Accepted for the Council:

Hilton A. Smith
Vice President for
Graduate Studies and Research

THE EFFECT OF PARATHYROIDECTOMY AND THYROIDECTOMY UPON THE SUBSEQUENT LEVELS OF CALCIUM AND PHOSPHORUS IN THE BLOOD OF SWINE

A Thesis

Presented to

The Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Barry Reit

March 1968

TO

my parents, Jean and Benjamin,

without whose unceasing confidence, support and love,

this thesis could not have been written.

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CHAPTER I

INTRODUCTION

Although considerable work has been conducted on thyroid and parathyroid function in several species of animals, thus far, such studies in swine are obscure. The results of early studies on the effects of thyroidectomy in other species were badly clouded because of the simultaneous removal of the parathyroids. It is now accepted that the two organs are anatomically and physiologically separate structures.

In most mammals the thyroid gland consists of two lobes--one on each side of the trachea close to its junction with the larynx. The lobes are usually connected by an isthmus crossing the ventral surface of the trachea. The pig is an exception in that the thyroid gland is a monolobed structure located on the ventral surface of the trachea just above the thoracic aperature.

The parathyroids, present in amphibians, reptiles, birds and mammals, are believed to have essentially the same function. There are usually two pairs of parathyroids. Marked variations in the position of the glands are seen in the different species, and, indeed, the position varies somewhat in animals of the same species. Location of these pyriform bodies is commonly on the posterior surface of the lateral lobes of the thyroid. Again, the pig proves to be an exception by having only two parathyroid glands--one in each anterior tip of the thymus gland (Schlotthauer and Higgins, 1934; Littledike, 1965).

Optimal activity in animals is expected to occur when there is a proper balance of secretory activity of all the endocrine glands. Both the thyroid and parathyroid glands play an important part in this balance. Thyroid effects on metabolism, growth, and sexual maturity are well known; parathyroid regulation of calcium and phosphorus metabolism in many species is also well documented. Extirpation of these glands produces a well-established syndrome of clinical symptoms; however, most of this knowledge is based on data compiled from research on dogs, cats, and rats. Relatively little attention has been devoted to the effects of the hormones of these glands on calcium and phosphorus metabolism in the pig.

The present investigation was conducted to determine the specific effects of parathyroidectomy and thyroidectomy on levels of calcium and phosphorus in the blood. The pig was chosen as the experimental animal because it is the only mammal that has an anatomically separated parathyroid and thyroid gland.

CHAPTER II

REVIEW OF LITERATURE

The following literature review is concerned primarily with outlining experimentation performed with various species as it relates to parathyroidectomy and thyroidectomy on subsequent calcium and phosphorus levels. No attempt is made to review the vast literature dealing with the broad aspects of calcium and phosphorus metabolism or general parathyroid and thyroid work. Such aspects of calcium and phosphorus metabolism are fully discussed, however, in a number of extensive reviews (Greenberg, 1939; Glass, 1952; Duckworth and Hill, 1953; and others). Several reviews of the literature on parathyroid and thyroid physiology have been published (Thomson and Collip, 1932; Grafflin, 1940; Anthony, 1950; Patten, 1952; Bartter, 1954; Greep and Kenny, 1955; Dukes, 1955; Ham, 1957; Nalbandov, 1958; Cole and Cupps, 1959; Gorbman, 1959; Turner, 1966; and Arnaud, Tenenhouse and Rasmussen, 1967).

I. EFFECTS OF PARATHYROIDECTOMY IN VARIOUS SPECIES

According to Turner (1966), the first anatomic description of the parathyroids was provided by Sandström, who dissected the dog, cat, rabbit, and ox, while Gley was the first to show that the parathyroids and thyroid gland had separate functions through experimentation with rabbits.

The parathyroids have been removed experimentally from animals of many species; however, review of the available literature revealed only four articles specifically related to the pig (Schlotthauer and Higgins, 1934; Littledike, 1965; Brown, Krook and Pond, 1966; and Care, Duncan and Webster, 1967).

The most significant changes after removal of the parathyroid glands in most species are:

1. Diminished levels of serum calcium, largely involving the diffusible fraction,

2. Reduced blood citrate,

3. Reduced urinary excretion of calcium,

4. Increased levels of inorganic phosphorus in the serum,

5. Diminished urinary elimination of inorganic phosphorus, and

6. Tetany.

These symptoms are best noted in the dog and cat; however, the latter species exhibits less severe symptoms post-operatively than does the dog (Dragstedt, Phillips and Sudan, 1923). Monkeys show chronic tetany, while lambs and most herbivores tolerate absence of the parathyroids and thyroid better than the carnivores. Goats usually do not exhibit tetany after parathyroidectomy, but definite muscular tremors have been seen in some cases. Tetany in rabbits develops earlier and is more severe than in dogs and cats. Parathyroidectomy in the rat is usually not fatal, but in long standing cases of hypoparathyroidism, cataractuous lenses have been reported (Evans and Kern, 1931; Turner, 1966).

Munson (1960) reported that the serum calcium level of rats fell from 8 mg. to about 4 mg. percent in a period of 8 hours following surgical excision of the parathyroid glands. When Munson (1955) injected parathormone (PTH) intravenously into parathyroidectomized rats (500 U.S.P. units) and took blood samples from 3 to 74 hours post injection, he noted a sharp rise in serum calcium from 0 to 18 hours after injection. Shah and Draper (1966) conducted an *in vivo* study of the effect of parathyroidectomy on the intestinal absorption of calcium by growing rats. They concluded that the parathyroid hormone was one of the factors involved in the phenomenon of adaptation to changing dietary intakes of calcium.

Results of parathyroidectomy in young laboratory animals were reported by Bartter (1954), Greep and Kenny (1955), and McLean and Urist (1955); they indicated occurrence of tetany shortly after parathyroidectomy. Similar results in young calves and mature dairy cattle were noted by Stott and Smith (1957) and Mayer, Ramberg and Kronfield (1966), respectively.

Smith and Stott (1956) reported that mature parathyroidectomized cattle were capable of survival and that parathyroidectomized dairy cows maintained lactation and calved normally. This is contradictory to results obtained by McLean and Urist (1955), Neuman and Neuman (1958), Munson (1960), and Mayer, Ramberg and Kronfield (1966), who indicated that work on other species resulted in tetany, impaired lactation and possible abortion. Todd *et al*. (1962) reported that a severe drop occurred in serum calcium in mature Jersey cows 2 days following thyroparathyroidectomy. The calcium level in these animals returned to normal within a 2 week period following surgery.

II. EFFECTS OF THYROIDECTOMY IN VARIOUS SPECIES

The role of the thyroid gland in domestic animals has been extensively reviewed. The manifestation that follows thyroidectomy regardless of age or species is a decided lowering of body metabolism. Gross external manifestations vary with the age of the animal. In young animals of all species there is stunting of physical, mental and sexual development. In mature animals, especially herbivores (Dukes, 1955), clinical symptoms may be slight and confined to dryness and thickening of the skin, loss of hair, physical and mental sluggishness, or they may be practically absent (Leblond and Eartly,1952). Until recently, little attention has been given to the possibility of an effect of the hormone (s) of the thyroid gland on calcium metabolism.

Experimental evidence in support of a parathyroid-calcitonin releasing factor. Much experimental evidence has accumulated since 1961 indicating that the thyroid-parathyroid complex of mammals is the source of a fast-acting polypeptide hormone, which lowers the level of serum calcium. This hormone, calcitonin, is released in response to hypercalcemia and opposes the action of PTH of the parathyroid gland. The main point of controversy at present is whether the calcium-lowering principle originates in the parathyroid gland or in the thyroid gland or in both glands. Copp et al. (1962) and Copp and Henze (1964) reported that a parathyroid extract (PTE) increased serum calcium much more in parathyroidectomized and thyroparathyroidectomized rats than in intact rats. Since this PTE response in thyroparathyroidectomized rats was no greater than it was in the parathyroidectomized rats, it appeared to be a parathyroid rather than a thyroid factor that acted to oppose the hypercalcemic effect of PTE in intact rats. These results supported the calcitonin concept introduced by Copp et al. (1962) that the parathyroid gland secreted a hypocalcemic hormone.

Experimental evidence in support of a thyroid-thyrocalcitonin releasing factor. In contrast to the above results, Talmage, Neuenschwander and Kraintz (1965) found no evidence in support of a parathyroid hypocalcemic hormone. In a series of experiments designed to detect the effect of administered calcium in intact, parathyroidectomized, autotransplanted parathyroid rats, thyroidectomized and thyroparathyroidectomized rats, they demonstrated the importance of the thyroid gland in counteracting hypercalcemia. These results indicated evidence for the existence of thyrocalcitonin, the hypocalcemic principle of thyroid origin (Hirsch, Gauthier and Munson, 1963; Hirsch, Voelkel and Munson, 1964; Hirsch and Munson, 1966; Foster et al., 1964; Care, 1965; Care, Keynes and Duncan, 1966; and Care, Duncan and Webster, 1967).

Further evidence that the thyroid is involved in the control of hypercalcemia comes from work by MacIntyre, Foster and Kumar (1965), who deduced from the results of thyroid perfusion studies in dogs that only the thyroid secretes a calcium-lowering principle. Bernstein, Kleeman and Pine (1965) reached a similar conclusion.

Evidence supporting a thyroid-parathyroid release mechanism. Evidence that both the parathyroid and thyroid glands play a role in preventing hypercalcemia was reported by Gittes and Irvin (1966), who suggested that the parathyroid secreted a humoral factor, which in turn released thyrocalcitonin from the thyroid. These experiments, similar to those conducted by Copp et al. (1962) and Copp and Henze (1964), consisted of testing the hypercalcemic responses to PTE. The response was greatest in thyroparathyroidectomized or thyroidectomized rats, intermediate in parathyroidectomized rats, and smallest in intact rats. They proposed that, in rats, a thyrocalcitonin-releasing factor was secreted from the parathyroid glands in response to a hypercalcemic stimulus.

Tashjian and Munson (1965), Care (1965), and Care, Duncan and Webster (1967) reported that hypocalcemia induced by thyrocalcitonin administration was followed by the return of the plasma calcium concentration towards the normal level in the pig. This action, they postulated, was due to a substance in the plasma that quickly destroyed the biological activity of thyrocalcitonin, while the parathyroid hormone simultaneously mobilized calcium to maintain a normal plasma concentration.

In vitro experiments by Friedman and Raisz (1965) pointed to an increased net accretion of bone salts as the basic mode of action of thyrocalcitonin. This agrees with reports by MacIntyre, Foster, and Kumar (1965) and MacIntyre and Parsons (1966), who used perfusion techniques with cat tibia in vivo, and suggested bone as the principle target organ for thyrocalcitonin.

More recently, Copp, Cockcroft and Kueh (1967) reported that acid extracts of thyroid glands from a dogfish shark (Squalus suckleyi) and domestic fowl (Gallus domestica) contained no detectable calcitonin activity, while a very potent hypocalcemic-inducing fraction was obtained from the ultimobranchial glands of these two species. The calcitonin concentration was 4 to 40 times that present in hog thyroid, which, as in most other mammals, contains ultimobranchial tissue. The evidence suggests that calcitonin is a fundamental calcium-regulating hormone present in all higher vertebrates and that it is an ultimobranchial rather than a thyroid hormone. This is consistent with the interpretation that calcitonin is secreted by the C cells of the mammalian thyroid (Bussolati and Pearse, 1967) and that these cells are of ultimobranchial origin (Pearse and Carvalheira, 1967).

Thyrocalcitonin is believed to be a polypeptide of a size and amino acid composition similar to, but distinct from, the classical calcium-mobilizing parathyroid hormone (Tenenhouse, Arnaud and Rasmussen, 1965).

In summary, there is general agreement among investigators, that parathyroidectomy results in a precipitous decrease in blood calcium, and a corresponding increase in inorganic phosphorus with concomittant reduction in urinary calcium and phosphorus. Recent evidence indicates that specific cells located in the thyroid gland secrete a hypocalcemic-inducing-factor which normally limits hypercalcemia. Relatively little is known about the mechanism or action of this factor.

The following experiment was conducted to determine the effect of parathyroidectomy and thyroidectomy on calcium and phosphorus blood levels in the pig. The pig was chosen as the experimental animal for this study because the separate anatomical location of the parathyroid and thyroid glands would permit studies on the effect of extirpation of each gland on subsequent calcium and phosphorus blood levels.

CHAPTER III

EXPERIMENTAL ANIMALS AND PROCEDURES

Two separate experiments using Duroc pigs were conducted to study (1) the effects of parathyroidectomy on blood serum calcium and phosphorus concentration, and (2) the effect of parathyroidectomy and subsequent thyroidectomy on hypercalcemia induced by the intraperitoneal injection of a hypertonic calcium solution. Sixteen Duroc pigs (eight males and eight females) from the Animal Husbandry Department swine herd were used in this study. The pigs were about six weeks of age and averaged 10 kg. in body weight when assigned to this study. They were randomly divided, within sex, into two comparable groups and fed, *ad libitum*, the rations shown in Table I (N.R.C., 1964). The only difference in the two rations was the calcium content (0.32 percent versus 0.59 percent). Within each group, pairs of pigs (one male and one female) were placed in separate pens equipped with self-feeders and automatic waterers. Pigs in Group I received the normal calcium ration (ration I), while those in Group II received the low calcium ration (ration II). All pigs were weighed at three-week intervals and total feed consumption for the entire experimental period was recorded for each group.

The experimental design is shown in Table II. Phase I was concerned with the effect of parathyroidectomy on calcium and phosphorus blood levels. The same pigs were subsequently used in Phase II, which

TABLE I

EXPERIMENTAL RATIONS

Ingredients (Percent)	Ration No.[a] I Normal Calcium	Ration No.[a] II Low Calcium
Corn	72.2	72.2
Soybean oil meal	25.2	25.2
Salt (non-iodized)	0.5	0.5
Dicalcium phosphate	1.2	0.8
Monobasic sodium phosphate	---	0.9
Ground limestone	0.5	---
Trace mineral salt (70 gms/114 kg.)[b]	---	---
Vitamin supplement[c]	0.4	0.4
Totals	100.0	100.0

[a]Rations I and II contained 0.59 percent and 0.32 percent calcium, respectively; both rations contained 18 percent protein and 0.6 percent phosphorus.

[b]Contained 10 percent manganese; 10 percent iron; 10 percent calcium; 10 percent zinc; 1 percent copper; 0.3 percent iodine; 1 percent cobalt.

[c]This supplement added 27 mg. vitamin B_{12}; 10.8 mg. riboflavin; 20.0 mg. pantothenic acid; 4.9 mg. niacin; 6.2 mg. choline chloride; 5.7 mg. terramycin; 5632 I. U. vitamin A; 198 I. U. vitamin D_2; per kg. of ration.

TABLE II

EXPERIMENTAL DESIGN SHOWING CALCIUM LEVEL IN RATIONS AND SURGICAL TREATMENT

Calcium Level (Percent)	Animal Number	Phase I Parathyroidectomy	Phase II Thyroidectomy
0.32	5-9	C[c]	C
	7-15	C	C
	6-3	C	Tx[c]
	8-2	C	Tx
	6-8	Px[c]	C
	8-14	Px	C
	5-5	Px	Tx
	7-3[a]	Px	--
	7-4[b]	Px	Tx
0.59	1-8	C	C
	3-2	C	C
	2-14	C	Tx
	4-12	C	Tx
	2-3[a]	Px	--
	2-1[b]	Px	C
	4-3	Px	C
	1-9[a]	Px	--
	1-6[b]	Px	Tx
	3-10	Px	Tx

[a]Indicates animals that died within 26 hours subsequent to parathyroidectomy.

[b]Indicates animals replacing those that died after surgery.

[c]C indicates controls; Px indicates parathyroidectomy; Tx indicates thyroidectomy.

was concerned with the effects of thyroidectomy on calcium and phosphorus blood levels. Prior to surgery (parathyroidectomy at 7 weeks of age and thyroidectomy at 13 weeks of age), feed and water was withheld for 24 hours. After recovery from anesthesia, the animals were returned to their pens. Three animals that died following parathyroidectomy were replaced with three others of comparable age and weight.

Initially, equal numbers of males and females were assigned to each treatment; however, since no differences due to sex were detected after analyses of the data, no further reference to this variable will be made.

I. COLLECTION OF BLOOD SAMPLES AND CHEMICAL ANALYSES

The procedures for collection of blood samples and chemical analyses of calcium and phosphorus were the same for Phases I and II. The pigs were restrained in the supine position and blood samples were obtained from the anterior vena cava with plastic 12 ml. disposable syringes fitted with 18 gauge x 38 mm. disposable needles. The blood samples were held overnight at room temperature and then centrifuged. The serum was decanted into glass tubes, corked and stored in the refrigerator at 5° C. until analyses of calcium and phosphorus were initiated. Calcium was determined by the potassium permanganate procedure (Clark and Collip, 1925) and phosphorus was determined by the photometric method of Fiske and SubbaRow (1925). A Beckman DU-2 Spectrophotometer was used for the phosphorus analyses instead of the Evelyn Colorimeter.

Initially it was planned to take samples 24 hours before and at 18, 24, 26, 42, 48, and 168 hours after parathyroidectomy. However, due to the death of three parathyroidectomized pigs following blood collection (Phase I) at 26 hours after surgery, the 18, 26, and 42 hour intervals were omitted for the remaining pigs. In Phase II, which was concerned with the effect of thyroidectomy on calcium and phosphorus levels, blood samples were taken 24 hours before intraperitoneal injection of calcium chloride ($CaCl_2$) and at intervals of 0.5, 1, 3, 6, 10, 24, and 48 hours post injection.

II. SURGICAL PROCEDURES

After a thorough study of surgical techniques as described by Markowitz (1949) and Littledike (1965), a preliminary trial was conducted using miniature pigs, to gain surgical experience. Anesthesia was induced by injection, into the anterior vena cava, of a solution of secobarbital sodium[1] (50 mg. per ml.) at the rate of 0.45 ml. per kg. of body weight, to effect. Subsequent to anesthesia, the following procedure was used for parathyroidectomy. A midline incision was made in the skin of the neck from the suprasternal notch anteriorly to the level of the mandibular salivary gland. The incision was continued through the fascia. Blunt dissection was then used to expose both of the thymus glands anteriorly, near the omohyoid muscle, and posteriorly, to the point of

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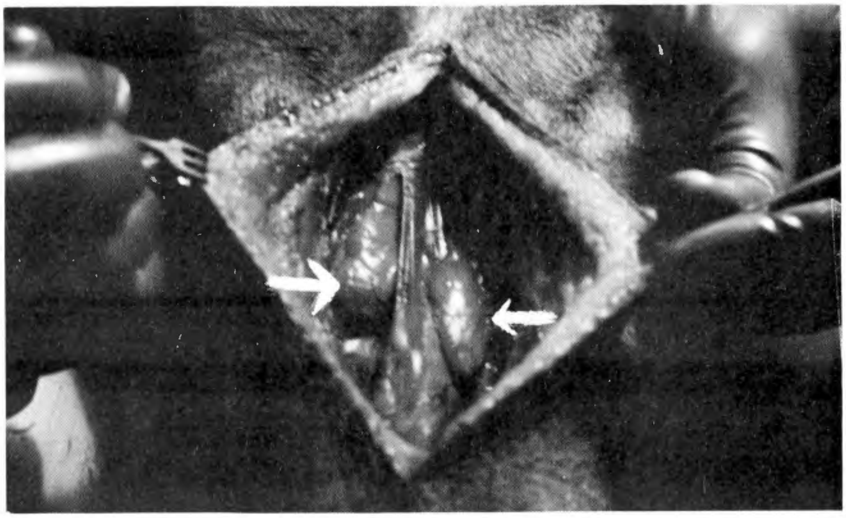
[1]Myothesia, distributed by the S. E. Massengill Company, Veterinary Division, Bristol, Tennessee.

entrance into the suprasternal notch, where they were ligated (Figure 1a). The glands were then removed. Chromic catgut was used to make the internal continuous suture and a nylon mattress suture closed the skin.

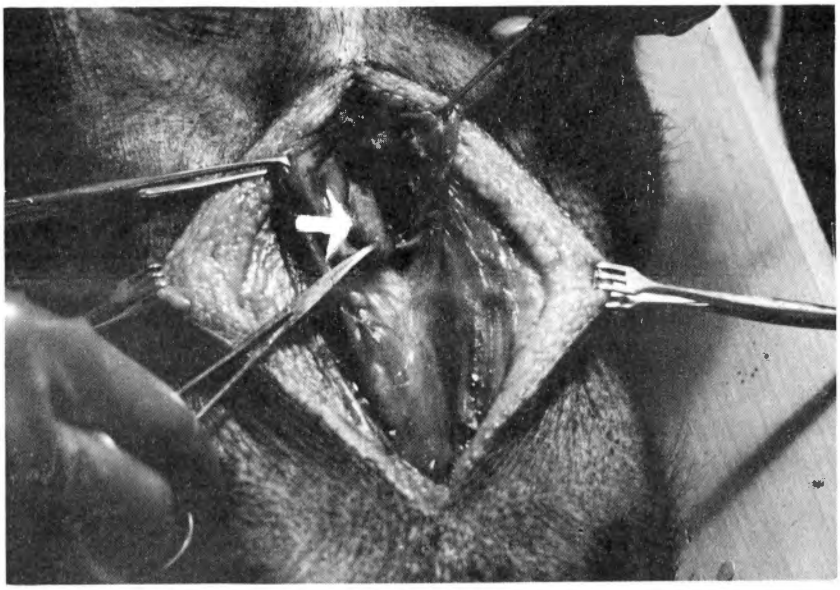
The thyroidectomy procedure was similar to the one described for parathyroidectomy. After intravenous anesthesia (see footnote 1, page 15), the incision was made in the skin of the neck from the suprasternal notch anteriorly to the thyroid cartilage. The sterno-hyoideus muscle ventral to the trachea was incised and then separated by blunt dissection. Palpation of the trachea helped to locate the carotid sheath and third tracheal ring. After further blunt dissection, the thyroid gland, appearing as a single median line structure anterior to the sternum, was grasped with forceps (Figure 1b). The superior and inferior thyroid arteries were ligated. The gland was then excised. After surgery, 200,000 units of procaine penicillin G[2] were administered intramuscularly. Sutures were removed one week post operatively.

Two weeks after thyroidectomy (animals 15 weeks of age), a solution of $CaCl_2$ containing 50 mg. per ml. was injected intraperitoneally at the rate of 4.4 ml. per kg. of body weight into all the animals. The strength of the solution used was determined as the result of preliminary trials with one miniature and one Duroc pig. After a solution of 25 mg. per ml. (Gittes and Irvin, 1966) failed to induce hypercalcemia in the miniature pig, the Duroc pig was dosed with a

[2]Procaine Penicillin G, distributed by the Agricultural Division, Charles Pfizer Company, Incorporated, New York, N. Y.



(a)



(b)

Figure 1. Location of thyroid and thymus glands.

(a) Thymus glands indicated by arrows.
(b) Thyroid gland indicated by arrow.

solution containing 50 mg. per ml. An increase of about 60 percent in serum calcium was detected in blood drawn 30 minutes after injection. This solution was subsequently used in both the control and thyroidectomized animals. Blood samples were taken 24 hours prior to and 0.5, 1, 3, 6, 10, 24, and 48 hours following injection. Procedures for handling and analysis of blood samples were similar to those used in Phase I. All the animals were observed for aberrant behavior post operatively for the duration of the experimental period. The t test was used to determine significant differences between treatment means (Steel and Torrie, 1960).

CHAPTER IV

RESULTS AND DISCUSSION

I. PHASE I--PARATHYROIDECTOMY

The serum calcium values after parathyroidectomy are summarized in Figure 2 and the individual values are shown in Table III. The parathyroidectomized pigs had less serum calcium than the controls at 18 hours after the operation ($P < .1$). By 26 hours after surgery, the serum calcium increased to a higher level than the control group ($P < .1$). This difference was due both to the increase in the parathyroidectomized pigs and a decrease in the control pigs on the low calcium ration. There were no changes in serum calcium levels in the control and parathyroidectomized pigs on the normal calcium ration. From 42 to 168 hours, there was no difference between the two groups. The decrease at 18 hours agrees with the findings in other species (Gittes and Irvin, 1966; Turner, 1966). However, the reason for the increase in the parathyroidectomized animals and the concomittant decrease in the controls by 26 hours post operatively is unknown. Care, Duncan and Webster (1967), working with pigs, and Hirsch and Munson (1966), working with rats, reported that after the initial drop, the serum calcium levels in the parathyroidectomized animals stayed below the control animals.

Four pigs died after parathyroidectomy, one in the low calcium group (7-3) died within an hour after surgery, and 7-4, which replaced

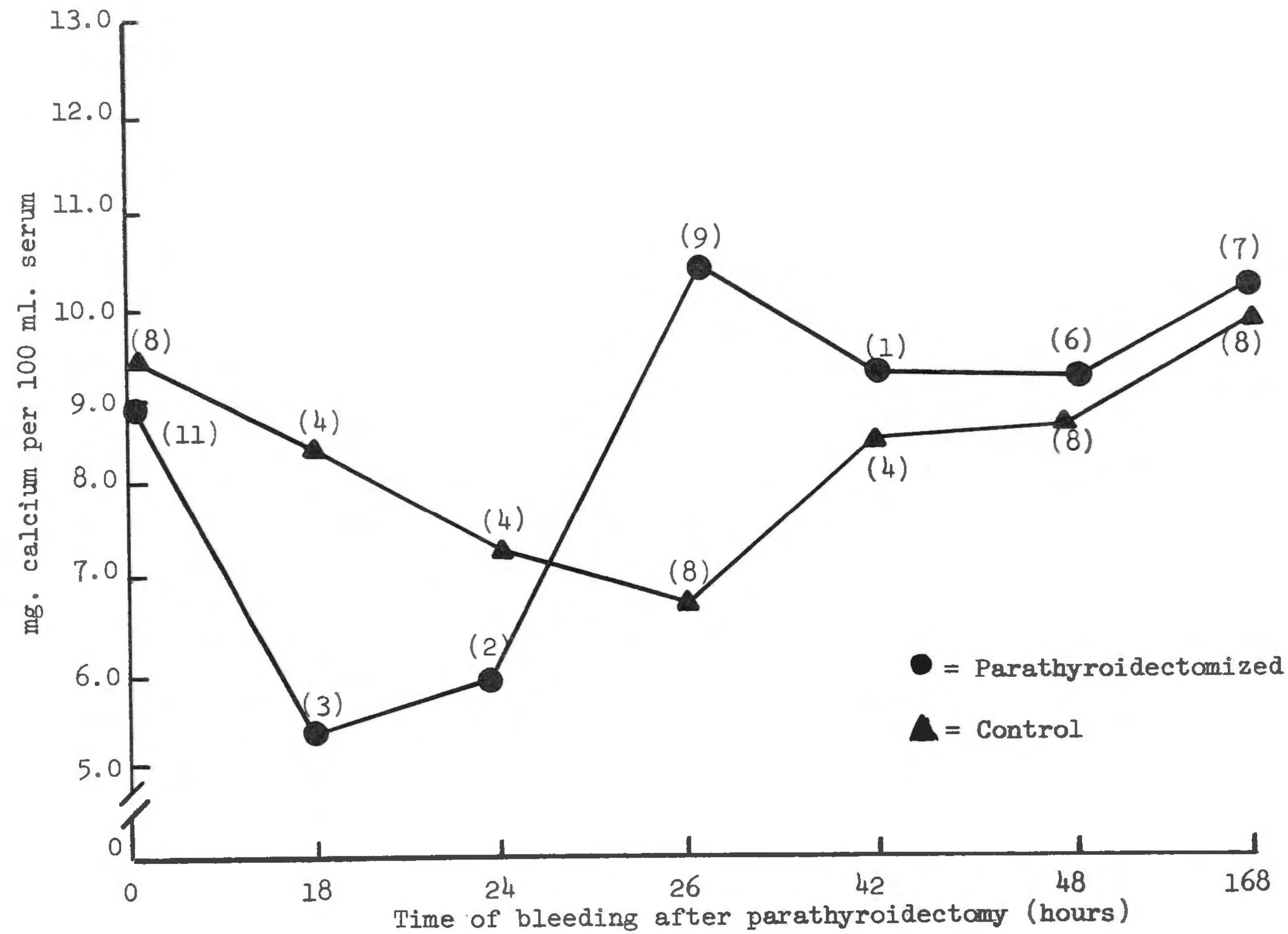


Figure 2. Effect of parathyroidectomy on serum calcium concentration in control and parathyroidectomized pigs (Phase I).

This includes pigs receiving both levels of calcium (0.32 percent and 0.59 percent) in the ration. Numbers of animals bled at each interval are indicated by numbers in parentheses.

TABLE III

EFFECT OF PARATHYROIDECTOMY ON SERUM CALCIUM CONCENTRATION IN PIGS (PHASE I)

Animal Number	Previous Treatment: Calcium Level in Ration (Percent)	Previous Treatment: Surgery	Intervals After Parathyroidectomy (Hours): -24	18	24	26	42	48	168
						Control[a]			
5-9	0.32	C	8.4	---	---	3.4	---	3.8	10.7
6-3		C	10.1	---	---	5.8	---	8.5	10.4
7-15		C	11.6	11.8	5.8	4.5	8.8	10.1	11.3
8-2		C	10.7	9.5	8.0	6.8	9.2	8.1	11.7
1-8	0.59	C	10.5	5.0	7.8	7.0	8.0	10.5	9.4
2-14		C	9.0	7.7	8.0	9.3	8.9	11.7	9.7
3-2		C	7.6	---	---	10.6	---	11.0	9.1
4-12		C	7.2	---	---	7.5	---	7.5	8.4
Average			9.4	8.5	7.4	6.9	8.7	8.9	10.1
Standard Deviation			±1.6	±2.9	±1.1	±2.4	±0.5	±2.5	±1.1
						Parathyroidectomized[b]			
5-5	0.32	Px	7.2	---	---	13.8	---	14.0	13.7
6-8		Px	8.4	---	---	20.5	---	8.5	10.7
7-3[c]		Px	10.6	---	---	---	---	---	---
7-4[d]		Px	9.1	---	---	9.3	---	---	---
8-14		Px	10.6	5.6	5.2	8.8	9.3	9.7	12.4
1-9[d]	0.59	Px	9.7	4.5	6.8	6.4	---	---	---
1-6		Px	9.9	---	---	9.5	---	---	9.1
2-1		Px	8.8	---	---	9.3	---	8.1	8.3
2-3[d]		Px	10.1	6.1	---	---	---	---	---
3-10		Px	7.8	---	---	7.5	---	7.2	9.8
4-3		Px	6.2	---	---	9.1	---	8.2	8.5
Average			8.9	5.4	6.0	10.5	9.3	9.3	10.4
Standard Deviation			±2.8	±0.8	±1.1	±4.3	±0.0	±2.4	±2.0

[a]Includes all control (C) animals in Phase I.

[b]Includes all animals parathyroidectomized (Px) in Phase I.

[c]Died within 1 hour after parathyroidectomy. [d]Died within 26 hours after parathyroidectomy.

7-3, died 26 hours after parathyroidectomy. Two parathyroidectomized pigs in the normal calcium group (1-9 and 2-3) died during blood sampling at 26 hours post operatively. The postmortem examination of these animals revealed no apparent cause of death for the one dying immediately after surgery. However, in the latter three pigs, large amounts of extravasated blood were found surrounding the trachea. It was surmised that death was due to asphyxiation. No sex difference in response was noted in the entire experiment.

None of the classical symptomology resulting from parathyroidectomy (anorexia, vomiting, hyperpnea, diarrhea or tetany), as reported by Dukes (1955) and Turner (1966), were observed in these animals. Smith and Stott (1956) and Mayer, Ramberg and Kronfield (1966) reported the occurrence of tetany after parathyroidectomy in young and mature dairy cattle, respectively. Todd et al. (1962), also working with dairy cows, reported a maximum decrease in serum calcium at 48 hours after parathyroidectomy. However, calcium levels in their animals returned to the normal level 2 weeks post operatively. Their animals did not show the classical symptoms reported by others.

It is possible that not all of the parathyroid tissue was removed from the pigs in this study. Although several investigators (Schlotthauer and Higgins, 1934; Littledike, 1965; Care, Duncan and Webster, 1967) have described the parathyroid gland as a discrete structure, others (Brown, Krook and Pond, 1966; Brown, 1967; Notzold, 1967) state that it is dispersed throughout the thymic tissue. Brown (1967) indicated that gross identification of the parathyroid tissue during postmortem examination, which permitted greater freedom in incision and manipulation than was possible in the living animal, was difficult. He stated that serial examination of histological sections showed disseminated islands of parathyroid parenchyma throughout the thymus gland. The major portion of the gland, however, was found on the anterior medial aspect of the head of the thymus. This part of the thymus is usually found just posterior to the mandibular salivary gland. Consequently, it would appear that to obtain a complete parathyroidectomy, all of the thymic tissue must be removed. If these latter observations are true, it might account for the lack of post surgical symptomology in this study. Some parathyroid tissue may have remained after surgical ligation of the posterior end of the thymus gland, near the suprasternal notch. In fact, part of the parathyroid tissue might have been located completely outside the thymus. The gross appearance of the thymus gland varied between animals.

The pig may have parathyroid rests, as are implicated in cats, which also could account for the lack of symptoms usually described. Kidney excretion patterns and regenerative power of the parathyroid tissue may also prevent tetany from arising in the pig.

Calcium levels were in the range discussed by many (Markowitz, 1949; Dukes, 1955; Turner, 1966). Serum phosphorus values, however, were inordinately high when compared to values reported in the literature.

A study was conducted, therefore, to determine if holding blood at room temperature rather than under refrigeration prior to serum removal affected the serum calcium or phosphorus levels. Three 10 ml.

blood samples were drawn from the anterior vena cava from each of three miniature pigs and put into three separate test tubes. One sample from each pig was centrifuged immediately and serum calcium and phosphorus determined. The other six samples were held overnight at room temperature and centrifuged the next day. After centrifugation, three more samples--one from each pig--were analyzed and the remaining three were stored in the refrigerator for 1 week at 5° C. The results are summarized in Table IV. Those samples analyzed immediately fell within the normal phosphorus range, whereas those held overnight and for 1 week, respectively, yielded hyperphosphatemic values. This indicated the necessity for proper handling of blood samples prior to analysis if valid phosphorus estimates were to be obtained. Calcium values, however, were not changed by the methods of storage prior to analyses.

The small number of animals involved in this study make it hard to draw definite conclusions regarding the effects of parathyroidectomy upon blood levels of calcium and phosphorus and general behavior. It is well known that there is great species variation that results from parathyroidectomy, including severe symptoms in dogs, cats and rabbits, to slight intermittent tremors in goats (Dukes, 1955; Turner, 1966). The pig seems to be an exception to parathyroidectomy effects reported thus far.

A different calcium and phosphorus control mechanism in the pig is suggested. The pig appears to be very efficient in its ability to rapidly mobilize calcium after surgery so that no tetany or other symptomology results.

TABLE IV

EFFECT OF METHOD OF HANDLING BLOOD PRIOR TO ANALYSIS ON SERUM CALCIUM AND PHOSPHORUS VALUES

Pig Number	Determination					
	1[a]		2[b]		3[c]	
	Ca[d]	P[d]	Ca	P	Ca	P
1	11.3	10.6	11.8	15.0	11.7	15.4
2	11.9	7.8	12.2	15.0	12.1	15.2
3	12.4	7.8	12.5	11.4	12.2	11.9

[a]Blood samples centrifuged and minerals analyzed within 1 hour after blood was drawn.

[b]Blood samples were held at room temperature for 24 hours before centrifugation and analyses of calcium and phosphorus.

[c]Blood samples stood at room temperature for 24 hours before centrifugation and refrigerated for 1 week before analysis of minerals.

[d]Ca (calcium); P (phosphorus).

II. PHASE II--THYROIDECTOMY

The effect of intraperitoneal injection of $CaCl_2$ on subsequent serum calcium levels are shown in Figure 3 and Table V, respectively. Serum calcium increased in both the control and thyroidectomized animals (control $P < .1$; thyroidectomized $P < .02$) at the 0.5 interval after $CaCl_2$ injection. Thereafter, at no time during the remainder of the experimental period did serum calcium levels of either group decrease to the pre-injection level.

It should be emphasized that, although this work was done to study the effect of parathyroidectomy on subsequent serum calcium levels in the blood, no differences resulted, regardless of surgical treatment performed. For this reason, the control and parathyroidectomized animals from Phase I and the thyroidectomized and thyroparathyroidectomized animals from Phase II were evaluated together.

Both groups showed increases in calcium after injection with $CaCl_2$. The thyroidectomized animals (includes thyroidectomized and thyroparathyroidectomized animals) evidenced a greater increase than the control animals (includes control and parathyroidectomized animals). In neither group did the serum calcium return to the pre-injection level during the remainder of the 48 hour experimental period. There was, therefore, no indication that thyrocalcitonin caused a withdrawal of calcium from the blood to prevent hypercalcemia, regardless of treatment. These results might be due to the comparatively slow rate of $CaCl_2$ absorption that could have caused the lingering high calcium levels.

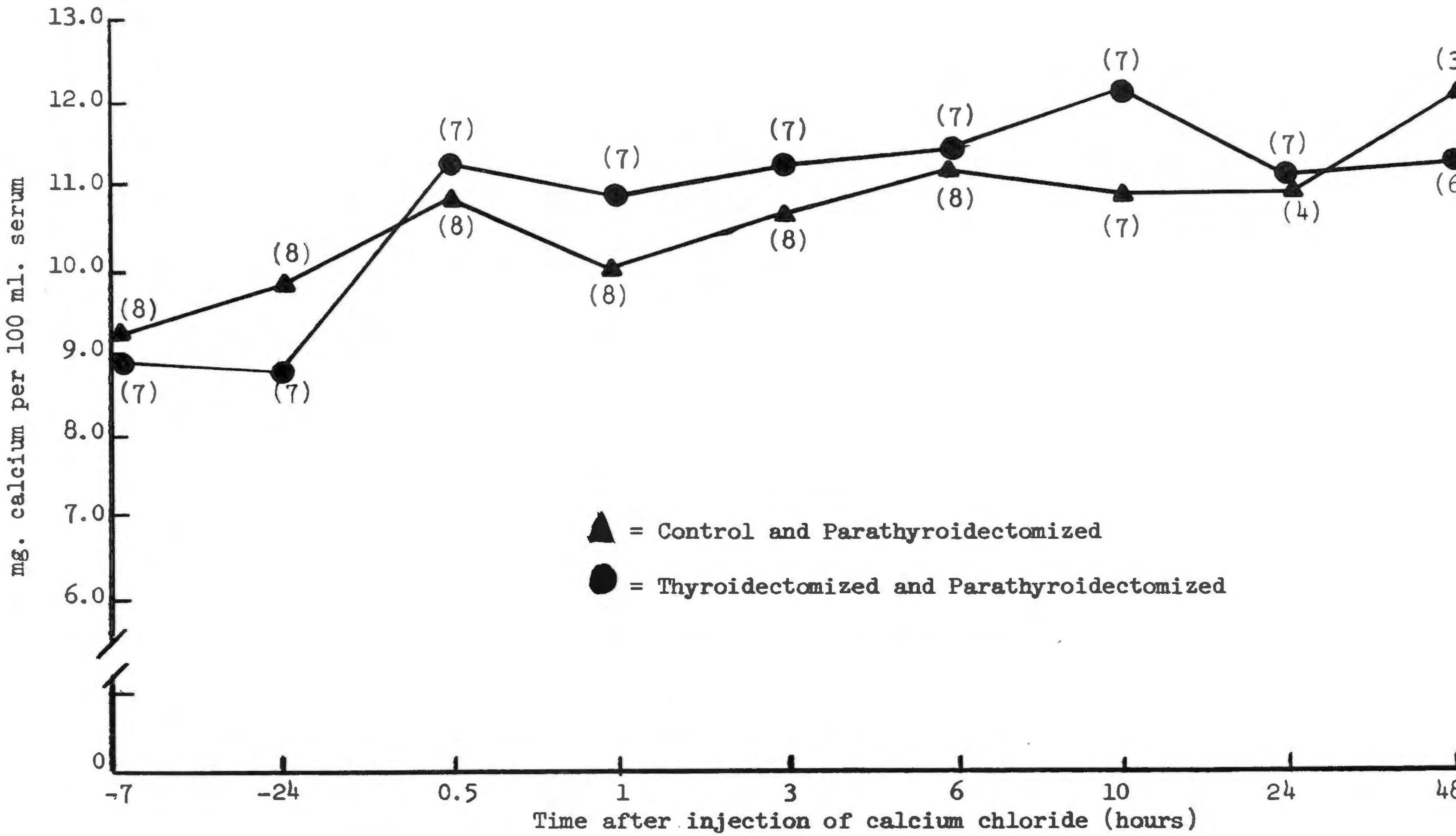


Figure 3. Effect of introperitoneal injection of calcium chloride on subsequent serum calcium concentration (Phase II).

This includes pigs receiving both levels of calcium (0.32 percent and 0.59 percent) in the ration. Numbers of animals bled at each interval are indicated by numbers in parentheses.

TABLE V

EFFECT OF INTRAPERITONEAL INJECTION OF CALCIUM CHLORIDE ON SUBSEQUENT SERUM CALCIUM CONCENTRATION (PHASE II)

Animal Number	Previous Treatment: Calcium Level in Ration (Percent)	Previous Treatment: Surgery	Intervals After Injection of CaCl2 (Hours): -24	0.5	1	3	6	10	24	48
			Control[a]							
5-9[b]	0.32	C	7.4	9.4	9.9	13.3	11.2	9.6	11.9	---
6-8[c]		Px	9.9	10.5	8.8	10.9	12.4	8.1	---	---
7-15[d]		C	10.5	12.7	13.9	9.7	11.2	---	---	---
8-14		Px	12.7	9.3	8.1	9.0	10.8	11.0	9.5	11.0
1-8	0.59	C	8.9	12.3	9.5	9.3	10.4	11.7	10.2	12.2
2-1[c]		Px	7.6	12.3	11.0	9.0	11.6	10.9	---	---
3-2[c]		C	11.2	9.4	11.1	10.7	10.5	12.6	---	---
4-3		Px	10.5	11.2	7.7	14.0	11.3	12.5	11.9	13.1
Average			9.8	10.9	10.0	10.7	11.2	10.9	10.9	12.1
Standard Deviation			±1.8	±1.4	±2.0	±1.9	±0.2	±1.6	±1.2	±1.1
			Thyroidectomized[e]							
5-5	0.32	TxPx	7.9	9.9	8.6	12.5	10.3	11.6	11.4	9.8
6-3[b]		Tx	8.3	9.7	8.5	11.7	10.4	11.8	10.3	---
8-2		Tx	8.2	12.9	12.5	11.8	11.9	12.0	10.6	12.2
1-6	0.59	TxPx	9.2	9.7	10.3	10.2	10.8	10.2	10.8	12.6
2-14		Tx	9.7	15.3	14.6	11.9	11.5	11.2	12.0	12.0
3-10		TxPx	8.9	10.5	10.2	9.7	12.6	16.0	11.4	11.5
4-12		Tx	8.7	10.1	10.6	11.0	12.5	11.9	10.9	9.6
Average			8.7	11.2	10.8	11.2	11.4	12.1	11.1	11.3
Standard Deviation			±0.6	±2.2	±2.2	±1.0	±1.0	±1.8	±0.6	±1.3

[a]Includes both control (C) and parathyroidectomized (Px) animals from Phase I.

[b]Died 24 hours after $CaCl_2$ injection. [c]Died 10 hours after $CaCl_2$ injection.

[d]Died 6 hours after $CaCl_2$ injection.

[e]Includes both thyroidectomized (Tx) and thyroparathyroidectomized (TxPx) animals.

Five of the control pigs (includes Phase I parathyroidectomized pigs) died within 24 hours after intraperitoneal injection of $CaCl_2$ (one after 6, three after 10, and one after 24 hours; one of the thyroidectomized pigs died after 24 hours post injection). Three of the control pigs and the thyroidectomized pig that died were fed the low calcium ration. From all the literature, one would expect that the control animals having an intact thyroid gland could more readily combat the hypercalcemic effect of the $CaCl_2$ injection due to the presence of thyrocalcitonin. This was not the case. Consequently, the pig may need both the parathyroid and thyroid glands, as suggested by Gittes and Irvin (1966) to respond to hypercalcemia.

Death was attributed to toxicity from the intraperitoneal injection of $CaCl_2$. A post injection syndrome was noted in all of the pigs. Within approximately one half-hour after the injection, the animals appeared very weak and started to vomit. Dyspnea, ataxia, and anorexia were also evident. They became hyperexcitable and died after severe tremors and convulsions. Bloat rapidly developed after death. Some of the surviving animals had a necrotic area after 24 hours at the sight of intraperitoneal injection. Subsequently, two of the animals developed severe abdominal hernias at the injection sight (8-3 parathyroidectomized; 18-14 parathyroidectomized). These were attributed to the irritating $CaCl_2$ injection. At the end of the experiment (4 days after $CaCl_2$ injection), a postmortem examination was conducted on one of the control animals for necropsy purposes. Lesions were confined to the abdominal cavity and abdominal wall. The intestines and urinary bladder were covered with a white exudate on the serous surface of the various viscera. No abnormal accumulations of fluid were noted. The body wall at two injection sites was inflamed, edematous and necrotic. Samples of kidney, liver, urinary bladder and intestine were sent to the Animal Disease Laboratory, Nashville, for analyses. Results of the autopsy revealed tubular hemorrhagic areas of severe fatty degeneration and overall necrosis of the tubular epithelium in the kidney. The liver evidenced uniform fragmentation of the hepatic cells and the intestinal mucosa was grossly infiltrated with eosinophils; the serosal surface was edematous; blood vessels were dilated; and necrosis of the abdominal muscles was evident. The urinary bladder exhibited diffuse hemorrhage in the subepithelial area. The $CaCl_2$, being highly irritating, caused the muscle necrosis (Turner, 1967). The white exudate on the surface of the organs was probably resultant calcium deposits from the injection.

All the animals that died subsequent to $CaCl_2$ injections were examined for indications of atrophic rhinitis. The turbinates were viewed at the level of the first premolar tooth, since this is believed to be the site of maximum development and, therefore, affords the best examination of the turbinate scrolls for positive signs of atrophic rhinitis (Brown, Krook and Pond, 1966). All animals were normal. This suggests that feeding a low calcium ration may not be influential in causing atrophic rhinitis, as reported by Brown, Krook and Pond (1966).

Those pigs previously parathyroidectomized in Phase I exhibited increased vascularity and scar tissue, which were observed during thyroidectomy (Phase II). The thyroid gland in these animals appeared grossly smaller and was situated more posteriorly (under the sternum) than those previous control animals that were only thyroidectomized. In the thyroidectomized animals, it was more difficult to get sufficient separation of serum from the formed blood elements after centrifugation of the blood for subsequent mineral analyses.

Hirsch and Munson (1966) and Care, Duncan and Webster (1967) reported that in rats and pigs, respectively, if the calcium level in the diet was low prior to thyroidectomy, one would not necessarily expect the same effect from induced hypercalcemia as one would if the animals were on normal calcium rations. They, however, used calcium gluconate and PTE, respectively. Care (1965) and Care, Duncan and Webster (1967) believed the hypocalcemic effect was due to thyrocalcitonin, while the rise back to normal was due to PTH. This was evidenced in both acutely and chronically parathyroidectomized pigs.

Gittes and Irvin (1966), however, reported the need for both the parathyroid and thyroid gland in order to get thyrocalcitonin release in rats. This does not agree with Foster et al. (1964), MacIntyre, Foster and Kumar (1965), Talmage, Neuenschwander and Kraintz (1965), MacIntyre and Parsons (1966), Care, Duncan and Webster (1967), and Hirsch and Munson (1966), who believed only the thyroid gland controlled the release of thyrocalcitonin.

Tashjian and Munson (1965), using rats, monkeys and calves, reported that injections of thyrocalcitonin were followed first by a decrease and then an increase in blood calcium. No evidence of this in the present study was observed.

Copp *et al.* (1962) and Copp and Henze (1964), however, reported the parathyroid gland as the source of calcitonin after injecting PTE into sheep, which resulted in an increase in serum calcium over the intact animals.

Gittes and Irvin (1966) used $CaCl_2$ intraperitoneally in thyro-parathyroidectomized, thyroidectomized and parathyroidectomized rats at the rate of 2.5 mg. per ml. of 0.9 percent sodium chloride, with no deleterious effects and indicated that thyrocalcitonin controlled the hypercalcemic effect of the injection. In the experiment reported herein, the $CaCl_2$ solution was, in fact, toxic to the animals and caused the death of six.

III. POST-EXPERIMENTAL OBSERVATIONS

Upon completion of Phase II (thyroidectomy) and $CaCl_2$ injection, the remaining eight pigs were returned to the University herd, where they were all fed a normal ration. The animals were observed periodically for clinical symptoms of thyroidectomy, as reported by Markowitz (1949), Leblond and Eartly (1952), Dukes (1955) and Turner (1966). Nine weeks after thyroidectomy (7 weeks after the injection with $CaCl_2$ solution), photographs were taken of three of the animals (5-5 thyro-parathyroidectomized; 18-2 thyroidectomized; and 18-14 parathyroidectomized), ranging in weight from 50.9 kg. to 70.9 kg. These animals are shown in Figure 4. Except for the decrease in weight gains in the thyroidectomized pigs, no characteristic symptomology such as myxedema, rough hair coat, wrinkling of facial epidermis, abnormal breathing or

Figure 4. Photograph taken nine weeks after thyroidectomy and fifteen weeks after parathyroidectomy.

Left--Thyroparathyroidectomized pig (wt. 50.9 kg.).
Center--Parathyroidectomized pig (wt. 70.9 kg.).
Right--Thyroidectomized pig (wt. 51.8 kg.).

ataxia were noted (Markowitz, 1949; Dukes, 1955). The absence of symptomology (Leblond and Eartly, 1952) could have been due to the age of these animals at time of surgery (13 weeks of age) or the short interval between surgery and photography (9 weeks).

The growth of the pigs receiving two levels of calcium in the ration, irrespective of surgical treatment, is summarized in Figure 5. Weights were taken every 3 weeks. After 3 weeks on experiment, the low calcium group consistently ate less than the normal calcium group. Those on the normal calcium ration (eight animals) for 62 days had an average initial weight of 9.6 kg. and an average final weight of 39.5 kg. for an average gain of 29.9 kg. This was an average daily gain of 0.48 kg. with a feed efficiency of 1.3 kg. The low calcium group (seven animals) had an average initial weight of 9.8 kg. and an average final weight of 34.9 kg. for an average gain of 25.1 kg. and an average daily gain of 0.4 kg. with a feed efficiency of 1.6 kg. These figures were recorded only from the start to the end of the experiment and do not include growth or consumption figures after return to the University farm.

The reasons for the failure of the thyroidectomized and presumably parathyroidectomized pigs to exhibit any of the classical symptoms anticipated in the Phase I parathyroidectomized animals (Dukes, 1955; Turner, 1966) are not clear. Two alternatives, however, must be considered. First, it is entirely possible that not all of the parathyroid tissue in the pig is localized in the anterior tip of the thymus glands (Schlotthauer and Higgins, 1934; Littledike, 1965; Care, Duncan and

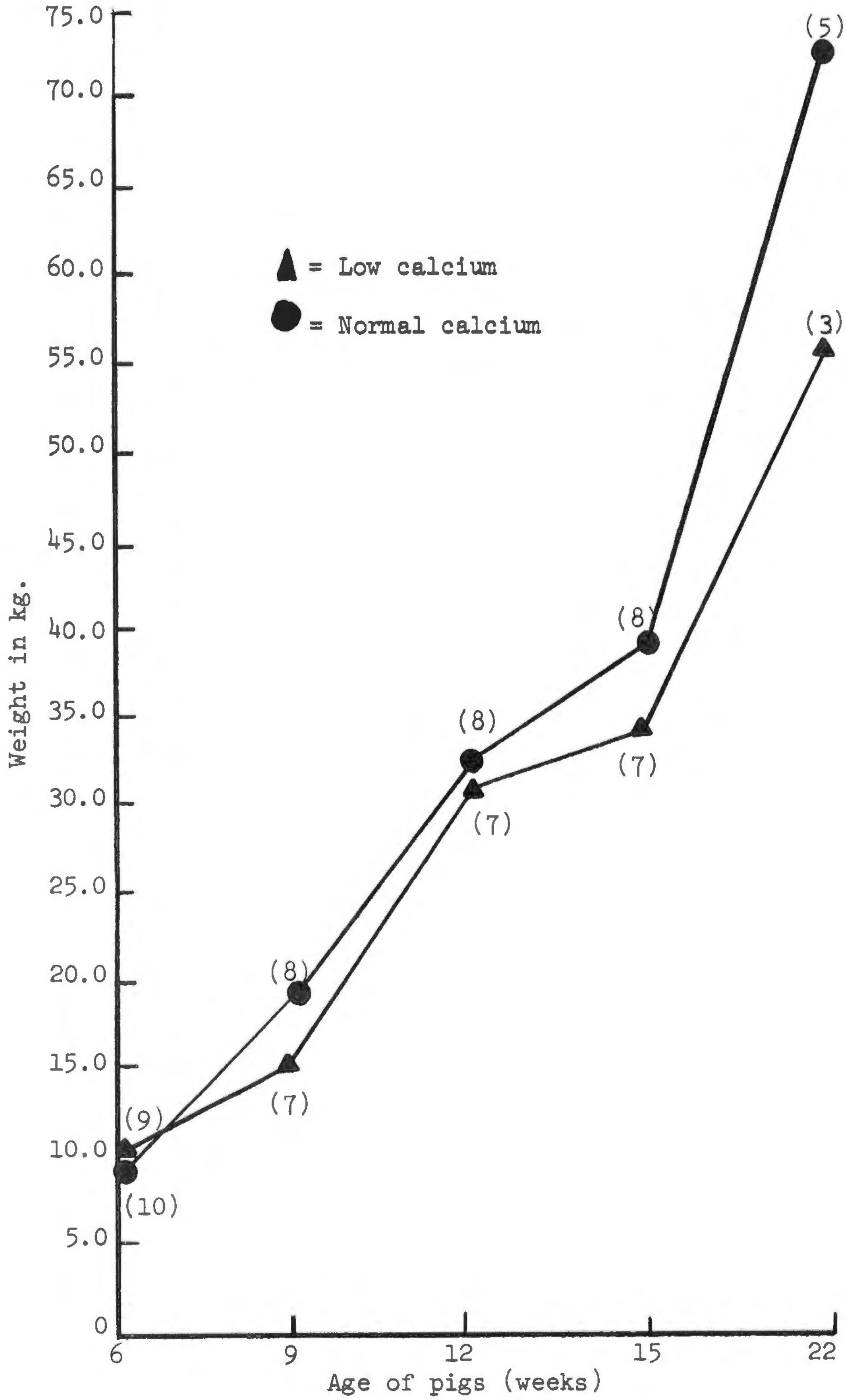


Figure 5. Growth curves of animals during experimental period.

Numbers of animals weighed at each interval are indicated in parentheses.

Webster, 1967). In fact, a dispersed gland as described by Brown, Krook and Pond (1966), Brown (1967) and Notzold (1967) may be of significance in subsequent swine parathyroid studies. Second, the pig may have some other regulatory mechanism, not yet recognized, for maintaining serum calcium levels within sufficient physiological ranges to prevent the appearance of tetany.

In Phase II (thyroidectomy), the characteristic increase and subsequent decrease in serum calcium levels reported in laboratory animals (Hirsch, Gauthier and Munson, 1963; Hirsch, Voelkel and Munson, 1964; Tenenhouse, Arnaud and Rasmussen, 1965; Care, Duncan and Webster, 1967) might have failed to appear in this study due to the slow absorption of $CaCl_2$ from the peritoneal cavity following intraperitoneal injection.

IV. RECOMMENDATIONS FOR FURTHER EXPERIMENTAL INVESTIGATION

In retrospect, on the basis of the experience gained in this study, the following recommendations should be considered if future experiments are conducted in this area:

1. Establish a preliminary feeding period so the animals become adjusted to the specified rations,
2. Vary age of pigs at time of surgery,
3. Vary levels of calcium in the ration,
4. Separate and concurrent parathyroidectomy and thyroidectomy; perform sham operations on control animals,

5. Histological examination of tissues (especially of the parathyroid gland), and

6. Use of radioactive compounds, histo-chemical and immunological techniques for more definitive identification of the distribution of the parathyroid tissue.

CHAPTER V

SUMMARY

The objectives of this study were to determine the specific effects of (1) parathyroidectomy and (2) thyroidectomy on the subsequent levels of calcium and phosphorus in the blood.

Sixteen Duroc pigs used in this study were randomly divided within sex into two groups of eight animals each. Group I received a normal calcium ration (0.59 percent calcium), while Group II received a low calcium ration (0.32 percent calcium) ad libitum. The other components of both rations were the same.

Following parathyroidectomy (Phase I), of four pigs from each ration group, the parathyroidectomized pigs had lower serum calcium levels than the control animals at 18 hours after surgery ($P<.1$). By 26 hours after surgery, serum calcium increased to a level higher than in the control group ($P<.1$). Thereafter, for the duration of the Phase I experimental period (168 hours post operatively), there was no difference between the two groups in serum calcium levels. Except for the decrease in calcium at 18 hours post operatively, none of the classical symptomology described for other species was seen in the parathyroidectomized pigs.

In Phase II, half of the control pigs and half of the parathyroidectomized pigs (from Phase I) were thyroidectomized. Subsequently, all pigs received an intraperitoneal injection of calcium injected as a $CaCl_2$ solution and administered at the rate of 4.4 mg. per kg. of body weight. The serum calcium increased in both the control and thyroidectomized animals (control $P < .1$; thyroidectomized $P < .02$) at the 0.5 interval after injection. Thereafter, at no time during the remainder of the experimental period (48 hours post-injection) did serum calcium levels of either group return to the pre-injection level. No evidence of a thyrocalcitonin effect was observed in this study.

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