SOME EFFECTS OF VITAMIN C ON ADRENALECTOMIZED GUINEA PIGS

by

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INTRODUCTION AND REVIEW OF LITERATURE

For many years the possibility of a connection between vitamin C and the adrenal glands has been suspected. Several of the facts that point toward this connection are the large amounts of vitamin C present in the adrenals (Turner, 1948, p. 226) and that vitamin C-deficiency and adrenalectomy cause many similar symptoms. Turner (1948, p. 230) reported that adrenalectomy causes a decrease in the volume of blood plasma and an interruption in reproductive functions while similar results were reported for vitamin C-deficiency by Harmon and Kordisch (1945), Goettsch (1935), and Kramer, Harmon and Brill (1933).

This problem was planned to determine if guinea pigs are influenced by high intake of vitamin C after adrenalectomy. Five main phases were considered during this study: 1. A technique for the removal of the adrenal glands. 2. A study of the survival time of adrenalectomized guinea pigs receiving extra amounts of vitamin C as compared to those on regular normal diets. 3. A study of the comparative weight lost and the per cent of weight lost from the time of operation until death. 4. A study of the hypertrophy of remaining adrenal glands, through a period of several weeks, after those from the opposite side had been removed. 5. A study of the testes of adrenalectomized guinea pigs in contrast to those from normal animals.

MATERIALS AND METHODS

The animals used in this study were 38 guinea pigs. They were obtained from Dr. H. L. Ibsen of the Department of Animal Husbandry, Kansas State College. Thirty-four of the animals were males and four were females. The experiments were begun in September, 1951 and were concluded in April, 1952. There were several phases in this study, each of which will be discussed in turn.

The first part of the work consisted of developing a method for the complete and total adrenalectomy of the guinea pigs.

The complete adrenalectomy of guinea pigs is a major undertaking and relatively little of this work has been done. The first available record of successful adrenalectomy in guinea pigs was performed by Simmons and Whitehead (1937). Their procedure resulted in very little success due to the tearing or cutting of the inferior vena cava each time. Schachter and Bebbe (1939) also report the removal of adrenals in guinea pigs using a method similar to that of Simmons and Whitehead. Bruzzone, Borel and Schwarz (1946) developed a technique similar to previous workers but they separated the adrenal from the kidney on the right side first then applied traction to the gland posteriorly and dorsally and by use of Pean forceps isolated the gland from the inferior vena cava and then were able to remove the gland in one piece.

A somewhat different technique was followed which will be described in detail for the right side, but both sides were removed similarly.

The guinea pigs were anesthetized with diethyl ether. The hair was cut away with clippers and the side washed with an antiseptic solution. After the pig was completely anesthetized an opening 2-3 centimeters long was made over the anterior part of the last rib. An incision was then made between the last two ribs, opening the abdominal cavity. A mastoid retractor was placed in the incision and spread to a convenient working width. Care was taken not to cut into the dorsal muscles and also not to perforate the diaphragm where it is attached to the ribs.

The dorsal and lateral sides of the adrenal gland were separated from the liver and body wall and surrounding fascia. Then by careful picking and teasing with forceps, the adrenal was loosened from the inferior vena cava on the median side. After this the gland was pushed aside to allow working space on the lateral edge of the gland to free it from the inferior vena cava. A pair of slightly curved pointed forceps was used and found to be excellent for the dissection. After both sides were loosened the forceps were inserted between the gland and the inferior vena cava and then allowed gradually to spread apart thus breaking the connections between the base of the gland and the inferior vena cava. Spreading was allowed to continue until the adrenal vein was reached anteriorly and the anterior edge of the kidney was reached posteriorly. At that time the forceps were removed and the adrenal was then separated from the kidney and fatty tissue around it. When the fatty tissue had been cleared away close to the inferior vena cava, a locking haemostat with slightly curved tips was used by inserting one point between the adrenal gland and the inferior

vena cava and the other point outside the connective tissue still holding it in place. The haemostat was closed and a sharp scalpel or knife was used to separate the gland from the haemostat by scraping close along its edge. After this end had been freed the haemostat was applied around the adrenal vein and connective tissue at the anterior end of the gland. This was then cut off and the gland removed from the body. Haemostats and sponges were removed and the retractor was released and removed from the body. The body wall was then sutured and sulpha diazine powder sprinkled over it to control infections. The skin was closed with a continuous suture and similarly sprinkled with sulpha diazine powder.

A recuperation period of one to two weeks was allowed before attempting to remove the left adrenal. It was removed in a manner similar to the right side except that it was not adhered to the inferior vena cava and careful separation was not necessary. Each animal after the second operation received an injection of one-half cc of cortisone acetate (Merck) solution to carry it over the shock period.

In several cases the left adrenal was removed first to have both normal left and right adrenals with which to make comparisons in weight.

After removal of the adrenals they were weighed and fixed in Bouin's fluid. They were later prepared for embedding and sectioning in the following manner:

Place in isopropyl alcohol 60%	1 hr.
Place in isopropyl alcohol 99%	1 hr.
Place in isopropyl alcohol 99%	2 hr.

Place in isopropyl alcohol 99% (40-45° C.)	3 hr.
Place in melted tissuemat (M.P. 50-52° C.)	1 hr.
Place in melted tissuemat (M.P. 56-58° C.)	overnight
Embed and block in tissuemat (M.P. 56-58° C.)	next day

The tissues were then sectioned with a microtome and prepared on slides. The slides were prepared for microscopic study in the following manner:

Egg albumin in glycerine was placed on the slide and rubbed in with the finger. After the sections were placed on it, water was added and the slide was warmed to expand and stretch the sections. The water was allowed to evaporate to dryness. The slides were then stained and counter stained with Mallory's triple stain by the following method:

Remove paraffin with xylol	5 minutes
Place in xylol	5 minutes
Place in 95% alcohol	1-2 minutes
Place in 85% alcohol	1 minute
Place in 70% alcohol	1 minute
Place in 50% alcohol	1 minute
Place in 35% alcohol	1 minute
Place in distilled water	5 minutes
Place in Mallory's solution #1	5 minutes
Place in Mallory's solution #2	3 minutes
Place in rinse 95% alcohol	2 minutes
Place in 95% alcohol for destaining	until destained to desired hue
Place in xylol	1 minute

The sections were mounted with piccolite, covered with number one cover slips and allowed to dry. The sections were then examined under high and low power of a compound microscope.

The second phase of the experiment consisted of determining survival time for adrenalectomized guinea pigs receiving extra amounts of vitamin C and those receiving normal maintenance dosage of vitamin C.

The vitamin C was administered to one-half of the pigs. The dosage was 200 mg of vitamin C twice daily. This was done by dissolving vitamin C tablets in water, putting the liquid in a syringe and forcing the liquid into their mouths. This was necessary only for the first and last few days. After they became accustomed to the taste they drank it readily. The pigs were weighed and weights were recorded immediately before each feeding.

The third phase of this experiment concerned the effect of adrenalectomy on the testes. As soon as possible after death from the effects of adrenalectomy the testes were removed from the body and fixed in Bouin's fixative solution. After 24 hours they were transferred to 70 per cent alcohol and changed three times to remove as much as possible of the fixative. The testes were prepared for embedding and staining in the same manner as were the adrenals.

The fourth phase of this experiment was concerned with the hypertrophy of the remaining adrenal gland when only one side was removed at a time. Due to the extreme shock of losing the adrenals, it was felt that a recuperation period of one to two weeks would help them overcome the effects of the first operation.

It was noted that after removal of the right adrenal gland the left one apparently enlarged to greater than normal size during the recuperation period. The adrenals were removed and a record of their weights kept. In the greater number of animals the right gland was removed first since it was not known whether or not it would be successful on that side. However, a few were removed on the left side first to give some normal weights of left adrenals. Weights of a number of other glands from guinea pigs not on experiment were added to make a more representative record.

RESULTS

Adrenalectomy

The complete adrenalectomy of guinea pigs is a major undertaking and many complications can arise during the operation or as a result of it. Infections developed in the earlier phases of the work but were later prevented by using sulpha diazine in the incisions. Post mortem examinations showed that adhesions had been formed. In some the adhesions were quite extensive especially of the liver and kidney.

Survival Time

The survival time of the guinea pigs after adrenalectomy appeared to depend upon at least two main points, the sex of the animal and whether or not it received extra amounts of vitamin C.

Each animal received an injection of five mg of cortisone acetate (Merck) immediately after the second gland had been removed to tide it over the shock period and thereby could have influenced the survival time also.

In Group I were four male guinea pigs about four months of age. Two were given vitamin C plus diets and two were fed regular diets. Those receiving regular diets survived for an average of nine and one-half days while those receiving 200 mg of vitamin C twice daily in addition to regular diet, survived for a period of 15 days.

Group II consisted of four females two to three months of age, two of which received regular diets and two received vitamin C plus diets (200 mg per day). The average survival time for these on regular diet was 14 days while those on vitamin C plus diet was more than double this length of time.

Group III consisted of one male guinea pig about two months old. It was fed a regular diet.

Group IV consisted of two male guinea pigs about two months of age, one of which was fed a regular diet and the other a vitamin C plus diet.

Group V consisted of one male guinea pig about two months of age which was fed a regular diet.

Table 1 shows a condensation of the facts concerning survival time after adrenalectomy.

Table 1. Summary of the survival time of the guinea pigs showing sex, approximate weight at time of operation, treatment and survival time in days.

Group	Animal	Sex	Approx. weight in gm	Treatment	Survival time in days
I	1	Male	750	Extra vit. C	15
	2	Male	740	Extra vit. C	16
	3	Male	560	Regular diet	-
	4	Male	500	Regular diet	12
II	5	Female	440	Extra vit. C	45
	6	Female	480	Extra vit. C	36
	7	Female	370	Regular diet	13
	8	Female	420	Regular diet	15
III	9	Male	680	Regular diet	7
IV	10	Male	440	Extra vit. C	12
	11	Male	600	Regular diet	10
V	12	Male	490	Regular diet	12

The right adrenal was removed first and survival time was counted from the time of removal of the left adrenal. Number three the survival time was not noted because when it was killed some two months later than the others had died, a fragment of adrenal tissue was found growing on the right side. It had grown to a sizable structure, about a fourth the size of the original gland. This was a case of regeneration of tissue since after the first operation no noticeable fragments were observed. This piece of tissue was fixed and identified microscopically as adrenal tissue.

Numbers five and six were females fed vitamin C for 31 days. These had shown no signs of dying except slight loss in weight. The vitamin C treatment was stopped and death occurred shortly afterward. However, it is not known whether they might have died

even if the vitamin C had been continued for a longer period of time. Number nine showed a short survival time but during the operation it lost considerable blood and this could have influenced the survival time.

Comparative Weights

This portion of the experiment consisted of keeping recorded weights twice a day from the time of the last operation until death. It was found that the total weight lost by each animal varied to a large degree depending mostly on the size of the animal to start with. When the per cent of weight lost for each animal was determined it was shown that the majority of them fell in the 10 to 20 per cent loss of weight column as shown in Table 2.

The average per cent lost was found to be 19.7. In determining the average per cent loss, the first group was not included as the weight after the operation was not recorded for several days. The extreme variants from the average, numbers five and eleven, were not explained from the work that was done in this experiment. Figures 1 and 2 show the graphic relationship of body weight to survival time.

Hypertrophy of Adrenals

Although the number of adrenal glands considered is small, the results of this part of the experiment were regarded as worthy of mention.

Table 2. Summary of weight lost for each animal and the per cent of body weight lost.

Animal	Weight lost in gm	Per cent of weight lost in gm
1	70	9.7
2	88	12.6
3	-	-
4	28	5.9
5	194	40.2
6	48	9.6
7	48	13.0
8	70	16.8
9	72	10.6
10	84	18.9
11	178	29.4
12	94	19.2

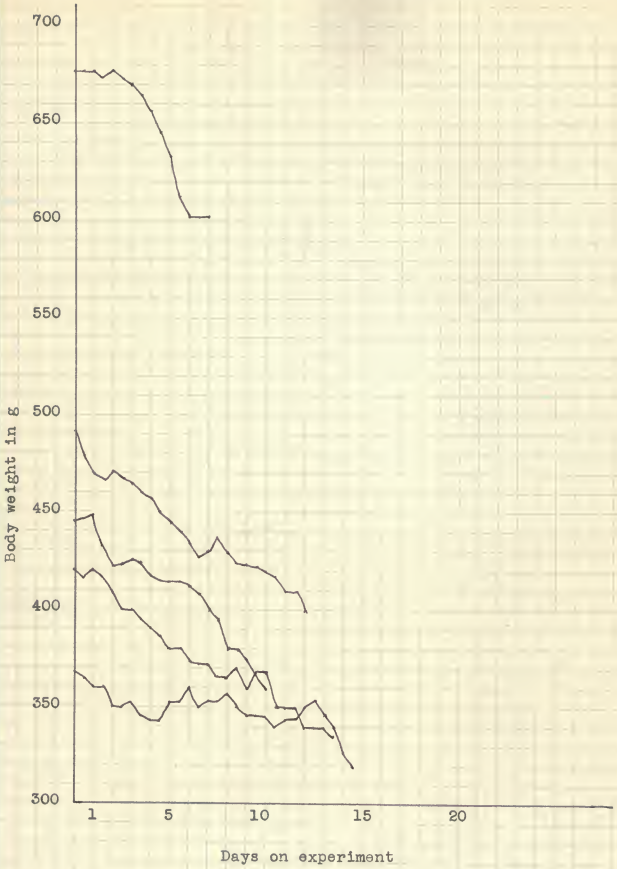


Fig. 1. Weight curves of animals receiving regular diets after complete adrenalectomy.

Fig. 2.. Weight curves of animals receiving extra amounts of vitamin C after complete adrenalectomy.

Table 3. Adrenal weights of right and left glands in which right gland was removed first.

Left adrenal weight in mg	Right adrenal weight in mg
350	113
252	105
228	104
205	75
202	73
192	54
160	53
144	50
133	49
103	39

Due to the extreme difficulty of these operations a number of animals were used for operations that were not put on experiment. Each of the adrenal glands was saved as it was removed from the animal and weighed. In this first series a number of adrenals were saved but the right and left glands of the same animal were not designated. These ten glands of each side were used as a representative group from each side.

The average weight of those on the right side was 71.5 mg. The average weight of those on the left side was 186.9 mg.

In three of the animals studied for adrenal weight but not put on experiment, the left adrenal was removed first and the right one removed about three weeks later.

Table 4. Adrenal weights of right and left glands in which the left gland was removed first.

Left adrenal weight in mg	Right adrenal weight in mg
75	234
55	188
52	180

Table 5. Comparative weights of the right and left adrenals of the six guinea pigs with body weights.

Animal	Adrenal first out	Weight in mg	Adrenal last out	Weight in mg	Body weight
1-1	left	148	right	170	520
2-1	left	80	right	136	524
10	right	86	left	138	444
11	right	48	left	115	604
12	right	70	left	105	516
13	right	82	left	182	396

In six of the animals studied the weight of right and left adrenals were kept as well as body weights. Two of the animals had the left adrenal taken out first and then the right one. The other four had the right adrenal removed first.

These two tables point out the fact that no matter which adrenal was removed first the other one always weighed more when it was removed.

A study of the tissues microscopically showed that in the glands removed last there was a higher degree of vacuolization in the cells than in the others.

Plate I, Fig. 1 shows a section of hypertrophied tissue as compared to a section of normal adrenal tissue, Plate I, Fig. 2.

The ability of the adrenal cortex to regenerate was also noted. In two of the animals studied it was thought at the time of the operation that all of the gland had been removed. However, as the experiment progressed the animals continued to gain weight and not show any signs of deficiency as did the other animals and it was suspected that a small tag had been left in the body. After about two months the animals were sacrificed and a post mortem performed. They were found to have a sizable piece of tissue where the original adrenal had lain on the right side. This tissue was observed to be about one fourth the size of the original adrenal. It was fixed and stained like the other tissue, studied microscopically and found to be adrenal cortical tissue. No medullary cells were found to be present. Throughout the entire work examination of dead animals was made to determine if fragments of adrenal cortical tissue remained in operated animals. No fragments were found except in the two animals as noted.

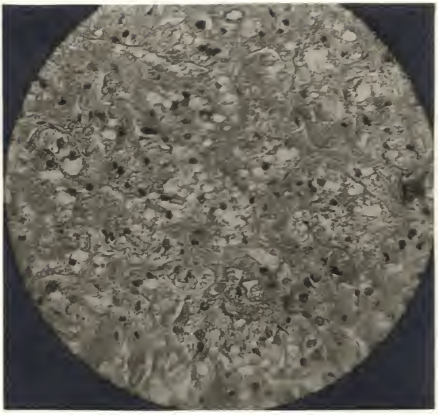
Testes

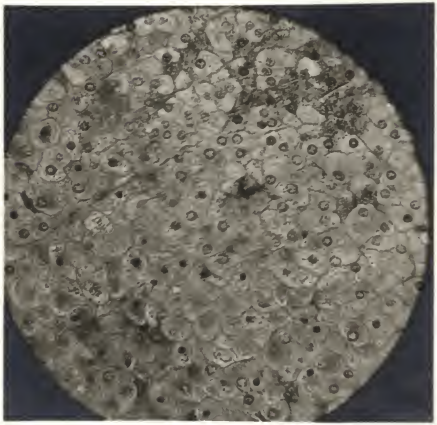
It has been shown by McKinely and Fisher (1926) and Vincent (1917) that feeding cortical tissue to experimental animals has produced earlier than usual maturity of the testes. The removal

EXPLANATION OF PLATE I

Fig. 1. Photomicrograph of hypertrophied adrenal tissue.

Fig. 2. Photomicrograph of normal adrenal tissue.

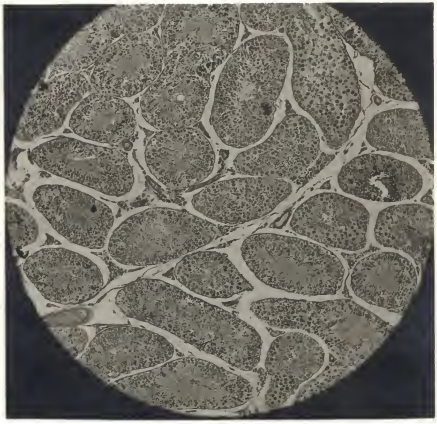


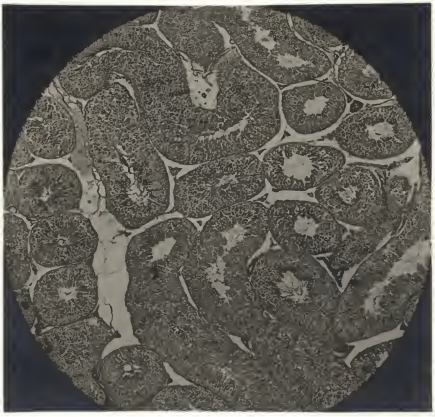


EXPLANATION OF PLATE II

Fig. 1. Photomicrograph of testes after adrenalectomy.

Fig. 2. Photomicrograph of normal testis.





of the adrenal gland has been described by Britton and Howard (1935), Carr (1931), and Martin (1932) as resulting in low fertility and degeneration of testes in rats. Herrick and Torstveit (1938) showed that adrenalectomy of fowls led to a degeneration of the testes. Harman (1950) has shown that vitamin C-deficiency of guinea pigs results in a degeneration of testicular material.

In this experiment the testes were removed as soon as possible after death, fixed and prepared as were the adrenals. Degeneration of the testes was noticeable in all the animals. Those that lived more than ten days showed an increased amount of degeneration. In practically all cases there was a marked closing of the lumena of the seminiferous tubules indicating degeneration. In the animals that died in less than ten days the lumena of some of the tubules were still present. If spermatozoa were present they were greatly reduced in number from those in normal tissue. There was no marked difference in the testes of those that received vitamin C and those that did not receive it.

Herrick, Mead, Egerton and Hughes (1952) have shown that injections of cortisone protected vitamin C-deficient guinea pigs from the degeneration of bone and testis tissue. Harman's (1930) work indicated that vitamin C-deficient guinea pigs showed degeneration of testicular material with the adrenal glands present. Lockwood and Hartman (1933) determined in their experiments that a definite relationship existed between vitamin C and the adrenal gland.

The work of this experiment indicates that large doses of vitamin C had no effect in preventing degeneration of the testes

in adrenalectomized guinea pigs. This work along with that of preceding workers leads to the postulation that vitamin C is necessary for normal secretion of the adrenal cortex which in turn affects the testes. If this were true, a deficiency of vitamin C would result in inadequate cortical secretion with subsequent degeneration of the testes.

Plate II, Fig. 1 shows the comparison of normal testes to those after adrenalectomy, Plate II, Fig. 2.

SUMMARY

The results of this investigation may be summarized as follows:

1. A procedure for total and complete removal of the adrenal glands of guinea pigs was developed.

2. Removal of one of the adrenals causes the other one to hypertrophy.

3. The addition of extra amounts of vitamin C to the diet of adrenalectomized guinea pigs lengthened the survival time. Females had a longer survival time than the males.

4. There is a possible relationship between adrenal hormones, vitamin C and female sex hormone.

5. At the time of death it was found that the animal had usually lost 15 to 20 per cent of its body weight.

6. The testes of adrenalectomized guinea pigs showed degeneration and extra vitamin C added to their diet did not prevent the degeneration.

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For many years the possibility of a functional connection between vitamin C and the adrenal glands has been suspected. Several of the facts that point toward this connection are the large amounts of vitamin C present in the adrenals and that vitamin C-deficiency and adrenalectomy cause many similar symptoms. It has been reported that adrenalectomy as well as vitamin C-deficiency causes a decrease in blood volume, of blood plasma and an interruption in reproductive functions.

A series of guinea pigs were subjected to complete and total adrenalectomy. These operations were performed only after a time-consuming technique had been developed. In a few cases it was found on post mortem examination that a minute fragment had been left in the body which had regenerated to a sizable tissue in several weeks. Results from these animals were not included in this experiment.

After complete adrenalectomy there are many phases of the endocrine system which could be studied. Five points are to be considered here: 1. A technique for adrenalectomy of guinea pigs. 2. Survival time of adrenalectomized animals. 3. Comparative weights lost to normal body weight. 4. Effects of adrenalectomy on the testis. 5. Effect of a single adrenalectomy on hypertrophy of the remaining gland. In all of these tests two groups of animals were run, those using regular diets and those receiving extra vitamin C (200 mg daily).

The survival time was determined by keeping daily records of weights and day of death. There appear to be at least two main

factors involved in survival time, the sex and whether or not they received extra amounts of vitamin C.

Table I shows a condensation of facts concerning survival time after adrenalectomy.

Table 1. Summary of the survival time of the guinea pigs, showing sex, approximate weight at time of operation, treatment, and survival time in days.

Group	Animal	Sex	Approx. weight in gm	Treatment	Survival time in days
I	1	Male	750	Extra vit. C	15
	2	Male	740	Extra vit. C	16
	3	Male	560	Regular diet	-
	4	Male	500	Regular diet	12
II	5	Female	440	Extra vit. C	45
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V	12	Male	490	Regular diet	12

The right adrenal was removed first and survival time was counted from the time of removal of the left adrenal. For number three the survival time was not recorded due to a regeneration of an adrenal tag.

Numbers five and six were females fed vitamin C for 31 days and still had showed no signs of dying except a slight loss in weight. The vitamin C treatment was stopped and death occurred shortly afterward. However, it is not known whether or not they

might have died even if the vitamin C had been continued for a longer period of time. Number nine showed a short survival time but during the operation it lost considerable blood and this could have influenced the survival time.

The weights of the animals were recorded twice a day from the time of second adrenalectomy to death. The total weight lost varied in each animal a great deal but in relation to per cent of original body weight they were found for the most part to fall into the 10-20 per cent group. The average per cent lost was 19.7.

It was observed during the operation that the last gland to be removed was always larger two to three weeks after the first operation. Without exception the last adrenal removed weighed more than did the first one. This would indicate a condition of hypertrophy and when sections were studied microscopically it was proved to be so.

The testes were removed as soon as possible after death following adrenalectomy and were studied microscopically. A definite degeneration of the seminiferous tubules was observed in all cases. Animals living more than ten days showed an increased amount of degeneration. Those that died in less than ten days the lumena of some of the tubules were still present but the spermatozoa were greatly reduced in number as compared with normal tissue. There was no marked difference in the testes of those that received excess vitamin C and those that received usual amounts of it.

It has been shown that injections of cortisone protected vitamin C-deficient guinea pigs from degeneration of testis tissue. Other work has indicated that vitamin C-deficient guinea pigs

showed degeneration of testicular tissue with the adrenal glands present.

The work of these experiments indicates that large doses of vitamin C had no effect in preventing the degeneration of the testes in adrenalectomized guinea pigs. This work along with that previously done leads to the postulation that vitamin C is necessary for normal secretion of the adrenal cortex which in turn affects the testes. If this were true, a deficiency of vitamin C would result in inadequate cortical secretion with subsequent degeneration of the testes.

The results of this investigation may be summarized as follows:

1. A procedure for total and complete removal of the adrenal glands of guinea pigs was developed.

2. Removal of one adrenal gland results in hypertrophy of the remaining one.

3. That the addition of extra amounts of vitamin C to the diet of adrenalectomized guinea pigs lengthened the survival time and for a still greater period in females.

4. There is a possible relationship between adrenal hormones, vitamin C and female sex hormone.

5. Death usually occurred after the animal had lost 15 to 20 per cent of its body weight.

6. The testes of adrenalectomized guinea pigs showed degeneration and extra vitamin C added to their diet did not prevent the degeneration.