THE EFFECT OF ESTROGEN (THEELIN) UPON THE SURVIVAL TIME OF ADRENALECTOMIZED GUINEA PIGS

by

BERNEY LOU GOLDEN

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INTRODUCTION

This study has been made to determine if a functional relationship exists in the guinea pig between the adrenal gland and the ovarian hormone, estrogen. Survival time following complete ablation of these glands was used as a criterion to measure this possible relationship.

Several workers have attempted investigations of the relationship of the adrenal glands to the ovaries and the use of ovarian hormones to off-set the effects of adrenal insufficiency. The most common approaches to this problem which have been reported in the literature were by making ovarian transplants in different sites, or by direct hormone therapy using injections of progesterone or estrogen. In the investigations in which estrogen was used, both the naturally occurring crystalline hormone and diethylstilbestrol were used.

Because of certain difficulties encountered in adrenalectomizing guinea pigs, this standard laboratory animal has been used less in investigations of this nature than the rat. Since the guinea pig is more typical of man in the sense of its inability to synthesize vitamin C as the rat can do, and since there is such a close interrelationship between this gland and vitamin C (Harman and Bascom, 1951) (Turner, 1948), it was decided that this problem would be undertaken using guinea pigs.

The literature contains numerous articles in which methods of increasing the survival time of bilaterally adrenalectomized animals are

discussed. In some animals the presence of accessory organs capable of secreting adrenal cortical hormones is sufficient to permit the animals a normal life span (Martin, 1932). Grollman (1952) found that a salt solution containing a multiplicity of compounds would likewise keep dogs alive after complete ablation; and too, extracts of the adrenal cortex in measured doses overcame the insufficiency brought on by removal.

REVIEW OF LITERATURE

Throughout the literature are numerous reports of work done in which the author attempted to determine if a relationship exists between the products of the gonads and an increase in the survival time of bilaterally adrenalectomized animals. Much controversy prevails concerning the gonad-adrenal cortex relationship and what substances are actually involved in the prolonging of the survival time of animals suffering from adrenal insufficiency is still a matter of theory.

Kar (1947) presented the hypothesis that after adrenalectomy the gonads share in the vital function of the adrenal cortex and after castration the opposit is true. The following review deals primarily with the ovary-adrenal cortex relationship.

In work done involving ovarian transplants to both ear and spleen (Lichton, et al. 1953) it was noted that there was an increase of survival time in adrenalectomized albino rats. Inasmuch as immature ovaries afford as much protection as those from mature animals it was assumed that the survival prolonging function was apparently independent of the usual

ovarian function. In animals which survived, it was found that the ovaries developed luteinization far beyond the limits of the corpora lutea; but in those cases in which the rats died, large quantities of luteal tissue was found which was restricted within the limits of the corpora lutea. This work presents the possibility of the formation of two hypotheses: (1) that the ovaries because of their ectopic position secrete an ovarian substance qualitatively different than the other ovarian secretions and life prolonging in action; (2) that the grafted ovaries highly luteinized secrete sufficient progesterone to sustain life. There is evidence to indicate that progesterone does assist survival for a limited length of time.

However, Hill (1949) while working with adrenalectomized mice noted that spleen-grafted and ear-grafted ovaries gave an increased survival period. Whatever the active substance was it was neither estrogen nor progesterone since it has been ascertained (Biskind and Biskind, 1942) that the liver serves to remove (inactivate) circulating estrogen; nor was it progesterone since the spleen-grafted ovaries had no corpora lutea present. Rather he suggests that the loss of the nerve control may be the primary factor in governing ovarian secretion.

Some researches have found that pregnancy and pseudopregnancy will extend the survival period of adrenalectomized animals while other workers discount it. Corey (1928) found that the survival time of bilaterally adrenalectomized pregnant cats was the same as that of adrenalectomized non-pregnant females and males. He further concluded that the presence of the corpora lutea had no influence in the lactating or pregnant females. This work was confirmed by Carr (1931) working with adrenal-

ectomized white rats. Swingle, et al. (1937) cited that an adrenalectomized bitch in heat would survive the adrenalectomy for as long as sixty-five days or that a pseudopregnant bitch would live for the interval of the pseudopregnancy. Pseudopregnancy as a factor for increasing the survival time of adrenalectomized animals was promulgated also by Cavanaugh and Gaunt (1937) and Swingle and Remington (1944).

Emery and Schwabe (1936) in work done to illustrate pituitary effectiveness in prolonging the lives of adrenalectomized rats by the influence of the pituitary gland on the ovary found that the ovaries can ameliorate the effect of adrenalectomy for a period of time. They concluded that the effective agent was not Theelin but a secretion from the active corpus luteum; but Swingle, et al. in work done a year later (1937) found that corpora lutea hormone did nothing for adrenalectomized cats. They also found that large amounts of ovarian (and pituitary hormones) did not increase the survival time of bitches which were not in heat. Schwabe and Emery (1939) ascertained that progesterone in sufficient dosage will extend the survival time of adrenalectomized rats. This work was a duplication of that done by Gaunt, et al. (1938). The conclusion which was reached concerning progesterone by Greene, et al. (1939) was that progesterone is "cortin-like" in that it will maintain adrenalectomized male rats. They point to the similarity in chemical structure between progesterone and certain of the cortical hormones (Turner, 1948), and to the fact that crystalline progesterone produces a sodium retaining action in adrenalectomized dogs.

Androgens cannot substitute for the principles of the adrenal cortex

but testosterone propionate causes sodium retention in normal dogs (Spurr and Kochakian, 1939). Work done by Greene, (1939) determined that progesterone in large quantities was androgenic.

Estrogen is a product about which is found a degree of diversity. However, most of the authors who have worked with this hormone find it is in no way beneficial to the adrenalectomized animal. Martin (1932) thought that theelin injections neither prolonged nor saved the life of animals in which the adrenal glands had been removed. Schacher, et al. (1937) stated that estrin was toxic to hypophysectomized rats; while in work done with adrenalectomized mice (Pfeiffer and Hooker, 1940) it was concluded that estradial benzoate is toxic in anything but the most minimal of doses. However, certain qualifications have been made concerning the toxicity of estrogen. Selye and Masson (1939) found that estrin had a toxic effect upon adrenalectomized mice if treatment followed immediately after complete ablation. This work was duplicated several years later (Bruzzone, et al. 1946) when they found that the survival time of adrenalectomized mice and rats was shortened if natural or artificial estrogen was given after adrenalectomy but that if therapy is started before the removal of the glands then the toxic action does not occur.

In no way have any of the preceding authors attempted to explain the toxic manifestations produced by estrogen. However, one paper attempts to explain this by the hypothesis that the toxic action of estrone may actually be an inhibition of the anterior lobe of the pituitary and that this insufficient supply of anterior pituitary hormone is actually the cause of the reduced period of survival. It has been established that

pituitary substance extends the survival time of adrenalectomized animals (Cavanaugh and Gaunt, 1937; Swingle and Remington, 1944; Emery and Schwabe, 1936; Swingle, et al., 1937).

MATERIALS AND METHODS

Simmons and Whitehead (1937) reported a method for the complete bilateral adrenalectomy of guinea pigs. This technique involved the crushing of ribs and the displacement of the kidneys. As a result the traumatic effect upon the animals was considerable, and the results of the investigation carried out were inconclusive according to some authors. Both Schaefer and Beebe (1939) and Bruzzone, et al.,(1946) described a technique somewhat similar to the method that was employed in this problem and less drastic than those employed by Simmons and Whitehead (1937).

In guinea pigs, the adrenal glands are located anterior and dorsal to the kidney and attached to the body wall, vena cava, kidney, and on occasion the lateral lobes of the liver. Connections are usually of soft connective tissue but at times they were highly fibrous and elastic in nature. The gland lies immediately adjacent to the ascending vena cava and while they share no common tissue, it is only the thickness of a fraction of a millimeter which indicates the independence of these structures. Previous workers (Colburn, 1952) have determined that the right adrenal gland is more difficult to remove than the left one since more of the gland adheres to the surface of the vena cava.

Three small arteries constitute the blood supply to the gland but

their distribution appears to be more or less unique to each individual guinea pig. Blood is returned from the gland via the adrenal vein which leads directly into the vena cava.

An attempt was made to remove the entire gland intact, to lessen the possibility of leaving fragments which might regenerate and produce sufficient adrenal (cortical) hormones to negate the response being sought.

Since the effects of adrenalectomy are considerable, the complete adrenalectomy is done in two stages with an interim of at least 14 days between the time of removal of the first gland and that of the second. Since there is greater difficulty encountered in removing the right adrenal gland than the left one, the sequence of operations was to attempt the removal of the right one first.

Twenty-four hours prior to surgery, the guinea pigs were fasted though they were permitted all the water that they desired. At all times, prior to, between, and after surgery, the diet consisted of Purina Rabbit Chow Checkers and occasionally lettuce leaves or other types of greens.

At the time of the operation, the side of the guinea pig to be operated upon was shaved and washed with a solution of two Sterotab tablets dissolved in approximately 500 cc. of tap water. It was in this same solution that the surgical instruments were kept during the operation and in which all cotton swabs, sponges and sutures were immersed.

The operating instruments used were as follows:

fenestrated loop	mastoid retractor
straight operating scissors	angular operating scissors

scalpel
heavy straight forceps
fine curved forceps
needle holder
hemostat
heavy curved forceps
suture needle

Surgical technique varied only slightly in meeting individual variations in each pig and was the same whether it was the right or left gland that was being removed. Complete anesthesia was produced with diethyl ether (anhydrous). The technique was as follows:

A three to four centimeter incision was made through the skin of the guinea pig on the dorso-lateral surface in the area between the last two ribs. Another incision was then made through the body wall between the last two thoracic ribs which was bounded dorsally by the longitudinal back muscles and ventrally by the end of the ribs. A mastoid retractor was used to enlarge the intercostal area and expose the underlying viscera. The peritoneum was slit, thus permitting free access to the abdominal organs. Large, moist, cotton swabs were used to restrain the small intestine and likewise swabs were used to prevent the lobes of the liver from slipping down over the gland and interferring with the work of removal. Occasionally if the situation warranted it, a thin layer of moist cotton was placed over the kidney to prevent damage to it from the pressure of the surgical instruments.

With the aid of the fenestrated loop and fine curved forceps the gland was freed from its surrounding tissue and carefully teased loose from its close connection with the vena cava. However, at all times, care was taken to prevent rupturing the adrenal vein. When the entire gland was free, then the adrenal vein was clamped and severed.

If bleeding persisted after ablation, a small wad of Oxycel, oxidized cellulose (Parke, Davis and Co.), was placed in the area from which the gland was extirpated, the cotton swabs were removed and the operative area covered with a sulfa compound to reduce infection. At different times sulfadiazene and sulfamerazine were used with the former giving better results. The body wall and the skin were sutured independently of each other with standard surgical silk thread and the animal was then placed in a recovery cage until it became fully conscious. Bilaterally adrenalectomized animals were isolated in small groups. An attempt at keeping the temperature of the cage constant was made by the use of two lamps which were kept burning constantly and which raised the temperature to a level slightly higher than normal room temperature.

On occasion, penicillin in an aqueous solution (Penicillin G. Potassium, Squibb and Co.) was given by subcutaneous injection in an attempt to prevent infection. At times, too, a dose of one cc. of Cortone (25 mg., Cortisone Acetate, Merck) was administered to the guinea pigs to help tide them over the period of shock following surgery, particularly after complete ablation. This practice ceased when it became apparent that this treatment might be affecting the survival time of these animals.

Four types of dosing schedules were established during the duration of this problem. Type "A" involved a dosage schedule which began on the day following the second operation; type "B" began the day of the second operation following surgery; type "C" was one in which the guinea pigs received an injection of estrogen immediately prior to the second

phase of the complete adrenalectomy; and type "D" involved an injection of estrogen daily for four days preceding the second adrenalectomy.

In all cases, regardless of the time of the initial dosage, those guinea pigs which received hormone therapy were given one mg. of estrogen by subcutaneous injection at intervals of twenty-four hours for the entire period of survival. The estrogen used was Theelin in aqueous solution (Parke, Davis and Co.) containing two mg. of estrogen per cc. of solution. Frequently the diet of these experimental animals was augmented by the addition of vitamin C in varying quantities ranging from 100 mg. per day to approximately fifty mg. per day. At first the vitamin was administered by dissolving each ascorbic acid pellet in five cc. of water. However, this technique proved to be highly unsatisfactory and was discontinued. Subsequently the method was devised whereby the pellet was placed into the back of the mouth of the guinea pig by means of a pair of forceps and the animal was held until it became apparent that the pellet either intact or in fragments had been swallowed. If an animal was receiving vitamin C, the quantity at the time of each dosage was constant and the schedule the same as that of the estrogen treated animals with the exception of those receiving estrogen prior to the second operation.

The weight of the animals was recorded every day at the time of dosing and upon death as soon afterwards as possible.

An autopsy was performed upon all animals which died any time following bilateral adrenalectomy regardless of the interim between surgery and death, and a careful macroscopic examination was made to ascertain

the existence of any remnants of the adrenal gland which might not have been removed at the time of surgery. Any apparent extenuating circumstances which might have been causative in leading to a premature death other than adrenal insufficiency was noted.

In almost all animals it was noticed that the lungs, on macroscopic examination, had lost their usual pale pink appearance and showed areas that appeared mottled with congested blood. This condition varied from cases of light, indiscriminate mottling to completely "bloody" lungs. This abnormal condition was particularly true of animals dying within a short period of time following complete ablation. Sections of this atypically appearing tissue were removed and regular histological technique was used in preparing them for examination. The stain used was Delafield's haematoxylin and the counterstain was Eosin B.

Since it was necessary to ascertain whether a physiological dosage of estrogen had been administered to the treated animals, two female guinea pigs were oophorectomized and after a lapse of seven days were treated with one mg. of estrogen peritoneally. The nature of the guinea pig is such that unless the female is in oestrus the vagina is sealed. This condition is true in the castrate female. A physiological dosage of estrogen is considered to be a quantity greater than the minimal requirement necessary to unseal the vagina of castrated female guinea pigs.

RESULTS

Approximately 85 guinea pigs were initially adrenalectomized, once

the surgical technique had been developed to any degree of proficiency. Of these, fifty-two survived the effects of unilateral adrenalectomy and were operated upon a second time during which the second gland was removed. Nineteen bilaterally adrenalectomized animals survived less than twenty-four hours following the second operation and are included in the averages of the survival time except in one case as will be discussed later.

Of those animals which died within twenty-four hours following surgery, it is difficult to determine whether the cause of death should be considered the result of typical surgical trauma or as a result of adrenal insufficiency or as trauma resulting from adrenal insufficiency. Agate and Zwemer (1935) reported the deaths of adrenalectomized rats within twenty-four hours after adrenalectomy caused by adrenal insufficiency; while Kramar and Kramar (1953) discuss "capillary crisis" caused by lack of adrenal cortical secretion after anesthesia or surgical trauma in albino rats. A manifestation of this "crisis" involves capillary leakage with widespread vascular dilation and increased capillary permeability. Upon histological examination of prepared material it was noted that there was an accumulation of fluid within the alveoli of the lung.

Corvie (1949) has determined that in rats there is a direct relationship between survival time following complete ablation, sex and age. In this problem it was noted that survival following unilateral adrenalectomy was greater in guinea pigs whose weight ranged between 400 grams and 550 grams; and that there was greater facility in removing the glands since they appeared less confined by surrounding organs and hidden by fat.

Of the fifty-two animals used, thirteen were controls, twelve were treated only with estrogen, fourteen received estrogen and vitamin C and seven received vitamin C only. Of the remaining animals six received cortone immediately following ablation. The results concerning their survival time have been treated separately since the mean survival period of the animals treated thus showed an increase over those animals not so treated; and that those control animals receiving cortone alone (12.5 mg. Cortisone Acetate, Merck) following complete ablation with no further therapy also illustrated an increase of survival time over the non-treated controls. Therefore, this group has been discarded from most of the following analysis.

All groups regardless of treatment showed deaths occurring within the twenty-four hour period immediately following complete ablation. But within the varying dosage schedules involving estrogen or estrogen and vitamin C there is considerable variation.

Among the animals receiving only estrogen in which administration of the hormone began the day following bilateral adrenalectomy, the longest survival period was found to be six days. However, there was a difference of four days between this and the period of the shortest life span, two days (Tables 2 and 4). Those animals treated with estrogen the same day as the second stage operation showed less difference between the longest and shortest survival time, tree days and two days respectively (Tables 2 and 4). But the most significant occurrence involving the groups of guinea pigs receiving just estrogen therapy can be noted amongst those animals which received estrogen immediately prior to the

Table 1. A composite review of the types of replacement therapy used in an attempt to counteract adrenal cortical insufficiency.

	Estrogen*	Estrogen & Vit. C**	Vitamin C	Controls	Cortisone - Estrogen & Vit. C	Cortisone
Total number of animals involved	12	14	7	13	3	3
Total number of males	8	13	6	10	3	3
Total number of females	4	1	1	3	0	0
Animals surviving less than twenty-four hours	7	2	1	8	0	1*
Shortest survival time following ablation	-24 hrs.	-24 hrs.	-24 hrs.	-12 hrs.	5 days	8 days
Longest survival time following ablation	6 days	8 days	6 days	7 days	6 days	12 days"
Average survival time following ablation	1.79 days	3.35 days	3.41 days	1.38 days	5.33 days	10 days*

* average excludes animal which died in less than twenty-four hours since it died of a hemorrhage.
** average is composite of all dosing schedules.
" physical decline in animal apparent at tenth day.

Table 2. Showing the results of the three types of dosing schedules upon the survival time in days the animals survived following complete ablation.

Animal's Number	Estrogen immediately prior to second operation (Dosing schedule "C")	Estrogen the day following the second operation (Dosing Sched. "A")	Estrogen same day as second operation (Dosing Schedule "B")
# 161	-24*		
# 250 F	-24*		
# 169 F	-24*		
# 162	-24*		
# 171 F	-24*		
# 175 F	-24*		
# 157	-24*		
# 243		6	
# 220		2	
# 132		5	
# 214			2
# 217			3
Average survival time in days following complete ablation	-1	4.33	2.5

F - female.
* - hours.

Table 3. Showing the results of three types of dosing schedules upon the survival time in days the animals survived following complete ablation.

Estrogen & Vit. C same day following second operation (Dosing Schedule "B")		Estrogen & Vit. C day following second operation (Dosing Schedule "A")		Cortisone-Estrogene & Vit. C(Cortisone given immediately after the second operation; dosing Schedule "A")		Estrogen four day period prior to second operation; Estrogen & Vit. C after second operation (Dosing Schedule "D")	
Animal	Days	Animal	Days	Animal	Days	Animal	Days
# 248	2	# 119	2	# 2	6	A 15.2	8
# 225	2	# 134	3	# 1	5	0	.5
# 19	4	# 233	2	# 4	5		
# 7	.5	# 229	2				
# 21	7	# 164	3				
		B-W*	8				
		# 152	2				
Average survival time in days following comp. ablation	3.10		3.14 2.33**		5.33		4.0

* excluding B-W.
** Female.

Table 4. Showing comparative results between individual types of therapy upon the survival time.

Therapy	-12 hours	-24 hours	2 days	3 days	4 days	5 days	6 days	7 days	8 days	12 days	21 days
VITAMIN C	0	1	2	0	0	2	1	0	0	0	1*
CONTROLS	2	6	2	0	1	0	1	1	0	0	0
ESTROGEN											
"A"	0	0	1	0	0	1	1	0	0	0	0
"B"	0	0	1	1	0	0	0	0	0	0	0
"C"	0	7	0	0	0	0	0	0	0	0	0
ESTROGEN & VITAMIN C											
"A"	0	0	4	2	0	0	0	0	1	0	0
"B"	0	1	2	0	1	0	0	1	0	0	0
"D"	0	1	0	0	0	0	0	0	1	0	0
CORTISONE-ESTROGEN & VITAMIN C	0	0	0	0	0	2	1	0	0	0	0
CORTISONE	0	1	0	0	0	0	0	0	1	1	0

* Tissue tag.
"A" Dosing started day following second operation.
"B" Dosing started same day as second operation.
"C" Dosing started immediately prior to second operation.
"D" Dosing began four days prior to second operation.

second operation. Here it was found that no animal lived through the entire twenty-four hours following complete ablation (Table 2 and 4).

Within the group receiving dosages of estrogen and vitamin C there was variation encountered with the individual dosing schedules as well as from schedule to schedule. Among the animals receiving their initial injection of estrogen on the day following ablation followed by subsequent estrogen and vitamin C therapy, it was found that death occurred most frequently on the second or third day following surgery (Tables 2 and 4). There was only one exception to this and that was a female guinea pig which survived for eight days after bilateral adrenalectomy.

Those guinea pigs which received estrogen on the same day as surgery followed by daily administration of estrogen and vitamin C also showed considerable variation in life span. The shortest period of survival recorded was less than twenty-four hours, while one animal remained alive for seven days (Tables 3 and 4).

The least variation in survival time was found among those animals which received cortisone immediately following ablation and estrogen and vitamin C therapy begun on the day following surgery. They lived for four or five days after complete ablation (Tables 3 and 4).

Of the two animals which received estrogen for four days prior to the second stage of adrenalectomy, one lived for eight days following complete adrenalectomy while the other survived for less than twenty-four hours, (Tables 3 and 4).

Among those on vitamin C therapy, survival ranged from less than

twenty-four hours to six days. One female remained alive for twenty-one days at which time she was sacrificed and it was found that a tissue tag of adrenal cortex was present which accounted for her continued survival. Among the controls, the survival period ranged from less than twelve hours to seven days (Table 4).

The largest number of deaths occurred in the first three days following adrenalectomy among the controls, estrogen therapy (schedules "B" and "C") and estrogen and vitamin C (schedules "A" and "B") as seen by a comparison of the relative survival time of the animals within a group and from group to group (Table 4). Among the animals receiving vitamin C exclusively, there was an even distribution in those animals which lived three or fewer days and those which lived longer; while in the case of estrogen (schedule "A") more animals lived longer than three days than did not. An even, but unaccountable distribution was found among the two animals treated with estrogen four days prior to the second operation.

CONCLUSION

Why the wide variations occurred within the various dosage schedules and groups was unknown. Some of it may be caused by individual variations among the animals themselves. However, certain trends were evident.

It was noted that those animals receiving estrogen immediately prior to the second operation died within a twenty-four hour period following surgery. While it was found that certain of the control animals did not

survive for twenty-four hours following ablation, this phenomenon was not consistent to all the animals in the group.

A comparison of the animals receiving estrogen and vitamin C regardless of the dosing schedules and those comprising the group receiving just vitamin C show very little difference in the average survival time. However, a comparison of the average survival time of these groups and the controls show an increase of the average life span of those animals receiving vitamin C, with or without estrogen, over the control group.

Among those guinea pigs receiving just estrogen there was great variance of results in the average survival period of the three dosing schedules. Some of this may be due in part to the number of animals involved. However, dealing with the group in its entirety, it can be noted that the average survival time is closest to the average survival time of the controls. Even though one dosing schedule ("A") shows an average survival time greater than any of the other dosing schedules or groups, no conclusions can be drawn because of the number of animals involved.

But from the results of this study, it can be noted that estrogen apparently does not increase the survival of adrenalectomized guinea pigs. And in the case where it was administered immediately prior to complete ablation it proved fatal in less than twenty-four hours. In those animals receiving vitamin C and estrogen, even though they showed a greater average life span than the controls, it may be assumed that this was caused by the vitamin C that they received because of the closeness of the average survival time between this group and that in which the animals received only vitamin C.

SUMMARY

From the data accumulated in the determination of this study, the following summary may be made:

1. Estrogen does not shorten the survival period of adrenalectomized guinea pigs except when the initial dosage is given immediately prior to the second stage of bilateral adrenalectomy.

2. When it is given at this time, it proves fatal in less than twenty-four hours.

3. Estrogen does not increase the survival time of adrenalectomized guinea pigs.

4. Evidence of adrenal insufficiency was apparent in some of the guinea pigs in less than twenty-four hours.

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THE EFFECT OF ESTROGEN (THEELIN) UPON THE SURVIVAL TIME OF ADRENALECTOMIZED GUINEA PIGS

by

BERNEY LOU GOLDEN

B. A., Hunter College of the City of New York,
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ABSTRACT

This study has been made to determine if a functional relationship exists in the guinea pig between the adrenal gland and the ovarian hormone, estrogen. Survival time following complete ablation of these glands was used as a criterion to measure this possible relationship.

In guinea pigs, the adrenal glands are located anterior and dorsal to the kidney and attached to the body wall, vena cava, kidney and on occasion the liver. While the adrenal glands and the vena cava share no common tissue, their proximity makes removal of the glands difficult.

Since the effects of adrenalectomy are considerable, the complete operation was done in two steps with an interim of at least fourteen days between the two. The sequence of operations was the removal of the right gland first and a subsequent operation two weeks later in which the left gland was removed.

Four types of dosage schedules were established for the groups receiving estrogen: (1) estrogen therapy begun on the day following the second operation; (2) estrogen therapy begun on the day of the second operation; (3) estrogen given immediately prior to the second operation; (4) injections of estrogen daily for four days prior to the second operation.

In all cases, regardless of the time of the initial dosage, those guinea pigs receiving hormone therapy were given one mg. of estrogen subcutaneously at intervals of twenty-four hours for the entire period of survival. In some cases the diet of the animals was augmented by

pellets of vitamin C in quantities of either fifty mg. or 100 mg. per day.

In many of the animals, particularly those which died within a 48-hour period following complete ablation, there was a change in the macroscopic appearance of the lungs. Upon histological examination it was apparent that there was fluid accumulation in the alveoli.

There were variations of survival period between the animals in each schedule as well as some variation in the average survival time of the different groups.

Those animals receiving estrogen immediately prior to the second operation all died within a twenty-four hour period following complete ablation. Some of the control animals also died within this period but it was a phenomenon not consistent to the entire group.

The animals receiving estrogen and vitamin C regardless of the dosing schedule, and those animals receiving only vitamin C showed little difference in the average survival time. However, a comparison of these groups and the controls showed an increase of the average life span of those animals receiving vitamin C with or without estrogen over the control group.

Among those guinea pigs which received only estrogen there was great variance of results in the average survival period of animals within the three dosing schedules. Some of this may have been due in part to the numbers of animals involved. However, dealing with this group in its entirety, it could be noted that the average survival time was closest to the average survival period of the controls. Even though animals in schedule (1) showed an average survival time greater than that of any other

schedule or group regardless of treatment, the number of animals involved was such that no conclusions could be drawn.

Those animals receiving estrogen and vitamin C showed a greater average life span than the controls, but it may be assumed that this was caused by the vitamin C which they received rather than the estrogen therapy, because of the proximity in the average survival time between this group and that in which the animals received only vitamin C.

From the results of this study it was concluded that estrogen does not increase the survival time of adrenalectomized guinea pigs. Less than twenty-four hours was the maximum survival time for any animal receiving estrogen immediately prior to the second operation.