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The Structure of the Lymphoid Tissues In the Adrenalectomized Albino Rat

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THE STRUCTURE OF THE LYMPHOID TISSUES
IN THE ADRENALECTOMIZED
ALBINO RAT

by

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A Thesis Submitted to the Faculty of the Graduate School
of Loyola University in Partial Fulfillment
of the Requirements for the Degree of
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LIFE

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CHAPTER I

INTRODUCTION

The problem of alterations in the lymphoid tissues attributed to bilateral adrenalectomy in the albino rat has not suffered from a want of investigators. The association was first noticed in Addison's disease in man (1869), and before the nineteenth century had closed (1899), similar changes had been achieved in the experimental animal. Notwithstanding the great abundance of research data which has accumulated in the past fifty years, there is still a dearth of facts concerning the exact histological modifications produced in all types of lymphoid tissues exposed to the altered physiological state in adrenalectomy.

Therefore, it is the purpose of this problem to make a gross and microscopic study of the lymphoid tissues (spleen, thymus, systemic lymph nodes and the lymphatic follicles of the small intestine) in the albino rat following bilateral adrenalectomy and subsequent maintenance with sodium chloride, sodium 1
citrate and dietary regulation. It is the intent that an answer be elicited to the question, "What is the anatomical and functional basis for this generalized lymphoid hyperplasia?"
CHAPTER II

A. HISTORICAL BACKGROUND-GENERAL

The first convincing report of the adrenals was given by Bartholomeus Eustachius Sanctoseverinatus, in 1563, in his "Opuscula Anatomica" where the sixth chapter was entitled "De glandulis quae renibus incumbunt." The claims that the adrenals are mentioned in the Bible appears to have no historical basis, and is based on an error in the Vulgate translation. The appearance of the term "renunculus" is in reference to the kidney and not to the adrenal gland. Eustachius described and pictured the adrenals ("glandulae renibus incumbentes") in their situation and general configuration, but he offered no explanation as to their function. Despite the clear exposition of Eustachius, many of the anatomical and medical works of the sixteenth and seventeenth centuries contained no mention of these organs. In the early days, when public opinion condemned the dissection of the human body and animal dissection had not attained popularity, autopsies had
to be performed in great secrecy and with a considerable lapse of time following the death of the organism. Therefore, the medulla was usually identified as a central cavity or as a viscous, dark fluid enclosed within the more resistant cortex, and this gave rise to the term "suprarenal capsules."

The findings of Eustachius were soon followed by many more contributions to the anatomy of the adrenal glands, and the differences of opinion soon became as diverse and wide-spread as the ever mounting theories in regard to the functions of the glands. There was a hearty disagreement in regard to the gross form and structure, the arterial and venous blood supply, the innervation and the lymphatics. The major discussions centered around the question of a cavity in the adrenal and of an excretory duct. Morgagni, 1706, noted the presence of accessory adrenal bodies in man and, also, the large size of the glands in the fetus. In 1752, Winslow published an accurate description of the human adrenal gland, but subsequent writers frequently disregarded his work or failed to adjust their concepts of the physiological function of the glands with the anatomical facts. Within the next one hundred years striking advances were made in the comparative morphology and in the microscopic anatomy.
Meckel, 1815, examined the adrenals of many species, and described the variations in birds and mammals but failed to observe glands in the Reptilia. Retzius, 1819, and Stannius, 1839, described the adrenal homologues in the various fishes. Rathke, 1825, noted the true nature of the glands in the Amphibia, and Nagel, 1836, first recognized the adrenal glands in the birds. Other landmarks of this period would include Ecker's microscopic anatomy (1846), Leydig's comparative anatomy (1851-1853) and Kölliker's microscopic anatomy (1854).

Concurrently with these advances in the anatomical knowledge of the adrenals, there was no paucity in the theories advanced to explain the functions of the glands. A brief discussion of the main proponents of the various theories might serve to illustrate the fantastic nature of many of them and their total incompatibility with the anatomical facts of the day. Spigelius, 1627, and Highmore, 1651, considered that the adrenals had a space occupying function in their position between the kidneys and the diaphragm and also aided in the support of the stomach. Riolan, 1628, believed that the adrenals served to support the nerve plexus in the vicinity of the kidneys and had a function in the generating of kidney fat in the fetus. T. Bartholinus, 1668, opposed the above theory and compared kidney fat with the "wheyish black
juyce" contained within the central cavity of the gland. Wharton, 1656, and Glisson, 1657, concluded that the adrenals most important feature was the connection with the nearby nerve plexus, and therefore, a nutritious juice was extracted from the nerves and passed into the venous circulation as the body needs demanded.

A large number of workers attempted to correlate the adrenals, in an indirect or direct fashion, with the elaboration of urine. Casserius, 1600, considered that they served as an aid in the secretion of urine, and Collins, 1685, speaks of a "fermentative liquor" which tended to regulate renal blood flow and affect the urinary output. Molinetti, in 1675, stated that the adrenals functioned only as diverticulae of blood, diverting most of the arterial blood from the kidneys and thereby preventing the secretion of urine in the fetus. Since urinary secretion in the fetus was considered to be disastrous, the adrenals would divert the blood into the maternal circulation via the placenta. Between 1732 and 1791, numerous workers remarked on the size of the adrenals in the fetus and young subjects and in the adult; and Vaughan, 1791, admitted that their function was unknown but postulated one purpose may be served in the fetus and another in the adult. Leyman in 1813 included the glands in his waste-gate system, and he believed that "whenever the blood was propelled to
the kidney with undue severity" the adrenals would divert the flow. The early American anatomist, John Coxe, 1827, revived and enlarged Molinetti's theory and considered that they lessened the secretion of urine in the fetus but that the descent of the diaphragm in the adult retarded the growth and function of the adrenals. He felt, further, that the adrenals perhaps continue to serve in their original capacity in adult life whenever unusual situations arise.

In total disregard to the circulatory details, C. Bartholinus, 1641, postulated that the adrenals absorbed an "atrabiliary juice" from the blood returning from the liver and spleen and conveyed it to the kidneys. Sylvius, 1663, considered that the adrenals derived a liquid from the blood which could be mixed with the blood returning from the kidneys, and thereby dilute it and prevent its coagulation. To Kerkringius, 1670, the function of the adrenals was to secrete a juice to color and animate the blood and produce a fermentation in the heart. This fermentation served in some manner to excite the heart to action.

Severinus and Valsalva, 1719, held to the tenet that an excretory duct connected the adrenal gland to the testis or epididymis in the male or to the ovary in the female. A foreshadowing of the real endocrinological function of the adrenals was
proposed by Goodsir, 1846, who related the thyroid, thymus and adrenals on a common embryological origin and considered that they elaborated some substance into the bloodstream.

As has been seen, most of the ideas concerning the function of the adrenals, bizarre as they were, were formulated shortly after the discovery of the glands. Relatively little was added during the succeeding century. The year 1855, almost three centuries after the work of Eustachius, marks the turning point in the advance of science over the problems of adrenal physiology. Numerous investigators had promulgated hypotheses, some of which had a sound basis in the anatomical and comparative morphological investigations, but the problem of the function of the adrenal gland and its role in the internal environment still remained unanswered. In 1855, Thomas Addison published his monograph, "On the Constitutional and Local Effects of Disease of the Suprarenal Capsules." This was the first clear cut correlation of clinical symptomatology and pathological findings. Earlier workers, especially Bright in 1831, had presented a fairly accurate account of the clinical and pathological findings, but the work of Addison was more comprehensive, and he was the first to express the causal relationship between specific symptoms and the underlying adrenal dysfunction as shown by the autopsy findings.
All of the cases which he reported showed destruction of the adrenals due either to tuberculosis, inflammatory atrophy or metastases, and with the exception of the latter cause, these are still considered the major etiological factors in the production of the disease which now bears his name. Addison's clinical observations are as modern today as they were in the year 1855, and they provided the stimulus whereby we have amassed a wealth of information in regard to the physiology, chemistry, pharmacology and endocrine inter-relationship of the adrenals. The work of Addison prompted Brown-Sequard, 1856, to extirpate the adrenals from a number of laboratory animals and to conclude that these glands are necessary for the maintainence of life.

Vulpian, 1856, had described the green coloration which occurred when ferric chloride was applied to the adrenal medulla, and Henle, 1865, observed the "chromaffin reaction" when the medulla of the adrenal was exposed to dilute solutions of potassium bichromate. The use of this method enabled Wiesel, 1904, to describe the chromaffin system as we know it today.

The observations of Oliver and Schaefer in 1894 of the pressor effects of extracts of the adrenal medulla turned attention to the dual character of the glands. In 1897, Abel isolated the active principle of the adrenal medulla, in the form of its
very insoluble benzoylate and gave the name "epinephrine" to this ester. The eventual crystallization of the hormone was first accomplished by Aldrich, 1901, and Takamine, 1901, with the latter using the term "adrenaline" to identify this hormone. The definitive character of the chemical structure of the hormone was established by Friedmann, 1906, following the synthesis of the hormone by Stolz and Dakin in 1906.

In 1910, Biedl was able to remove the interrenal material (cortical substance) of the Selachii and found that its destruction was incompatible with the continuation of life and that death occurred under conditions similar to those which followed extirpation of the adrenals in the higher forms. This was followed by the observations of Wheeler and Vincent in 1917 that demedullation of the gland led to no serious symptoms and was compatible with life. Numerous workers then showed that adrenalectomized dogs and cats could be kept alive by the continued administration of adrenal-cortical extracts. In the subsequent years between 1925 to 1935, purification of the crystalline fractions showed that the cortical compounds belonged to the general group of steroid hormones. (Stewart and Rogoff, 1929, Hartman, Hartman and MacArthur, 1929, Swingle and Pfiffner, 1931, Kendall, 1932, 1935, Wintersteiner and Pfiffner, 1935)

In 1937, the first pure adrenal cortical compound
(corticosterone) was prepared almost simultaneously by Reichstein, et al. and Mason, Kendall, et al. Since that time at least twenty-five more crystalline compounds and an amorphous fraction have been added to the list of substances obtained by various fractionating procedures involving the adrenal cortex. When we consider the complex functioning of the adrenal gland in its dual role in the body, it becomes apparent that the early investigators faced insurmountable obstacles which required concomitant advances in the field of chemistry before any solution could be resolved.

B. HISTORICAL BACKGROUND-SPECIFIC

1. Addison's disease and the lymphatic system:

Changes in the lymphatic system were first noted in dysfunction of the adrenals in Addison's disease. During life one may observe enlargement of the lymphatic nodules at the base of the tongue, of the tonsils and other palpable lymphatic tissues. At autopsy there is marked enlargement of Peyer's patches and isolated follicles in the gastro-intestinal tract and mesentery. In the original description of the disease, Addison did not list any hyperplastic changes in the lymph nodes or in the thymus. Averbbeck, 1869, reviewed sixty-one cases of Addison's disease and
noticed a local or general hyperplasia of the lymphatic system. The mesenteric, retroperitoneal and intestinal lymph nodes were markedly swollen with little change in the axillary, cervical and inguinal group of nodes. No mention was made of any demonstrable changes in the thymus gland.

An unusual case of Addison's disease was reported by Star, 1895, who found that the mesenteric and retroperitoneal lymph nodes were enlarged, and the thymus gland was compatible in size with that of a pre-puberal individual. This correlation was definitely stated by Pappenheimer in 1910 who found that in conditions attending an atrophic state of the adrenal gland there is an enlarged or persistent thymus. Jaffe, 1927, in his review of the adrenals, found that with loss of the adrenal cortical function due to disease or experimental ablation there is an associated hypertrophy in the tissues of the lymphatic system.

2. Adrenalectomy and the lymphatic system:

Concomitant with the advances made in the study of the pathological changes in Addison's disease, we find a rising interest manifested in the experimental extirpation of the gland and its associated effects. Auld, 1899, partially removed the adrenals in dogs and noticed there was a consistent hypertrophy of
the thymus and enlargement of the spleen at autopsy. Boinet in 1899 and Calogero in 1901 reported thymic enlargement in rats following adrenalectomy. However, this work was not carefully controlled, the life history of the animals was not known and the enlargement was stated to have occurred within one to two days post-operatively. After removal of the adrenals and the spleen in rats of one month of age, Schaefer, 1908, found that the rats had a normal growth for four months and that at autopsy the lymph nodes were enlarged and hyperplastic.

In 1914, Crowe and Wislocki undertook a careful and comprehensive study of the effects of partial and total adrenalectomy in the dog. After prolonged periods of chronic insufficiency, they noted large mesenteric, retroperitoneal and mediastinal lymph nodes, marked involvement of the solitary lymph follicles of the small intestine, frequent hyperplasia of the thymus, a slight increase in the size of the spleen and persistent, enlarged tonsillar masses. They concluded that the "increased size of the lymph nodes was due to a hyperplasia of the cells lining the medullary sinuses and in the germinal centers" and that there was no increase in the numbers of lymphocytes.

In working with the rabbit, numerous investigators (Marine and Bauman, 1921, Take and Marine, 1923, Marine, et al.,
1924) have demonstrated that the animals subjected to bilateral adrenalectomy show an increase in the size and weight of the thymus and generalized lymphoid hyperplasia. In a series of three papers, Jaffe, 1924, covers the correlation of bilateral adrenalectomy in rats of varying age groups. In the first group, it was shown that thymic enlargement reached a maximum within three to five weeks post-operatively and then there was a gradual decline in size, but involution was markedly delayed. Notwithstanding the fact that 52 per cent of the animals in the experimental class lost weight, their thymic weights were 16 to 117 per cent above the normal. The second group consisted of rats of an older age who were subjected to a partial thymectomy prior to the adrenalectomy. The partial thymectomy served as a control, and it was noted that following the adrenalectomy the remaining thymic lobes were regenerated and enlarged. This process was initiated within twenty-four hours and reached its completion in two weeks. Rats of a younger age were included in the final group, and the thymus glands of the experimental animals averaged 49 per cent heavier than the controls.

Seitz and Leidenius in 1925 adrenalectomized rabbits and reported an enlargement of the thymus glands in their offspring. In 1925, Hammett was able to describe the reciprocal
relationship between the size of the adrenal cortex and the thymus. The finer microscopical details of the lymph node hyperplasia in adrenal insufficiency in dogs were reported by Banting and Gairns in 1926. The lymph nodes were enlarged and edematous with compressed germinal centers, proliferated endothelial leukocytes and increased reticular tissue, but the spleen exhibited little alteration from the normal. In cats, there is marked lymphoid hyperplasia in the spleen and lymph nodes with an increase in the size of the germinal centers and in the numbers of lymphocytes and mitoses. (MacMahon and Zwemer, 1929)

By maintaining the adrenalectomized rat with sodium chloride, Rheinhard and Holmes, 1940, were able to demonstrate definite changes in the weights of the systemic and mesenteric lymph nodes and of the thymus. Both the experimental animals and the controls with sodium chloride in their diet showed an increase in the weight of the thymus. The controls with sodium chloride had a decrease in both groups of lymph nodes over the plain control weights, and the systemic nodes of the experimental animals were far increased in weight over the mesenteric nodes. The histological picture was reported to be an increase in the "bulk of the elements" with no specific morphological change.

Comparable work by Gatz, 1941, showed an increase in
the body weight and in the weight of the spleen and thymus with
the larger nodules in the spleen exhibiting a thickened periph­
eral ring of lymphocytes. The cortex of the thymus gland in the
adrenalectomized rat contained numerous cells with large nuclei
and lilac stained cytoplasm. A possible explanation for the add­
ed growth of the experimental animals was revealed in the study
of the anterior pituitary cytology where the eosinophils had in­
creased by 10 per cent, the chromophobes were decreased by 10 per
cent and the basophils were unaltered.

Several South American investigators have carried on
similar investigations in attempting to correlate the adrenals
and the testes with the thymus and the lymph nodes. Houssay, et
al., 1941, concluded that the adrenal gland exercises an action
over the growth of the thymus and to a lesser degree over the
lymph nodes. Hyperplasia of the thymus in the adrenalectomized
rat occurred only during the period of adrenal cortical insuffi­
ciency with involution following in the course of growth of the
purely cortical accessory masses. The maximum weight of the thy­
mus was attained thirty days post-operatively. In 1944, Rapelo
made a study of the thymus gland in rats which were adrenalectom­
ized and/or castrated. He demonstrated that either operation
would cause a hyperplasia of the thymus with a summation of the
effect in the dual procedure. The maximum increase in the thymic weight was noted by the twenty-fifth to thirtieth day post-operatively with subsequent hypertrophy of accessory adrenals, made up exclusively of cortical tissue, producing atrophy of the thymus by the fiftieth day.

Contrary to most observers, Sörek, 1944, believed that if one compared thymus weights in relation to body weight rather than age, there was no difference between adrenalectomized male rats and optimally growing normal rats. He concluded that the normal course of thymic development is not changed after castration or adrenalectomy. If the condition are sub-optimal, the thymus shows less "underdevelopment" or atrophy than does the thymus of non-operated animals under similar conditions. This could be considered to be a part of the general picture of Sel-ye's "alarm reaction" in which the typical thymic atrophy does not occur in the adrenalectomized animal.

3. Adrenal extracts, ACTH and the lymphid system:

The clinical antithesis of adrenal insufficiency, namely, increased adrenal cortical secretion, had its humble inception in the work of Hoskins and Hoskins, 1916, who fed desiccated adrenal material to rats for two to nine weeks and noted no
alteration in the growth rate and an inconstant, variable decrease in the size of the spleen. McKinley and Fisher in 1926 fed fresh adrenal cortex and whole gland to rats and found that they were 9.10 per cent heavier in body weight and the spleens averaged 18.9 per cent heavier than the controls. With the increase in the purification of the cortical extracts, Ingle, 1938, administered cortin to intact rats and produced a marked involution of the thymus. In 1940, he was able to obtain similar results by the use of 17-hydroxy-11-dehydrocorticosterone (Kendall's compound E). A qualitative difference in the effect of adrenal cortical compounds was noted by Wells and Kendall, 1940, in the regression of the thymus as produced by corticosterone and dehydrocorticosterone. Kendall, 1941, reviewed this adrenal cortex-thymus relationship and concluded that corticosterone and related compounds produce profound thymic atrophy while desoxycorticosterone produced no atrophy.

With the realization and subsequent identification of an anterior pituitary-adrenal relationship, it became possible to study the effects of adrenal cortical hypersecretion as mediated through the adrenal stimulation by the adrenocorticotropic hormone. In the preliminary work, Crede and Moon, 1940, observed that this hormone engendered an acute thymic atrophy in the normal twenty-one to twenty-three day old rats with no effect noted
in the adrenalectomized animal. Castration did not prevent this atrophy. In the hypophysectomized rat, ACTH yielded thymic atrophy but to a lesser degree. Later, Moon, 1940a, duplicated some of the above work and found that ACTH administered to the four-day old rat resulted in thymic atrophy with loss of the lymphocytes and a relative increase in the number of epithelial cells. When this work was attempted with the adrenalectomized animal, (Moon, 1940b) no signs of thymic atrophy were evident. Simpson, et al., 1943, observed the similarity of response of the thymus and lymph node to the administration of ACTH in the rat by noting the reduction in the weight and size of the thymus and the cervical lymph nodes.

A long series of investigations by Dougherty and White has revealed more detailed information in regard to the functional alterations in lymphoid tissue, and in the circulating blood cells, as produced by the injection of ACTH into mice. In 1943, it was noted that pure pituitary ACTH would produce a decrease in the weight of the thymus and in the axillary, inguinal and mesenteric lymph nodes; the spleen showed no weight decrease. By utilizing repeated injections of the ACTH, (1945) the lymph nodes in mice showed disintegration of the lymphocytes and "washing-out" phenomena of the cytoplasmic contents and in the nuclear portion
of the cells, edema, phagocytosis and an increase in the size of the reticular cells with their coalescence into giant cells. Edematous changes occurred in the spleen with degeneration of the lymphocytes, erythrophagocytosis and polymorphonuclear cell infiltration. Within three hours, the macrophages had increased in number, phagocytosis occurred and there was a lack of mitoses. As the gland became atrophic, the thymic corpuscles increased in number.

4. Thymus-adrenal relationship:

A great deal of research has been expended in the attempt to clarify the definitive relationship between the thymus and the adrenal gland. Wiesel, 1905, and Marine, 1928, have presented reviews of human status thymicolymphaticus with the consistent autopsy findings of an enlarged thymus associated with hypoplastic adrenals. Contrariwise, Soli, in 1909 was able to detect a slight, although definite, adrenal hypertrophy following thymectomy in the experimental animal. With the advent of a thymus extract (Hanson), Rowntree, 1935, found that its administration led to a hypoplasia of the rat adrenal, but on the other hand, Tislowitz and Chodkowska, 1936, claimed that thymus
extracts produced somatic growth and adrenal hypertrophy in the rabbit.

The problem was attacked from a new angle by Anderson, 1935, who found that extreme muscular exercise in the rat induced an enlargement of the adrenals and a marked atrophy of the thymus. Selye, 1937, confirmed and elaborated upon the above work to show that it was a part of the phenomenon of the "alarm stage" of adaptation, and it could be induced by a variety of noxious agents (cold, morphine, formalin, adrenaline, etc.). During this stage there was a hypertrophy of the adrenals and a sudden involution of the thymus and a less regular involution of the lymph nodes and the spleen. Adrenalectomy prevented thymic involution, and the treatment of these animals with adrenal extracts allowed the involution to occur. Gershon-Cohen, et al., 1938, found that the adrenals were consistently enlarged in young male rats whose thymus glands had undergone atrophy after the administration of large doses of x-ray.

In 1940, Segaloff and Nelson demonstrated that thymectomy completely failed to produce any alteration in the course of adrenal insufficiency in the bilaterally adrenalectomized rat. Therefore, they concluded that despite any effect the thymus may have upon adrenal-controlled physiological processes, it is
incommensurate with the acute situation in the adrenalectomized animal.

Research, similar to the adaptation study of Selye, by Dohan, in 1942, revealed that if rats are exposed to a low atmospheric pressure for more than two days there is an absolute increase in the adrenal weight with an attendant decrease in the weight of the thymus and testes.
CHAPTER III

MATERIALS AND METHODS

The advantages and ready availability of the common laboratory rat (Rattus norvegicus albinus) has made it the subject of many investigations concerning the effects of adrenal extirpation. Immediately after Addison published his epic-making monograph on adrenal disease, Brown-Sequard, 1856, and others studied the consequences of adrenalectomy in animals. Although the death of the animals ensued, adrenal insufficiency as the cause, was promptly questioned, since even a unilateral adrenalectomy was fatal in certain species. This could be attributed to the quality of the operative technique and the failure to observe aseptic measures. An additional shadow was cast upon the problem by Philipeaux, 1856, who produced evidence that the albino rat could withstand a bilateral adrenalectomy for a prolonged period of time.

In the following three-quarters of a century, a multitude of investigators attacked the puzzle in the albino rat, and
it was the general view that 50 per cent of the rats would survive indefinitely after bilateral adrenalectomy. Jaffe reviewed the matter in 1926 and reached the conclusion that the majority of the workers had not studied their animals for a sufficient length of time. He followed the course of ninety adrenalectomized rats for as long as one year after the operation. Thirty-five per cent died within thirty days post-operatively, with most of the deaths occurring before the thirteenth day, 46 per cent were chronically insufficient but died within seven months and 19 per cent showed no demonstrable effects and exhibited large accessories at autopsy. Pencharz, et al., 1930, found that the average survival time following adrenalectomy was less than twenty days. Freed, et al., in 1931, had a varying survival time of seven to fourteen days, and this was conditioned by the thoroughness of the operation and the length of adrenal pedicle removed. On the other hand, Agate, 1935, stated that 90 per cent of the albino rats will survive the bilateral procedure. However, he utilized McCollum's lactation diet which contains 1 per cent sodium chloride, and this factor could be responsible for his high rate of survival.

Therefore, it can be assumed that the albino rat is a suitable animal for this problem despite the controversial role
which the animal has played in the past history of the subject.

The animals to be utilized in the project were selected with an attempt to discard any litters in which gross signs of sickness were evident, e.g., 'snuffles', pulmonary disorders, diarrhea, etc. An effort was made to accommodate the animals to the prepared diet by introducing them to it at least two weeks prior to the onset of the experimental period. This diet, which was modified slightly from the original of Kendall, consisted of:

- Casein--------- 20 per cent
- Lard---------- 20 "  "
- Starch-------- 36 "  "
- Yeast--------- 10 "  "
- Cod liver oil-- 2 "  "
- Cellu flour---- 7 "  "
- Calcium lactate- 1 "  "

The modification was produced by the addition of ten grams of yeast and five cc. of Mazola oil to every one hundred grams of diet. It was felt that these substances would aid in attaining a high appetite level and obviate any possibility of a vitamin E deficiency, respectively. No difficulty was encountered with the diet, for the animals accepted it readily, demonstrated a normal growth rate and were free from any digestive tract dysfunction.

After the preliminary period of diet-feeding, the
litter mates were segregated into control and experimental units. The latter group was subjected to a single stage, bilateral adrenalectomy. The operative technique was as follows: (1) Ether seemed to be the anesthetic agent of choice and was used exclusively during this problem. Prolonged exposure to the anesthesia was avoided in an attempt to curtail post-operative complications. (2) The anesthetized animal was secured to the operating board with the legs being attached to small hooks. (3) The operative site, mid-dorsal line at the level of the kidneys, was prepared by clipping the hair and scrubbing the skin with tincture of green soap and washing with 70 per cent ethyl alcohol. A sterile gauze pad was then draped around the area to minimize contamination. (4) The instruments, which had been sterilized in a steam sterilizer, were placed in a 70 per cent ethyl alcohol solution. (5) A single, dorsal, mid-line incision was made through the skin at the approximate level of the kidneys. By cutting through the sub-cutaneous fascia, the right or left dorsal muscle mass was exposed, and this was incised along its lateral border from the mid-kidney level toward the angle created by the last rib and the dorsal muscle mass. The adrenal gland is situated at the upper pole of the kidney, and it is usually embedded within a mass of adipose tissue. This entire mass was carefully dissected free from the surrounding structures, and the pedicle was clamped by
a small, curved forceps, making sure that the immediate region of the adrenal was not disturbed. Electric cautery was then applied to the pedicle, and the entire mass was removed from the body. It is of major importance that the entire adrenal with its adherent adipose tissue be removed intact and without any loss of cortical cells. The right adrenal lies slightly more anterior in the body cavity than its left counterpart. (6) Suturing of the muscle incision is a matter of choice and can be deleted without impairing the success of the operation. (7) The skin incision was closed by means of metal clips or interrupted catgut sutures. A final painting of merthiolate was applied to the operative site. (8) It was found advisable to have the animals conscious and recovering from the anesthesia prior to the time that they were returned to the recovery cages. (9) In the succeeding twenty-four to forty-eight hours, the animals were maintained in an atmosphere free from drafts and precipitous temperature deviations.

In addition to the prepared diet, the experimental animals received 0.5 per cent sodium chloride and 0.5 per cent sodium citrate in their drinking water. Certain of the control groups were divided, also, with some receiving plain and some the saline drinking water. The amount of diet furnished daily to the control animals was regulated by the corresponding amount consumed by the
experimental litter mates. Several control animals were furnished with an unlimited amount of diet in an attempt to contrast rates of growth. Daily observations were made for general behavior and condition, and bi-weekly checks were made on the body weight.

Animals dying because of the operative procedure or of an evident sickness were autopsied, but the tissues were not included in the study. At varying lengths of time after the initiation of the experimental period, the experimental animals and their litter mate controls were sacrificed. The animals were placed under deep ether anesthesia, the thoracic cage was opened and three to five cc. of a 10 per cent formaldehyde solution was injected into the right and left ventricular cavities of the heart. The animals were then dissected and the tissues (spleen, thymus, cervical lymph nodes and intestinal lymph follicles) placed into Bouin-Allen and Zenker-formol fixing fluids. Prior to cutting, the spleen was measured (length, width and thickness), and the thymus was sketched and measured. A search was also made in an attempt to locate any accessory adrenal bodies, and the operative site was studied for any adrenal cortical masses remaining coincident to a faulty operative technique. Any suspicious tissue was removed, fixed in Zenker-formol and studied histologically. After fixation, all tissues were embedded in paraffin and sectioned at seven to eight micra; the sections were mounted
and stained with Harris' hematoxylin and eosin, Kornhauser and MacFarland connective tissue stains and the Dublin reticular stain.

A total of sixty-eight animals were bilaterally adrenalectomized with a 25 per cent mortality in total experimental deaths. Of the latter, 4.3 per cent of the deaths occurred within forty-eight hours post-operatively, 8.6 per cent occurred within seventy-two hours and the remainder ranged from seven days to forty days post-operatively. Therefore, fifty-one animals survived the designated experimental periods and were available for study. Fifty-five litter mates were included as controls, and four died during the course of the experiment.
CHAPTER IV

OBSERVATIONS

A. General observations and body weight:

The experimental animals maintained a healthful appearance, but their activity was definitely limited and the animals seemed to be sleeping a greater proportion of the time than the litter mate controls. Paired feeding of the experimental and control animals was retained. The results indicate that the adrenalectomized rats were slightly heavier than their litter mate controls. The control animals showed a more rapid growth only in the short-term, older age groups. (Table I)

The female experimental animals appeared to maintain a fairly normal estrous cycle, but a number of them failed to carry a pregnancy through to parturition, as was evidenced by the uterine findings at autopsy. However, the fertility of the two groups seemed to be equal, but was reduced over that normally exhibited by the colony.

When subjected to fluctuating room temperatures or to
the temporary withdrawal of the drinking water, the experimental animals could not withstand the imposed stress and death would ensue. Several of the experimental animals died during the course of the study and exhibited rather massive urogenital tract infections.

B. Lymph nodes:

The distinction between the experimental and the control animal was not manifested to a great degree in the histological study of the cervical lymph nodes. There was a moderate increase in the cellularity of the medullary cords and in the apparent number of macrophages in the experimental nodes. Phagocytosis, as indicated by the number of ingested erythrocyte fragments, was also increased in the same nodes. Mitotic figures were more frequent in the nodes removed from the experimental animals. (Figure I) The general character of these lymph nodes is such that very little could be determined pertaining to the size of the cortex and medulla, the lymphatic follicles and the germinal centers.

C. Intestinal lymph follicles:

Grossly, it was evident that the lymphoid follicles of the small intestine in the experimental animal were larger and
slightly more frequent. However, there were frequent exceptions to the above findings, especially if the animal had exhibited a digestive tract disorder at any time during the course of the project. Microscopically the evidence was rather sparse with only a few experimental animals showing hypertrophied follicles that were covered internally by a simple epithelium devoid of glands and projected externally where a thin, two to three cell-layer of muscle formed the outer wall. (Figures II and III)

D. Spleen:

The gross measurements of the spleen immediately after its removal from the body revealed a moderate but consistent increase in the experimental animal. This appeared to be absent in the short-term animals. Histologically, the chief feature was an increase in the width of the peripheral band of closely-packed cells surrounding the splenic nodule. In the control animals this area averaged 0.271 mm. in diameter as compared to 0.302 mm. in the experimental animals. (Figure IV) A thin, fibrous band derived from the connective tissue serves to demarcate the peripheral band from the main body of the splenic nodule. The spleen from the experimental animal also shows a relative increase in the proportion of white pulp as compared to the red pulp. Another
interesting feature is the presence of large numbers of megakaryocytes which are distributed throughout both groups of animals.

E. Thymus:

The most striking effect of the adrenal cortical insufficiency-thymus relationship was found in the short-term, older age group of animals. (Table I, litters 16 and 17) On gross examination, it was evident that the thymus of the control animals had a high content of fat, whereas the thymus of the experimental animals appeared firm and fleshy with a much reduced fatty infiltration. This impression was substantiated by microscopic examination which showed the lobules of the control gland to be small and widely separated by connective tissue and fat and showed extensive connective tissue ingrowths along their peripheral margins. Apparently there was greater involution in the controls on plain drinking water than those receiving saline drinking water. (Figures V and VI) The thymus gland in the experimental animals contained larger lobules with a reduced amount of interlobular connective tissue and fat. (Figure VII) The experimental animals which were maintained for prolonged periods of time showed little deviation from the control configuration.
CHAPTER V

DISCUSSION

From the observed results, it appeared that the bilaterally adrenalectomized rat with the supportive therapy of sodium chloride and sodium citrate was able to lead an apparently normal life in so far as bodily growth and functions are concerned. The major expression of the chronic adrenal insufficiency was seen in the alterations of the lymphoid tissues. The systemic lymph nodes and the intestinal lymph follicles gave evidence of increased mitotic activity and phagocytosis, the peripheral band of the splenic nodule was enlarged and the atrophic thymus regenerated. The added sodium chloride in certain of the control diets apparently had little effect except that it may have retarded the involutionary process in the thymus. Perhaps the focus of action of the sodium chloride in the normal, intact animal is in the anterior lobe of the pituitary where Koneff, et al. have reported some modifications. The mechanism involved in the lymphatic tissue changes in the adrenalectomized animal has been suggested by
White and Dougherty, 1944, to center around the blocking of the humoral agents which normally cause the dissolusion of the lymphocytes. The lack of this control then results in an increase in the total lymphocyte count (Dougherty and White, 1944) and hyperplasia of the lymphoid tissues (Rheinhardt and Holmes, 1940).

In the absence of a definite testing agent, it would seem that the macrophages have increased in number in the systemic lymph nodes and that erythrophagocytosis has occurred. However, Gordon and Katsch, 1949, have utilized thorium dioxide in a colloidal solution and demonstrated a decrease in the size and number of the macrophages in the liver and spleen.

The lymphatic tissue modifications which have been produced in the rat are similar in nature and extent to the autopsy findings in human adrenal insufficiency. Conversely, the thymic changes observed in status thymicolymphaticus are associated with an adrenal hypoplasia.

The present findings would seem to indicate a degree of correlation with the conclusions of Houssay, et al. and Rapelo who feel that maximum effects of adrenalectomy in the rat are observed by the twenty-fifth day. The hyperplasia of purely cortical accessory masses then takes effect and nullifies the pre-existing alterations in the lymphoid tissues. This is most strikingly seen in the thymus gland. Even the work of Jaffe in 1924 would
indicate that there was a maximum period of action with a subsequent period of plateau and slow decline. The question of the adrenal accessory masses in the rat might be answered more completely if routine chemical tests of the blood and urine were performed in the pre- and post-operative states of the animal and the time of any significant changes noted.
CHAPTER VI

SUMMARY

The influence of adrenalectomy upon the structure of the spleen, thymus, systemic lymph nodes and intestinal lymph follicles has been examined in detail in the albino rat. General observations were made in regard to bodily growth, behavior and fertility; detailed study was made of the microscopic appearance of the above mentioned tissues.

1. The adrenalectomized animals maintain a normal, or even accelerated, rate of growth when sodium chloride and sodium citrate are added to the drinking water.

2. General activity is retarded, but fertility falls within a normal range.

3. The lymph nodes and follicles exhibit an increased lymphocytic activity.

4. The peripheral ring of the splenic nodules proliferates.

5. The atrophic thymus regenerates.

6. The maximum reaction is attained at about forty days.

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CHAPTER VII

BIBLIOGRAPHY


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</tr>
</tbody>
</table>
Figure III

Figure IV
APPROVAL SHEET

The thesis submitted by James C. Beyer has been read and approved by three members of the Thesis committee.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Arts.

[Signature of Adviser]

Date: June 1, 1950