COMPARATIVE LEVELS OF 17-KETOSTEROIDS IN NORMAL AND ADRENALECTOMIZED FOWLS

by

PAUL FREDRICK BRANDE

B. A., Augustana College, Sioux Falls, South Dakota, 1953

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Zoology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1955

TABLE OF CONTENTS

INTRODUCTION

In 1937 and 1938 Herrick and Torstveit performed what was probably the first successful adrenalectomy in birds. In this work they found a regression in size of both the comb and testes following adrenalectomy, thus indicating an interrelationship between the adrenal glands and the reproductive system.

Much work has been done to substantiate the fact that the primary and secondary sex characters are maintained by the sex hormones (Turner, 1948, and Jaap and Robertson, 1953). Herrick's work along with that of others of the same nature, suggests the possibility that the adrenals may produce certain sex hormones or precursors of these hormones. Most of the work devoted to this problem has been done in the males and the sex hormones involved were androgen-like and androgenic in nature.

It was the purpose of this work to determine relatively what quantity of normal androgen-like secretions are contributed by the adrenal cortical tissue in birds. The method used was that of the 17-ketosteroid titer in the urine since the hormones which are androgenic in nature are primarily of the 17-ketosteroid type. The titer was determined in normal and adrenalectomized birds. It is believed that the difference between the two titers was indicative of the amount of 17-ketosteroid or androgenic-like compounds contributed by the adrenal glands.

It is known that the 17-ketosteroids present in the urine also may come from other sources than the testes and the adrenal glands (Dorfman, et al., 1947; Paschkis, et al., 1954), but it is believed that the

difference in the levels of the 17-ketosteroids found between the normal and the adrenalectomized birds was great enough to attribute it to the lack of adreno-cortical material.

Hamilton et al. (1954) concluded, and a few others indicate, that the 17-ketosteroid titer is not conclusively indicative of the andrcgenic level in the urine. Zarrow, et al. (1950) and other workers, (Dingemanse, et al., 1952; Callow and Callow, 1940; Frazer, 1941, Munson, et al., 1954) have found that the opposite situation exists; that the 17-ketosteroid titer indicates the relative androgen or androgenic levels in the urine. Consequently it is believed that the criteria used in this work to indicate the amount of androgenic contribution of the adrenal gland was satisfactory.

REVIEW OF LITERATURE

Much of the early work concerning androgenic activity of the adrenal glands was conducted by administering portions of, or extracts from, either the adrenals or the testes and noting the effect upon the other.

Grollman and Howard (1933) injected adrenal cortical extract into rats and noted that the testes averaged 20 percent heavier per body weight than in the control animals. They found no apparent effect upon the female reproductive system.

Corey and Britton (1932) injected an adrenal cortical extract into young albino rats and noted a precocious maturation of the sex glands.

In 1929, Eaton, et al. observed that chicks fed desiccated adrenal

cortex developed testes of greater size than did control birds.

McKinley and Fisher (1926) found that rats which were fed on adrenal cortices had 21 percent heavier testes than control rats.

Hoskins (1933) observed hypertrophied testes in white rats which were fed adrenal cortical tissue.

A stimulation of testicular growth when young rats were fed substance from the whole adrenal gland was noted by Vincent (1917).

According to Mason and Engstrom (1950), Butenandt in 1931 was the first to describe the isolation of a crystalline steroid from male urine. In 1934, it was identified as androsterone. This was the first of a group of compounds known as 17-ketosteroids. Since then several compounds belonging to this group have been isolated. A number of methods for their quantitative determination also have been described. The quantitative distribution of urinary 17-ketosteroids is important for the understanding of certain diseases, and their quantitative determination is often used as a clinical diagnosis for those diseases. The 17-ketosteroid levels are often believed to indicate the functional state of the adrenal cortex and the testes.

The 17-ketosteroids comprise a group of steroid compounds which have in common a ketone group at the C_{17} position of the steroid nucleus. They may differ in structure in other ways, but because of the 17-keto group they are characterized by certain color reactions. Some of the urinary 17-ketosteroids have androgenic properties, but others such as etiocholanolone appear to be biologically inactive.

Lieberman, et al. (1948) have shown that in humans 42 apparently homogeneous and non-identical steroid substances have been encountered

in urine. Of this group, 26 have been fully characterized and identified. The other 16 have been somewhat characterized and described as new steroids. From the group of 42 substances, 35 are alpha hydroxy compounds, and seven are thought to be beta hydroxy steroids. The diagnostic difference in identification of these two fractions is the fact that the alpha compounds are not precipitated by digetonin, whereas the beta fractions are. The significance of the fractionation can be seen in that some workers, Lieberman et al. (1948) and others, consider the alpha steroids to be largely of testicular origin, whereas the beta fractions are believed to arise in the adrenal cortex.

That the adrenal cortex is in some manner a source of urinary 17-ketosteroids is generally accepted. Mason and Engstrom (1950) cite evidence to support the conclusion that when the adrenal cortex is destroyed, there is a marked decrease in the amount of urinary 17-ketosteroids. These men also stated that when adreno-cortical tumors were present, large amounts of 17-ketosteroids appeared in the urine.

Salter, et al. (1946) and McCullagh, et al. (1948) have demonstrated that patients with Addison's disease usually show a total excretion of 17-ketosteroids which is one-half the level found in normal humans.

Callow and Callow (1940) described situations in which the urine of castrate human males contained at least some androsterone and etiocholanolone. They reasoned that there must be an extra gonadal source of urinary 17-ketosteroids, the adrenal cortex.

Dingemanse and her co-workers (1952) have stated that part of the 17-ketosteroids found in urine come about as metabolites of both gonadal

hormones and cortical secretions.

Some of the first to describe a ratio between the urinary 17-ketosteroids in relation to their source were Fraser, et al. (1941). This group stated that urinary 17-ketosteroids are derived from male gonads and adrenal cortex in a ratio of about 9 mg from the adrenal cortex and 5 mg from the testes. This indicates that the adrenal cortex is a greater contributor of neutral 17-ketosteroids than are the testes. This is contrary to the accepted belief.

Dorfman, et al. (1947) suggested the possibility of a source of urinary 17-ketosteroids other than the testes and the adrenal cortex. These men found that after adrenalectomy and gonadectomy in monkeys of both sexes, the urinary 17-ketosteroid level was about one-third of the preoperative level.

Substantiation of the theory that the adrenal cortex is an extra gonadal source of urinary 17-ketosteroids is the fact that administration of adrenocorticotropic hormone (ACTH) will influence the 17-ketosteroid titer in the urine.

Lieberman, et al. (1950) have shown that the intravenous administration of ACTH causes a noticeable increase in the urinary 17-ketosteroid level. Forsham, et al. (1948) have also shown that ACTH stimulated increased excretion of 17-ketosteroids and corticosteroids. If the ACTH administration is great enough the amount of 17-ketosteroids may increase considerably. Also demonstrating that ACTH causes an increase in the urinary 17-ketosteroid titer was the work done by Sandburg, et al. (1952) in human beings.

While testosterone is undoubtedly a precursor of 17-ketosteroids, the nature of the adrenal cortical precursors is largely unknown. Most workers agree that 17-ketosteroids as such are not formed by the adrenal cortex. Some precursor probably is formed and the 19-carbon, 17-ketosteroid compounds found in the urine are degradation products of the precursor.

Lieberman, et al. (1948) isolated in the urine a 19-carbon compound which had been previously labeled at C_{11}. They believed this strongly suggested that the adrenal cortical precursor was a 21 carbon atom compound which was degraded to the 19 carbon metabolite which may have been another precursor or the actual 17-ketosteroid found in the urine.

Work done by Reichstein and Shoppee (1943) and Samuels, et al. (1947) led to the belief that 17-ketosteroids or related 19 carbon steroids are elaborated by the adrenal cortex. These compounds have become altered by participation in metabolic activities from 21 carbon atom compounds.

Sayers, et al. (1949) suggested that urinary 17-ketosteroids in humans are breakdown products of 21 carbon atom adrenal cortical steroids.

Callow (1939) working with humans has suggested that 17-ketosteroids may be conversion products of other steroid substances. She and her coworkers administered a wide variety of C_{19} steroids, some of which were believed to be degradation products of 21 carbon atom compounds. These compounds caused an increase in the output of urinary 17-ketosteroids, especially etiocholanolone.

Dingemanse, et al. (1952) have shown that regardless of the precursors of the 17-ketosteroids excreted in the urine, they were chiefly in

the form of esters of sulfuric acid and glucuronides. Only a small amount were unconjugated.

That the known cortical hormones act as precursors to the C_{19}, 17-ketosteroids has raised controversy among many workers. Those cortical hormones which have been isolated in crystalline form have a two carbon side chain at C_{17} of the steroid nucleus. This group is commonly known as the adrenal corticosteroids. It would be necessary to remove this side chain in order to convert one of them to a 17-ketosteroid. From the work of some, it would appear that this is not readily accomplished.

According to Cuyler, et al. (1942) it is not possible to affect the excretion of 17-ketosteroids by the administration of desoxycorticosterone. It seems to be converted in some part to pregnanedial.

The main controversy concerning the corticosteroid effect upon 17-ketosteroid excretion seems to be concerning cortisone.

Sprague, et al. (1950) has shown that large amounts of cortisone administered to patients with Addison's disease showed an increase in 17-ketosteroid excretion.

Munson, et al. (1954) performed both orchidectomy and adrenalectomy on men. Following orchidectomy the 17-ketosteroid level dropped considerably, but after adrenalectomy, the titer was still lower. They administered cortisone in varying amounts and found that 300 mg daily (6 to 12 times the maintenance requirement) would raise the amount of urinary androgen to a level only slightly higher than it was before adrenalectomy. However, the same dosage of cortisone caused a marked increase in the urinary 17-ketosteroid level. These men believed their findings added support

to the hypothesis that the adrenal cortex normally produced as yet unidentified steroids which were androgenic or precursors of androgens. They believed that cortisone was intimately related to this androgen production.

Sokel and Bondy (1954) administered cortisone to patients with carcinoma of the prostate and to a group of control individuals. They found an increase of 17-ketosteroids in the normals. In the patients with cancer, however, the increased increment of 17-ketosteroid production following cortisone administration was five times as great as in the normal ones. These men indicated that cortisone was involved in 17-ketosteroid production and somehow related to prostatic cancer.

In complete opposition to the theory that the corticosteroids affect 17-ketosteroid excretion, Homburger, et al. (1948) reported no increase in ketosteroid excretion upon administration of cortisone or 11-dehydrocorticosterone (an isolated corticosteroid).

Wilkins (1952) found a negative effect of cortisone upon 17-ketosteroid excretion. He found that cortisone administered orally or intramuscularly caused a suppression of 17-ketosteroid excretion.

Kupperman, et al. (1953) also found that cortisone administered in the same way depressed, within one week, the elevated ketosteroid excretion noted in a patient with a 22 year history of adreno-genital syndrome.

Sprague, et al. (1950) found a depression of urinary 17-ketosteroids following cortisone therapy. However, they suggested that this may have been due to a depression of some of the general functions of the

cortex by cortisone, and indirectly affected the 17-ketosteroid excretion. These men did, however, report an increase in 17-ketosteroid excretion when a patient with Addison's disease was treated with cortisone.

The effect of corticosteroids other than cortisone and desoxycorticosterone on 17-ketosteroid excretion has been reported. Conn, et al. (1951) using compound F(17-hydroxycorticosterone) and Polley and Mason (1950) using 11-dehydrocorticosterone found that in humans, these compounds caused an increase in the urinary 17-ketosteroid excretion.

The metabolism of the 17-ketosteroids themselves has been studied. Mason and Kepler (1947) found that when dehydroisoandrosterone was injected intramuscularly into humans, not only was there an increase in 17-ketosteroid excretion, but androsterone and etiocholanolone could be isolated from the urine in relatively large amounts. In some cases, a small part of the dehydroisoandrosterone was recovered unchanged in the urine.

Miller and Dorfman (1945) isolated dehydroisoandrosterone from the urine of male guinea pigs after they were given injections of (delta) 5-androstene-3(beta), 17(alpha)-diol. This is a substance which was isolated from the urine of humans as a metabolite of dehydroisoandrosterone.

It would appear as though factors other than hormonal have an effect upon the 17-ketosteroid excretion.

Hamilton, et al. (1954) have shown that aging in men is accompanied by a decrease in the titer of androgenic activity. Most of the decline in values is due to decline in the alpha fractions (testicular

source). The beta fractions also decrease but to a lesser extent than the alpha fractions.

Another apparently extra glandular influence on 17-ketosteroid excretion is that of starvation. Landau, et al. (1948) has shown that in humans, starvation for four days produced a decrease of fifty percent in the excretion of total neutral 17-ketosteroids.

Work done by Pazos and Huggin (1945) with dogs demonstrated that androgen production by the testes is reduced during inanition. This suggested that "undernutrition" alone may often contribute to the depression of 17-ketosteroid excretion.

Attention has been directed by Selye (1946) to the effect of stress upon adrenal cortical activity in his description of the "alarm reaction" and the "adaptation syndrome". He stated that there is evidence of increased cortical activity in response to the stimulus of stress. In view of the evidence of increased cortical activity, the effect of stress on urinary 17-ketosteroids is somewhat paradoxical. An increase in cortical activity would be expected to result in an increase of 17-ketosteroid excretion.

Mason and Engstrom (1950) stated that the immediate effect of stress in previously normal individuals was to increase the amount of urinary 17-ketosteroids for one to three days. However, after this brief initial rise, the levels excreted decreased to below control values. After the stress had completely passed, the levels would then rise to normal.

Forbes, et al. (1947) substantiated this evidence by demonstrating that a rise in 17-ketosteroids followed surgery, fever, or sudden onset

of an acute illness. This was followed by a decline which reached subnormal levels.

Work done by Chou and Wang (1939) showed that chronic illness associated with malnutrition or evidence of infection, often led to depression of urinary 17-ketosteroid values.

The technique established by Zimmerman (1936) was probably the first valid chemical determination of 17-ketosteroids. It was a long involved process which required that much of the work be done in reduced light and darkness. Since then many modifications have been made on Zimmerman's original work (Callow, et al., 1938; Langstroth and Talbot, 1939; Talbot, et al., 1940; Friedgood and Whidden, 1939; Nathanson and Wilson, 1943; Consalazio and Talbott, 1940; Sloan and Lowrey, 1948; Drekter, et al., 1952). A slight modification of the Drekter method was employed in this work for the determination of 17-ketosteroids.

Most of these methods differed from the Zimmerman method in the concentrations of reagents used and the solvent used for the neutral 17-ketosteroids. The Drekter method differed from most of the given modifications in that none of the work had to be performed in either reduced light or darkness.

MATERIALS AND METHODS

The birds used for this work were from the Kansas State College poultry farm. They were a breed known as the Delaware Cross. The birds were obtained as chicks and kept in wire batteries until they were approximately six months old. By this time surgery had been completed and col-

lections of urine were made.

Many birds were used in establishing the technique of the surgery and the chemical determinations. Eight birds were used for the series of collections described in this work of which four were normal and four were adrenalectomized.

The collection of uncontaminated specimens of avian urine presented considerable difficulty because the urine of birds flow from the ureters into the cloaca where it is freely mixed with fecal matter. Previous investigators have devised a variety of methods for obtaining avian urine free from feces.

The method used in this work was suggested from work done by Hart and Essex (1942) in which an artificial anus was constructed in order to collect uncontaminated urine from the cloaca. Hart's method was modified somewhat for this work.

With the bird under pentobarbitol sodium anesthesia, an incision was made in the skin approximately half-way between the cloaca and the cartilage on the posterior end of the sternum on the ventral side of the bird. This incision was from two and one-half to five centimeters long, depending upon the deposition of fat beneath the skin in this region. Any fat which was present was removed by cutting it out in small pieces. An incision was then made in the peritoneum to the extent of the incision in the skin. By probing in the abdominal cavity, the colon was located and brought to the outside of the body through the incision. A silk thread was used to tie off the rectum as close to the cloaca as possible. A suitable instrument, usually a forceps, was inserted between the colon

and the skin to keep the colon from slipping back into the abdominal cavity. With a pair of forceps between the colon and the skin, the colon was cut only three-fourths of the way around so that if it did slip back into the abdominal cavity there would be no free end for which to search. The cut edge of the colon was sutured to the peritoneum and to the skin in one suture. The sutures were continuous around the free edge of the colon and when a sufficient amount of it was attached to the peritoneum and skin to prevent slipping back, the forceps were withdrawn and the remainder of the colon severed. The sutures were then continued completely around the colon. The portion of skin which was not sutured to the colon was then closed usually with one suture above and below the artificial anus. The length of the rectum emptying into the cloaca was cleaned out with a cotton swab.

It was absolutely necessary to keep the new anus and the surrounding area well covered with vaseline to prevent hardening and drying of the tissue before the area had healed. It was advantageous to keep this area well greased at all times after surgery even during the collection periods to prevent drying.

After the skin above and below the anus was closed, a large cotton swab which was covered with vaseline was inserted into the anus for a distance of about two centimeters and left there until it was forced out by fecal material. This usually took about twelve hours. This cotton swab served two functions. It prevented any adhesions of the free edges of the colon to each other; and it prevented drying in the colon until the area was passing fecal material freely.

Upon post mortem it was found that the area where the colon was sutured to the peritoneum and skin healed smoothly inside the abdominal cavity and no complications could be seen so far as the surgery was concerned.

After the surgery, a gauze collodion cannula of original design was attached to the skin around the cloaca for the collection of urine.

The cannulae were approximately twenty millimeters in diameter and thirty to forty millimeters long. They were constructed by applying collodion to a test tube of proper diameter and wrapping a single thickness of gauze around the area. Collodion was then applied over the gauze layer and another layer of gauze wrapped around the first. Collodion was again applied to the second gauze layer and the process was continued until the thickness of the gauze layers was approximately one millimeter. The collodion soaked cannula was slipped off the test tube and the shape maintained by the fingers. Into one end of the cannula was inserted a small plastic ring of the proper diameter and about eight millimeters long. A silk thread was used to hold this ring in place until the collodion dried. The ragged edges were trimmed and the cannula was allowed to dry for about twelve hours. A single thickness of gauze had to be used each time it was wrapped around the test tube, otherwise in the drying process the cannula would shrink and bend and not be of the correct shape for the optimum usage.

After the cannula had dried, one end was flanged outward and holes were punched in the flange to provide for the sutures. Immediately after surgery, the proximal end of the cannula was sutured to the skin around

the cloaca with continuous sutures which could be drawn up tightly to provide a watertight connection between the cannula and the skin (Plate I). The birds were allowed to carry the cannula for several days before any collection was made to allow them to become accustomed to it.

After three days the cannula was cleaned out and a suitable collecting bag was slipped over the plastic ring in the distal end. The bag was held to the cannula by means of a rubber band (Plate II).

A harness of original design made of cloth was used to support the weight of the bag and urine. This harness slipped over the bird's head with the front part resting on the breast. It contained an opening for each wing and continued over the back where it crossed and was pinned. The two flaps then passed over the tail and down beneath the cannula where they were sewn together to form a pocket into which the collecting bag was inserted (Plate III). In this way any pressure which was put on the harness by the weight of the urine was supported by the back of the bird. When the harness was not in use, that portion holding the collecting bag was drawn back up over the bird's tail and pinned to the cross on the back.

With this procedure, 24 hour collections were made and the bird was used for several collections. The cannula remained in place at all times.

After the colostomy, the birds passed fecal material of their own accord for about ten days to two weeks if they were given drinking water which contained about two percent sodium chloride. This appeared to hasten the healing process and also helped to maintain the bird. After

EXPLANATION OF PLATE I

Photograph showing the artificial anus and cannula ready for collection.

PLATE I

EXPLANATION OF PLATE II

Photograph showing the collecting bag attached to the cannula.

PLATE II

EXPLANATION OF PLATE III

Photograph showing bird wearing the saddle which supported the collecting bag.

PLATE III

two weeks the muscle tonus of the colon began to decrease and the fecal material was passed with difficulty. The birds could be used for another week or ten days only if the colon was flushed out every day using a large rubber bulb pipette.

The urine was collected for 24 hours. When the urine of birds leaves the ureters and enters the cloaca, the uric acid precipitates. This precipitate was passed on into the collecting bag.

Whether the analysis should be made with the urine specimen emulsified so that the uric acid was evenly distributed, or to allow the precipitate to settle out before removing the samples had to be determined. It was not known whether the presence or absence of the uric acid would affect the analysis of the 17-ketosteroids. In order to determine this, five separate collections were made from each of three normal birds. In each case the urine was mixed well in a Waring Blender and two samples were removed. The urine was then allowed to settle for about twenty minutes until most of the uric acid had reached the bottom of the container. Two more samples were taken from the same collection. All four samples were analyzed and the difference between the readings was not considered great enough to indicate that the distribution of uric acid was a factor which would affect the level of 17-ketosteroid determination. The readings obtained were not converted to 17-ketosteroid values since the similarity of readings was all that was being considered.

To facilitate pipetting, the samples for all the remaining determinations were removed after the uric acid had been allowed to settle.

The adrenalectomy of the birds was performed after the method des-

cribed by Herrick and Torstveit (1938). In that method, the adrenal, because of its extremely close association with the post caval vein, was destroyed by cauterization rather than the usual procedure of removing the gland used in other animals.

The adrenal gland on one side was destroyed several weeks before the second adrenal was extirpated. This wait of about seven days after the second adrenal was removed gave ample opportunity to determine whether or not the bird had been successfully adrenalectomized. The criteria used for this determination was the regression of the comb. If the adrenalectomy had been complete the comb would pale noticeably within three days, and within four more days the edges of the comb would turn black and become hard and dry. During the waiting period the birds were given access to sufficient amounts of sodium chloride which were present in the drinking water. The wait of about a week after the second adrenal was destroyed also gave the bird sufficient time to recover from the adrenalectomy before the colostomy was attempted.

The colostomy was performed about three days before the first collection. This provided ample time for the recovery from surgery and also allowed the bird to become accustomed to the cannula.

From each of the four normal birds at least three collections were made. From each collection at least three samples were taken. The collections were made on three successive days whenever possible. This resulted in a minimum of nine determinations from each bird over three successive days. A total of thirty-six determinations resulted from the normal birds. The same procedure was followed for the adrenalectomized birds.

The technique used for the determination of the urinary 17-ketosteroids was the Drekter, et al., (1952) modification of the Zimmerman (1936) reaction. The Zimmerman determination was probably the first valid chemical determination for 17-ketosteroids. It was based upon a colorimetric reaction. The Drekter modification, also a colorimetric determination, differs from the original Zimmerman primarily in the concentration of the reagents used, the solvent for the 17-ketosteroid, and the fact that much of the Zimmerman determination had to be done in darkness; whereas the Drekter method can be done in the light.

The Drekter modification was followed closely in this work, but because of the character of the photoelectrometer used, some modifications were made. A discussion of the modifications will follow.

After the quantity of the twenty-four hour collection of urine was measured, three 10 ml samples were quantitatively pipetted into 30 ml . ground glass stoppered pyrex bottles (Aloe & Co.). Along with these samples of urine, a method blank was prepared by substituting 10 ml of triple distilled water. This method blank was treated exactly as the urine samples during the entire procedure. For hydrolysis, 3 ml of concentrated hydrochloric acid were added to each sample of the method blank. They were placed in a water bath at 100 degrees centigrade for ten minutes, after which they were removed and allowed to cool. Ten ml of ethylene dichloride were added. The bottles were stoppered and the mixture was then centrifuged for five minutes. The top aqueous layer was aspirated. The material was then filtered through Watman #1 filter paper. Approximately 20 pellets of sodium hydroxide C.P. were added

to the filtrate and it was shaken for fifteen minutes. It was filtered again through Watman #1 filter paper. Theoretically the filtrate now contained only the neutral ketosteroids in the ethylene dichloride solution. An exact 4 ml aliquot of the solution was pipetted into the small vials which were used in the Fischer photoelectrometer. At this point a reading was taken in the photoelectrometer using triple distilled water set at 100 percent transmission using a green filter of 525u. This reading was to determine the amount, if any, of chromogenic material which was inherently present in the urine and could not be removed. This reading was known as the urine blank. The extract was dried in a water bath at 100 degrees centigrade until no trace of the solution remained.

According to the Drekter method, a solution containing a known concentration of the standard, dehydroisoandrosterone, should also be dried in this water bath. (This step will be further discussed).

The tubes were removed, cooled, and into each was placed .8 ml of one percent of purified metadinitrobenzene. The tubes were rotated to dissolve the dried material. To each tube was added .6 ml of 8N potassium hydroxide spaced at three minute intervals. After exactly twenty five minutes, 4 ml of 75 percent ethyl alcohol were added to stop color development. The exterior of the tubes were cleaned with ether. Readings were then obtained from the unknown extract using the same tube of triple distilled water set at 100 percent transmission that was used for reading the urine blank. The green filter of 525u was used.

The metadinitrobenzene used had been previously crystallized in alcohol and dried. Into each of a series of dry test tubes was placed .1 gm. In this way it could be dissolved in 100 percent ethyl alcohol as

needed and thus provided a fresh solution for each usage. The 8N potassium hydroxide was checked by titration against a standard acid. The absolute ethyl alcohol was purified according to the method of Drekter to remove interfering substances which might produce a false positive Zimmerman reaction.

The technique used in this work differed somewhat from the Drekter method. According to the Drekter method, the method blank should be set at 100 percent transmission and the unknowns read against it. However, because of the character of the photoelectrometer, the method blank could not be set at 100 percent transmission. Instead, the same sealed tube of triple distilled water was set at 100 percent transmission. The method blank was read against it in the same manner as the unknowns; and its reading subtracted from the unknowns.

The Drekter method makes no provision for a urine blank to account for the inherent color in the urine. A urine blank was introduced in this work.

Instead of drying a solution of the standard, dehydroisoandrosterone, along with each series of unknowns and reading the standard known against the Zimmerman blank (metadinitrobenzene and 8N potassium hydroxide), the technique used in this work was modified slightly.

The readings of sixteen separate samples of exactly the same concentration of standard were determined and a mean was calculated. In this way, each reading for a given concentration of the standard was based on sixteen separate determinations instead of only one. This was done for three different concentrations. If the reading determined from an unknown fell between the readings which were determined from the stan-

dard, the readings from the unknown were interpolated between the standard readings and the exact concentration on the unknown was calculated.

Because the Zimmerman blank could not be set at 100 percent transmission in the photoelectrometer used, the same tube of triple distilled water was again used. It was set at 100 percent transmission and the reading for the Zimmerman blank was determined in the same way as the reading for the known standards. Since the reading obtained from the blank indicates the color development only from the Zimmerman reagents, and the higher reading of the standard solution indicates the color development from both the Zimmerman reagents and the 17-ketosteroids, the reading obtained from the Zimmerman blank was simply subtracted from the reading obtained from the known. The result was the value from the 17-ketosteroids only.

It was determined that urine analyzed after storage by freezing did not give the same results as analysis from fresh urine. A more satisfactory method of storage was found when the filtrate obtained after the first filtration was refrigerated. It could be kept without change for as long as two weeks. This filtrate theoretically, contained only neutral 17-ketosteroids in an ethylene dichloride solution.

The level of 17-ketosteroids was determined on the basis of 10 ml of urine. These values were not converted to the amount present in a twenty-four hour sample, since the primary purpose of this work was to ascertain the relative contribution of the 17-ketosteroids from the adrenal glands.

RESULTS AND CONCLUSIONS

It has been found that those birds successfully adrenalectomized had a urinary 17-ketosteroid titer of less than one-half the amount excreted by normal birds. With the exception of one bird, (bird E, Table 2) the adrenalectomized birds excreted an average of 43 percent of the amount of 17-ketosteroids excreted by the normal birds. Bird E, first thought to be completely adrenalectomized, revealed upon autopsy, what was believed to be a remnant of adrenal material. Due to laboratory accident this tissue was lost, and histological examination could not be made. However the 17-ketosteroid values for this bird were twice the values obtained for any of the three adrenalectomized birds, and also fell into the higher range of values obtained from normal birds, (Bird B, Table 1). Because of the high values obtained from Bird E, it was presumed that this bird was not completely adrenalectomized.

The values for each of the three samples from each collection are given in Tables 1 and 2. These values are photoelectrometer readings which were derived after the method blank reading had been subtracted from the reading of the unknown extract. The variation in these readings was due to error inherent in the method and to the human error involved in pipetting exactly the same amount of urine and reagents each time. This variation will be discussed further.

The average reading per collection was determined, and converted to milligrams of 17-ketosteroids expressed as dehydroisoandrosterone. This conversion was based upon the readings obtained from the different

concentrations of known standard (Table 3). The variation in the values from collection to collection was due to the difference in 17-ketosteroid excretion from day to day within the same bird. This variation will also be discussed further.

Table 1. Data obtained from normal birds.

Collection and Date	Values from 17-KS per 10 cc urine	Average value per collection from 10 cc urine	Average value per bird	Mg 17-KS per 10 cc urine expressed as Dehydroisoandrosterone	Average Mg 17-KS per bird from 10 cc urine expressed as Dehydroisoandrosterone
	Bird A				
I 11-17-54	U1 = 11.1 U2 = 10.1 U3 = 11.2	10.8		.0098	
II 11-18-54	U1 = 10.2 U2 = 10.4	10.3	10.5	.0092	.0094
III 11-19-54	U1 = 10.6 U2 = 10.5 U3 = 10.2	10.4		.0093	
	Bird B				
I 12-1-54	U1 = 7.9 U2 = 8.8 U3 = 8.4	8.4		.0078	
II 12-2-54	U1 = 7.8 U2 = 8.3 U3 = 8.3	8.1	8.3	.0074	.0075
III 12-5-54	U1 = 8.1 U2 = 8.5 U3 = 8.4	8.3		.0075	
	Bird C				
I 12-8-54	U1 = 9.3 U2 = 10.1 U3 = 10.1 U4 = 9.1	9.4		.0085	
II 12-9-54	U1 = 8.5 U2 = 8.5 U3 = 8.5 U4 = 8.5	8.5	9.0	.0077	.0081

Table 1. (Concl.)

Collection and Date	Values from 17-KS per 10 cc urine	Average value per collection from 10 cc urine	Average value per bird	Mg 17-KS per 10 cc urine expressed as Dehydroisosterone	Average Mg 17-KS per bird from 10 cc urine expressed as Dehydroisosterone
		(Bird C)			
III 12-10-54	U1 = 9.3 U2 = 9.1 U3 = 9.0 U4 = 8.7	9.0		.0081	
		Bird D			
I 12-11-54	U1 = 9.6 U2 = 10.2 U3 = 9.4	9.7		.0087	
II 12-12-54	U1 = 9.4 U2 = 9.1 U3 = 8.9	9.1	9.4	.0082	.0085
III 12-13-54	U1 = 9.7 U2 = 9.4 U3 = 9.3	9.5		.0086	

Table 2. Data obtained from adrenalectomized birds.

Collection and Date	Values from 17-KS per 10 cc urine	Average value per collection from 10 cc urine	Average value per bird	Mg 17-KS per 10 cc urine expressed as Dehydroisosterone	Average Mg 17-KS per bird from 10 cc urine expressed as Dehydroisosterone
		Bird E			
I 12-14-54	U1 = 10.0 U2 = 8.5 U3 = 9.5	9.3		.0084	
II 12-15-54	U1 = 7.0 U2 = 6.5 U3 = 6.9	6.8	8.3	.0063	.0075
III 12-16-54	U1 = 8.8 U2 = 8.7 U3 = 8.5	8.7		.0079	

Table 2. (Concl.)

Collection and Date	Values from 17-KS per 10 cc urine	Average Value per collection from 10 cc urine	Average value per bird	Mg 17-KS per 10 cc urine expressed as Dehydroisosterone	Average Mg 17-KS per bird from 10 cc urine expressed as Dehydroisosterone
		Bird F			
I 1-11-55	U1 = 4.0 U2 = 3.8 U3 = 3.5	3.8		.0037	
II 1-12-55	U1 = 3.8 U2 = 3.5 U3 = 3.2	3.5	3.7	.0034	.0036
III 1-13-55	U1 = 4.2 U2 = 3.6 U3 = 4.0	3.9		.0037	
		Bird G			
I 1-23-55	U1 = 4.9 U2 = 5.7 U3 = 4.8	5.1		.0049	
II 1-24-55	U1 = 2.6 U2 = 2.1 U3 = 2.6	2.4	3.7	.0023	.0036
III 1-25-55	U1 = 3.7 U2 = 4.0 U3 = 3.7	3.8		.0037	
		Bird H			
I 2-8-55	U1 = 3.9 U2 = 3.3 U3 = 3.6	3.6		.0035	
II 2-9-55	U1 = 4.6 U2 = 3.5 U3 = 3.9	4.0		.0038	
III 2-10-55	U1 = 4.1 U2 = 3.3 U3 = 3.9	3.8	3.6	.0037	.0035
IV 2-15-55	U1 = 3.5 U2 = 3.0 U3 = 3.0	3.2		.0031	
V 2-16-55	U1 = 3.5 U2 = 3.5 U3 = 3.5	3.5		.0034	

Table 3. Standardization.
Results obtained from the different concentrations of standard dehydroisoandrosterone.

Concentration	Readings	Average Reading	Reading due to 17-KS Zimmerman minus std. reading	Maximum deviation from mean	Range
	18.3 16.9				
Zimmerman	16.8 16.9				
Blank	16.6 17.1				
	17.0 16.7	16.8		1.45	2.2
	16.5 16.9				
	16.4 17.2				
	16.1 16.8				
	16.6 16.8				
	23.5 23.0				
	20.5 22.9				
	22.9 22.6				
.1 ml	22.7 22.5	22.1	5.3	1.40	2.8
	22.6 21.9				
.0051 mg	21.5 21.9				
	21.5 21.5				
	21.5 20.7				
	29.8 29.7				
	29.8 29.5				
	28.5 28.8				
.2 ml	28.2 28.4	28.1	11.5	1.50	2.7
	27.5 28.1				
.0102 mg	27.5 27.5				
	27.5 27.1				
	27.3 27.1				
	36.0 35.9				
	36.7 35.9				
	35.4 35.6				
.3 ml	35.0 34.8	35.1	18.3	.60	1.5
	34.8 34.8				
.0153 mg	35.0 34.7				
	34.5 34.6				
	34.5 34.5				

An average value was obtained for each bird from the values obtained from each collection. The value per bird was converted to milligrams of 17-ketosteroids (expressed as dehydroisoandrosterone) using the same table of known standard values (Table 3). The variation from bird to bird was due to the individual variation of each bird. This variation too will be further discussed.

From the milligrams of 17-ketosteroids determined per bird, the average amount was calculated for all the normal birds. This amount was found to be .0084 mg of 17-ketosteroids per 10 cc of urine, expressed as dehydroisoandrosterone.

It was found that the amount of 17-ketosteroids from the adrenalectomized bird F (Table 2) was .0036 mg per 10 cc urine. This is 43 percent of the amount of 17-ketosteroids established for the normal birds. The same condition is true for the adrenalectomized bird G, (Table 2). The 17-ketosteroid titer for bird H (Table 2) which was based on five collections instead of three, was found to be .0035 mg per 10 cc of urine. This was equivalent to 42 percent of the titer determined for the normal birds.

Thus it can be seen, discounting bird E (Table 2) because of its abnormal results, that the 17-ketosteroid titer in the urine of adrenalectomized birds is approximately 42.5 percent of that found in normal birds.

Upon statistical analysis of the results, all collections from all birds were considered, but only three determinations were used in those collections containing more than three samples.

It was of interest to note that while the mean squares of the variance from bird to bird and from collection to collection was greater for the adrenalectomized birds, the mean squares of the variance from sample to sample were comparable between the two groups.

The variation attributable to each of the sources was computed by the method of variance components. The estimates obtain were as follows:

S^2d (determinations)	.106 (Normal)	.138 (Adrenalectomized)
S^2c (collections)	.119 "	.711 "
S^2b (bird to bird)	.796 "	11.162 "

From these results it could be seen that the contribution of variation from bird to bird was about seven times as great as that due from collection to collection, and that from determinations in the normal birds. The differences in the adrenalectomized birds were even greater. The large amount of variation from bird to bird in those birds was due primarily to the effect of bird E. The variation from collection to collection in adrenalectomized birds was greater than in the normal birds. However, it was noteworthy that the amount of variation between the two groups due to the actual determinations was similar and in both cases much less than the other two sources.

In terms of the normal birds as a group, the variation of the mean was .2119, and the coefficient of variation of the mean was 4.9 percent. For adrenalectomized birds the respective values were 1.777 and 23.2. These larger values for the latter group were attributable to the effect of bird E.

The data indicated that the variability due to determinations was

relatively small when compared to the overall variation. Under these conditions it was believed possible to detect differences in 17-ketosteroid levels between birds and groups of birds with a high degree of reliability and precision with the technique used.

The greatest objection to the Zimmerman technique or its modifications has been the variance of readings obtained from two samples within the same collection. Known concentrations of standard would show wide variation in readings on different determinations of the same standard. Although that situation existed to a certain extent in this work, it was believed that much of the variation was decreased by using the same tube of triple distilled water against which all the readings were taken. This provided a blank which did not change from determination to determination as did the Zimmerman blank and the method blank, against which, according to the Drekter method, the known and unknown respectively should have been read.

The use of only one blank to be set at 100 percent transmission also facilitated a minimum of handling of the blank. In this work, this was found to be a significant factor in causing a variety of readings from the same concentration.

The use of the urine blank in each determination to account for inherent chromogenic material was also believed to be a factor in bringing about a similarity of readings from the same material.

From a search of the literature, it is believed that this is the first determination of 17-ketosteroids in birds. The results are in agreement with many of the reports in mammals; that the adrenal cortex provides a definite contribution to the urinary 17-ketosteroids.

SUMMARY

1. Successfully adrenalectomized birds excrete approximately 42.5 percent of the amount of 17-ketosteroids excreted by normal birds.

2. This is in agreement with much of the work done with the 17-ketosteroid levels in mammals.

3. It is believed that the use of a urine blank such as was introduced in this work tended to reduce the error caused by non-steroid chromogenic substances inherent in the urine.

4. The use of a number of samples of the same concentration of standard gave a more valid standard value.

5. The use of the same single blank of triple distilled water set at 100 percent transmission, against which all readings were made, reduced the error due to blank differences. This procedure also reduced the amount of handling of the blanks which was found to be a significant factor contributing to error in the method.

6. It is believed that this work was the first 17-ketosteroid determinations made in birds.

7. The use of the described cannula and saddle holding the collecting bag made possible satisfactory twenty-four hour urine collections.

8. By using the procedure described to perform the colostomy, the birds passed fecal material satisfactorily for an ample length of time to make the necessary urine collections.

ACKNOWLEDGMENTS

The author gratefully wishes to acknowledge Dr. E. H. Herrick for his advice and criticism during this study, for performing the surgery pertinent to the problem, and for the design and construction of specialized equipment necessary in this work; Mr. R. R. Baron for his assistance during the surgery; Dr. W. S. Ruliffson for his advice concerning the chemical determinations; and The Schering Corporation for supplying the dehydroisoandrosterone used in this study.

BIBLIOGRAPHY

Callow, N. H.
The isolation of two transformation products of testosterone from urine. Biochem. J. 33:559, 1939.

Callow, N. H. and R. K. Callow.
Excretion of androgen by eunuchs: isolation of 17 KS from urine. Biochem. J. 34:276, 1940.

Callow, N. H., R. K. Callow and C. W. Emmens.
Chlorimetric determination of substances containing the grouping $-CH_2-CO-$ in urine extracts as an indication of androgen content. Biochem. J. 32:1312, 1938.

Chou, C. Y. and C. W. Wang.
Excretion of male sex hormone in health and disease. Chin. J. Physio. 14:151. 1939. Original not seen. Discussion in J. Clin. Endocrinol. 8 (2), 1948.

Conn, J. W., L. H. Lewis and S. S. Fajans.
The probability that compound F (17 hydroxycorticosterone) is the hormone produced by the normal human adrenal cortex. Science 113:713, 1951.

Consolazio, W. V. and J. H. Talbott.
The extraction and determination of 17 KS in urine. Endocrinol. 27 (3):355, 1940.

Corey, E. L. and S. W. Britton.
The induction of precocious sexual maturity by cortico-adrenal extract. Amer. J. Physiol. 99:33, 1932.

Cuyler, W. K., D. V. Herst, J. M. Powers and E. C. Hamblen.
Effects of desoxycorticosterone acetate on 17-KS and pregnanediole excretions. J. Clin. Endocrinol. 2:373, 1942.

Dingemanse, E., L. G. Huis in't Veld and S. Lore Hartogh-Katz.
Clinical method for the chromatographic colorimetric determination of urinary 17 KS. II. Normal adults. J. Clin. Endocrinol. 12 (1) Jan. 1952.

Dorfman, R. I., B. N. Horwitt, R. A. Shipley, W. R. Fish and W. E. Abbot.
Metabolism of the steroid hormones: studies of 17 KS and androgens. Endocrinol. 41:470, 1947.

Drekter, I. J., A. Heisler, G. R. Scism, S. Stern, S. Pearson and T. H. McGavack.
Urinary 17-KS: Pigment free extracts and simplified procedure for estimation. Endocrinol. 12 (1): 55, Jan. 1952.

Eaton, A. G., W. M. Insko, G. P. Thompson and F. E. Chidester.
The influence of suprarenal cortex and medulla on the growth and maturity of young (White Leghorn) chicks. Amer. J. Physiol. 88: 187, 1929.

Engstrom, W. W. and H. L. Mason.
A study of the colorimetric assay of urinary 17 KS. Endocrinol. 33(4):229, Oct. 1943.

Forbes, A. P., E. C. Donaldson, E. C. Reifenstein and F. Albright.
The effect of trauma and disease on the urinary 17KS excretion in man. J. Clin. Endocrinol. 7:264, 1947.

Forsham, P. H., G. W. Thorn, F. T. G. Prunty and A. G. Hills.
Clinical studies with pituitary adrenocorticotropin. J. Clin. Endocrinol. 8 (1), 1948.

Fraser, R. W., A. P. Forbes, F. Albright, H. Sulkowitch and E. C. Reifenstein.
Colorimetric assay and 17-KS in urine. J. Clin. Endocrinol. 1 (3): 234, March 1941.

Friedgood, H. B. and H. L. Whidden.
Determination of crystalline androsterone with the Evelyn Photoelectric Colorimeter. Endocrinol. 25:919, 1939.

Grollman, A. and E. Howard.
The effect of adrenal cortex extract on the reproductive system. Amer. J. Physiol. 105:42, 1933.

Hamilton, J. B., H. B. Hamilton and G. E. Mestler.
Aging in normal men - androgenic activity of urinary KS and of their alpha and beta fraction. J. Clin. Endocrinol. 14 (2), Feb. 1954.

Hart, W. M. and H. E. Essex.
Water metabolism of the chicken (Gallus domesticus) with special reference to the role of the cloaca. Amer. J. Physiol. 136, March - July 1942.

Herrick, E. H. and O. Torstveit.
Some effects of Adrenalectomy in Fowls. Endocrinol. 22 (4): 469, 1938.

Homburger, F., J. C. Abels and N. F. Young.
Observation on a normal young woman given synthetic 11 dehydrocorticosterone acetate. Am. J. Med. 4:163, 1948.

Hoskins, R. G.
The tides of life. New York: Norton and Co., 1933, pp. 25-63.

Jaap, R. G. and H. Robertson.
The chick comb response to androgen in inbred brown leghorns. Endocrinol. 53 (5), 1953.

Kupperman, H. S., R. Finkler and J. Burger.
Quantitative effects of cortisone upon KS excretion and clinical picture of adrenogenital syndrome. J. Clin. Endocrinol. 13 (9) Sept., 1953.

Landau, R. L., K. Knowlton, D. Anderson, M. B. Brandt and A. T. Kenyon.
The effect of starvation on urinary 17 KS excretion. J. Clin. Endocrinol. 8 (2):133, 1948.

Langstroth, G. O. and N. B. Talbott.
The absorption spectra of the compounds formed by androsterone and testosterone in the m Dinitrobenzene reaction. J. Biol. Chem. 128: 759, 1939.

Lieberman, S., K. Dobriner, B. R. Hill, L. F. Fieser and C. P. Rhoades.
Studies in steroid metabolism. II. Identification and characterization of KS isolated from urine of healthy and diseased persons. J. Biol. Chem. 172:263, 1948.

Lieberman, S. K., L. B. Hariton and K. Dobriner.
Alteration in steroid excretion after administration of ACTH in man. Fed. Proc. 9:196, March, 1950.

Mason, H. L. and W. W. Engstrom.
The 17-KS: Their origin, determination, and significance. Physiol. Review. 30:321, 1950.

Mason, H. L. and E. J. Kepler.
Urinary steroids isolated after administration of dehydroisoandrosterone to human subjects. J. Biol. Chem. 167:73, 1947.

McCullagh, J., K. Perry, F. Schneider, H. Bowman and E. Smith.
Adrenal and testicular deficiency. A Comparison based on similarities in androgen deficiency, androgen and 17-ketosteroid excretion and on differences in their effects upon pituitary activity. J. Clin. Endocrinol. 8:275, 1948.

McKinley, E. B. and N. F. Fisher.
Effects obtained from feeding fresh adrenal cortex, medulla, and whole gland to the standard white rat. Am. J. Physiol. 76:268, 1926.

Miller, A. M. and R. I. Dorfman.
Metabolism of the steroid hormones: The conversion of delta (5) androstenediol - 3(beta), 17(alpha) to dehydroisoandrosterone. Endocrinol. 37:217, 1945.

Munson, P. L., F. C. Goetz, J. C. Laidlaw, J. H. Harrison and C. W. Thorne.
Effect of adrenocortical steroids on androgen excretion by adrenalectomized, orchidectomized men. J. Clin. Endocrinol. 14 (5), May, 1954.

Nathanson, I. T. and H. Wilson.
Factors affecting colorimetric urinary 17-KS determination. Endocrinol. 33 (4):189, Oct., 1943.

Paschkis, K. E,, A. E. Rakoff,and A. Cantarow.
Clinical Endocrinology. New York: Paul B. Hoeber, Inc., 1954.

Pazos, R. and C. Huggins.
Effect of androgen of prostate in starvation. Endocrinol. 36: 416, 1945.

Polley, H. H. and H. L. Mason.
Rheumatoid arthritis: effects of certain steroids other than cortisone and some of the adrenal cortex extracts. J. A. M. A. 143: 1474, 1950.

Reichstein, T. and C. W. Shoppee.
Vitamins and hormones. New York: Academic Press Inc., 1943. Vol. I, p. 345.

Salter, W. T., R. L. Cahen and T. S. Sappington.
Urinary 17-ketosteroids in metabolism. II. Partition of gonadal and adrenocortical hormonal derivatives of normal, endocrine and cancerous patients. J. Clin. Endocrinol. 6:52, 1946.

Samuels, L. T., C. McCauley and D. M. Sellers.
Metabolism of steroids by tissue. I. The enzymatic destruction of testosterone by liver tissue. J. Biol. Chem. 163:477, 1947.

Sandburg, A. A., D. H. Nelson, E. M. Glenn, F. H. Taylor and L. T. Samuels.
17 hydroxy corticosteroids of 17 KS in human urine by beta glucuronidase hydrolysis. J. Clin. Endocrinol. 13 (12):1445, 1952.

Sayers, G., T. W. Burns, F. H. Tyler, B. V. Jager, T. B. Schwartz, E. L. Smith, L. T. Samuels and H. W. Davenport.
Metabolic actions and fate of intravenously administered adrenocorticotropic hormone in man. J. Clin. Endocrinol. 9:593, 1949.

Selye, H.
The general adaptation syndrome and the disease of adaptation. J. Clin. Endocrinol. 6(2):117, 1946.

Sloan, C. H. and O. H. Lowrey.
The elimination of common error in the determination of total 17KS by the m-dinitrobenzene reaction. Endocrinol. 48:384, 1948.

Sokol, J. E. and B. K. Bondy.
Effect of cortisone on 17-ketosteroid excretion of cancer patients. Yale. J. Biol. and Med. 26:345, April, 1954.

Sprague, R. G. , M. H. Power, H. L. Mason, A. Albert, D. R. Mathieson, P. S. Hench, E. C. Kendall and C. H. Slocum.
Observations on the physiologic effects of cortisone and ACTH in man. Arch. Int. Med. 85:199, 1950.

Sprague, R. G., M. H. Power, H. L. Mason and H. E. Cluxton.
Physiological effects of cortisone and pituitary adrenocorticotropic hormones (ACTH) in man. J. A. M. A. 144:1341, Dec., 1950.

Talbot, N. B., A. M. Butler and E. MacLachlan.
The colorimetric assay of total, alpha and beta - 17-ketosteroids in extracts of human urine. J. Biol. Chem. 132:595, 1940.

Turner, C. D.
General Endocrinology. Philadelphia: W. B. Saunders Company, 1948.

Vincent, A.
Recent views as to the function of the adrenal bodies. Endocrinol. 1:140, 1917.

Wilkens, L.
The diagnosis of the adrenogenital syndrome and its treatment with cortisone. J. Pediat. 41:860, 1952.

Zarrow, M. X., P. Munson and W. T. Salter.
A comparison of Androgens determined biologically and 17-KS determined chemically in urine. J. Clin. Endocrinol. 10 (7):692, 1950.

Zimmerman, W.
Colorimetrische bestimmung der Keimdrüsenhormon. Ztschr. Physiol. Chem. 245:47, 1936.

COMPARATIVE LEVELS OF 17-KETOSTEROIDS IN NORMAL AND ADRENALECTOMIZED FOWLS

by

PAUL FREDRICK BRANDE

B. A., Augustana College, Sioux Falls, South Dakota, 1953

AN ABSTRACT

of

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Zoology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1955

It has been generally accepted that the adrenal cortex possesses androgenic properties. However the exact nature of this action is not known. Since those compounds which are androgenic in nature are all characterized by a ketone group on the C_{17} position of the steroid nucleus, they can be measured by colorimetric methods.

In order to study the amount of androgen-like compounds contributed by the adrenal cortex, the 17-ketosteroid levels were determined in the urine of normal and adrenalectomized birds.

The collection of urine from birds presents considerable difficulty since the urine flows from the ureters directly into the cloaca where it is freely mixed with fecal matter. In order to collect uncontaminated urine, an artificial anus was constructed which brought the feces to the exterior without entering the cloaca. In this way, uncontaminated urine was collected from the cloaca.

In order to facilitate the collection of urine, a gauze collodion cannula was used. This cannula was prepared by wrapping gauze around a test tube of the proper diameter and saturating it with collodion. After drying for twelve hours, the cannula was sutured to the skin around the cloaca. The birds carried the cannula for several days before any collections were made. A suitable collecting bag was attached to the cannula. The collecting bag was supported by a harness which the bird wore over his neck and back, with a pocket to carry the weight of the collecting bag containing the urine. Twenty-four hour collections were made, and the bird was used for several collections since there was no pressure on the cannula.

After the colostomy the birds passed fecal material freely for about ten days to two weeks if they were given drinking water which contained about two percent sodium chloride. This appeared to hasten the healing process and also helped to maintain the bird.

The adrenalectomy was performed after the method of Herrick and Torstveit (1938). In that method the adrenal, because of its extremely close association with the post caval vein was destroyed by cauterization rather than the usual procedure used in other animals of removing the gland.

The adrenal gland on one side was destroyed several weeks before the second adrenal was extirpated. A wait of about seven days after the second adrenal was removed gave ample opportunity to determine whether or not the bird had been successfully adrenalectomized. If successful, the comb would pale noticeably within three days, and within four more days, the edge of the comb would turn black and become hard and dry.

From each bird at least three collections were made, and from each collection at least three samples were taken. The collections were made on three successive days whenever possible.

The technique used for the determination of the urinary 17-ketosteroids was the Drekter, et al. (1952) modification of the Zimmerman reaction. It is based on the formation of a color complex between the 17 keto group and the Zimmerman reagents (1 percent metadinitrobenzene and 8N potassium hydroxide). Because of the character of the photoelectrometer used, several modifications were made on the Drekter method.

Instead of using the Zimmerman blank and method blank set at 100

percent transmission, against which the known standard and the unknown respectively should be read, a single sealed tube of triple distilled water was set at 100 percent transmission and all the readings were made against it. This provided a blank which did not change from determination to determination.

According to the Drekter method, a known concentration of standard should be run with each series of unknowns. Instead, sixteen samples of exactly the same concentration of standard were read and a mean was calculated. This provided a more valid value for each concentration of standard.

The Drekter method makes no provision to account for inherent chromogenic material in the urine extract. A urine blank was introduced in this work to compensate for that factor.

The level of 17-ketosteroids was determined on the basis of 10 ml of urine. These values were not converted to the amount present in a 24-hour sample, since the primary purpose of this work was to ascertain the relative contribution of the 17-ketosteroids from the adrenal glands.

The average value per collection was determined and converted to milligrams of 17-ketosteroids expressed as dehydroisoandrosterone. From the three collections, an average value per bird was obtained and converted to milligrams of 17-ketosteroids. From the milligrams of 17-ketosteroids per bird, the average amount was calculated for all the normal birds. This amount was found to be .0084 mg of 17-ketosteroids per 10 cc of urine expressed as dehydroisoandrosterone.

The average for all of the adrenalectomized birds was obtained in the same way. It was found that those birds successfully adrenalectomized had a urinary 17-ketosteroid titer which was 42.5 percent of the amount excreted by the normal birds.

A statistical analysis of the results was made. The variation from bird to bird was about seven times as great as that from collection to collection and from determination to determination. The variation from collection to collection in the adrenalectomized birds was greater than in the normal birds, however the amount of variation between the two groups due to the actual determinations was similar and in both cases smaller than the other sources.

The data indicated that the variability due to determinations was relatively small when compared to the overall variation. Under these conditions it was believed possible to detect differences in the 17-ketosteroid levels between birds and groups of birds with a high degree of reliability and precision with the technique used.

SUMMARY

1. It is believed this is the first determination of 17-ketosteroids in birds. The results are in agreement with many of the reports in mammals; that the adrenal cortex provides a definite contribution to the urinary 17-ketosteroids.

2. Successfully adrenalectomized birds excrete approximately 42.5

percent of the amount of 17-ketosteroids excreted by normal birds.

3. It is believed that the use of a urine blank such as was introduced in this work tended to reduce the error caused by non-steroid chromogenic substances inherent in the urine.

4. The use of a number of samples of the same concentration of standard gave a more valid standard value.

5. The use of the same single blank of triple distilled water set at 100 percent transmission, against which all readings were made, reduced the error due to blank differences. This procedure also reduced the amount of handling of the blanks which was found to be a significant factor contributing to error in the method.

6. The use of the described cannula and saddle holding the collecting bag, made possible satisfactory twenty-four hour collections.

7. By using the procedure described to perform the colostomy, the birds passed fecal material satisfactorily for an ample length of time to make the necessary urine collections.