A TECHNIQUE OF ADRENALECTOMIZING LAMBS AND A STUDY OF THE ASSOCIATED PATHOLOGICAL CHANGES

by

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INTRODUCTION

It was first thought that the main or essential hormone of the adrenal gland was epinephrine. This thought persisted for about 20 years and delayed greatly the study of the cortex of the gland. In 1917 it was shown that demedullation led to no serious symptoms. Ten years later it was shown that cortical extracts could maintain life in adrenalectomized animals. This discovery greatly stimulated the study of the adrenal cortex. This has led to a considerable understanding of the cortical portion of the gland.

Many workers have described technics for the removal of the adrenal glands in the common laboratory animals, such as the dog, cat, rat, and guinea pig. The literature reviewed, however, failed to show where any worker had removed the adrenals from sheep. The purpose of this study was to perfect a technic for the removal of the adrenal glands, to observe what pathological conditions accompany the operation and to find the necessary procedure to maintain the animals after the removal of the glands. This was done so that future workers would have a technic for operating on and maintaining sheep for hormonal studies, particularly those associated with the adrenal gland.

REVIEW OF LITERATURE

The early workers, who attempted adrenalectomizing animals, recorded in the literature difficulties of technic and maintaining the animals for a sufficient period of time to study a true adrenal insufficiency. The vast majority of the first animals surely died of shock, hemorrhage, or infections, for surgical technic was not sufficiently advanced to do such a delicate operation. There was then little known of asepsis. In fact the first

operations caused death when only one gland was removed (Hartman and Brownell 1949).

Later the technics for the operation became more refined. It was demonstrated that by removal of one gland at a time, with a two week interval, the animals would live longer than if both were removed at the same operation.

Elliot (1914) showed that with proper care one gland may be removed from the cat and the animal survive for an indefinite period of time. He further showed that after the removal of the second gland the cat would die in a matter of a few days. The nature of the symptoms before death were the same if both glands were removed at once or if they were removed in two phases. He noted that the symptoms shown were loss of weight, ulceration of the stomach, weakness, difficulty in walking with no paralysis of muscles until just before death. His observations were that if one gland was removed the other would hypertrophy.

Sometime after the work on cats by Elliot, Rogoff and Stewart (1926) adrenalectomized 73 dogs; 34 being males and 39 non-pregnant females. They used a two-phase operation of from one week to two months. Their technic was an extra-peritoneal lumbar operation. After the second phase of this operation, the first and most constant symptom noted was loss of appetite with gastro-intestinal irritation. Ulceration of the stomach and congestion of the pancreas, kidneys and liver were also observed. Vomiting was a common symptom in dogs. They found that after the appearance of the first symptoms the dogs would live for approximately three days. Their male dogs lived 6.9 days and their females 6.4 days. The results showed that there is very little tendency to differ in the survival time of the males and non-pregnant females. They found that the survival period was greatly

increased in pregnant females. In some of their later studies on dogs, they found that a fairly common symptom was a loss of hair. This usually occurred about eight days after the removal of the second adrenal gland.

Rogoff and Stewart (1929) in their work on the survival period of untreated adrenalectomized cats found that certain conditions were conducive to a longer survival period. These were that the quarters that the animals were housed in be well heated and that the operation be as short as possible. By far the most important of the two is the well heated quarters. They found that vomiting was not common in cats as it was in dogs and a definite period of anorexia was not present. In fact some of the cats ate up to a few hours before death. They did not find the congestion and hemorrhage that was so prominent in the dog. The survival time for the 46 cats which they operated on ran up to a maximum of 31.5 days. The average for males being 11 days and for non-pregnant females 10.75 days. Their results again showing that there was very little difference in the male and the non-pregnant female.

Britain (1931) ran a series of experiments on rats, opossums, squirrels and ground hogs on their survival time. He used a one-phase operation. In six cases where he used a two-phase operation on rats, the survival time was about a week or less. Muscular weakness was the first sign of adrenal insufficiency. This was manifested by weakness in the rear legs. These rats also showed lesions in the gastric mucosa and pancreas. In his one-phase operation on the opossum and squirrel, both showed symptoms similar to the rat, but lived for a maximum period of 33 days. In his experiments on ground hogs in the summer, it was found that after adrenalectomy they lived for a period not exceeding 10 days; however, on the same animals

operated on in winter they lived from 29 to 128 days. This was explained on the basis of their lowered metabolic rate during hibernation.

In 1933 Firor and Grollman did further work with white rats in determining the survival time of adrenalectomized animals. They found that the following factors influenced the length of time which an animal lived: (1) the specie of animal (2) the type of anesthesia and its duration (3) the age of the animal (4) the pre-operative treatment (5) the completeness of extirpation (6) and the post-operative treatment.

Reid (1933) studied the survival time and symptoms of adrenalectomized rabbits. He used 32 rabbits of the Albino Hymalayan variety. He used a two-phase operation through the lumbar region at intervals of not less than 16 days. Four died at two to three days, nine died at nine to 17 days, and 19 survived over 100 days. The animals appeared normal until two to three days before death, at which time there was a loss of appetite, weakness, depression, loss of weight and a slow and feeble heart rate. He also demonstrated that sex of the rabbit had no effect on the survival time. He also produced an adrenal insufficiency by the removal of one gland and the innervation of the other.

Using rats Sisson and March (1935) attempted to discover some of the factors making for individual differences in survival time. They found that survival time was longer in a two-phase operation. These animals were divided into six groups according to age at the time of operation beginning at 20 days of age and proceeding at 10 day intervals through 70 days of age. Daily weight records were kept for each rat beginning with the day of operation. Analysis of the data showed the following: (1) Age was a predominant factor in determining the survival period. The older they were the longer they lived. At 20 days of age and 50 grams weight, they lived 16 days. (2) Contrary

to earlier work they found that their females lived longer than their males. (3) The weight loss in the survival period was directly correlated with the length of the survival period. The more weight they lost the quicker they died.

Gaunt, et al. (1935) found that the administration of a 2.5 percent solution of sodium chloride would assist adrenalectomized rats in maintaining life. This was also true for the dog and cat, but would not indefinitely do so. He found that animals thus treated would continue to grow but at a subnormal rate.

Agate and Zwemer (1935) described a method for the removal of adrenal glands from rats. They used a one-phase operation. They used ether for anesthesia and were very careful to maintain strict asepsis. They made an incision at the angle of the last rib and the erector spinae muscle. After exposure, the gland was picked up by the surrounding tissue and removed. All glands removed were examined under the microscope. If there was any doubt that the gland was intact, the animal from which they were removed was discarded. Autopsies performed on some of the living rats showed that they had accessory cortical tissue. They also found that the rats raised in their own laboratory and maintained on a special diet containing a high salt content survived for a much longer period of time than those from outside sources. Loss of weight in adrenalectomized rats is an indication of adrenal insufficiency.

Zwemer and Truszkowski (1936) showed that not only was sodium chloride beneficial to adrenalectomized animals but that potassium was exceedingly harmful. They further demonstrated that the adrenal cortex was directly correlated to the sodium and potassium balance of the body. They found that rest had a tendency to lower the potassium content of the body. From

these experiments they concluded that a diet for adrenalectomized animals should be low in potassium and high in sodium.

Simmons and Whitehead (1937) presented a technic for the removal of the adrenal glands from guinea pigs. They used a compound urethane anesthetic at the rate of one milligram per gram of body weight subcutaneously at the back of the neck. This made the guinea pig drowsy, and ether was administered just before the skin was incised. The right adrenal was the most difficult to remove and therefore was done first. The incision was made over the last intercostal space parallel to the ribs extending from the outer border of the lumbar muscles to the midline. The penultimate rib was crushed and removed and the adrenal glands plus the kidneys were lifted through this opening. The glands were dissected loose, the adrenal veins ligated, and the adrenal glands removed.

The first complete adrenalectomy that was done on guinea pigs showed that they lived only a few hours. Later a bilateral operation in two phases enabled the guinea pigs to live up to 28 days. These two-phase operations were performed at seven day intervals. After the operation they were placed in a room of 24 degrees Centigrade over night and then returned to the animal house.

Freud, et al. (1938) described a technic for the removal of the adrenal from dogs. They used a two-phase operation. The skin was prepared by clipping and shaving and sterilizing with picric acid. The dog was anesthetized with morphine and ether. A three inch incision was made from the costo vertebral angle parallel to the last rib. A one inch incision was then made into the extra-internal oblique muscle long enough to admit one finger of each hand. This was enlarged by traction until a soft bulge of peritoneum was felt across the bottom of the wound. The peritoneum was opened and the

kidney and adrenal exposed. The large adrenal vein was dissected free and ligated. The gland was shelled free of surrounding tissue with the aid of sticks wrapped at each end with cotton. The sticks were wrapped to avoid fragmentation to the gland. The glands were thus removed. Out of the 30 dogs adrenalectomized, 20 survived longer than 48 hours and most of them lived for several months or years. Special care must be taken to avoid injury to the nerve ganglia. The authors were unable to maintain dogs after a one-stage operation. They considered skill and care to be of the utmost importance.

Herrick and Torstveit (1938) described a method of removing adrenal glands from birds. They found that the adrenals were situated so closely to the aorta and vena cava that hemorrhage was a great problem to overcome. The slightest attempt to break the gland away from its attachment caused a fatal hemorrhage. Ligation of the blood vessels seemed to be impossible. Because of this difficulty, they decided to use thermal cautery. Voltage to the tip of the wire cautery was controlled so that a dull red glow was produced. The temperature control was necessary in order to control the cutting. Birds were prepared for surgery by withholding food for 48 hours. Anesthesia was intravenous sodium pentobarbital. The body was opened between the last two ribs as far dorsally as possible. A screw controlled retractor was used to spread the ribs apart. After the gland was destroyed the ribs were pushed back together and sutured. The wounds healed in a few days. Only one gland was removed at the time and a week or longer elapsed between operations. Immediately following the removal of the second gland, one cubic centimeter of cortical extract was given intramuscular. This treatment was repeated for the next two to three days. Drinking water with 16

grams of sodium chloride per liter was available to the birds at all times. After the cortical extract was discontinued, injections of normal saline were begun, giving 12 to 15 cubic centimeters intramuscularly daily. Birds thus treated lived as long as 82 days. Mature birds usually lost a few grams after the operation but later regained their normal weight. Young birds continued to grow but not so rapidly as the controlls. If cortical hormones or sodium salts were not administered, chickens died in from six to fifteen hours. The typical symptoms of cortical insufficiency in mammals did not occur in chickens. They were quite normal until shortly before death.

Schachter and Beebe (1939) further advanced the technic for operating on guinea pigs. The pigs were fasted for 24 hours previous to the operation. Pigs were anesthetized with ether. The incision was parallel to the last rib and about an inch long. The liver and intestine were removed out of the way with sterile packs. The gland was freed from the adjacent tissue by blunt dissection, leaving only the adrenal vein. The vein was clamped with forceps for about 30 seconds, and then the gland torn free. All non-cortical treated guinea pigs died within three to four days. The operation was performed in two stages.

Hartman, et al. (1943) used a two-stage operation in adrenalectomizing opossums. They used the lumbar approach. The second gland was removed only after complete healing from the first operation. After the removal of the second gland they gave adrenal extracts for three days and kept the animals in a constant temperature room. They used 13 female opossums and found that they had a survival time of from 30 to 603 days. At autopsy no adrenal glandular tissue was found.

Bruzzone, et al (1946) did further work in perfecting the technic of removing adrenal glands in guinea pigs. Their technic was very similar to that of Schachter and Beebe.

Colburn (1952) also did work on the adrenalectomizing of guinea pigs. He used one-half cubic centimeter of cortisone daily after the second phase of the operation. He found that no matter which gland was removed first the second one was always larger.

Grollman (1952) described a procedure for maintaining bilateral adrenalectomized dogs in good condition for prolonged periods of time by intraperitoneal injections of the inorganic materials of the blood in concentrations equal to the normal blood plasma.

Golden (1954) reported a technic for the removal of adrenal glands from guinea pigs. She used a two-phase operation with an interim of at least 14 days. Her technic was similar to that of Colburn.

MATERIALS AND METHODS

The 20 lambs used in this experiment were obtained from a local farm. The weight of the lambs was from 45 to 50 pounds. They were closely selected as to age and size so as to have a uniform group. They were picked from approximately 200 to 300 lambs. The lambs were nursing at the time of purchase and, therefore, it was necessary to delay the operations until the lambs were adjusted to natural food stuffs.

A diligent search of the literature has revealed no technic reported to date for the removal of adrenal glands in sheep.

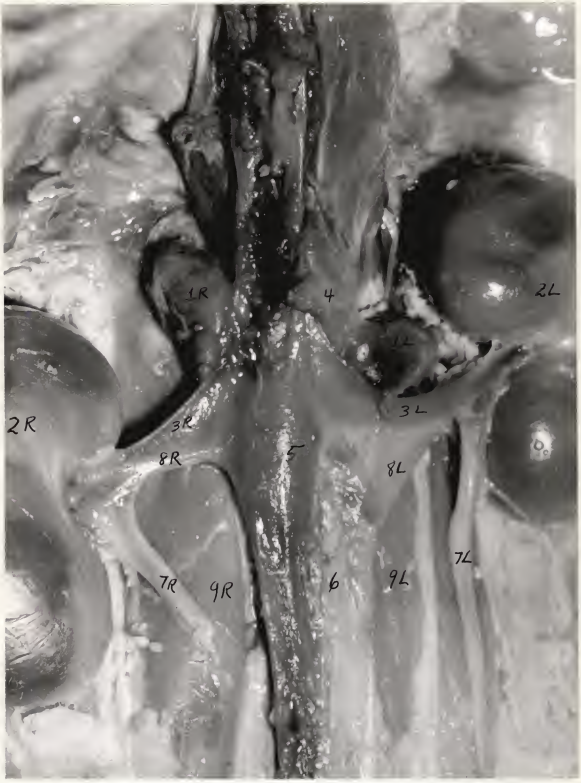
In sheep the adrenal glands are located anterior and slightly medial to the anterior end of the kidney. The relationship of these glands to other structures and organs is given in Plate I. The left gland is considerably

EXPLANATION OF PLATE I

Sublumbar region of the sheep, with liver removed, showing the adrenal glands and associated structures.

1R, 1L	-	Right and left adrenal glands
2R, 2L	-	Right and left kidneys
3R, 3L	-	Right and left renal veins
4	-	Anterior mesenteric artery
5	-	Posterior vena cava
6	-	Aorta
7R, 7L	-	Right and left ureters
8R, 8L	-	Right and left renal arteries
9R, 9L	-	Sublumbar muscles

PLATE I



further forward from the kidney than the right one. Also it must be borne in mind that the left gland is situated considerably further to the right than it would be in a non-ruminant type animal. The size of the rumen has a tendency to shove both the kidney and the gland to the right of the median plane. Previous authors have described the right adrenal gland as being the most difficult one to remove. This does not hold true in the sheep. The right one is more difficult to locate but its attachments are less extensive than the left. The right gland is attached to the body wall and the vena cava and the adjacent fatty tissues. It is located below the renal notch of the liver. Here one has difficulty in separating the tissues that buries it under the lobe of the liver. Care must be exercised when dissecting in this area for the capsule of the liver is easily damaged. The left gland has similar attachments except that it does not attach to the liver and it is attached also to the renal vein and to the anterior mesenteric artery. The arterial blood supply of both glands is from small arteries arising from the aorta. The venous attachments are by way of the renal veins to the vena cava on the right of the gland and to the large renal vein on the left gland. The nerve supply is from the coeliac and renal plexuses and is quite abundant (Teitelbaum 1942, Sisson and Grossman 1938).

Two methods for the removal of the adrenal gland were attempted. One by way of a mid-line abdominal incision, and the other by way of a flank incision.

The first animals to be operated on were used primarily to establish a technic. An effort was made to remove the gland intact. The anesthesia used was a commercial solution of sodium pentobarbital. Instruments used were the type ordinarily used for performing a laperotomy. The sheep were fasted 24 hours before the operation was performed.

The anesthesia was administered intravenously at the rate of about one grain per five pounds of body weight to effect. After the animal was completely anesthetized, it was placed on its back and the entire abdominal region was clipped. Then the area of incision on the midline was shaved and was sterilized with a skin disinfectant. About an eight inch incision was made parallel to the penis and slightly sagittal. The peritoneum was opened exposing the abdominal viscera.

Because the glands were inaccessible with the viscera over them, the intestines were rolled onto sterile trays, exposing the lumbar region. The kidneys were sited and by palpating anteriorly and medially, the adrenal glands were located. The peritoneum over the glands was incised and the adrenals exposed. By blunt dissection, they were freed from the surrounding tissue. With the aid of fine forceps, the gland was torn from its attachments. The closeness of its attachment to the vena cava made it impossible to ligate. Small pieces of surgical cellulose were placed over the area of ablation to control the hemorrhage. Both glands were removed by this technic. During the operation, the intestines were kept moist with warm sterile saline solution. The intestines were replaced in the abdominal cavity and the muscle and peritoneum sutured with catgut. The skin was closed with linen, and the incision was sprayed with a collection type skin dressing in an effort to keep down infection.

When the operation was completed the animals were given injections of penicillin daily until fully recovered. In addition to this, 25 to 75 milligrams of cortisone, depending upon the size of the animal, was given intramuscularly daily. After about three days if the animal seemed to be in good health, the cortisone was discontinued. These animals would remain in good health until about the fifth day after the operation, then there

would be a complete anorexia followed on the same day by a profuse watery diarrhea. The animal appeared depressed and continued to strain and show signs of muscular weakness, manifested by a weakness in the rear legs and a subsequent inability to stand. All of the animals bleated during attempt of defecation during the latter stages. On the sixth day, the animal died. Autopsies revealed varying degrees of inflammatory changes in the area of operation. In addition there was some degree of peritonitis. The most prominent change observed, however, was a marked engorgement of the mesenteric vessels and a severe gastro-enteritis. The survival period was from two days to six days for the animals operated upon by this method. Various efforts were undertaken to keep these animals alive in addition to cortisone therapy; such as, whole blood, glucose, and saline injections, but once symptoms developed, it seemed impossible to prevent death regardless of treatment or therapy. After operation on several sheep by this method, it was decided to attempt a different surgical technic to see if the animal could be saved after adrenalectomy.

Careful observations on the first group of sheep revealed that more emphasis had to be placed on the diet, housing and surgery. Gaunt, et.al. (1935) showed that the salt balance of an animal was very important in maintaining adrenalectomized animals. They found that a two percent solution of salt would maintain rats for a considerably longer period of time than those with a lower salt content. Zwemer and Truszkowski (1936) also showed that potassium is harmful to adrenalectomized animals. They also demonstrated that the adrenal cortex is directly related to the sodium and potassium balance of the body. It was decided that a simple diet consisting of a one percent solution of sodium chloride be used in the drinking water. Prairie

hay was selected as the feed of choice because of its low potassium content. The sheep had free access to this at all times. The grain portion was corn gluten meal at the rate of 1.5 pounds per day. The animals were kept on this diet for three weeks before the operation. The sheep were kept in an indoor stall that was well and evenly heated at all times.

Since the effects of a double adrenalectomy were so severe, it was decided that this operation would be done in two stages with an interim of at least three weeks. If there were any signs or symptoms of sickness or infection at the end of this period, the operation was further delayed. Since there was more difficulty involved in the removal of the left gland than the right, it was decided to remove the left one first. After the removal of the first gland, penicillin was administered intramuscularly at the rate of 600,000 units daily. This was to combat any infection from the operation. It was felt that no hormonal therapy at this stage of the operation was necessary. Twenty-four hours before the operation, all feed was removed from the animals, but they were permitted to drink all the salt water they desired. The operative site was in the para-lumbar or flank region. Prior to the operation an area along the side of the neck was clipped to expose the skin covering the juglar vein. Anesthesia was a commercial solution of sodium pentobarbital and was administered in the juglar vein to effect. Care was taken in administering the anesthesia, because it was easy to give an overdose. The flank area was clipped and shaved after the animal was anesthetized. The skin was sterilized as noted before. The instruments and suture materials were autoclaved for sterilization. In addition to the previously mentioned instruments, a tonsilectomy snare and a metal speculum, with a lumen of about two inches and a length

of about four inches, were used. The speculum was used to maintain the opening and act as a retractor to the intestines.

The technic of the operation was as follows: A six to eight inch incision was made through the skin beginning as close to the transverse processes of the lumbar vertabrae as possible. This was about one inch posterior to the last rib and parallel to it. The muscle layers were then incised until peritoneum was visible. The peritoneum was picked up with a small pair of forceps and opened with a pair of scissors for the length of the incision. This exposed the abdominal viscera. Hemorrhage during the operation was controlled by the use of hemostats. A speculum was inserted at this stage and the viscera pushed aside until the kidney could be seen through the speculum. The gland was visible with the aid of a strong light directed into the speculum. The operation was performed through the lumen of the speculum. The peritoneum over the gland was incised with the point of the scapel. Once exposed the gland was picked up gently with a pair of forceps. Then by careful blunt dissection with another pair of pointed forceps, the surrounding tissue was torn away from the gland until the only attachment was the adrenal vein. The tonsil snare was slipped over the forceps and gland and down around the vein. Being careful not to damage the vena cava, the tonsil snare was closed and the gland removed. This caused considerable hemorrhage, but due to the glands close attachment to the vena cava, it was impossible to do any ligating. A small piece of surgical cellulose was placed over the area of ablation to control the hemorrhage. If this procedure was unsuccessful, four or five cubic centimeters of a commercial intravenous hemostatic was given, which controlled the hemorrhage successfully. The peritoneum was sutured with number zero catgut and the musculature with number two catgut. The skin was closed with linen and covered with an antiseptic collodion dressing.

The second gland was removed in a like manner after a 14 day interval. At this time 25 milligrams of cortisone and 600,000 units of penicillin were administered intramuscularly daily. These were continued along with the diet for four or five days, then the penicillin was discontinued and the dosage of cortisone was lowered to 12.5 milligrams daily for another two days. If, after this time, the animal was eating and in good health, the cortisone was discontinued and the animal maintained only on its special diet. Otherwise both cortisone and penicillin were continued until the animal appeared normal.

DISCUSSION

This experiment was conducted on 20 castrated male sheep. It was decided that these animals all be of one sex due to the small number used. A one-phase bilateral adrenalectomy was performed on 12 of the 20 animals. Out of these 12, all but three survived for six days. Three died within 48 hours. Of the three animals that died within 48 hours following surgery, it was difficult to determine whether the cause of death was due to surgical trauma and shock or as a result of an adrenal insufficiency. Previous workers, Agate and Zwemer (1935), have reported the death of adrenalectomized rats within 24 hours as a direct result of an adrenal insufficiency; however, all workers were not in agreement with this. Kramer and Kramer (1953) contributed such an early death to what they termed "capillary crisis". However, they concluded this was caused by a cortical insufficiency. Sisson and March (1935) determined that there was a direct relationship between the survival time following adrenalectomy and the age of the animal. In this experiment it was noticed that the more mature lambs seemed to withstand the effects of adrenalectomy better than young ones. No effort was made to study this problem in any detail however.

The eight remaining sheep were operated on in two phases; all of them lived longer than those operated on in one phase. This group of eight animals contributed most to the knowledge of maintaining sheep after adrenalectomy. It was found in the first group of sheep that both glands cannot be removed successfully at one time. Other workers also found one-phase operations unsuccessful. These eight sheep lived from the time of the removal of the second gland or complete bilateral adrenalectomy for a minimum period of 19 days. They were at all times on a special diet of one percent sodium chloride in the drinking water, prairie hay and corn gluten meal.

The first of these eight animals was number 16; this was a mature castrated male sheep. At the time of the operation the animal had been on the special diet for three weeks. Using sodium pentobarbital intravenously for anesthesia and the flank approach as previously described, the left adrenal gland was removed first. Considerable difficulty was encountered in the surgery in this sheep due to the obesity of the animal. Immediately after the operation 600,000 units of penicillin only was given intramuscularly. This dosage of penicillin was given daily for about two weeks. The sheep developed a severe peritonitis and enteritis. The penicillin was continued until the sheep had recovered. Forty-nine days were allowed to lapse between the first operation and the removal of the second gland in order that the sheep could recover completely. The second operation revealed considerable adhesions of the abdominal viscera; however, the second gland was removed successfully. Immediately after the operation, the sheep received 600,000 units of penicillin and 25 milligrams of cortisone intramuscularly. The same dosage of penicillin and cortisone was given for a period of five days, then the penicillin was stopped and the cortisone

continued at one-half the above dosage for two days. Then it was stopped and the animal was maintained only on the special diet for a period of three months after the removal of the second gland. This animal was kept alive for future experimentation.

The second sheep was number 345. It was a mature castrated male sheep. The first gland removed was the left. Penicillin, 600,000 units, was given intramuscularly daily for four days after the operation. The animal completely recovered in a week as no ill effects were noted. The animal then received 600,000 units of penicillin and 25 milligrams of cortisone intramuscularly daily for five days. The penicillin was stopped and the cortisone reduced to 12.5 milligrams daily for two days, then this was discontinued and the animal maintained only on the special diet. No ill effects were noted. After 114 days the sheep was slaughtered. There were some adhesions of the intestines, but no evidence of adrenal tissue could be found.

The third sheep was number 22 and was a mature castrated male sheep. The operation and subsequent treatment and maintenance of this sheep was the same as number 345. This sheep was slaughtered 114 days after the removal of the second gland. A small piece of tissue found in the area of the adrenal gland was sectioned and identified as active adrenal tissue. This was evidently a small piece of capsule and gland left in the animal at the time of the operation.

The following sheep were all young lambs, approximately two months of age. The fourth sheep was number 2888. The left gland was removed first by the same technic previously described. Penicillin, 600,000 units, was given intramuscularly daily for three days. There were no ill effects noted

after the first operation. Thirty days after the first operation, the second gland was removed. There was extensive hemorrhage as the gland was freed from its venous attachment and it was necessary to give an intravenous hemostatic to control the hemorrhage. When the operation was completed, the lamb appeared to be in shock as the respiration was shallow, the mucous membranes pale and cyanotic and the pulse rapid and weak. One cubic centimeter of epinephrine was given subcutaneously to overcome these symptoms. Six hundred thousand units of penicillin and 25 milligrams of cortisone were given intramuscularly. The lamb did not respond too well to the treatment so the penicillin and cortisone were continued for a period of eight days. At this time the penicillin was discontinued, but the cortisone in 12 milligram doses was continued for two more days. The cortisone was discontinued and this animal remained in good health and also was kept alive for future experimentation.

The fifth and sixth animals were young castrated males. They were numbers 2886 and 2887. The left glands were removed first in these two animals. They were given 600,000 units of penicillin for four days after the operation and no illness was noted. Twenty-eight days later the right glands were removed on these same animals. There was extensive hemorrhage following the surgery on lamb 2886. This lamb was given five cubic centimeters of a hemostatic solution, one cubic centimeter of epinephrine, 600,000 units of penicillin and 25 milligrams of cortisone. The hemorrhage in lamb number 2887 was not as severe as in the previous animal. Penicillin, 600,000 units, and 25 milligrams of cortisone were administered after the surgery. Both animals received penicillin and cortisone at the above doses for a period of six days. Penicillin was then discontinued and the dosage of the cortisone

lowered to 12.5 milligrams for two more days; and then the cortisone was stopped, and the animals maintained only on the special diet previously mentioned.

Lamb number 2886 lived 43 days after the removal of the second gland and 33 days after the cortisone was discontinued. This lamb was in good health up until three days before death, at which time it stopped eating and drank only very little water. The most prominent symptoms were the complete loss of appetite and extreme depression. The lamb had no apparent interest in its surroundings and lost weight rapidly. There was extreme muscular weakness and just before death the animal was unable to stand. When the animal was autopsied, there was no evidence of adrenal tissue.

Lamb number 2887 lived 23 days after the removal of the second gland and 13 days after the cortisone was discontinued. Three days before death the animal stopped eating. When autopsied a careful examination was made of the area where the adrenal gland is normally located, but no evidence of adrenal tissue was found. There were large adhesions in the area where the abdominal wall had been opened. Several large abscesses were found in the abdominal cavity, and these were filled with a creamy greenish pus. Abscesses as large as an inch in diameter were also found in both lungs. In addition both lungs showed moderate pneumonia. It was concluded from these findings that the cause of death was a generalized septicemia.

The seventh lamb, number 2890, was a young castrated male. The same surgical technic was employed as previously described for the removal of the adrenals. There was a 19 day interval between the first and second operations. The lamb was given 600,000 units of penicillin and 25 milligrams of cortisone for four days after the operation. The penicillin was discontinued after that

date and the cortisone was lowered to 12.5 milligrams for two more days. This animal lived for 19 days after the second operation and 13 days after the cortisone was discontinued. The first symptom noted occurred three days before death. There was loss of appetite followed by weakness and depression. When autopsied the carcass showed marked emaciation and dehydration, but no other significant findings. There was no evidence of adrenal tissue in this animal.

The eighth lamb was number 2892 and was treated just as the one above but survived for the duration of the experiment.

Of the eight sheep operated on by the two-phase technic, three survived the entire time of experimentation and two were slaughtered while still apparently normal. In one of these latter two a small amount of adrenal tissue was found.

SUMMARY AND CONCLUSIONS

Considerable interest has arisen since estrogen-like substances have been added to feeds for fattening lambs. The undesirable effects have created this interest, and attempts were, therefore, undertaken to establish a technic for adrenalectomizing sheep.

The literature on adrenalectomizing laboratory animals was reviewed. The lack of a technic for adrenalectomizing sheep was noted. A series of operations were performed to establish a technic for adrenalectomy in sheep.

The first sheep were adrenalectomized completely in one operation. These animals remained alive from two to six days. Of the animals that lived for a full six days, there was adequate time to develop a true adrenal insufficiency. This was manifested by a complete loss of appetite that occurred on the fourth or fifth day. On that day or the day following, there would be a

profuse watery diarrhea that was sometimes blood tinged. These animals all exhibited extreme pain when attempting to defecate as many would bleat during the act of defecation. There was complete depression; the animal showing no interest in any of its surroundings. There were signs of muscular weakness manifested by weakness in the rear quarters; muscle tremors were present in the back and rear legs. Just before death the animal would be unable to stand. There was noted at autopsy varying degrees of inflammatory changes at the site of the operation. The most prominent lesion was a severe gastro-enteritis. Efforts to keep these animals alive consisted of whole blood, saline, glucose injections and cortisone therapy. None of these treatments prevented death once the symptoms developed.

In the second series of operations, via the paralumbar area, the left adrenal gland was removed first and after normalcy returned, the right gland was removed. A retal speculum two inches in diameter and about four inches long greatly facilitated the operation. This speculum afforded easy access to the glands as well as keeping the abdominal viscera from obliterating the area. Hemorrhage from the operation was controlled by placing surgical cellulose on the vessel or in more severe cases by intravenous administration of a hemostatic solution. Three of the eight animals died after the operation; the first in 19 days, the second in 22 days and the third in 43 days.

Before the operation these animals were put in a stall where the temperature was evenly maintained at 72 degrees Farenheit. They were placed on a regimen of one percent saline water as the only drinking water, corn gluten meal at one and one-half pounds per head per day, and free access to prairie hay. This supplied a low potassium, high sodium diet which favors survival

of adrenalectomized animals. The sheep were kept on this diet for three weeks before the operation. The left gland was the first gland removed. No ill effects were noted after this phase of the operation. Three weeks later or when the sheep had fully recovered from the effects of the first operation, the second gland was removed. The animals were given penicillin intramuscularly at the dosage rate of 600,000 units per day after the operations, and, when indicated, for a few days and longer. Cortisone at the rate of 25 milligrams per day was administered after the second operation for about six days and then gradually decreased. The animals were then maintained only on the special diet as mentioned above. Animals thus adrenalectomized lived from 19 days to an indefinite period of time. Symptoms of the animals that died were very similar to those described for the first group except that there was a longer period of anorexia in this group. The main difference in the pathology exhibited by the first and second group was the absence of gastro-enteritis in the latter.

It was demonstrated that castrated male sheep may be adrenalectomized in a two phased operation and maintained on a high sodium, low potassium diet for an indefinite time. Animals treated in this manner lived from 19 days to an indefinite time when adrenalectomized by the two-phase method. Whereas, the longest survival time from the one-phase method was six days.

A practical method of adrenalectomising and maintaining castrated male sheep was developed.

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A TECHNIQUE OF ADRENALECTOMIZING LAMBS AND A STUDY OF THE ASSOCIATED PATHOLOGICAL CHANGES

by

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This study was made to develop a technic for the removal of the adrenal glands in sheep, to observe pathological conditions that accompany the operation and to find the necessary procedures in maintaining animals for a sufficient period of time for study. This study was undertaken so that future works would have a technic for adrenalectomizing and maintaining sheep for the study of those hormones associated with the adrenal gland.

In the literature reviewed, various technics were described for the adrenalectomizing of the common laboratory animals. It was found that the cat would survive for an indefinite period of time after the removal of one adrenal gland. If the second gland was removed, a few days later the animal would die. Symptoms shown before death indicated loss of weight, difficulty in walking and a generalized weakness. Autopsy revealed ulceration of the stomach and a generalized enteritis. Clinical observations showed that after the first gland was removed the other would hypertrophy.

Sometime later a technic for the removal of the adrenal glands from dogs was described. This was done in two phases. The site of operation was in the paralumbar region. After the removal of the first gland, there was an interval of a month before the second adrenal was removed. This resulted in a slightly longer survival period. Later it was found that certain conditions were condusive to a longer survival period. These were, the quarters in which the animals were housed be well heated and the duration of the operation be as short as possible.

In a series of experiments run on opossums and squirrels where a one-phase operation was used, it was found that they survived for a maximum period of 33 days. In experiments on ground hogs in the summer, it was

found that they lived for a period not exceeding 10 days; however, when they were operated upon in the winter, they lived from 29 to 128 days. This was explained on the basis of their lowered metabolic rate during hibernation. Work done with white rats and their survival time indicated that several factors influenced the length of time an animal may live following adrenalectomy. The factors were, specie of the animal, the age of the animal, the pre-operative treatment, the duration of anesthesia, the complete adrenalectomy and the post-operative treatment. It was found that the oral administration of a two percent solution of sodium chloride would assist adrenalectomized animals in maintaining life. It was also determined that a diet high in potassium was harmful. Previous work indicated that the adrenal cortex was directly correlated to the sodium and potassium balance of the body.

Technics for the adrenalectomizing of animals are described. The operation of choice was a two-phase operation where an interim of two to four weeks was allowed between operations. The technic for the operation was to make an incision in the paralumbar region parallel to the last rib and sufficiently large enough to allow easy access to the adrenal area. The gland was dissected free and the adrenal vein ligated. The abdomen was closed and the wound treated to facilitate healing. The more suitable work was where the animals were maintained on a diet high in sodium chloride. Cortisone administered for a few days after the removal of the adrenals seemed to keep the animals living for a longer period of time after the operation.

Twenty castrated male lambs were used in this study. A number of different surgical technics were attempted to find a satisfactory method for the removal of the adrenals in sheep. Out of this study two sterile

surgical technics were developed. On the first group both adrenal glands were removed at one operation. This operation was performed through a midline incision. These animals lived from two to six days after the operation. Various treatments were used such as saline, glucose, whole blood and cortisone injections in an effort to keep these animals alive. None of these treatments proved successful. The first symptoms noted were loss of appetite, depression, weakness and a severe diarrhea. These animals showed great pain while defecating.

The second technic was attempted on the next series of sheep. They were maintained on a special diet consisting of one percent sodium chloride solution in the drinking water, prairie hay and corn gluten meal. They were kept on this diet throughout the experiment. These animals were maintained at all times in an evenly heated stall. These animals were never subjected to stress of any kind during the experiment. The operations on these animals were in two phases. The first removed was the left gland. This procedure was followed because the left gland is considerably more difficult to remove than the right. An incision was made about eight inches long through the skin and muscle in the flank parallel to the last rib. The abdominal cavity was opened by incising the peritoneum and a speculum placed in the incision. This greatly facilitated the operation in that it prevented the intestines and other abdominal viscera from occluding a clear view of the adrenal gland. The gland was dissected free by blunt dissection and removed by means of a tonsil snare. Hemorrhage was controlled at this site by surgical cellulose and an intravenous hemostatic solution. Penicillin was administered after the operation to control infection. The second gland was removed after an interval of three weeks. Cortisone and penicillin were administered for approximately five days. The penicillin was discontinued

and cortisone dosage reduced to half for two more days. The animal was then maintained only on the special diet previously mentioned. These animals lived from 19 days to an indefinite period of time. Those that died exhibited symptoms of gastro-enteritis.

It was demonstrated that castrated male sheep may be adrenalectomized in a two-phase operation and maintained on a high sodium and low potassium diet for an indefinite period of time. A practical method of adrenalectomizing and maintaining castrated male sheep was developed.