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Determination of Minimal Maintenance Levels of Adrenal Steroids in the Bilaterally Adrenalectomized Goat.* (28187)

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Few reports appear in the literature on maintenance therapy in ruminants following bilateral adrenalectomy. Differences in hormone administration routes, criteria, species and environmental conditions probably account for the different dose levels that are reported. Cowie and Stewart(1) and Cowie and Tindal(2) reported that daily absorption from implanted tablets of 6-12 mg cortisone and 2.5 mg desoxycorticosterone acetate (DCA) is sufficient for maintenance of lactation in the adrenalectomized goat. Goding and Denton(3) were able to maintain adrenalectomized sheep on daily intramuscular injections of 25 mg cortisone and 5-10 mg DCA. Estergreen and Van Demark(4) reported that 25 mg of cortisone and 5 mg DCA per 100 lb body weight per day would successfully maintain adrenalectomized calves.

Materials and methods. To evaluate properly the minimal maintenance levels of adrenal steroids required in the bilaterally adrenalectomized animal, environmental and physiological stress should be reduced to a minimum.

The animals used in this study were 3 year old female goats of a mixed dairy type breed. Adrenalectomized and intact control animals were housed in separate pens in a controlled environment chamber. A temperature of 25°C ± 0.5°C, relative humidity of 50 ± 2% and alternating 12 hours of light and darkness were maintained throughout the study, thus minimizing environmental stress.

Bilateral adrenalectomy was performed in two stages. The first stage was removal of the right adrenal gland. After a 3 week interval the second stage, removal of the left adrenal, was performed.

Adrenalectomy in the goat is complicated by the absence of adrenal veins. The right adrenal is securely attached to the vena cava and the left adrenal gland is firmly attached to the renal vein near its junction with the vena cava. Previous investigators(1,2) reported the use of special clamps which were applied between the gland and the vessel wall. This procedure involved removal of a portion of the vena caval wall and suturing the vessel. Estergreen and Van Demark(4) performed successful adrenalectomies in calves using a Merrick kidney forceps applied between the gland and the vena caval wall. Following removal of the adrenal gland the forceps were left in place for approximately 5 minutes. This eliminated the need for suturing the caval wall. The technique developed for the present study consisted of freeing the gland from surrounding adipose tissue by blunt dissection. No ligation was needed to control

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TABLE I. Values for Blood Constituents of Control and Adrenalectomized Goats on 4 Cortical Hormone Regimes.

		Na^+, meq/l	K^+, meq/l	$Na^+ : K^+$ ratio	Blood glucose, mg %	Absolute eosinophil count/mm^3
9 control animals	Mean 1st wk	146	5.40	27.04	48	378
	" 2nd "	147	5.10	28.82	54	394
	" 3rd "	142	5.00	28.40	49	286
	Total sample means	144	5.16	27.90	51	353
Treatment	Goat No.					
Regime 1						
75 mg cortisone	17	143	3.60	39.72	77	78
15 mg DCA	19	143	3.20	44.69	68	56
	21	151	4.15	36.39	52	72
	Means	146	3.65	40.26	66	78
Regime 2						
50 mg cortisone	17	154	4.00	38.50	61	72
10 mg DCA	19	153	4.15	36.87	61	67
	21	151	4.30	35.12	57	139
	Means	153	4.15	36.83	60	93
Regime 3						
25 mg cortisone	17	144	5.20	27.69	52	172
5 mg DCA	19	142	4.65	30.97	61	150
	21	140	4.80	29.17	67	144
	Means	153	4.88	29.28	60	155
Regime 4						
12.5 mg cortisone	17	142	5.50	25.82	52	184
2.5 mg DCA	19	143	5.50	26.00	61	145
	21	144	5.60	25.71	47	189
	Means	143	5.53	25.84	53	173

bleeding from the small vessels around the gland, only pressure and hemostats were occasionally used. Once the gland was freed, except for the connections to the vena cava or renal vein, by carefully teasing the capsule away from the vessel, the gland was removed with minimal hemorrhage. The bleeding which did occur was controlled by immediate application of Oxycel® and gauze sponges. These were left for approximately 5 minutes. The sponges were then carefully removed leaving the Oxycel® in place and closure of the wound followed. Surgical preparation included an initial 0.5 mg/kg intramuscular injection of atropine sulfate which was continued during surgery at a dose level of 0.2 mg/kg at approximately one-half hour intervals. Anesthesia consisted of an initial intravenous dose of 20 mg/kg of thiopental sodium. A polyethylene 190 catheter inserted in the external jugular vein also allowed administration of thiopental during surgery and provided instantaneous control of anesthesia in emergencies. The desired level of anesthesia was maintained during surgery by ether-oxygen administered through an endotracheal tube connected to a closed system apparatus described by Booth *et al.*(5). Insertion of a stomach tube guarded against the harmful effects of regurgitation and bloating.

Following successful bilateral adrenalectomy the animals were placed on various hormone regimes as shown in Table I. Hormones were administered intramuscularly daily and each regime continued for 8 days. Approximately 12 ml of blood were drawn from the jugular vein on the eighth day and the following blood components were measured and used as criteria in establishing optimum hormone dose levels: serum sodium and potassium using a flame photometer, glucose according to Folin and Wu(6) and absolute eosinophil count as described by MacFarlane and Cecil(7).

Normal blood levels for the above criteria were determined in 9 additional intact goats. Blood was drawn from these animals at weekly intervals over a 3 week period.

TABLE II. Five Monthly Average Levels of Blood Constituents in 9 Adrenalectomized Goats Receiving 12.5 mg Cortisone and 2.5 mg DCA per 100 lb Body Weight Daily.

Month	Blood glucose (mg %)	Na^+ (meq/l)	K^+ (meq/l)	$Na^+:K^+$ ratio
June	55.3 ± 5.18*	142 ± 3.03	4.53 ± .38	31.5 ± 3.02
July	50.9 ± 4.12	146 ± 3.48	5.08 ± .22	29.3 ± 4.33
Aug.	48.2 ± 4.52	144 ± 4.84	4.70 ± .14	30.9 ± 3.36
Sept.	50.0 ± 3.39	146 ± 2.02	4.70 ± .45	30.9 ± 2.49
Oct.	51.2 ± 3.92	147 ± 2.29	5.2 ± .41	28.4 ± 2.07

* Standard deviation.

Results and discussion. The data obtained are summarized in Table I.

Administration of higher levels of hormones (Regime 1, 2 and 3) produced an increase in serum sodium and a decrease in serum potassium as compared to control values. The highest sodium-potassium ratio (40.26) appeared in Regime 1 with a progressive decrease toward the normal through Regimes 2 and 3 (36.83 and 29.28) respectively. Also at these higher levels there was a considerable increase in blood glucose concentration as well as a decrease in absolute eosinophil count. It can be concluded from these data that Regimes 1, 2 and 3 were too high for normal maintenance of the animals under controlled temperature and humidity conditions. The control value for the sodium-potassium ratio (27.90) falls between the mean values obtained with hormone Regimes 3 and 4 (29.28 and 25.84) respectively. From this it appears that the optimal dose level for the mineralcorticoid DCA could perhaps be adjusted to 3.5 mg/100 lb body weight. Blood sugar levels also indicate that Regime 4 approaches a more optimal level of cortisone administration. In view of these data it is felt that the minimal maintenance dose for bilaterally adrenalectomized goats should be Regime 4 (12.5 mg cortisone, 2.5 mg DCA/100 lb body weight/day). This is in agreement with a previous report by Cowie and Tindal(2) for the goat, using maintenance of lactation as a criterion and administration of the hormones by tablet implant. These data also agree with maintenance dose levels appearing in the literature for other ruminants insofar as the cortisone:DCA ratio (5:1) is concerned. However, the absolute values reported(3,4), (25 mg cortisone and 5 mg DCA /100 lb b.w.) could be reduced to 12.5 mg cortisone and 2.5 mg DCA/100 lb b.w. and still retain adequate maintenance. It should be noted that the animals used in this study were kept under more rigidly controlled environmental conditions than those employed in previously cited investigations. Furthermore, it is believed that a ratio approximating 4:1 (12.5 mg cortisone and 3.5 mg DCA/ 100 lb b.w.) could be used for adequate maintenance in adrenalectomized goats.

Following cessation of hormone treatment, 2 of the goats died after 5 days and the remaining animal died on the 6th day. The mean meq/l Na^+ concentration dropped from 143 (measured while on minimal maintenance dose) to 136 just prior to death, while meq/l of K^+ increased from 5.53 to a high of 7.43 then dropped to 6.73 during the same period. The $Na^+:K^+$ ratio decreased from 25.84 to a low of 18.91 on day 3 following cessation of treatment. Blood glucose levels also dropped from 53 to 48 mg% while absolute eosinophil count rose from 173 to 528/cubic mm. Overt symptoms of adrenal insufficiency in the goat just prior to death were muscular weakness, loss of appetite, dyspnea and coma.

Although 3 animals preclude statistical significance it should be mentioned that 9 additional bilaterally adrenalectomized goats have been maintained on Regime 4 for 5 months under the controlled conditions described earlier. These latter goats are part of another experiment to be reported later; however, measurements of blood constituents have been continued as a monitor of their well being. These data, contained in Table II, indicate satisfactory maintenance of glucose, sodium and potassium levels in the blood.

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Preparation of a Chronic Thoracic Duct-Venous Shunt in Calves.*† (28188)

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Good methods for collecting thoracic duct lymph are important for many studies concerned with fluid balance, absorption and transport of nutrients, and kinetics of lymphopoiesis. Cannulation of the thoracic duct with polyethylene or polyvinyl tubing has greatly facilitated the collection of lymph for prolonged periods in unanesthetized animals(1-6). However, inasmuch as these preparations do not provide for the conservation of lymph, the physiological state of the animal beomes greatly altered. The technique described below affords a preparation in calves whereby lymph is readily accessible, and an essentially normal physiological state is maintained by arranging for the return of lymph to the venous system. Calves are excellent animals for experimental work of this nature for they are easily restrained and their size permits removal of relatively large volumes of blood and lymph without physical deterioration. Similar preparations have been described in dogs(7,8).

Materials and methods. The polyethylene cannulas were prepared as shown in Fig. 1. Reinforcement of the thoracic duct cannula prevents kinking of the smaller tubing and also permits secure coupling with the venous cannula. Polyethylene tubing was easily molded into the desired shape by bringing it near a small flame. The ends were flared in a similar manner.

Female, Holstein calves, weighing 50 to 60 kg, were purchased on the open market and maintained until no clinical or hematological abnormalities were observed (generally 2 to 3 weeks). Prior to surgery, solid feed was withheld for 48 hours and liquid for 12 hours. One liter of cream fed slowly 1 hour before operation facilitated the location of the thoracic duct. Five mg of methyl atropine nitrate and 200,000 units of procaine penicillin were injected intramuscularly one-half hour preceding anesthesia. After rapid intravenous administration of 12 mg of pentobarbital sodium per kg of body weight, the calf was secured in a supine position with the aid of a trough-shaped table. Surgical anesthesia was obtained and maintained by supplementary intravenous injections of pentobarbital sodium or 5% chloral hydrate. Chloral hydrate was preferred as it resulted in a more rapid recovery from anesthesia. During the operation approximately 500 ml of normal saline were given intravenously.

The area over the left external jugular vein, immediately cranial to the first rib, was prepared for surgical intervention. An incision, 6 to 8 cm long, was made in the skin beginning at the first rib and extending anteriorly along the lateral edge of the left jugular vein. The inferior cervical artery and corresponding vein were then located 2 to 4 cm anterior to the first rib in the tissue between the ster-

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