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Adrenalectomy in Frogs and Toads.*

WILLIAM G. CLARK,† EDWIN L. BRACKNEY, AND ROBERT A. MILINER.
From the Department of Zoology, University of Minnesota.

A voluminous literature exists on the relationships of the adrenal cortex, carbohydrate metabolism, and electrolyte exchange in muscle. Much of this work has been done on muscles from adrenalectomized amphibians, because isolated surviving preparations from cold-blooded animals are more viable than those from mammals, and there is less chance of introducing complicating variables due to the effect of the adrenal insufficiency syndrome on general metabolism and the nervous and vascular supply to the muscle.

Successful, uncomplicated adrenalectomy in the amphibian is difficult due to the proximity of the uncapsulated diffuse adrenal gland to the vascular supply of the kidney. Damage to this vascular bed may lead to renal failure, edema, asthenia, and death. Most workers therefore have utilized "renal damaged controls" in their studies, and the supposition has been that differences between the control and the experimental groups are due solely to adrenal insufficiency.

More recent workers[1-6] (See for earlier bibliography) have reported variable results regarding survival periods, degree and time of appearance of asthenia, edema, and the relationship of adrenal insufficiency to muscle performance and metabolism.

Although adrenalectomy in amphibians has been worked on since 1891,[7] to our knowledge no one has been able to prove: (1) that his "adrenal insufficient" animals did not have histologically evident large rests of adrenal cortical tissue; (2) that renal failure and uremia did not complicate the syndrome; and (3) that successful replacement therapy was possible by adrenal cortical hormones, followed by a return of insufficiency symptoms which, if not ameliorated, culminate in death after subsequent withdrawal of hormone therapy. The extensive metabolic and work performance studies of muscles from cold-blooded animals in which these tests were not demonstrated may be open to criticism.

Methods and Results. Bullfrogs (*Rana catesbeiana*) and other frogs proved inferior to toads because of their poor wound healing and susceptibility to infections. Large species of toads proved more useful than small species because of the visualization of the adrenal and ease of operation. *Bufo marinus*[8] from Puerto Rico and Bermuda, and *Bufo peltacephalus* from Cuba were investigated next. The Cuban species proved inferior to the *Bufo marinus* for the same reasons given for frogs. The specimens ranged from 5 to 6 inches in body length, thus uniformly larger than most North American species.

The animals were kept in frequently cleaned galvanized iron tanks of 100 gallon capacity, with running water and wooden emergence platforms. They were force-fed every week

* Supported by grants from the Ella Sachs Plotz Foundation, The Carnegie Corporation of New York, and the Graduate School of the University of Minnesota.

† On leave of absence to Department of Physiology, School of Medicine, University of Southern California, Los Angeles. Other coauthors in armed forces.

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with 2-3 No. 000 gelatin capsules containing a balanced small animal ration consisting of 50% dextrinized starch, 30% milk casein, 8% corn oil, 2% cod liver oil, 6% yeast, and 4% USP salt mixture. The toads did well on this diet, maintaining or gaining weight, revealing large fat bodies when killed, and exhibiting great vigor, clasping habits, and general health. Twelve specimens were kept in good health for 3 years by this method.

In adrenalectomizing amphibians, most workers used thermal or diathermy cautery to destroy the adrenals. In the present study several methods were tried in over 100 animals. They were: diathermy cautery, thermal cautery (electro-cautery), freezing cautery, chemical cautery, and various surgical extirpation technics. Of these, only the latter were promising in our hands.

Single or two-stage adrenalectomies by diathermy and thermal cautery destroyed too much renal tissue and blood vessels if sufficient adrenal cortical tissue was destroyed to give insufficiency symptoms. Freezing cautery with ether and solid CO_2-chilled probes did not destroy sufficient tissue. Chemical cautery by saturated trichloracetic acid could not be localized sufficiently to prevent extensive renal damage when enough cortical tissue was destroyed.

Over 60 animals were operated upon using diathermy and thermal cautery and single or two-stage operations, the stages separated by 14-21 days. In one series, unilateral adrenalectomy by diathermy cautery was followed by histological studies to ascertain the extent of renal damage, existence of adrenal cortical rests, and to see if regeneration of renal or adrenal tissues occurred. Autopsy material removed 16-42 days after operation showed marked compensatory hypertrophy of the intact kidney of the unoperated side and a practically non-functional kidney on the operated side, with very few functional glomeruli, vacuolation of renal tubules and extensive lymphocytic infiltration in large areas. This occurred in all cases which did not reveal considerable adrenal cortical rests due to insufficient cautery, permitting indefinite survival.

The trypan blue method of Salmon and Zwemer[9] was employed as a possible test of regeneration of adrenal cortical cells. Six to 10 cc of 0.5% trypan blue were injected into the dorsal lymph sacs over a period of 3-12 days, various times after the operation, and the kidneys studied histologically. There was no indication of regeneration in contrast to the reports for the rat.

The osmic acid method developed by Flexner and Grollman[10] as an indicator of adrenal cortical activity, was useful in thick longitudinal sections of the kidney, for rapid and nearly macroscopic evaluation of the completeness of adrenalectomy, although the stain was poor for renal tissue.

Surgical removal was the only method which yielded successful results. The operative procedure finally evolved was as follows: The toad was anesthetized with Na-pentobarbital, 500 mg per kg in a 0.5% solution in 10% alcohol, thus 1 cc per 100 g of body weight, injected into the dorsal lymph sac or intraperitoneally. After about 10-15 minutes the toad was packed in ice and placed in a refrigerator for 30 minutes or longer, which induced a total hibernation state with no apparent respiratory movements, heart beat, blood flow, or reflexes. The operation was performed with the animal packed in ice to maintain this state.

A one-inch mid-dorsal incision was made through the skin and longissimus dorsi muscle slightly to one side of the transverse spinous processes, in the region of the kidney. The kidney was drawn out of the body cavity through the incision by traction on either the testes or the oviduct. The blood supply to the kidney was cut off by clamping off all the vessels to and from the kidney with delicate hemostatic mosquito forceps. The adrenal tissue was removed from one kidney by cutting the mesothelium covering the kidney and adrenal, around the border of the adrenal, with a sharp-pointed fragment of a razor blade held in forceps. One end of the adrenal was raised with very delicate forceps and the adrenal tissue separated from the kidney with

[9] Salmon, T. N., and Zwemer, R. L., *Anat. Rec.*, 1941, **80**, 421.

[10] Flexner, L. B., and Grollman, A., *Anat. Rec.*, 1939, **75**, 207.

a razor blade fragment or a very sharp, small scalpel. After removal of the adrenal, the hemostats were left clamped for 10-15 minutes or longer to allow clotting of blood exuding from the surface of the cut. With the animal in ice, clotting is retarded. Although its efficacy was not proved, sulfathiazole powder was dusted into the body cavity. The peritoneum and muscle wall were ligated with interrupted stitches of No. 00 gut. More sulfathiazole was dusted on the ligated muscle and the skin was sewn with black surgical thread. Successful procedure necessitated a unilateral adrenalectomy as described, followed no sooner than 5-7 days by unilateral extirpation of the remaining uninjured kidney with its contained adrenal, through a new incision. In performing the nephrectomy, all renal vessels were ligated with surgical silk. It usually required 3 ligatures to ligate all of the genital and renal vessels and the renal portal vein. The mesentery was then cut at the base of the kidney, the kidney removed and the body wall and skin sutured as before.

Two-stage bilateral adrenalectomies were unsuccessful. Death by hemorrhage occurred in all of 7 animals operated upon. Single stage bilateral adrenalectomy also resulted in death. No animals died in which unilateral adrenalectomy was followed later by the unilateral nephrectomy including the remaining adrenal.

Observations of these animals revealed asthenic symptoms which have been described by others.[1B] Examples of such symptoms are, an inability to right when overturned and locomotion by a crawling gait instead of hopping. If the toad was asthenic and edematous, it may have had adrenal insufficiency, uremia, or both. To distinguish these, desoxycorticosterone acetate in oil was injected for several days into the dorsal lymph sac in 3 doses of 0.15 mg 3 times daily. Disappearance of asthenic symptoms after treatment in addition to the ability to void before as well as after treatment indicates that the animal has a functional kidney and that sufficient adrenal cortical tissue was removed to cause an extreme adrenal insufficiency asthenia which probably would be followed by death. Following successful therapy, if the hormone is withheld until subsequent return of the insufficiency symptoms and death, the technic may be considered successful.

Of 12 animals so treated, 4 showed the desired results. The other 8 showed no improvement from the hormone and all died in 3-6 days, average 4. None voided urine, contrary to the 4 successfully operated animals. Autopsy uniformly showed marked edema, empty urinary bladders, vacuolated renal tubules, marked reduction in numbers of functional glomeruli, and extensive lymphocytic infiltration of renal tissue.

The 4 successes were given hormone 7 days. After withholding treatment they died in 3-6 days. Histological examination showed a great reduction in the amount of adrenal cortical tissue present, the remaining cells buried in scattered patches deep in the kidney. Renal damage was apparent, but functional glomeruli and tubules remained in sufficient number to correlate positively with the ability of the animals to void when handled.

It is believed that with experience with this surgical technic and hormone test method, the approximately 30% successes in the small series studied could be increased considerably. The utility of such final test animals in studies of the effect of the adrenal cortical hormones on muscle metabolism, in contrast with mammalian preparations, already has been pointed out in the introduction.

Summary. In a small number of the giant species of toad, *Bufo marinus,* approximately 30% were apparently successfully adrenalectomized without renal failure, by unilateral surgical extirpation of one adrenal followed later by removal of the remaining kidney with its contained adrenal. Hormone maintenance therapy was possible with desoxycorticosterone acetate and, when withheld, was followed by a return of adrenal insufficiency symptoms and death.