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STUDY OF THE ANDROGENIC ACTIVITY OF THE REGENERATING ADRENAL CORTEX

by

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ABSTRACT

The androgenic function of the adrenal cortex has been studied in adult male rats; circulating androgens were assessed through sexual accessory organ weights and zinc 65 uptake by the dorsolateral prostate. Acclimated intact and castrated animals were used as well as animals with regenerating cortices (bilateral - adrenal - enucleated) and dual operations (enucleation - castration).

At room temperature (22°C), the intact and enucleated appeared similar in androgen level but, exposure to severe cold (-5°C) depressed the level of circulating androgens. The cold exposed intacts tended to recover but enucleates remained depressed after 12 days of cold exposure; cold exposed castrastes did not display an increase in circulating androgens but the seminal vesicle weights of the cold exposed dual operated fell to basal castrate levels only after 4 days of exposure.

ACTH treatment in warm exposed intacts caused a significant increase in the androgenic indicators, as well as an increase in glucocorticoid activity, assessed by thymic involution. Similar treatment to enucleated animals caused a transient increase in the androgen level but failed to cause thymic involution.

ACTH treated warm exposed castrated and dual operated animals displayed a small non-significant increase in the level of androgens; the glucocorticoid output in castrated animals was similar to that of ACTH treated intacts, while thymolysis was absent in the ACTH treated dual operated. All cold exposed animals displayed a lesser androgenic response to the exogenous ACTH compared to the warm exposed counterparts.

The results 1) indicate a real androgenic capability of regenerating adrenal cortices; the increase in activity corresponds to the increase in the regenerating cortical tissue. 2) suggest that the transient androgenic response and lack of glucorticoid activity in enucleated animals is due to an impairment of steroid function within the regenerating gland and 3) suggest that castration removes a factor necessary for the expression of adrenal androgens in the adult male rat.

Acknowledgments

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To the memory of my Father and to my dear wife Sandra

INTRODUCTION

(i) Adreno-Genital Relations

The physiological relationship between the adrenals and the gonads has been the object of many previous investigations; the work has been reviewed by Parkes in 1945 (1), Courrier et al. in 1953 (2) and Zuckerman in 1953 (3). Yet the precise role of the adrenal cortex in the androgenic economy remains to be satisfactorily explained.

There is, however, sufficient biological evidence to indicate that the normal adrenal cortex of male rats secretes androgens or substances similar to those produced by the male gonads. Price and Ingle (4) have observed a local androgenic stimulation in the seminal vesicles and ventral prostates of animals carrying adrenal autotransplants in situ. Castration of immature rats results in adrenal hypertrophy suggesting a possible feedback mechanism; adrenal cortical extract maintains the sexual accessories of adrenalectomized male rats, while large doses cause precocious development of these organs (5). The reviews consistently point out that some changes in the sexual accessory organs are associated with both the activity or loss of activity of the adrenals or of the gonads.

The adrenals apparently produce extremely small amounts of androgens and while the literature does not indicate the amounts secreted by the rat, Liu et al. (6) estimate that the cortex of the male dog secretes less than 5 micrograms per day of androgenic material.

The ability of the adrenal cortex to synthesize androgens decreases as age progresses (7), even under ACTH stimulation, as demonstrated by Moore (8) and Deanesly (9). This fact appears to be significant in the failure of many workers to measure adrenal androgen production in adult animals.

Recent studies in this laboratory (10) suggest that in the mature rat, 93% of the total androgens emanate from the testes, while only 7% is of adrenocortical origin. In other studies on the adreno-genital axis, workers have used cold stress as a stimulus to the adrenal cortex on the assumption that the resultant elevation in adrenal activity would be accompanied by a proportionate increase in adrenal androgens detectable in the elevation of the weight of the sexual accessory organs. However, the testicular degeneration due to stress often masks any change in the extra-testicular contributions (11, 12, 13, 14, 15).

Tullner observed that ACTH induced the growth of accessory organs in castrated, but not in castrated-adrenalectomized animals (16); ACTH has also been found androgenically effective in cold exposed castrates (12). Other workers have been unable to elicit androgenic responses by means of an ACTH stimulation (17).

The observations on adrenal androgen production are equivocal and seemingly dependent upon the method of treatment of the animal groups.

(ii) Adrenal Enucleation and Cortical Regeneration

Adrenal enucleation was first described by Evans (18) in 1936 as a method of studying the carbohydrate regulation by the adrenal cortex; recently Schonbaum has used the procedure in studies of sympathetic function (19, 20).

The procedure consists in the surgical removal of the adrenal medulla and cortex, leaving the capsule intact; the cortex then regenerates from the cellular remnants adhering to the capsule. The operation has been described for the rabbit (21), mouse (22), dog (23), man (24) and rat (25).

Recent work by Schonbaum has shown that rats with regenerated cortices can survive cold (4°C) for many weeks and that the absence of the medulla does not preclude adaptation to cold, nor resistance to acute exposure to severe cold; residual sympathetic nerve activity is adequate to maintain life at 4°C (20).

Immediately following enucleation, the animal is essentially adrenalectomized and the initial deficiency in mineral corticoid function can be offset by providing 1% NaCl in the drinking water.

Ingle (31) demonstrated that the regeneration of the adrenal cortex appears to be related to a physiological need for the cortical hormones and is specifically controlled by ACTH. When one adrenal gland is enucleated and the other removed, the remnant of the enucleated gland regenerates rapidly (26); regeneration can be suppressed by the administration of

large amounts of cortical extract or by leaving the contralateral gland intact. Regeneration does not occur in hypophysectomized animals and repeated injections of ACTH have little effect on enucleated or transplanted glands. However ACTH infusion by continuous drip induces massive hyperplasia and hypertrophy of the glands and the secretion of abnormally high amounts of glucocorticoids; the animals ultimately die of adrenal hyperfunction.

At first, lipids cannot be detected; according to Pellegrino (30), this suggests a rapid release of all formed products. The cholesterol and ascorbic acid levels remain subnormal for as long as three months (32). Verne and Herbert have observed an initial augmentation of histologically demonstrable alkaline phosphatase (33).

Chester-Jones and Wright (34) and Brogi and Pellegrino (35) report normal production of mineralocorticoids during the regeneration process. Glucocorticoid production 1-2 weeks after enucleation has been reported adequate to maintain homeostasis and permit resistance to various stresses (27, 36, 37). Resistance to histamine is reduced, presumably because of the deficiency of the medullary hormones (38, 39).

The secretory ability of the regenerating cortex has been studied by measuring the adrenal effluent: the glands secrete both aldosterone and corticosterone after an initial deficient period, but the secretory rate remains subnormal for many months (35, 40); however, Fortier and DeGroot, report supranormal secretion from 16 days onward (41).

Hyde and Skelton (42) raise the point of functional secretion as opposed to functional ability. In rats with regenerating cortices, stressed by fracture of the tibia, the glands were not able to secrete as much corticosterone as those of animals with intact adrenals stressed in the same way. Macchi and Whyman (43) ascribe the difference in response to a retention of the hormone in the glands because of a decreased venous outflow, rather than to a decreased secretory ability. The in vitro work of Birminghal et al. (44) supports this contention by showing that incubated regenerated cortices are capable of a sustained steroid response to ACTH.

No mention has been made in the literature of the in vivo androgenic capability of the regenerating or regenerated adrenal cortex.

(iii) Statement of the Problem

Accepting the fact of the limited but real androgenic capability of the intact adrenal cortex, we thought that further elucidation of the adrenal-androgenic complex might result from a study of the regenerating adrenal cortex; this system would allow a time study of the secretory potential of the androgenic adrenal. In addition, cold exposure and exogenous ACTH could be used as ancillary stimuli.

The level of circulating androgens could be estimated from the accepted androgenic indicators; sexual accessory organ weights (45) and zinc 65 uptake by the dorsolateral prostate (46).

The present study was therefore designed to investigate the following:

(i) the androgenic capability of the regenerating adrenal cortex *in situ*;

(ii) the modification of this androgenic capability by

a) exposure of the animals to the stress of severe cold (-5°C)

b) administration of exogenous ACTH.

MATERIALS AND METHODS

Animals and Diet

Adult male albino Wistar rats were used in all experiments. The animals were cages individually in galvanized steel cages and supplied Purina Lab Chow and tap water ad libitum. Enucleated animals were given 1% NaCl in lieu of tap water throughout the post-operative period.

Temperature Environments

Three temperature environments were used in the experiment; all rooms were ventilated and artificially illuminated from 8:00 A.M. to 6:00 P.M.:

a) Room temperature - Air-conditioned animal quarters maintained at 22 ± 1°C; relative humidity adjusted at 45-55%;

b) "Acclimation" - cold room maintained at 2 ± 1°C;

c) Cold exposure - cold room maintained at -5 ± 1°C.

Treatment Schedule (Figure 1)

Grouping: - Upon arrival from the supplier*, the animals weighed between 130-140 grams and were subjected to a quarantine of 6-8 days at room temperature (22°C). The animals were then divided into the 3 following groups:

A. Absolute Control Group - these animals were kept at room temperature (22°C) for the entire experimental period.

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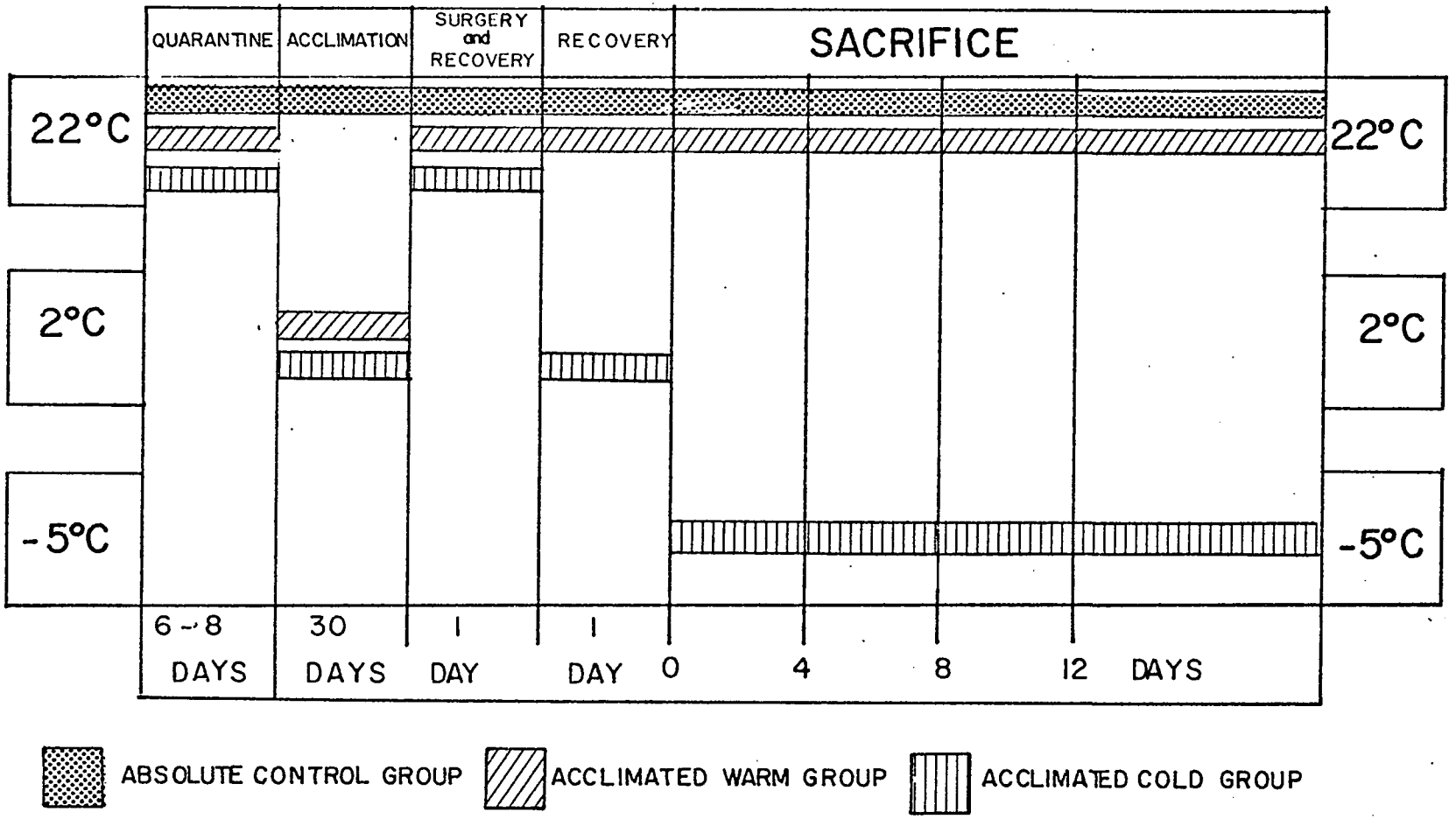
* Quebec Breeding Farms Ltd., St. Eustache, P.Q.

FIGURE I

TREATMENT SCHEDULE



B. Pre-Acclimated - Warm Group - these animals were "acclimated" at 2°C for 30 days, whereupon surgery at room temperature was performed; this group then remained at room temperature for the duration of the experimental period.

C. Pre-Acclimated - Cold Group - the animals were "acclimated" at 2°C for 30 days prior to surgery. A 48-hour recovery period was allowed: 24 hours at room temperature (22°C) followed by 24 hours at +2°C; the animals were then exposed to severe cold (-5°C) for the remainder of the experiment.

Animal Sampling:

Since the experiment involved the use of large numbers of animals in limited facilities, the experiment was divided into sub-experiments or stages of approximately 40 animals, comprising representatives of each experimental treatment class.

Surgery:

All surgery was done at room temperature (22°C) under light ether anaesthesia. Bilateral adrenal enucleation was performed through a single mid-dorsal skin incision, followed by a bilateral epimeric approach to the adrenals; the adrenal capsules were nicked with a scalpel and the contents "popped" with forceps pressure. The muscle incisions were sutured and the skin incisions were closed with wound clips. Castration was performed by abdominal approach; the entire spermatic cord was ligated and the testes and epididymes were removed. "Dual-operation" consisted in performing adrenal enucleation and

castration at the same time.

Recovery was rapid and complete in all cases; any animals displaying signs of abcess, perforation or prolonged surgical shock (less than 1%) were excluded from the experimental data.

ACTH Administration

ACTH[1] was administered to preacclimated warm- and pre-acclimated cold-exposed animals beginning on day + 2 post-operatively, corresponding to day 0 of cold exposure. However, for cold exposed enucleates and dual-operated animals, ACTH treatment began on day + 5 post-operatively (day + 3 of cold exposure)[2].

Autopsy

The animals were killed between the hours of 9:30 A.M. and 2:00 P.M. at 2, 6, 10 and 14 days post-operatively corresponding to 0, 4, 8 and 12 days of cold exposure. The animals were killed by stunning, followed by decapitation and exsanguination. Organs were quickly dissected out, examined, blotted and weighed wet on a Roller Smith torsion balance to the nearest 0.1 mg; organs weighing more than 500 mg were weighed on a CENT-O-GRAM balance to the nearest 0.5 mg.

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1 ACTH- generously supplied by K. Antoft, Nordic Biochemicals Ltd., Montreal, as non-sterile powder, with quoted potency of 57 I.U./mg. The powder was dissolved in 16% gelatin (U.S.P.) to a potency of 30 I.U./ml. Injection: 15 I.U./day/rat I.P. at 9:30 A.M.

2 Due to high mortality following I.P. injection into cold exposed enucleated and dual-operated animals, ACTH administration was delayed until day +5 postoperatively (day +3 of cold exposure).

The following data was obtained at autopsy:

1. Body weight
2. Organ Weights: thymus
   adrenals
   dorsolateral prostate
   ventral prostate
   seminal vesicles
   testes

Zinc 65 Uptake by the Dorsolateral Prostate

The Zinc 65 uptake by the dorsolateral prostate was determined by the method of Gunn et al. (46). Exactly 24 hours prior to sacrifice, the tracer[3] was administered into the external jugular vein by way of a small skin incision with the animal under light ether anaesthesia. At autopsy, the dorsolateral prostate was carefully dissected out, dried to constant weight at 85°C (24 hours) and counted whole in a low-background gas flow counter (Nuclear Chicago Model C115).

Histology

Samples of the testes, adrenals, ventral prostate and kidney were kept for histological examination. They were fixed in 10% formalin, Paraplast-imbedded and cut at 6-8 μ; the sections were stained with Hematoxylin, Phloxin and Orange G (H.P.O.).

Statistical Analysis

The arithmetical mean and the standard error of the mean were calculated for each experimental group. The data were then subjected to an analysis of variance for samples of unequal

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[3] Supplied by AECL as Zinc Nitrate in 2.7 N Nitric Acid. Neutralized with 1N NaOH and diluted with Normal Saline to 1 mCi/ml. Injection: 40 μCi/100 gm Body Weight in saline to a total volume of 0.3 ml (40 μCi/0.1 ml).

size (47). The analyses showing significant differences at the 1% level were tested at the 5% level, with Kramer's modification of Duncan's Multiple Range test (48).

Expression of Results

The data are submitted in the form of tables and graphs; in the graphs, the results are expressed as a percent of the absolute control (itself represented as a straight horizontal line at the 100% level). Since the standard errors are generally 10% or less of the mean, the errors are not shown on the graphs.

Exposure to Moderate Cold ("Acclimation")

Since the regeneration period of the adrenal cortex has been reported to be as short as 7 days after enucleation (41), with the production of steroids normal after 7 to 14 days (34), an examination of the androgenic activity of the regenerating cortex required the shortest possible recovery period between surgery and exposure of the animals to severe cold (-5°C).

Moreover, pilot studies on enucleated animals revealed that mortality consequent to cold exposure (-5°C) increased significantly when a recovery period of less than 5 days was permitted. Hence, exposure to moderate cold (+2°C) for 30 days prior to surgery was used to obtain a greater resistance to the stress of severe cold; the post-operative recovery period was reduced to 48 hours: 24 hours at +22°C, and 24 hours at +2°C.

All animals, except for the absolute controls (which remained at room temperature, +22°C, for the entire experiment) were thus

exposed to +2°C, and presumably acclimated. Comparison is made in all instances with the absolute controls (the non-acclimated controls) present in each surgical group.

RESULTS

The Effect of Exposure to Moderate Cold

The effectiveness of the exposure to moderate cold (30 days at + 2°C) is reflected in the survival of the enucleated animals after 12 days of exposure to severe cold (-5°C). Since this pre-operative period was successful in conferring a degree of resistance against the subsequent severe cold exposure, we can now refer to this pre-treatment as ACCLIMATION (49).

At day 0 (day +2 post-operatively), because of the exposure to moderate cold, the terminal body weights of all animals is depressed: the intacts and enucleated have significantly lower body weights, but the castrated and dual operated are not different from their non-acclimated counterparts (Table I and Plate I, fig. 1).

Furthermore, at day 0, the zinc 65 uptake by the dorsolateral prostate shows an androgenic depression greater by 20-30% than the depression observed in the weights of the sexual accessory organs.

Terminal Body Weights (Table I and Plate I)

In the non-acclimated (fig. 1), the surgical status causes a negligible loss (6%) of the terminal body weights compared to the absolute controls.

Following acclimation (fig. 2), there is a general depression of the body weights compared to the non-acclimated

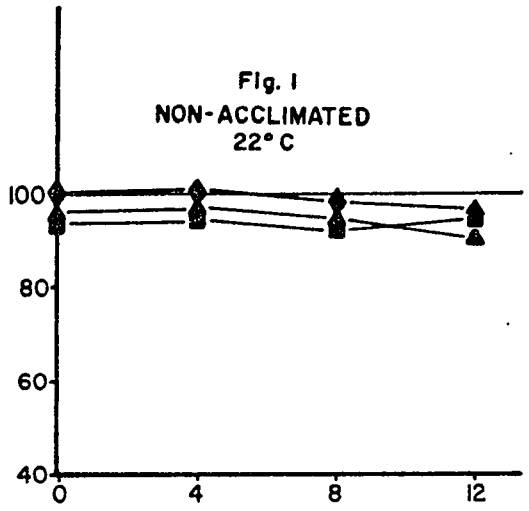
TABLE 1

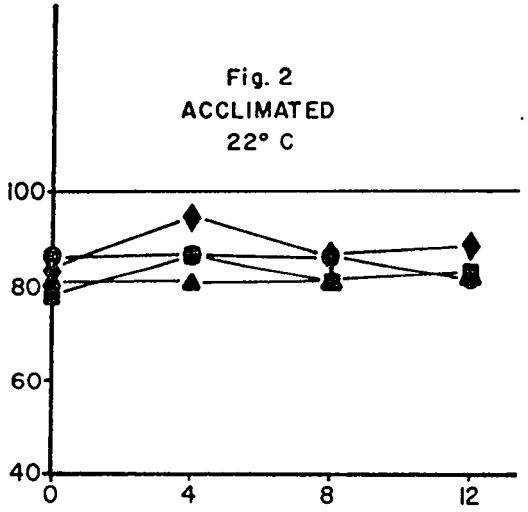
TERMINAL BODY WEIGHT (grams)

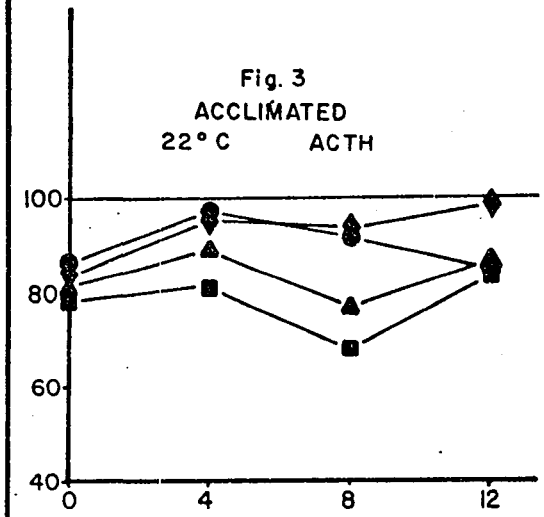
Class of Treatment		Day 0	Day 4	Day 8	Day 12
Absolute	Control[1]	336.8[2] ± 8.7[3] (8)[4]	330.1 ± 13.2 (10)	357.6 ± 8.4 (8)	361.4 ± 10.4 (8)
	Enucleate	336.2 ± 7.9 (10)	334.1 ± 10.7 (9)	348.7 ± 7.4 (10)	350.4 ± 22.3 (10)
	Castrate	316.8 ± 7.1 (10)	313.6 ± 7.3 (10)	330.1 ± 5.6 (10)	347.1 ± 9.2 (9)
	Dual	322.4 ± 7.2 (10)	320.2 ± 6.9 (9)	336.9 ± 11.0 (9)	326.2 ± 20.3 (9)
Preacclimated	Control	282.3 ± 6.2 (11)	284.0 ± 5.2 (6)	307.0 ± 11.5 (6)	298.0 ± 14.9 (7)
	Enucleate	279.2 ± 9.5 (11)	315.0 ± 6.7 (6)	309.0 ± 12.2 (6)	318.0 ± 6.5 (7)
Warm	Castrate	263.0 ± 7.4 (6)	283.0 ± 5.6 (7)	290.0 ± 5.2 (7)	300.0 ± 7.8 (7)
	Dual	273.0 ± 8.3 (6)	268.0 ± 7.4 (8)	289.0 ± 13.8¢ (8)	299.0 ± 8.6 (6)
Preacclimated	Control	269.8 ± 9.1 (14)	273.0 ± 8.3 (7)	258.0 ± 7.2 (6)	280.0 ± 10.1 (7)
	Enucleate	271.1 ± 5.8 (15)	259.0 ± 9.7 (8)	243.0 ± 11.6 (8)	245.0 ± 17.4 (7)
Cold	Castrate	257.0 ± 9.6 (7)	245.0 ± 10.3 (8)	251.0 ± 7.8 (8)	266.0 ± 10.9 (7)
	Dual	240.8 ± 13.4 (8)	243.0 ± 17.7 (6)	249.0 ± 18.0 (5)	241.0 ± 13.7 (5)
Preacclimated	Control		321.0 ± 18.2¢ (5)	328.0 ± 14.5 (5)	308.0 ± 12.1 (5)
Warm	Enucleate		312.0 ± 22.9 (3)	333.5 ± 11.7 (7)	357.0 ± 5.8 (4)
ACTH[5]	Castrate		269.0 ± 18.0 (4)	242.8 ± 10.2 (8)	303.0 ± 18.8 (5)
	Dual		295.6 ± 10.4 (7)	276.1 ± 9.5 (7)	311.8 ± 17.0 (4)
Preacclimated	Control		303.3 ± 6.4 (9)	318.1 ± 11.2 (10)	281.0 ± 14.0 (7)
Cold	Enucleate[6]		272.3 ± 12.9 (4)	284.5 ± 11.5 (4)	262.0 ± 9.6 (6)
ACTH[5]	Castrate		259.3 ± 14.6 (4)	277.3 ± 14.2 (7)	301.5 ± 8.4 (6)
	Dual[6]		288.0 ± 24.5 (4)	- - - - - - -	- - - - - - -

[1] Absolute Control
[2] Arithmetical mean
[3] Standard error of the mean
[4] Group Population
[5] ACTH - 15 I.U./Day in 16% gelatin I.P.
[6] ACTH began on day +3 of treatment

PLATE I



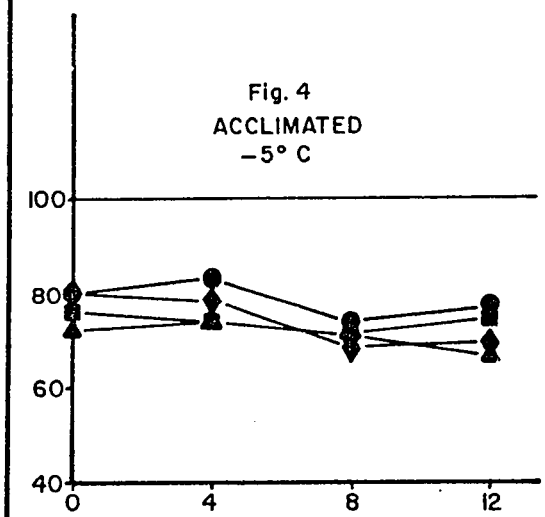


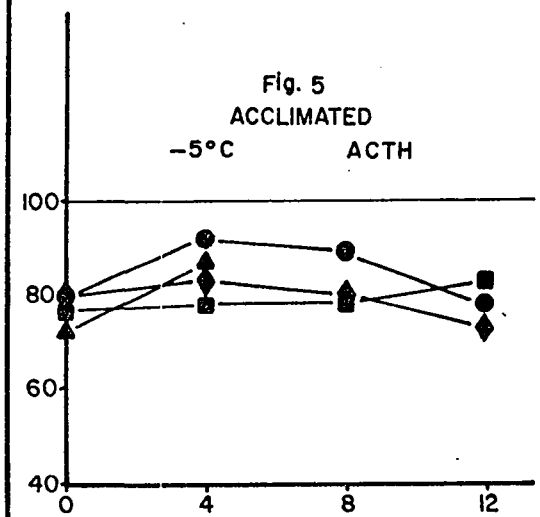


TERMINAL BODY WEIGHT

% OF CONTROL (100%)
VERSUS
DAYS OF TREATMENT

● INTACT
♦ ENUCLEATED
■ CASTRATED
▲ DUAL-OPERATED





counterparts. The intact acclimated animals weigh significantly less than the absolute controls at 0 and 4 days, the enucleates weigh less only at day 0, and both the castrated and dual operated do not differ from their non-acclimated opposites. Exposure to the warm environment following the acclimation period tends to cancel the effects of the moderate cold on the body weight and on the weight of the sexual organs.

On the other hand, exposure to severe cold aggravates the loss of body weight suffered during acclimation (fig. 4). The intact animals are significantly lighter than the absolute controls at 4 and 8 days of cold exposure; enucleated and dual operated show the greatest weight loss at the end of the 12th day of exposure.

The changes in the terminal body weights of the warm and cold exposed animals treated with ACTH are not significant (figures 3 and 5). Dual operated animals exposed to the cold and treated with ACTH do not survive beyond 4 days of cold exposure and hence are not followed beyond this point (fig. 5).

Testes Weights (Table 2 and Plate II)

The Multiple Range Test reveals no point of significant difference between the experimental groups; however, the depression of the testes weights of the animals exposed to severe cold at -5°C (fig. 4), and the apparent correction of this depression under ACTH treatment at day 12 (fig. 5) suggests biological significance. The depression of the testes weight appears to

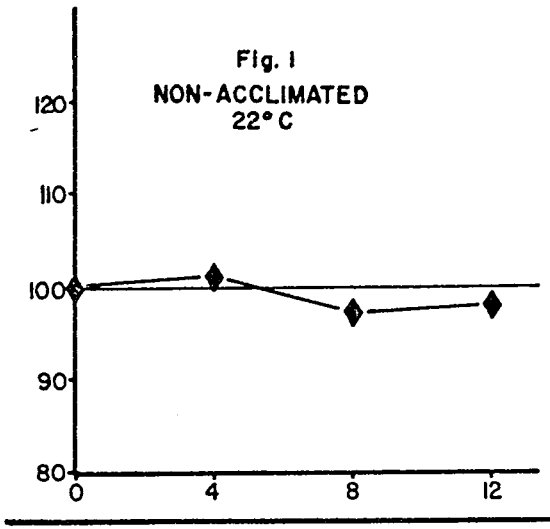
TABLE 2

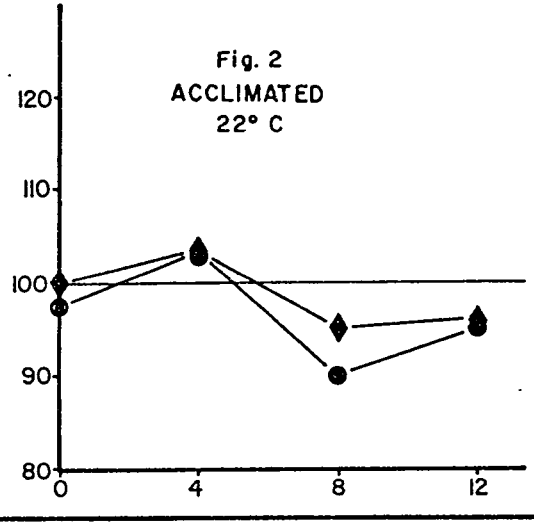
TESTES - WEIGHT (mgm)

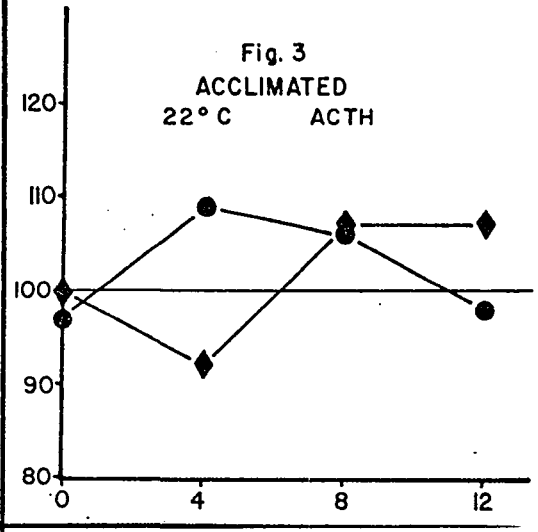
Class of Treatment		Day 0		Day 4		Day 8		Day 12	
Absolute	Control[1]	3.10[2] ± 0.17[3]	(8)[4]	2.94 ± 0.11	(10)	3.26 ± 0.43	(8)	2.95 ± 0.08	(8)
	Enucleate	3.11 ± 0.16	(10)	2.97 ± 0.22	(9)	3.17 ± 0.06	(10)	2.89 ± 0.19	(10)
	Castrate								
	Dual								
Preacclimated	Control	3.02 ± 0.1	(11)	3.02 ± 0.19	(6)	2.95 ± 0.11	(6)	2.80 ± 0.16	(7)
	Enucleate	3.07 ± 0.1	(11)	3.03 ± 0.20	(6)	3.11 ± 0.10	(6)	2.82 ± 0.19	(7)
Warm	Castrate								
	Dual								
Preacclimated	Control	3.02 ± 0.1	(14)	2.96 ± 0.08	(7)	3.09 ± 0.05	(6)	2.91 ± 0.19	(7)
	Enucleate	3.10 ± 0.1	(15)	2.99 ± 0.07	(7)	2.82 ± 0.11	(8)	2.43 ± 0.28	(7)
Cold	Castrate								
	Dual								
Preacclimated	Control			3.21 ± 0.08	(5)	3.47 ± 0.02	(5)	2.89 ± 0.22	(5)
Warm	Enucleate			2.71 ± 0.24	(3)	3.49 ± 0.14	(7)	3.17 ± 0.24	(4)
ACTH[5]	Castrate								
	Dual								
Preacclimated	Control			3.13 ± 0.06	(9)	3.18 ± 0.10	(10)	3.01 ± 0.11	(7)
Cold	Enucleate[6]			2.71 ± 0.03	(4)	2.85 ± 0.21	(6)	2.85 ± 0.21	(6)
ACTH[5]	Castrate								
	Dual[6]								

[1] Absolute Control
[2] Arithmetical mean
[3] Standard error of the mean
[4] Group Population
[5] ACTH - 15 I.U./Day in 16% gelatin I.P.
[6] ACTH began on day +3 of treatment

PLATE II





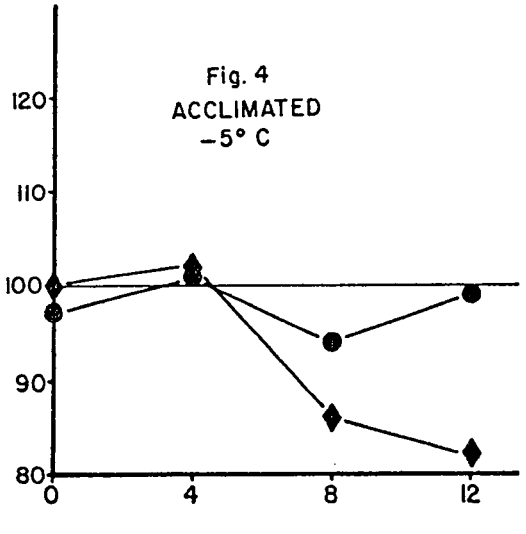


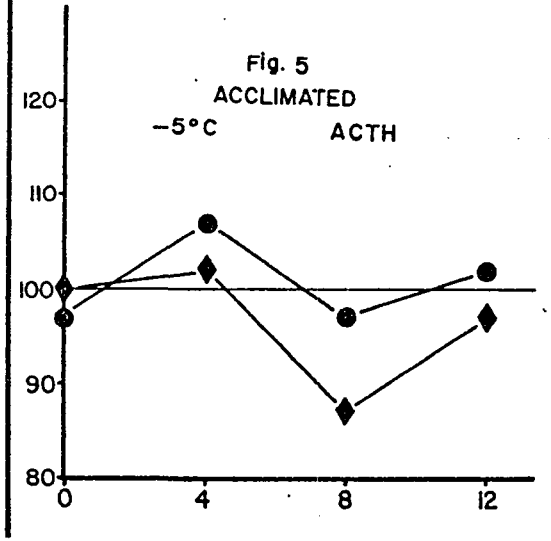
TESTIS

WEIGHT

% OF CONTROL (100%)
VERSUS
DAYS OF TREATMENT

- INTACT
- ENUCLEATED
- CASTRATED
- DUAL-OPERATED





parallel the loss of body weight and indicates a systemic effect in the cold exposed animals.

Ventral Prostate Weight (Table 3 and Plate III)

Non-acclimated enucleates do not differ significantly from the absolute controls, and the expected significant fall in sexual organ weight due to castration is observed in the castrated and dual operated animals (fig. I).

The effect of the exposure to moderate cold is significant (fig. 2); all animals have significantly lower ventral prostate weights on day 0. The enucleates and intacts return to normal by the 12th day, whereas the castrated and the dual operated are not different from the non-acclimated by the 4th day.

The intact and enucleated animals exposed to -5°C show a significant fall in their ventral prostate weight (fig. 4); the intacts recover, a significant increase, by day 12. At day 4 of cold exposure, the ventral prostate weights of the dual operated are heavier than those of the cold exposed castrates. Although the difference is not significant at the 5% level, this observation is seen in the other androgenic indices and suggests a higher level of androgenic activity in the dual operated at 4 days of cold exposure compared to the castrates.

ACTH-administration causes an androgenic stimulation of the ventral prostate in the intact and enucleated (fig. 3); the depression of the ventral prostate weight following accli-

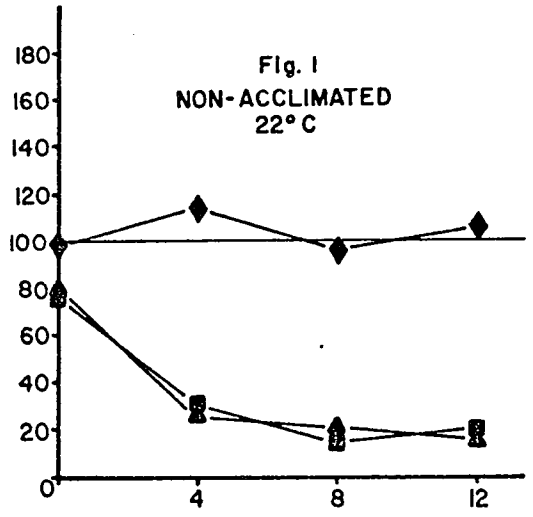
TABLE 3

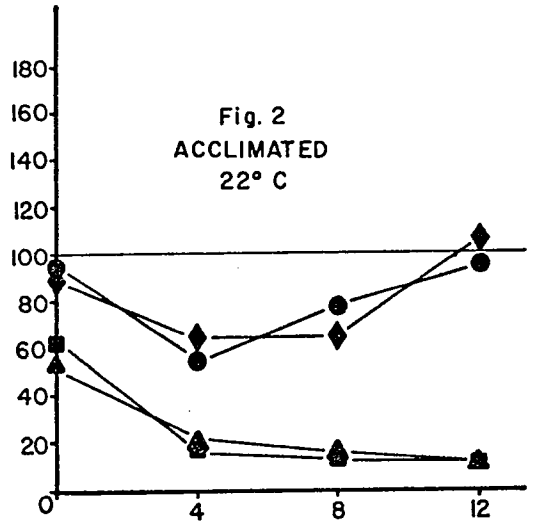
VENTRAL PROSTATE - WEIGHT (mgm)

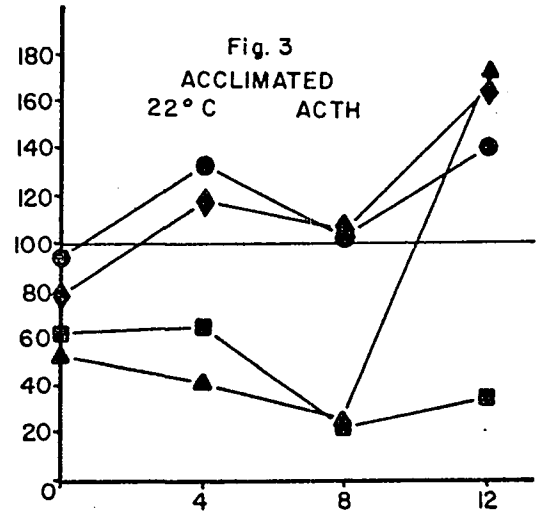
Class of Treatment		Day 0	Day 4	Day 8	Day 12
Absolute	Control[1]	311.9[2] ± 13.3[3] (8)[4]	316.7 ± 26.6 (10)	388.4 ± 31.2 (8)	310.5 ± 47.3 (8)
	Enucleate	305.2 ± 18.4 (10)	360.6 ± 32.9 (9)	367.6 ± 30.4 (10)	326.5 ± 37.5 (10)
	Castrate	234.2 ± 8.8 (10)	85.9 ± 8.3 (10)	59.2 ± 6.5 (10)	58.0 ± 7.1 (9)
	Dual	244.7 ± 12.1 (10)	77.2 ± 7.7 (9)	76.8 ± 6.9 (9)	45.4 ± 4.1 (9)
Preacclimated	Control	293.7 ± 21.6 (11)	172.6 ± 23.5 (6)	302.2 ± 27.6 (6)	295.4 ± 31.0 (7)
	Enucleate	273.2 ± 29.7 (11)	201.8 ± 23.7 (6)	252.9 ± 30.8 (6)	329.1 ± 25.6 (7)
Warm	Castrate	193.9 ± 13.0 (6)	57.3 ± 3.7 (7)	54.0 ± 7.7 (7)	38.8 ± 5.7 (7)
	Dual	164.2 ± 16.2 (6)	64.2 ± 8.9 (8)	58.6 ± 6.7 (8)	37.8 ± 6.9 (6)
Preacclimated	Control	264.6 ± 19.2 (14)	213.3 ± 26.9 (7)	183.0 ± 16.4 (6)	359.5 ± 42.0 (7)
	Enucleate	284.1 ± 15.6 (15)	217.2 ± 37.1 (8)	176.8 ± 16.0 (8)	187.1 ± 33.8 (7)
Cold	Castrate	161.9 ± 15.3 (7)	54.5 ± 4.2 (8)	48.6 ± 7.3 (8)	46.4 ± 4.5 (7)
	Dual	121.9 ± 11.3 (8)	75.8 ± 16.1 (6)	30.6 ± 5.7 (5)	52.2 ± 7.8 (5)
Preacclimated	Control		419.2 ± 63.8 (5)	395.2 ± 80.1 (5)	437.0 ± 32.7 (5)
Warm	Enucleate		378.3 ± 37.8 (3)	411.3 ± 12.2 (7)	507.0 ± 88.6 (4)
ACTH[5]	Castrate		202.1 ± 65.7 (4)	83.9 ± 6.9 (8)	106.0 ± 19.4 (5)
	Dual		129.0 ± 10.2 (7)	88.3 ± 8.9 (7)	537.5 ± 66.1 (4)
Preacclimated	Control		410.4 ± 25.9 (9)	439.0 ± 42.4 (10)	351.0 ± 28.2 (7)
Cold	Enucleate[6]		342.0 ± 34.2 (4)	348.0 ± 44.5 (4)	287.0 ± 35.4 (6)
ACTH[5]	Castrate		121.0 ± 10.9 (4)	121.0 ± 22.4 (7)	72.5 ± 5.4 (6)
	Dual[6]		201.2 ± 64.9 (4)	- - - - - - -	- - - - - - -

[1] Absolute Control
[2] Arithmetical mean
[3] Standard error of the mean
[4] Group Population
[5] ACTH - 15 I.U./Day in 16% gelatin I.P.
[6] ACTH began on day +3 of treatment

PLATE III





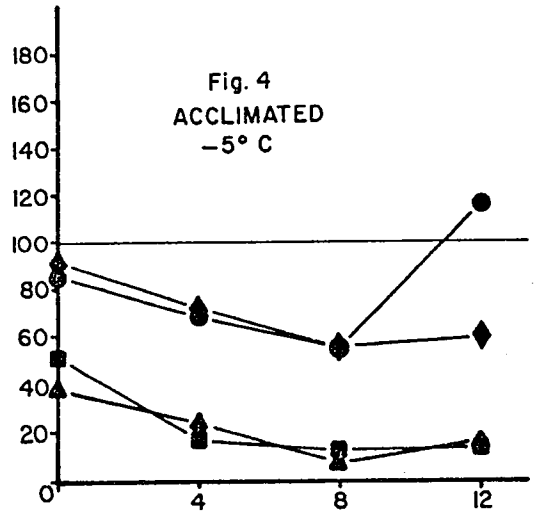


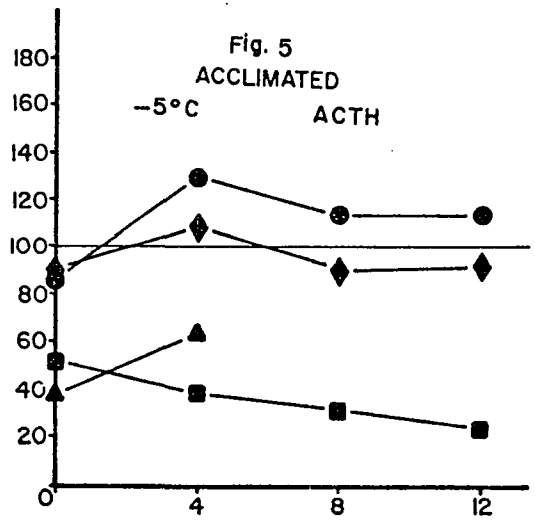
VENTRAL PROSTATE

WEIGHT

% OF CONTROL (100%)
VERSUS
DAYS OF TREATMENT

- INTACT
- ENUCLEATED
- CASTRATED
- DUAL-OPERATED





mation is negated and a significant elevation in weight occurs. At 4 days, the castrates show a 20% increase over the non-ACTH treated and remain above non-treated levels. The dual operated respond after 8 days of treatment with a significant gross increase in their ventral prostate weights; this is observed solely in this parameter of androgenic activity and may be indicative of the selective responsiveness of the sexual target organs to the circulating androgens.

In the cold exposed animals treated with ACTH (fig. 5), the intacts are more responsive than the enucleated, but not as sensitive as the ACTH treated warm exposed. Similarly, the castrated animals treated with ACTH in the cold show a decline in the expected regression of their sexual organ weights; those of the dual operated show an increase in androgenic activity following a single ACTH injection 24 hours previously.

Seminal Vesicle Weight (Table 4 and Plate IV)

Enucleation has no apparent effect on the seminal vesicle weight in the non-acclimated animals (fig. 1). Castration causes a weight reduction to 35% of control values by the 12th day (14 days post-operatively).

Acclimation to moderate cold (fig. 2) does not alter the response of the seminal vesicles in any of the animal groups exposed at room temperature, although the seminal vesicle weights are significantly less at day 0 compared to the non-acclimated.

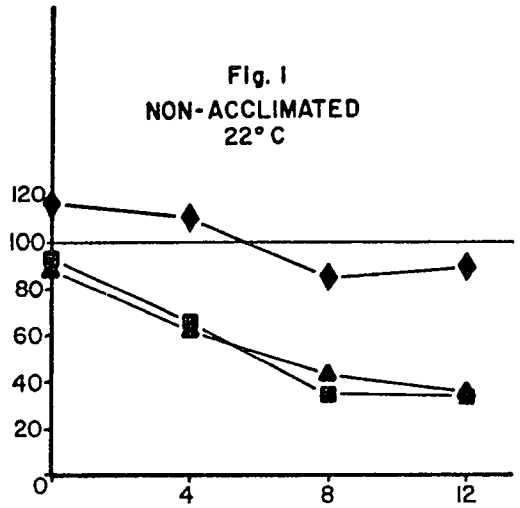
TABLE 4

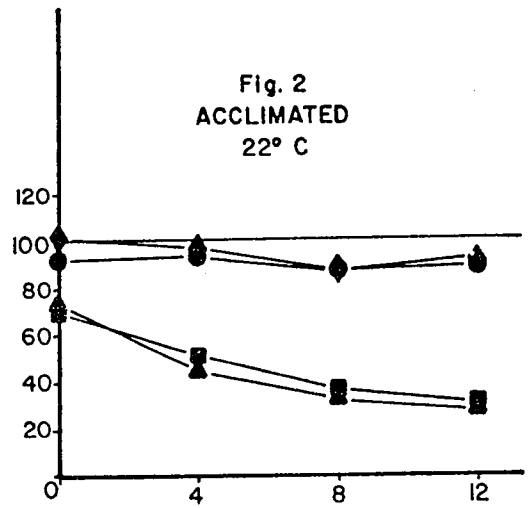
SEMINAL VESICLES - WEIGHT (mgm)

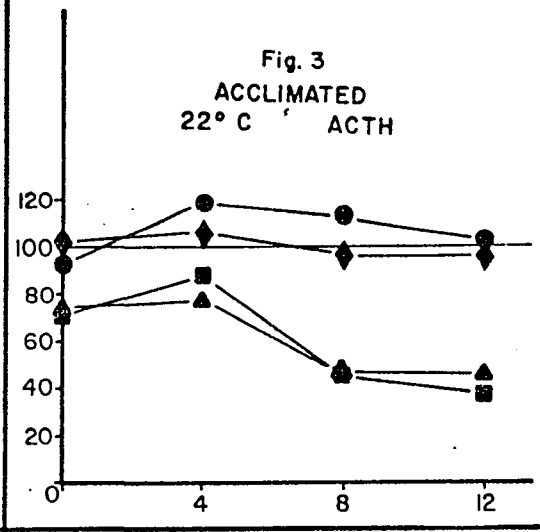
Class of Treatment		Day 0	Day 4	Day 8	Day 12
Absolute	Control[1]	463.2[2] ± 29.2[3] (8)[4]	443.4 ± 21.7 (9)	571.6 ± 22.3 (8)	516.2 ± 30.1 (8)
	Enucleate	537.9 ± 23.1 (10)	489.0 ± 21.8 (9)	485.0 ± 26.5 (10)	459.4 ± 44.2 (9)
	Castrate	432.4 ± 25.8 (10)	279.6 ± 14.9 (10)	197.1 ± 6.7 (10)	181.6 ± 22.8 (9)
	Dual	410.7 ± 19.8 (10)	270.7 ± 10.7 (9)	244.2 ± 22.5 (9)	183.1 ± 9.9 (9)
Preacclimated	Control	430.7 ± 16.4 (11)	416.3 ± 17.3 (6)	487.5 ± 17.5 (6)	459.3 ± 42.0 (7)
	Enucleate	468.3 ± 41.2 (11)	429.8 ± 32.0 (6)	499.9 ± 30.1 (6)	470.2 ± 31.0 (7)
Warm	Castrate	329.3 ± 25.6 (6)	225.5 ± 17.9 (7)	201.5 ± 14.1 (7)	153.7 ± 18.4 (7)
	Dual	335.7 ± 16.8 (6)	192.9 ± 8.3 (8)	182.9 ± 15.2 (8)	146.4 ± 22.5 (6)
Preacclimated	Control	401.6 ± 18.8 (14)	408.9 ± 16.6 (7)	368.2 ± 31.9 (6)	437.9 ± 20.0 (7)
	Enucleate	449.3 ± 20.5 (15)	365.6 ± 33.8 (8)	281.0 ± 30.8 (8)	316.1 ± 49.6 (7)
Cold	Castrate	273.1 ± 21.0 (7)	203.3 ± 17.0 (8)	187.7 ± 18.5 (8)	150.5 ± 16.3 (7)
	Dual	289.6 ± 22.2 (8)	264.4 ± 39.9 (6)	165.7 ± 15.8 (5)	154.0 ± 13.7 (5)
Preacclimated	Control		526.0 ± 32.3 (5)	648.0 ± 47.1 (5)	529.3 ± 49.6 (5)
Warm	Enucleate		473.7 ± 41.4 (3)	556.4 ± 55.4 (7)	497.0 ± 75.2 (4)
ACTH[5]	Castrate		389.0 ± 24.2 (4)	258.9 ± 12.8 (8)	202.0 ± 24.5 (5)
	Dual		347.0 ± 32.7 (7)	262.8 ± 12.6 (7)	232.0 ± 24.8 (4)
Preacclimated	Control		532.0 ± 32.7 (9)	572.0 ± 35.9 (10)	478.0 ± 48.7 (7)
Cold	Enucleate[6]		434.0 ± 19.7 (4)	376.5 ± 31.7 (4)	343.0 ± 19.3 (6)
ACTH[5]	Castrate		364.0 ± 32.0 (4)	303.2 ± 9.3 (7)	187.2 ± 11.2 (6)
	Dual[6]		303.0 ± 33.3 (4)	- - - - - - -	- - - - - - -

[1] Absolute Control
[2] Arithmetical mean
[3] Standard error of the mean
[4] Group Population
[5] ACTH - 15 I.U./Day in 16% gelatin I.P.
[6] ACTH began on day +3 of treatment

PLATE IV





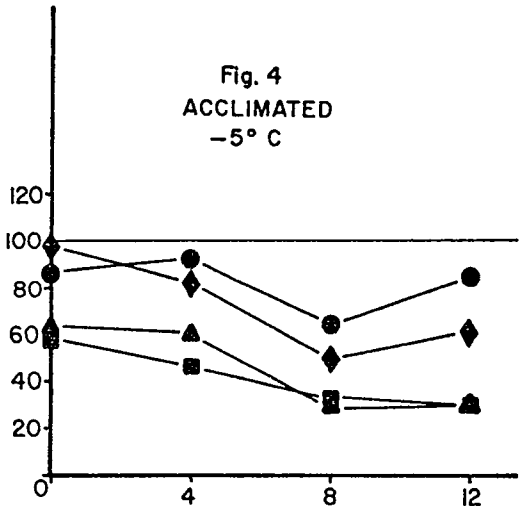


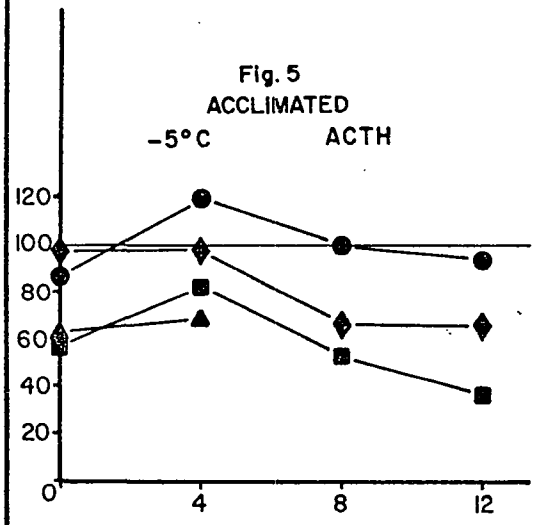
SEMINAL VESICLES

WEIGHT

% OF CONTROL (100%)
VERSUS
DAYS OF TREATMENT

- INTACT
- ENUCLEATED
- CASTRATED
- DUAL-OPERATED





The exposure of the intacts and enucleated to severe cold (fig. 4) causes a significant drop in the seminal vesicle weight of these animals. The organ weights of the intacts tend to recover after 12 days of exposure, while those of the enucleates do not recover as much. Cold exposure causes the accelerated regression of the seminal vesicle weight to a basal level (35% of the absolute control values) after 8 days of exposure. The dual operated, however, show a retardation of the expected organ weight regression at day 4, and their organ weights are significantly heavier than those of the both the warm and the cold exposed castrated as well as those of the warm exposed dual operated. This appears as the only point where exposure of castrated animals to severe cold results in a statistically significant suggestion of adrenal androgenic activity.

The warm exposed intacts treated with ACTH (fig. 3) respond with a gradual increase in the accessory weights, reaching a plateau 10-20% above the untreated levels by the 12th day. The enucleated respond similarly. Both the castrated and dual operated show a significant but, transient elevation in organ weight at the 4th day of ACTH treatment and remain above untreated levels.

The cold exposed intacts treated with ACTH react similarly to the warm exposed (fig. 5), but the enucleated do not respond as much as they do in the warm. Cold exposed castrates receiving ACTH show a small increase resulting in a temporary retardation of the seminal vesicle weight regression.

Dorsolateral Prostate Weight (Table 5 and Plate V)

The dorsolateral prostate weight shows responses similar to that observed in the other androgenic indices.

Non-acclimated enucleates do not differ from the absolute controls in their organ weight, and the castrated and dual operated animals show the expected 'castration' effects (fig. 1).

Following acclimation (fig. 2), the warm exposed intact and enucleated have similar prostate weights, although their weights are less than the non-acclimated controls at day 0 of treatment. The dorsolateral prostate weights of the castrated and dual operated animals do not differ from those of the non-acclimated counterparts.

Exposure of the animals to severe cold (fig. 4), causes a significant depression of the organ weights of the intact and enucleated animals; those of the intacts tend towards recovery of their organ weights, while those of the enucleated remain depressed after 12 days of cold exposure. The castrated show the castration response immediately upon exposure; the dual operated show a retardation of the expected organ weight regression at 4 days of cold exposure, but are not significantly different from the castrated, consistent with the retardation observed at this point in the other androgenic indices.

In the warm exposed ACTH treated, there is evidence of androgenic stimulation (fig. 3): intact animals display a gradual increase in organ weight, while those of the enucleates

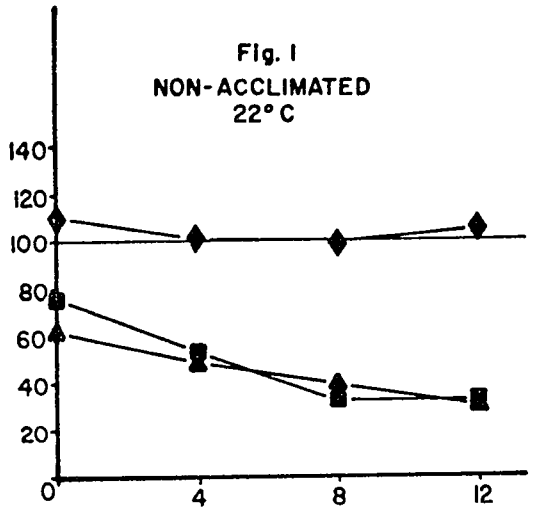
TABLE 5

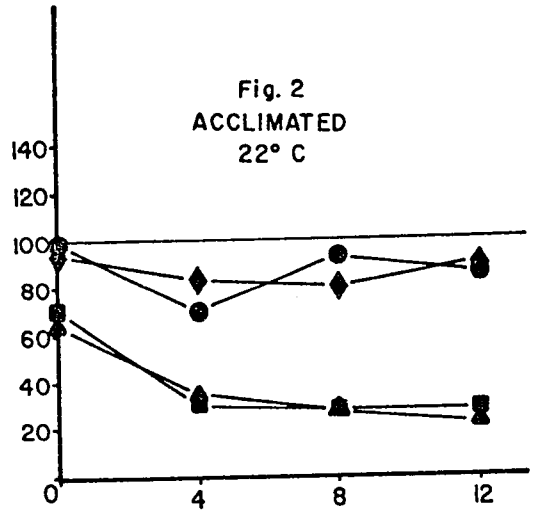
DORSOLATERAL PROSTATE - WET WEIGHT (mgm)

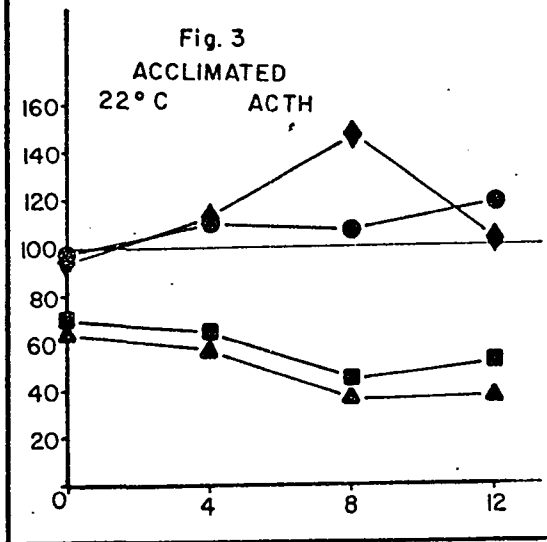
Class of Treatment		Day 0	Day 4	Day 8	Day 12
Absolute	Control[1]	235.8[2] ± 21.0[3] (8)[4]	265.1 ± 21.0 (10)	275.8 ± 17.9 (8)	278.9 ± 8.3 (8)
	Enucleate	258.7 ± 19.7 (10)	266.9 ± 18.6 (9)	273.7 ± 18.4 (10)	292.3 ± 27.3 (10)
	Castrate	175.7 ± 13.8 (10)	138.0 ± 6.1 (10)	89.0 ± 4.6 (10)	88.9 ± 5.6 (9)
	Dual	145.9 ± 7.9 (10)	127.2 ± 8.7 (9)	107.6 ± 7.5 (9)	86.2 ± 8.4 (9)
Preacclimated Warm	Control	229.8 ± 14.5 (11)	184.1 ± 17.6 (6)	255.9 ± 30.2 (6)	238.3 ± 27.6 (7)
	Enucleate	218.6 ± 28.2 (11)	218.7 ± 20.4 (6)	223.3 ± 20.8 (6)	246.4 ± 27.6 (7)
	Castrate	164.1 ± 16.7 (6)	79.6 ± 6.2 (7)	75.8 ± 8.8 (7)	77.4 ± 4.1 (7)
	Dual	150.1 ± 7.3 (6)	89.1 ± 7.7 (8)	75.7 ± 7.5 (8)	64.9 ± 4.7 (6)
Preacclimated Cold	Control	233.4 ± 14.4 (14)	171.7 ± 24.5 (7)	193.5 ± 21.3 (6)	245.3 ± 11.1 (7)
	Enucleate	223.8 ± 13.3 (15)	138.8 ± 16.7 (8)	136.1 ± 12.0 (8)	148.1 ± 24.5 (7)
	Castrate	161.1 ± 19.7 (7)	66.1 ± 3.7 (8)	57.1 ± 3.9 (8)	77.0 ± 9.2 (7)
	Dual	115.0 ± 15.7 (8)	120.4 ± 32.1 (6)	54.0 ± 2.3 (5)	74.0 ± 7.5 (5)
Preacclimated Warm ACTH[5]	Control		293.0 ± 37.4 (5)	294.0 ± 31.9 (5)	329.0 ± 18.7 (5)
	Enucleate		299.7 ± 22.9 (3)	406.0 ± 38.6 (7)	287.0 ± 31.8 (4)
	Castrate		170.2 ± 22.6 (4)	121.0 ± 7.6 (8)	144.0 ± 10.1 (5)
	Dual		150.8 ± 15.1 (7)	96.9 ± 13.6 (7)	107.0 ± 8.8 (4)
Preacclimated Cold ACTH[5]	Control		251.1 ± 18.2 (9)	353.0 ± 38.9 (10)	238.0 ± 18.9 (7)
	Enucleate[6]		285.2 ± 29.8 (4)	223.0 ± 57.6 (4)	239.7 ± 40.9 (6)
	Castrate		147.3 ± 15.7 (4)	114.4 ± 14.5 (7)	111.0 ± 11.5 (6)
	Dual[6]		204.4 ± 55.0 (4)	- - - - - - - -	- - - - - - - -

[1] Absolute Control
[2] Arithmetical mean
[3] Standard error of the mean
[4] Group Population
[5] ACTH - 15 I.U./Day in 16% gelatin I.P.
[6] ACTH began on day +3 of treatment

PLATE V





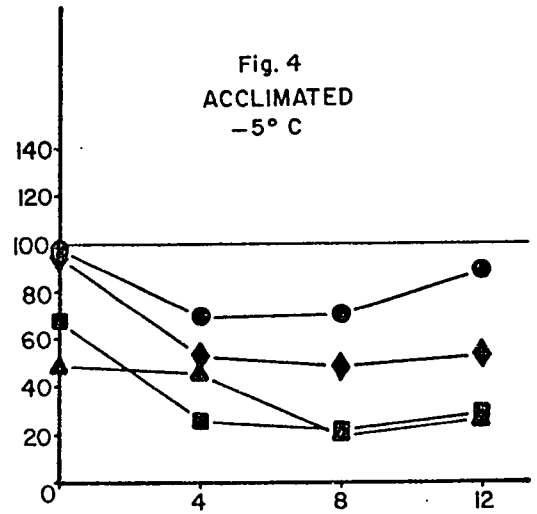


DORSOLATERAL PROSTATE

WET WEIGHT

% OF CONTROL (100%)
VERSUS
DAYS OF TREATMENT

- INTACT
- ENUCLEATED
- CASTRATED
- DUAL-OPERATED



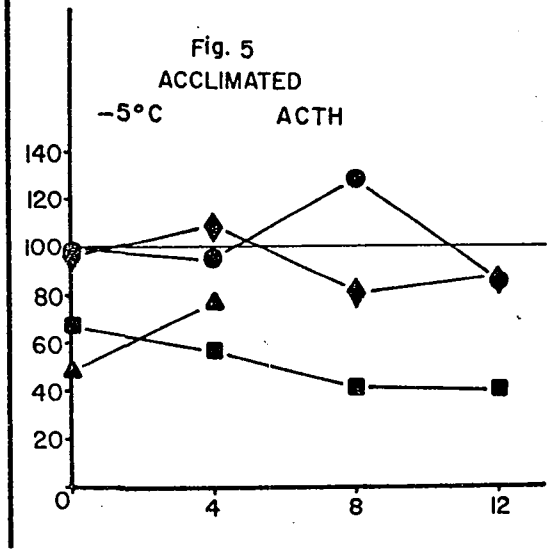


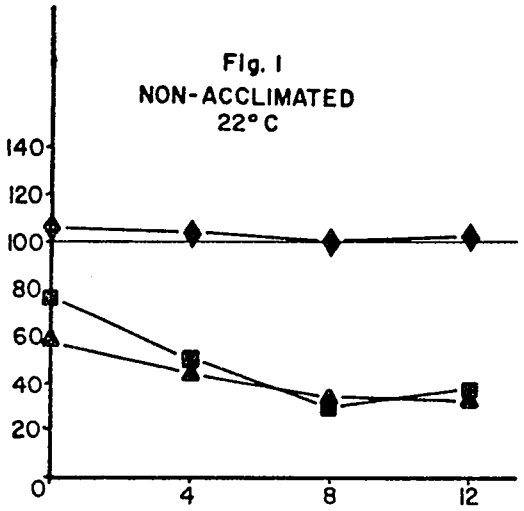
TABLE 6

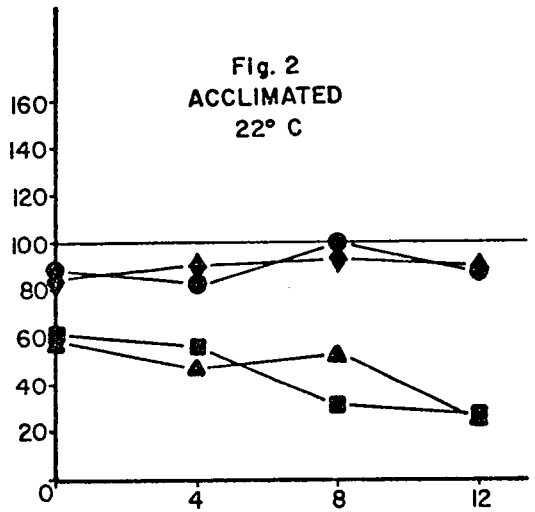
DORSOLATERAL PROSTATE - DRY WEIGHT (mgm)

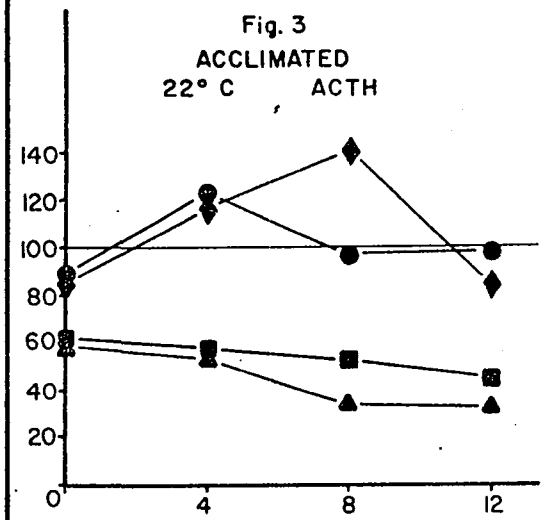
Class of Treatment		Day 0	Day 4	Day 8	Day 12
Absolute	Control[1]	53.6[2] ± 4.0[3] (8)[4]	48.2 ± 3.2 (10)	52.4 ± 2.7 (8)	58.1 ± 22.1 (8)
	Enucleate	56.8 ± 4.3 (10)	48.9 ± 3.6 (9)	52.4 ± 3.8 (10)	58.9 ± 5.0 (10)
	Castrate	40.6 ± 3.3 (10)	23.9 ± 1.2 (10)	15.7 ± 0.9 (10)	21.6 ± 7.8 (9)
	Dual	31.0 ± 1.4 (10)	21.6 ± 1.3 (9)	16.5 ± 0.8 (9)	19.0 ± 1.5 (9)
Preacclimated	Control	47.3 ± 3.0 (11)	39.4 ± 3.5 (6)	52.6 ± 5.0 (6)	50.4 ± 5.4 (7)
	Enucleate	44.8 ± 5.8 (11)	43.2 ± 6.0 (6)	49.0 ± 6.4 (6)	51.0 ± 4.7 (7)
Warm	Castrate	32.1 ± 2.2 (6)	26.7 ± 6.9 (7)	15.4 ± 2.6 (7)	15.3 ± 1.5 (7)
	Dual	31.8 ± 1.7 (6)	22.5 ± 2.8 (8)	27.8 ± 9.1 (8)	14.1 ± 7.0 (6)
Preacclimated	Control	44.5 ± 3.1 (14)	38.7 ± 5.0 (7)	41.3 ± 4.6 (6)	46.3 ± 3.5 (7)
	Enucleate	42.0 ± 2.4 (15)	28.2 ± 2.8 (8)	27.7 ± 2.3 (8)	31.4 ± 4.3 (7)
Cold	Castrate	34.3 ± 2.8 (7)	16.2 ± 1.8 (8)	12.8 ± 0.9 (8)	14.5 ± 1.4 (7)
	Dual	25.0 ± 3.2 (8)	20.1 ± 2.2 (6)	9.9 ± 0.9 (5)	14.4 ± 1.5 (5)
Preacclimated	Control		59.2 ± 8.9 (5)	51.1 ± 5.2 (5)	57.2 ± 3.6 (5)
Warm	Enucleate		56.1 ± 5.6 (3)	73.6 ± 7.7 (7)	48.7 ± 7.3 (4)
ACTH[5]	Castrate		27.3 ± 3.9 (4)	27.5 ± 2.7 (8)	25.5 ± 1.6 (5)
	Dual		26.1 ± 2.8 (7)	17.9 ± 2.5 (7)	18.8 ± 1.5 (4)
Preacclimated	Control		46.8 ± 3.1 (9)	64.2 ± 6.6 (10)	44.2 ± 3.7 (7)
Cold	Enucleate[6]		45.5 ± 5.5 (4)	41.9 ± 11.0 (4)	38.7 ± 6.1 (6)
ACTH[5]	Castrate		21.7 ± 1.1 (4)	20.9 ± 3.1 (7)	18.9 ± 1.6 (6)
	Dual[6]		36.7 ± 8.2 (4)	- - - - - - -	- - - - - - -

[1] Absolute Control
[2] Arithmetical mean
[3] Standard error of the mean
[4] Group Population
[5] ACTH - 15 I.U./Day in 16% gelatin I.P.
[6] ACTH began on day +3 of treatment

PLATE VI





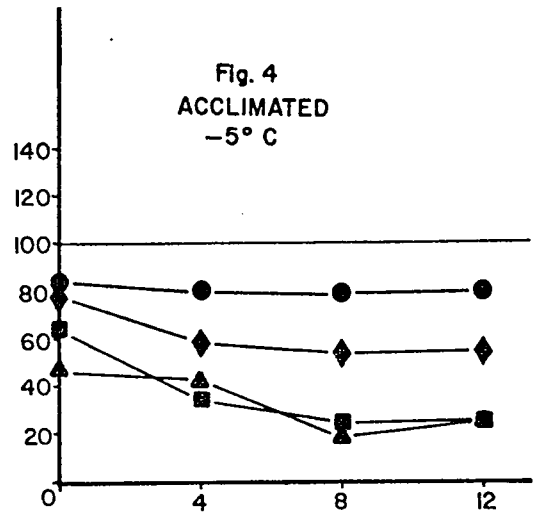


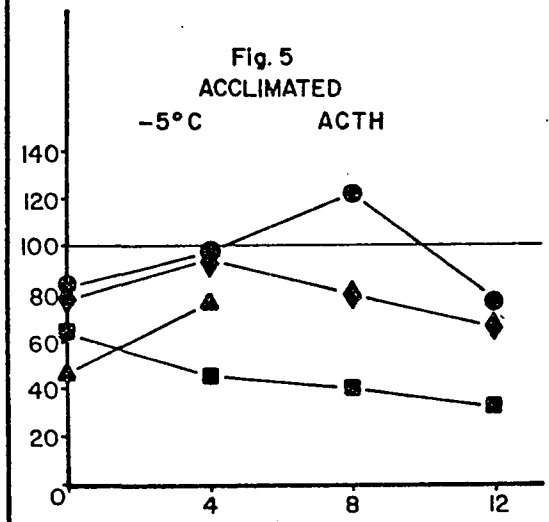
DORSOLATERAL PROSTATE

DRY WEIGHT

% OF CONTROL (100%)
VERSUS
DAYS OF TREATMENT

- INTACT
- ENUCLEATED
- CASTRATED
- DUAL-OPERATED





show a transient but, marked increase, reaching a peak at 8 days (140% of absolute control), and falling to untreated levels (90% of control) by day 12. Castrated and dual operated animals remain slightly above untreated levels (about 10%), but are not significantly different in their androgenic activity.

In the cold exposed animals (fig. 5), ACTH is transiently effective in the intacts and less so in the enucleates; their organ weights are slightly greater than the non-treated counterparts, but less than like-treated warm exposed. The castrated show a slight retardation in the expected regression of the dorsolateral prostate weight, but fall to untreated levels at the end of the treatment period. Dual operated animals have glands that suggest increased androgenic activity after a single ACTH injection.

Dorsolateral Prostate (Dry Weight) (Table 6 and Plate VI)

The dry weight of the dorsolateral prostate shows the same responses observed in the wet weight of the organ (Table 5 and Plate V).

Zinc 65 Uptake by the Dorsolateral Prostate

Absolute Counts Per Minute (Table 7 and Plate VII)

In the non-acclimated animals (fig. 1), enucleation results in a small fall of the zinc 65 uptake at day 0, a return to normal at day 8, and a fall to 65% of control at day 12. On day 0 of treatment, the zinc 65 uptake of both the castrated

TABLE 7

DORSOLATERAL PROSTATE - ZINC 65 UPTAKE (CPM)

Class of Treatment		Day 0	Day 4	Day 8	Day 12
Absolute	Control[1]	7019.5[2] ± 906.6[3] (8)[4]	4773.4 ± 449.7 (10)	5422.2 ± 529.2 (8)	6722.3 ± 876.7 (8)
	Enucleate	5598.9 ± 699.6 (10)	4031.9 ± 484.6 (9)	5551.7 ± 775.0 (10)	4398.4 ± 471.7 (10)
	Castrate	2250.7 ± 196.3 (10)	821.4 ± 105.0 (10)	596.4 ± 52.4 (10)	710.6 ± 395.6 (9)
	Dual	1995.6 ± 349.5 (10)	827.1 ± 157.3 (9)	471.0 ± 54.0 (9)	339.8 ± 78.4 (9)
Preacclimated	Control	3462.0 ± 533.8 (11)	2227.3 ± 188.7 (6)	3472.8 ± 341.2 (6)	3673.0 ±1014.8 (6)
	Enucleate	3038.6 ± 463.6 (11)	2335.2 ± 325.7 (6)	3996.4 ± 554.4 (6)	2519.5 ± 664.6 (7)
Warm	Castrate	879.8 ± 160.2 (6)	354.7 ± 60.3 (7)	276.4 ± 38.9 (7)	245.1 ± 28.0 (7)
	Dual	715.8 ± 133.1 (6)	365.9 ± 53.1 (8)	231.6 ± 32.9 (7)	476.2 ± 228.8 (6)
Preacclimated	Control	3631.3 ± 416.7 (14)	2006.7 ± 601.3 (7)	1401.4 ± 186.6 (6)	2874.5 ± 622.9 (7)
	Enucleate	4094.8 ± 491.6 (15)	1222.3 ± 202.2 (8)	1010.8 ± 262.3 (8)	1193.4 ± 656.2 (6)
Cold	Castrate	871.4 ± 84.5 (7)	247.8 ± 16.9 (8)	220.0 ± 23.4 (8)	204.3 ± 43.5 (7)
	Dual	664.0 ± 117.6 (8)	511.3 ± 211.0 (6)	163.0 ± 16.4 (5)	530.5 ± 338.6 (5)
Preacclimated	Control		4065.0 ± 604.5 (5)	4687.3 ± 787.6 (5)	9132.5 ±1411.3 (5)
Warm	Enucleate		2677.1 ±1733.3 (3)	544.3 ± 545.1 (7)	1544.8 ± 343.9 (4)
ACTH[5]	Castrate		1603.1 ± 111.5 (4)	493.4 ± 69.4 (8)	1103.2 ± 297.6 (5)
	Dual		1100.0 ± 175.5 (7)	502.4 ± 77.8 (7)	404.6 ± 76.5 (4)
Preacclimated	Control		3286.1 ± 312.0 (9)	3620.8 ± 473.8 (10)	3442.8 ± 577.3 (7)
Cold	Enucleate[6]		1893.4 ± 756.3 (4)	2046.6 ± 357.8 (4)	1179.5 ± 120.9 (6)
ACTH[5]	Castrate		800.1 ± 133.0 (4)	374.7 ± 55.4 (7)	314.8 ± 38.1 (6)
	Dual[6]		2347.0 ± 395.7 (4)	- - - - - - - -	- - - - - - - -

[1] Absolute Control
[2] Arithmetical mean
[3] Standard error of the mean
[4] Group Populations
[5] ACTH - 15 I.U./Day in 16% gelatin I.P.
[6] ACTH began on day +3 of treatment

PLATE VII

Fig. 1
NON-ACCLIMATED
22° C

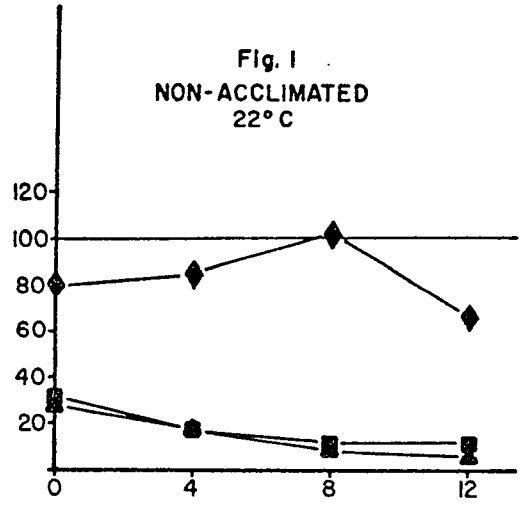


Fig. 2
ACCLIMATED
22° C

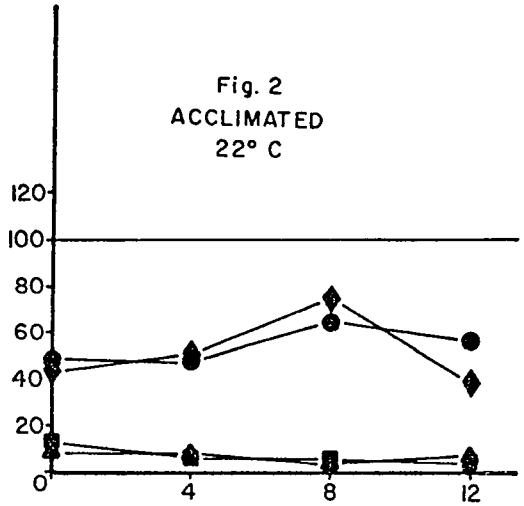
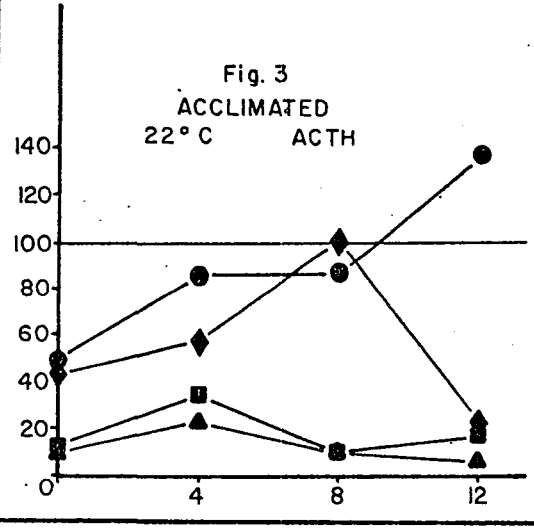


Fig. 3
ACCLIMATED
22° C ACTH



DORSOLATERAL PROSTATE

ZINC 65 UPTAKE (cpm)

% OF CONTROL (100%)
VERSUS
DAYS OF TREATMENT

- INTACT
- ENUCLEATED
- CASTRATED
- DUAL-OPERATED

Fig. 4
ACCLIMATED
−5° C

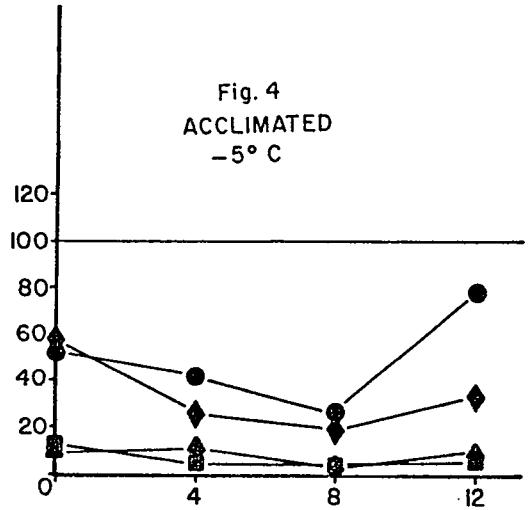
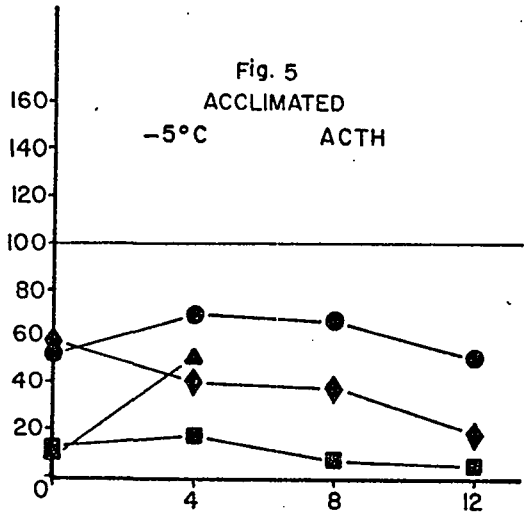


Fig. 5
ACCLIMATED
−5°C ACTH



and dual operated have already dropped to 30% of control, and gradually fall to a basal level of 6-10% of the absolute controls; both the castrated and the dual operated show the same level of androgenic activity.

Following acclimation (fig. 2), all animal groups show a decrease in their zinc concentrating ability. At day 0, the intacts and enucleates are 50% of non-acclimated control intact animals tend towards a recovery, while the enucleates show a transient increase, similar to that seen in the non-acclimated counterparts. The castrated and dual operated are similar: acclimation results in the establishment of the low basal level on day 0 of treatment, and remains at this level to the end of the experimental period.

Exposure to severe cold (fig. 4), causes a depression of the zinc 65 uptake ability of the intact and enucleated; the enucleated are affected more than the intacts, but, the intacts show a significant recovery at the end of 12 days of cold exposure. Exposure of the castrated and dual operated animals to severe cold does not further aggravate the loss of zinc 65 uptake; the zinc 65 level remains at the basal level of 6-10% of control. At day 4, the dual operated have a greater zinc 65 level than the castrated, but, the difference is not significant.

The zinc 65 uptake by the dorsolateral prostate also shows the effects of ACTH administration (fig. 3); intacts show a significant progressive increase, to 140% above the control

values after 12 days of treatment with no indication of having reached a peak. The enucleates, however, respond transiently to the ACTH administration, reaching a peak of zinc 65 uptake on day 8, and falling below untreated levels by day 12. This is similar to the trend observed in the warm non-acclimated and acclimated enucleates, although the response is of different magnitude. The castrated and dual operated show a slight non-significant increase, and remain above untreated levels throughout their treatment; the maximum response is seen on day 4.

The result of ACTH treatment in the cold is seen in fig. 5: cold exposed intacts receiving ACTH maintain a relatively constant level of zinc 65 uptake and show the absence of the depression seen in the untreated cold exposed (fig. 4) but do not increase as do the warm exposed ACTH treated (fig. 3). ACTH treatment to the cold exposed enucleates is androgenically ineffective; the prostates actually lose some zinc 65 compared to the untreated counterparts. The castrated show a slight, non-significant, increase in zinc 65 uptake, but, are essentially the same as the cold exposed untreated castrated. The dual operated hint at anincrease in their androgenic activity, as their zinc 65 uptake rises significantly at day 4, following a single ACTH injection.

Specific Activity (counts/minute/mgm. wet) Table 8 and Plate VIII)
(counts/minute/mgm. dry) Table 9 and Plate IX)

These parameters do not vary appreciably from the absolute uptake values (Table 7 and Plate VII) and indicate similar trends in androgenic activity.

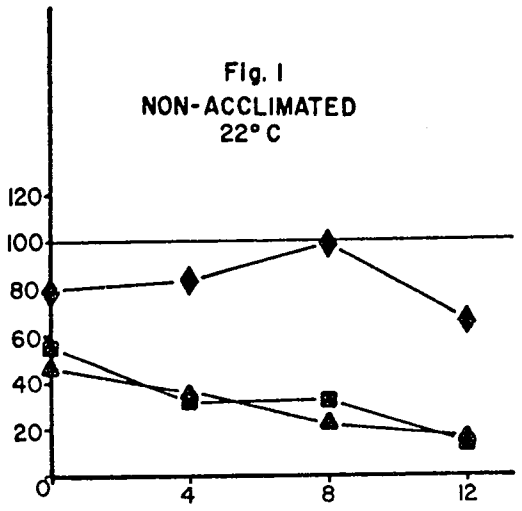
TABLE 8

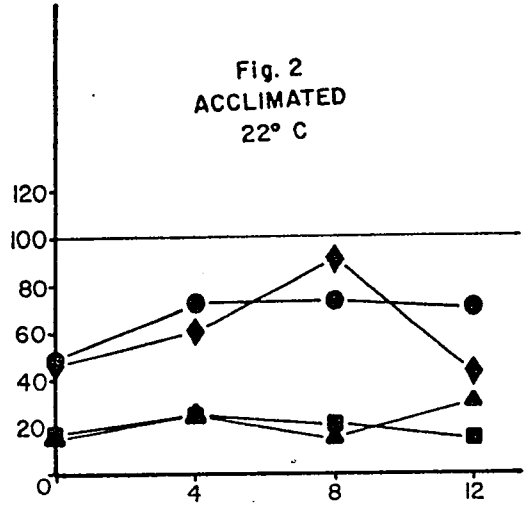
DORSOLATERAL PROSTATE - ZINC 65 UPTAKE (CPM/mgm wet)

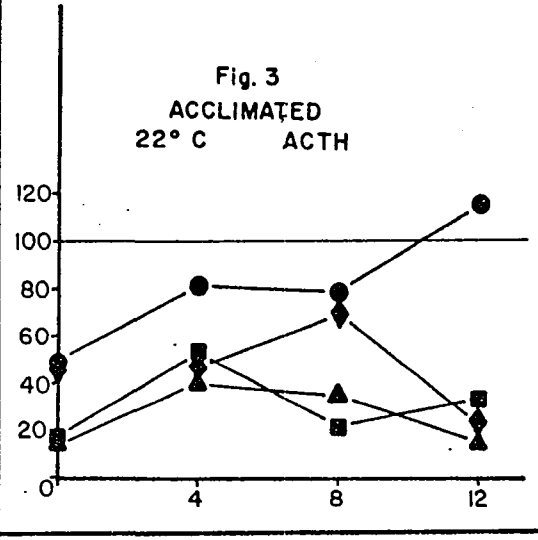
Class of Treatment		Day 0	Day 4	Day 8	Day 12
Absolute	Control[1]	30.2[2] ± 3.8[3] (8)[4]	18.1 ± 1.4 (10)	20.1 ± 2.3 (8)	24.0 ± 2.8 (8)
	Enucleate	23.5 ± 4.0 (10)	15.1 ± 1.6 (9)	19.8 ± 2.5 (10)	15.5 ± 1.8 (10)
	Castrate	16.5 ± 3.6 (10)	6.0 ± 0.8 (10)	6.7 ± 0.4 (10)	3.7 ± 0.3 (9)
	Dual	14.1 ± 2.4 (10)	6.3 ± 1.0 (9)	4.4 ± 0.5 (9)	3.9 ± 0.6 (9)
Preacclimated	Control	14.4 ± 1.5 (11)	13.0 ± 2.2 (6)	14.7 ± 2.5 (6)	16.1 ± 3.4 (6)
	Enucleate	14.1 ± 1.4 (11)	10.8 ± 1.2 (6)	18.0 ± 2.4 (6)	10.1 ± 2.7 (7)
Warm	Castrate	5.2 ± 0.5 (6)	4.4 ± 0.6 (7)	4.1 ± 0.8 (7)	3.3 ± 0.5 (7)
	Dual	4.8 ± 0.9 (6)	4.1 ± 0.4 (8)	3.1 ± 0.3 (7)	7.4 ± 3.7 (6)
Preacclimated	Control	15.0 ± 1.2 (14)	10.7 ± 1.9 (6)	7.3 ± 0.8 (6)	12.0 ± 2.9 (7)
	Enucleate	17.8 ± 1.5 (15)	8.7 ± 1.0 (8)	6.9 ± 1.2 (8)	6.2 ± 2.4 (6)
Cold	Castrate	5.7 ± 0.7 (7)	3.8 ± 0.3 (8)	3.9 ± 0.4 (8)	2.7 ± 0.5 (7)
	Dual	5.9 ± 0.8 (8)	4.0 ± 1.3 (6)	3.0 ± 0.3 (5)	7.0 ± 4.3 (5)
Preacclimated	Control		14.7 ± 2.7 (5)	15.7 ± 1.3 (5)	27.7 ± 3.9 (5)
Warm	Enucleate		8.5 ± 5.4 (3)	13.8 ± 1.3 (7)	5.4 ± 1.1 (4)
ACTH[5]	Castrate		9.9 ± 1.4 (4)	4.2 ± 0.6 (8)	7.7 ± 2.2 (5)
	Dual		7.2 ± 1.0 (7)	7.1 ± 0.8 (7)	3.7 ± 0.4 (4)
Preacclimated	Control		13.4 ± 1.2 (9)	11.1 ± 1.5 (10)	14.9 ± 2.6 (7)
Cold	Enucleate[6]		6.3 ± 2.2 (4)	9.7 ± 0.9 (4)	5.3 ± 0.8 (6)
ACTH[5]	Castrate		5.7 ± 1.2 (4)	3.3 ± 0.3 (7)	2.9 ± 0.3 (6)
	Dual[6]		14.8 ± 4.5 (4)	- - - - - - -	- - - - - - -

[1] Absolute Control
[2] Arithmetical mean
[3] Standard error of the mean
[4] Group Population
[5] ACTH - 15 I.U./Day in 16% gelatin I.P.
[6] ACTH began on day +3 of treatment

PLATE VIII





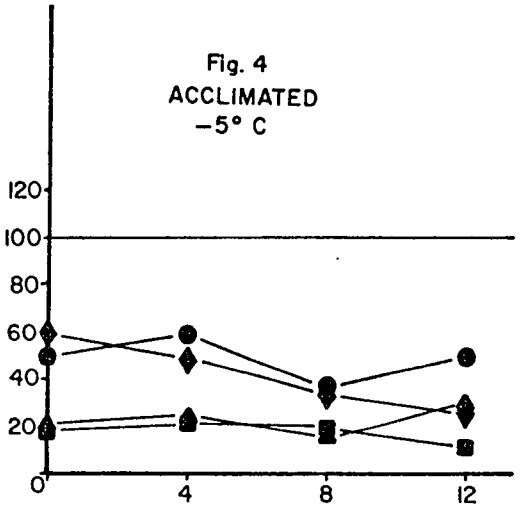


DORSOLATERAL PROSTATE

ZINC 65 UPTAKE (cpm/mg wet)

% OF CONTROL (100%)
VERSUS
DAYS OF TREATMENT

- INTACT
- ENUCLEATED
- CASTRATED
- DUAL-OPERATED



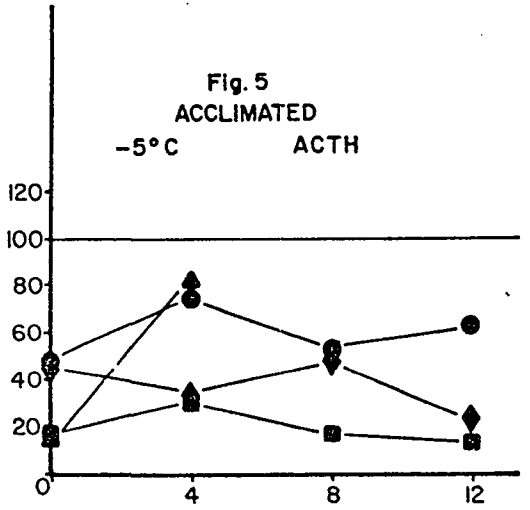


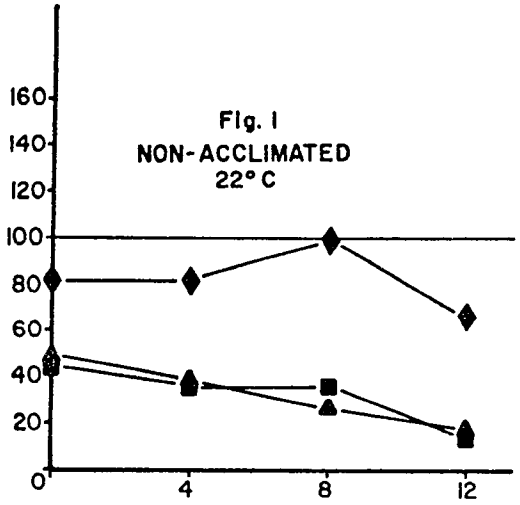
TABLE 9

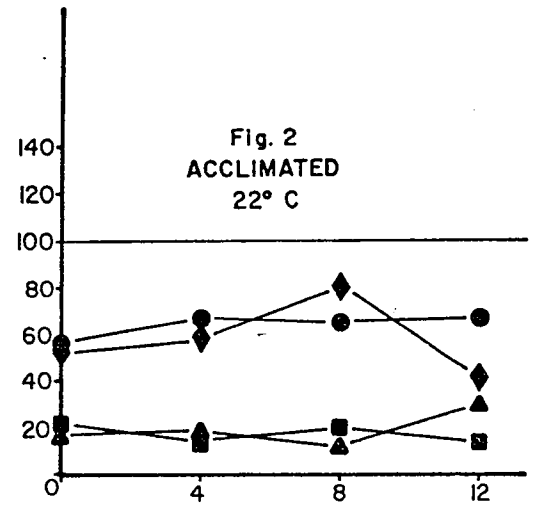
DORSOLATERAL PROSTATE - ZINC 65 UPTAKE (CPM/mgm dry)

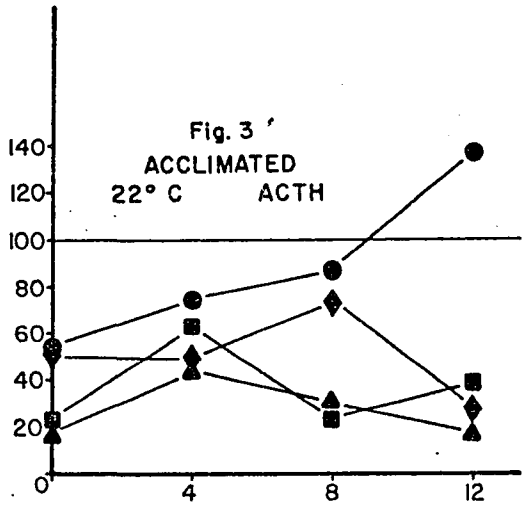
Class of Treatment		Day 0	Day 4	Day 8	Day 12
Absolute	Control[1]	132.0[2] ± 16.3[3] (8)[4]	99.3 ± 6.7 (10)	104.5 ± 11.0 (8)	115.2 ± 11.6 (8)
	Enucleate	106.8 ± 18.0 (10)	80.7 ± 7.8 (9)	104.7 ± 13.4 (10)	76.0 ± 8.6 (10)
	Castrate	57.6 ± 6.0 (10)	35.4 ± 4.9 (10)	38.0 ± 2.6 (10)	15.9 ± 1.8 (9)
	Dual	66.0 ± 11.1 (10)	36.7 ± 5.4 (9)	28.3 ± 2.7 (9)	17.8 ± 3.0 (9)
Preacclimated	Control	71.0 ± 8.1 (11)	66.6 ± 16.9 (6)	68.3 ± 8.8 (6)	78.3 ± 18.2 (6)
	Enucleate	70.2 ± 8.1 (11)	58.5 ± 10.1 (6)	84.9 ± 13.7 (6)	47.6 ± 12.5 (7)
Warm	Castrate	27.6 ± 4.7 (6)	15.8 ± 2.7 (7)	20.5 ± 4.2 (7)	17.5 ± 3.3 (7)
	Dual	22.6 ± 3.8 (6)	16.6 ± 1.9 (8)	11.6 ± 2.3 (7)	33.5 ± 14.3 (6)
Preacclimated	Control	79.9 ± 6.7 (14)	50.5 ± 10.1 (7)	34.2 ± 3.7 (6)	66.9 ± 17.8 (7)
	Enucleate	98.4 ± 11.3 (15)	52.3 ± 9.0 (8)	30.8 ± 5.5 (8)	31.6 ± 12.8 (6)
Cold	Castrate	26.4 ± 3.7 (7)	16.4 ± 1.8 (8)	17.7 ± 2.1 (8)	13.7 ± 1.9 (7)
	Dual	27.1 ± 3.8 (8)	21.9 ± 6.6 (6)	16.5 ± 1.2 (5)	30.5 ± 15.9 (5)
Preacclimated	Control		74.0 ± 14.4 (5)	90.8 ± 10.8 (5)	157.5 ± 19.8 (5)
Warm	Enucleate		46.2 ± 30.0 (3)	77.2 ± 21.3 (7)	31.9 ± 5.9 (4)
ACTH[5]	Castrate		62.3 ± 9.4 (4)	23.9 ± 4.1 (8)	43.6 ± 12.5 (5)
	Dual		42.3 ± 6.3 (7)	30.9 ± 4.8 (7)	21.2 ± 2.9 (4)
Preacclimated	Control		71.3 ± 6.0 (9)	59.8 ± 7.7 (10)	79.6 ± 13.3 (7)
Cold	Enucleate[6]		43.6 ± 19.7 (4)	51.9 ± 6.1 (4)	33.7 ± 6.1 (6)
ACTH[5]	Castrate		37.1 ± 6.5 (4)	18.5 ± 1.7 (7)	16.8 ± 1.7 (6)
	Dual[6]		79.9 ± 26.9 (4)	- - - - - - - -	- - - - - - - -

[1] Absolute Control
[2] Arithmetical mean
[3] Standard error of the mean
[4] Group Population
[5] ACTH - 15 I.U./Day in 16% gelatin I.P.
[6] ACTH began on day + 3 of treatment

PLATE IX





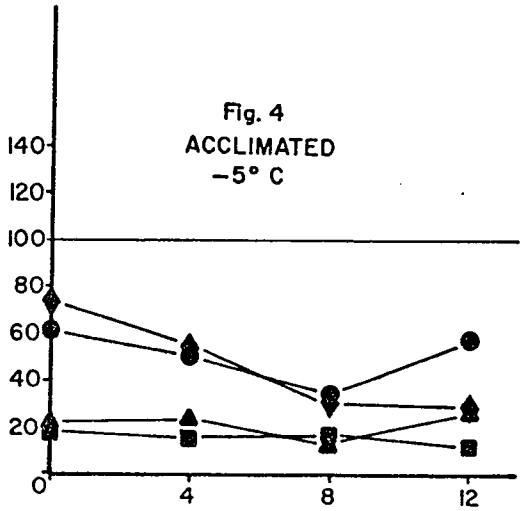


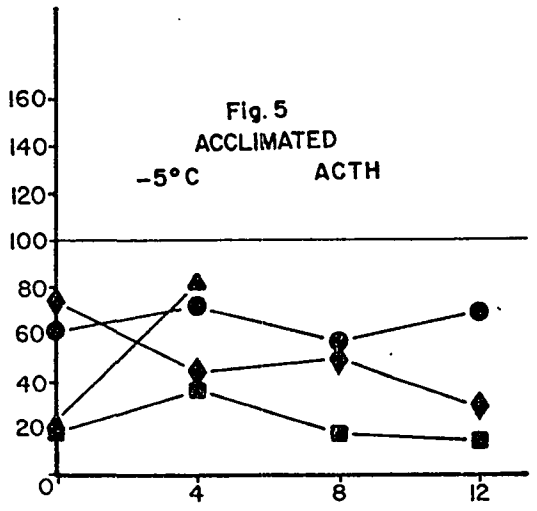
DORSOLATERAL PROSTATE

ZINC 65 UPTAKE (cpm/mg dry)

% OF CONTROL (100%)
VERSUS
DAYS OF TREATMENT

- INTACT
- ENUCLEATED
- CASTRATED
- DUAL-OPERATED





Adrenals

Weights (Table 10 and Plate X)

On day 0 (day +2 post operatively), non-acclimated enucleated and dual operated animals (fig. 1) have adrenal weights considerably above those of the castrated and intact animals; the difference, indicated by the photomicrographs, is due to a swelling of the gland with blood and scar tissue, and does not represent the actual weight of the cortical tissue. By day 4, the weights of the enucleated adrenal glands approach control values, and by the 12th day are not significantly different from the glands of the other groups.

The adrenal weights of the acclimated animals are similar to those of the non-acclimated (fig. 2), although they appear to be less in weight; the difference is significant only at day 8.

Consequent to the exposure to severe cold (fig. 4), the adrenal weights of the castrated and intact animals increase significantly. Those of the cold exposed enucleated and dual operated show a retardation of the adrenal weight regression which can be considered as an increase in the adrenal weights.

The adrenal weights show the expected response to the ACTH treatment in the warm (fig. 3): the intacts show a steady increase in their adrenal weights reaching about 240% above control values after 12 days of treatment, with no indication of having achieved the maximum response. The adrenal weight changes of ACTH treated intacts appear to correspond to the

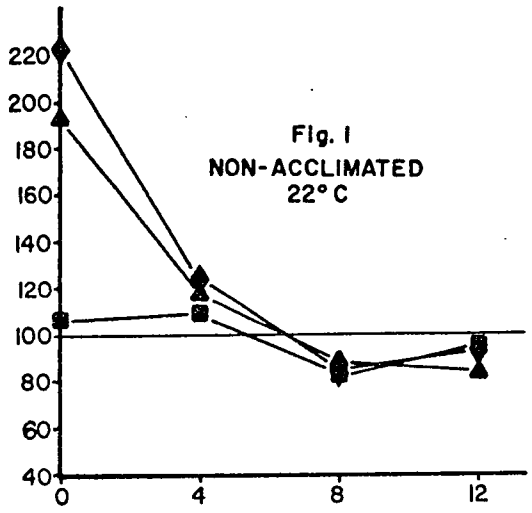
TABLE 10

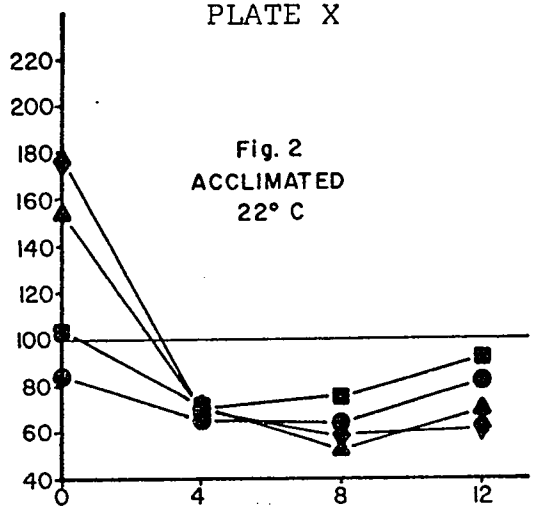
ADRENALS - WEIGHT (mgm)

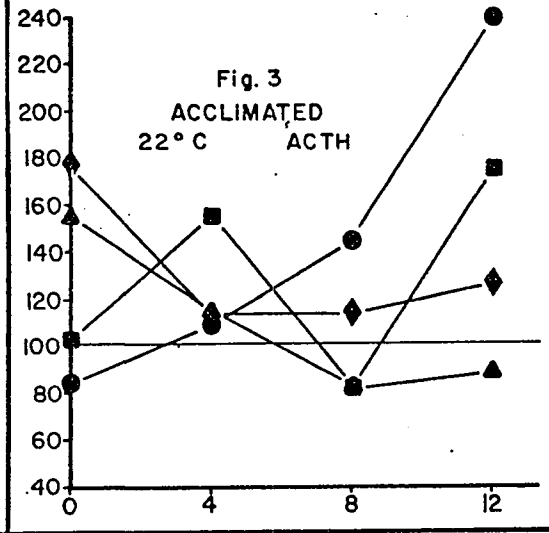
Class of Treatment		Day 0	Day 4	Day 8	Day 12
Absolute	Control[1]	64.7[2] ± 2.5[3] (8)[4]	75.6 ± 4.6 (10)	77.1 ± 4.5 (8)	65.1 ± 3.2 (8)
	Enucleate	144.0 ± 9.6 (10)	94.0 ± 7.2 (9)	64.7 ± 3.7 (10)	60.2 ± 6.2 (10)
	Castrate	69.3 ± 4.2 (10)	82.2 ± 5.6 (10)	64.2 ± 3.2 (10)	60.6 ± 3.1 (9)
	Dual	124.9 ± 7.7 (10)	88.9 ± 7.5 (9)	67.3 ± 4.1 (9)	54.0 ± 5.1 (9)
Preacclimated	Control	54.6 ± 2.1 (11)	50.8 ± 3.3 (6)	49.3 ± 4.1 (6)	53.2 ± 5.3 (7)
	Enucleate	114.3 ± 8.7 (11)	51.5 ± 6.9 (6)	45.8 ± 4.9 (6)	40.1 ± 4.9 (7)
Warm	Castrate	66.8 ± 5.0 (6)	52.9 ± 4.8 (7)	57.7 ± 3.7 (7)	59.3 ± 3.8 (7)
	Dual	100.2 ± 4.7 (6)	51.9 ± 5.6 (8)	41.1 ± 3.3 (8)	44.7 ± 7.1 (6)
Preacclimated	Control	78.7 ± 6.2 (14)	60.8 ± 1.9 (7)	52.8 ± 1.9 (6)	76.2 ± 6.8 (7)
	Enucleate	143.3 ± 5.5 (15)	69.6 ± 5.2 (8)	52.8 ± 5.3 (8)	52.4 ± 5.5 (7)
Cold	Castrate	59.4 ± 3.0 (7)	62.6 ± 3.2 (8)	56.5 ± 2.2 (8)	72.5 ± 5.7 (7)
	Dual	92.4 ± 6.1 (8)	77.2 ± 8.7 (6)	64.8 ± 5.1 (5)	56.7 ± 10.0 (5)
Preacclimated	Control		82.8 ± 8.3 (5)	111.0 ± 15.5 (5)	155.3 ± 11.6 (5)
Warm	Enucleate		85.1 ± 11.2 (3)	87.9 ± 9.2 (7)	81.9 ± 12.6 (4)
ACTH[5]	Castrate		117.0 ± 9.9 (4)	62.3 ± 3.4 (8)	114.0 ± 7.2 (5)
	Dual		88.3 ± 9.9 (7)	62.4 ± 4.6 (7)	57.2 ± 8.8 (4)
Preacclimated	Control		86.6 ± 5.1 (5)	99.1 ± 6.0 (10)	98.2 ± 6.3 (7)
Cold	Enucleate[6]		134.0 ± 17.3 (3)	93.8 ± 11.5 (4)	105.5 ± 14.4 (6)
ACTH[5]	Castrate		108.0 ± 14.4 (4)	77.3 ± 4.1 (7)	89.7 ± 4.9 (6)
	Dual[6]		88.1 ± 17.1 (7)	- - - - - - - -	- - - - - - - -

[1] Absolute Control
[2] Arithmetical mean
[3] Standard error of the mean
[4] Group Population
[5] ACTH - 15 I.U./Day in 16% gelatin I.P.
[6] ACTH began on day +3 of treatment

PLATE X





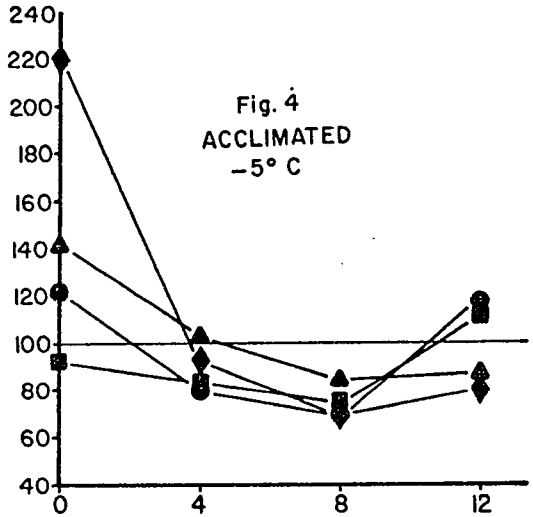


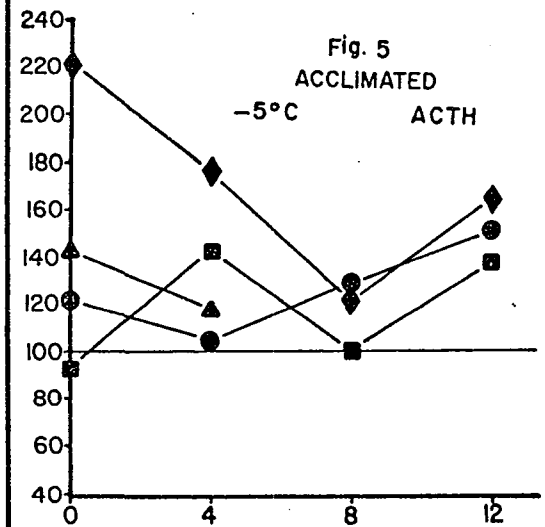
ADRENALS

WEIGHT

% OF CONTROL (100%)
VERSUS
DAYS OF TREATMENT

● INTACT
♦ ENUCLEATED
■ CASTRATED
▲ DUAL-OPERATED





changes in androgenic activity observed through the androgenic indices. The adrenal weights of the castrated animals respond with an alternance of increase and decrease, while the adrenal weights of the enucleated and dual operated show a slight response to the ACTH treatment and remain 60-80% above untreated levels after 12 days of treatment.

The cold exposed animals treated with ACTH show responses in their adrenal weights similar to those of the warm exposed

While there does not appear to be a correlation in all surgical groups between the androgenic activity and changes in the weights of the adrenal glands, the increase in adrenal weights of the cold exposed intacts and the concomitant increase in androgenic activity may be related. Furthermore, the ACTH treated intacts show a close correlation between the adrenal weight changes, and their androgenic activity.

Adrenals

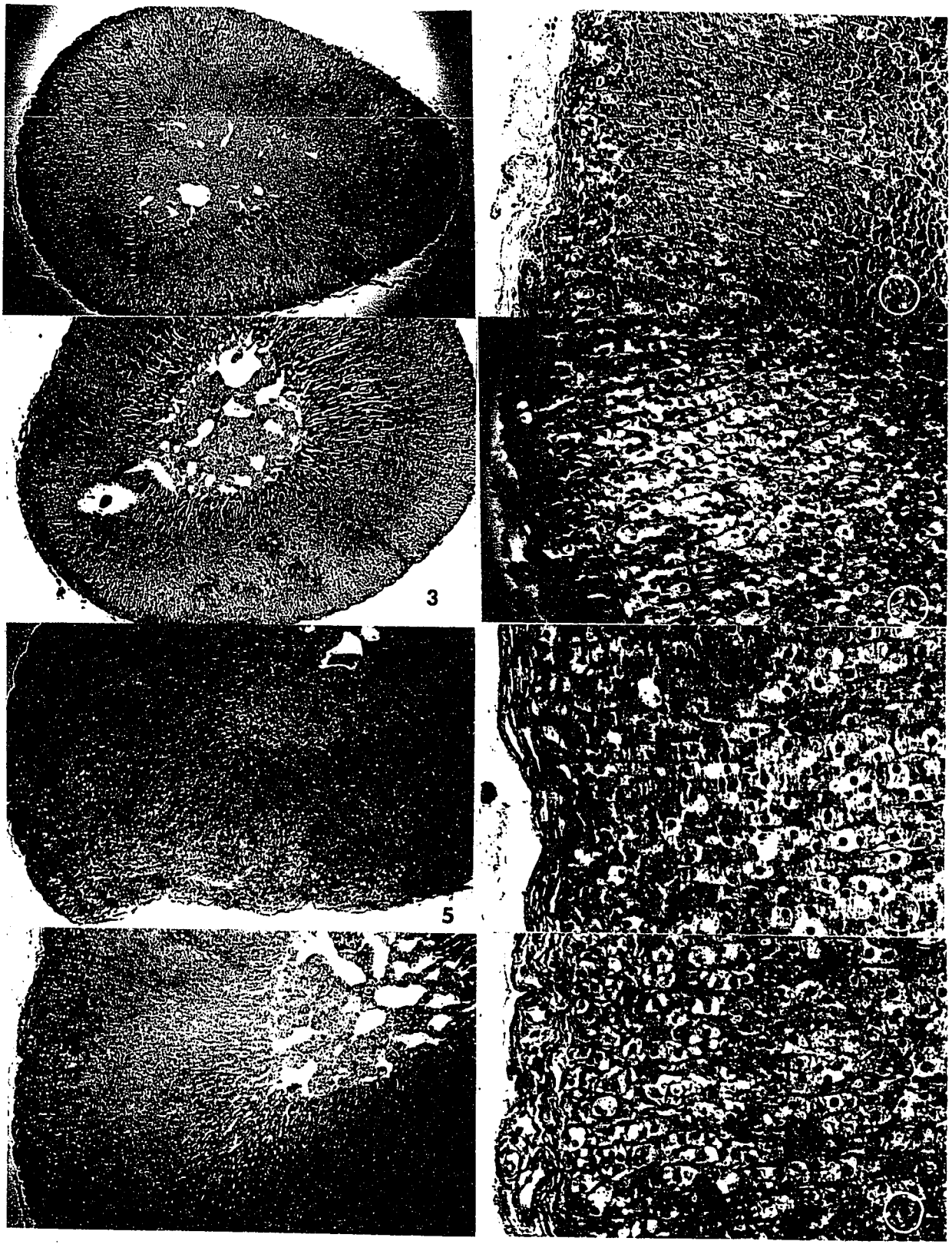
Histology (Figures 2 to 6)

The histology of the normal adrenal gland is shown in Figures 2-1, 2-2. Figures 2-3, 2-4 shows the effect of exogenous ACTH on the structure of the gland; after 12 days of ACTH, the cells of the zonae fasciculata and reticularis appear elongated, with some vacuolation of the cytoplasm. Cold exposure in the intact animals seems to have a similar effect upon the adrenal cortex (Figures 2-5, 2-6), as the cells are also elongated and enlarged compared to the controls. Similarly,

Figure 2

Adrenal Glands From Acclimated Intact Animals

1	Warm Exposed, 12 days,	whole gland		24 x
2		cortex		160 x
3	Warm Exposed, ACTH treated 12 days,	whole gland		24 x
4			cortex	160 x
5	Cold Exposed, 12 days,	whole gland		24 x
6		cortex		160 x
7	Cold Exposed, ACTH treated 12 days,	whole gland		24 x
8			cortex	160 x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the administration of ACTH to the cold exposed animals causes hypertrophy of the cells of inner zones of the cortex (Figures 2-7, 2-8).

The regeneration of the cortex of the enucleated glands is shown in Figures 3 to 6. Figure 3 shows the enucleated glands from the warm exposed pre-acclimated animals and corresponds to +2, +6, +10, and +14 days of regeneration following surgical enucleation of the adrenal cortex.

At day +2 (Figures 3-1, 3-2), the enucleated adrenal appears as a thin connective tissue capsule enclosing a loose aggregation of blood cells and connective tissue fibers; the cortical zonation cannot be readily defined.

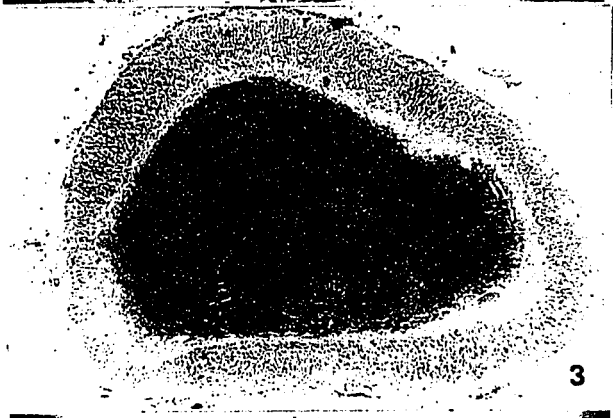
After 6 days of regeneration (Figures 3-3, 3-4), the cortex and its zonation become evident; the zona glomerulosa occupies the major portion of the cortex and the two inner zones are indistinct. The boundary between the cortical and the medullary portions of the gland is composed of connective tissue.

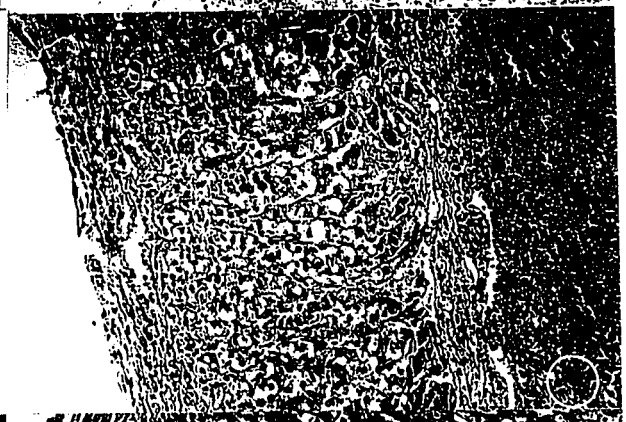
By the 10th regenerative day (Figures 3-5, 3-6), the cortex approaches normality in appearance; the zonae fasciculata and reticularis are prominent, but the zona glomerulosa is not very thick. There appears to be a substantial increase in the number of cells in the two inner zones, the cells presumably deriving from the zona glomerulosa (27, 50). On the 14th day of adrenocortical regeneration (Figures 3-7, 3-8), the cortex has reached normal proportions and resembles the cortex of

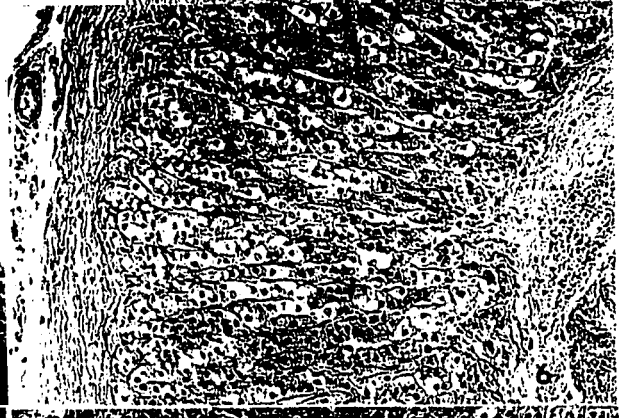
Figure 3

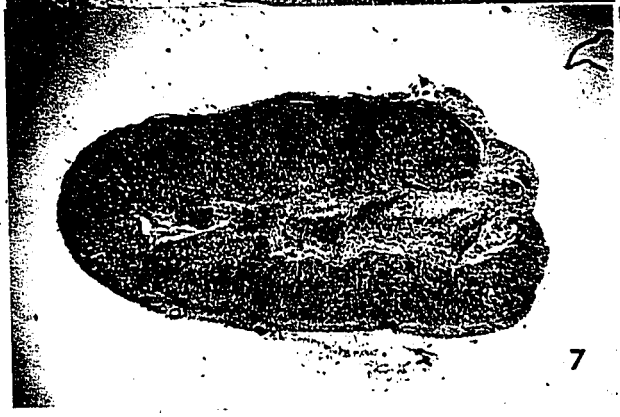
Regeneration of Enucleated Adrenals in Acclimated Warm Exposed Animals

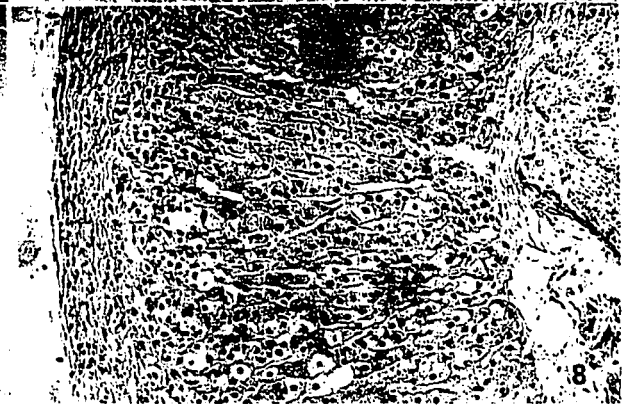
1	2 days post operatively	whole gland	24 x
2		cortex	160 x
3	6 days post operatively	whole gland	24 x
4		cortex	160 x
5	10 days post operatively	whole gland	24 x
6		cortex	160 x
7	14 days post operatively	whole gland	24 x
8		cortex	160 x











the intact animals (Figures 2-1, 2-2). The central portion of the gland is filled with connective tissue and a small amount of blood cells.

The regeneration of the adrenal cortex appears accelerated in the enucleated animals exposed to severe cold. At day 0 of exposure, corresponding to +2 days of regeneration, the cortex is a disorganized array of cells (Figures 4-1, 4-2), but the cortical zonation becomes evident by the 6th regenerative day (Figures 4-3, 4-4) with the cells resembling those of the ACTH treated intacts (Figures 2-3, 2-4). The cortex of the gland regenerated for 14 days possesses clear zonation and a broad cortex (Figures 4-7, 4-8).

Figures 5 and 6 show the regeneration of the glands from enucleated animals (warm and cold) treated with ACTH. It is evident that ACTH accelerates the regeneration process; the glands of the warm exposed ACTH treated enucleates are well developed by the 4th day of treatment (Figures 5-1, 5-2). The cells of the zonae fasciculata and reticularis are large and the cytoplasm often vacuolated, while the zona glomerulosa is thin and the cells appear normal. By the 12th ACTH treatment ie, the 14th day of regeneration, (Figures 5-5, 5-6), the cortex is normal in shape and size with well defined cortical zonation.

In the cold exposed enucleates treated with ACTH (Figure 6), the cortex assumes a normal appearance after the 5th ACTH treatment and a regeneration period of 10 days (Figures 6-1, 6-2). The cells of the cortex are large and somewhat vacuolated

Figure 4

Regeneration of Enucleated Adrenals in Acclimated Cold Exposed Animals

1	2 days post operatively	whole gland	24 x
2		cortex	160 x
3	6 days post operatively	whole gland	24 x
4		cortex	160 x
5	10 days post operatively	whole gland	24 x
6		cortex	160 x
7	14 days post operatively	whole gland	24 x
8		cortex	160 x

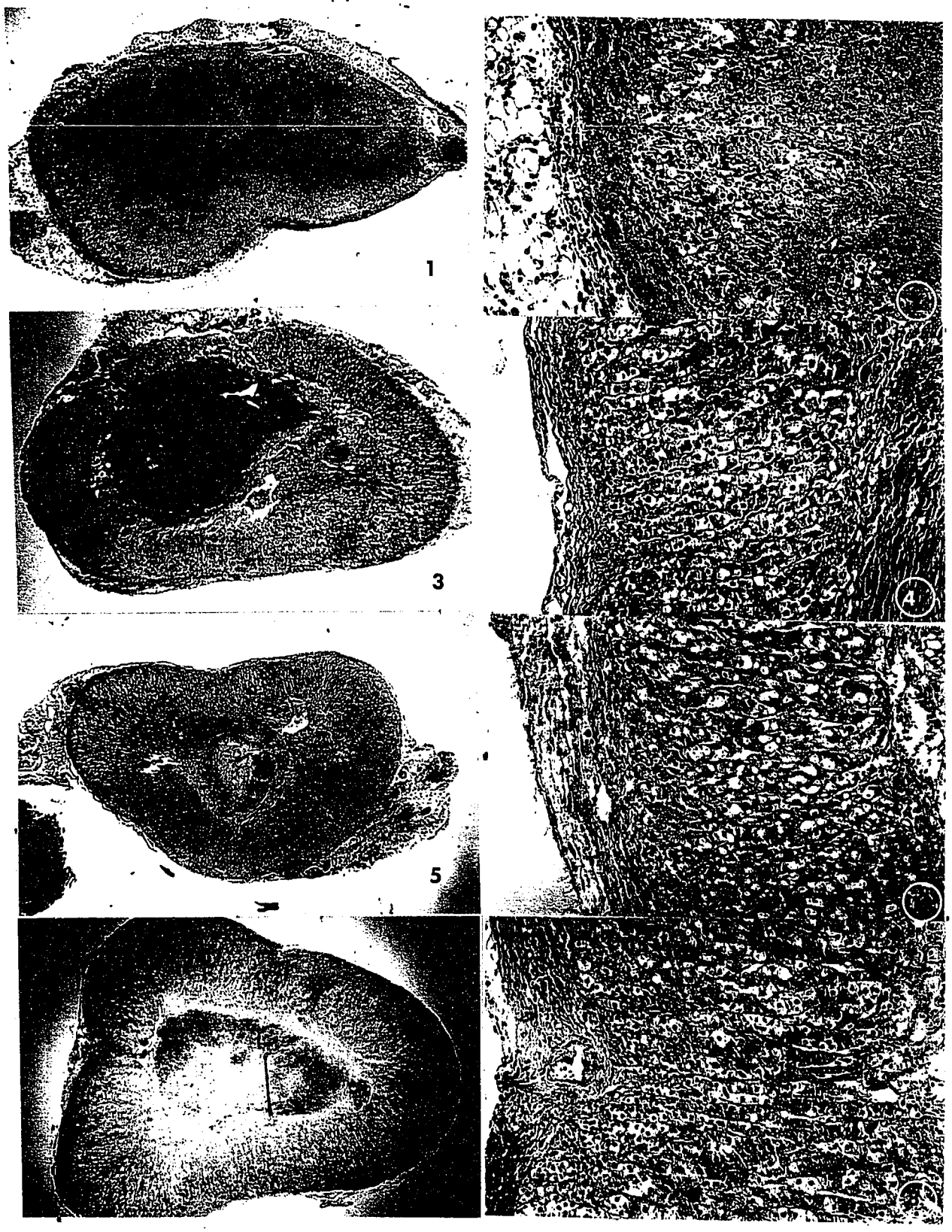
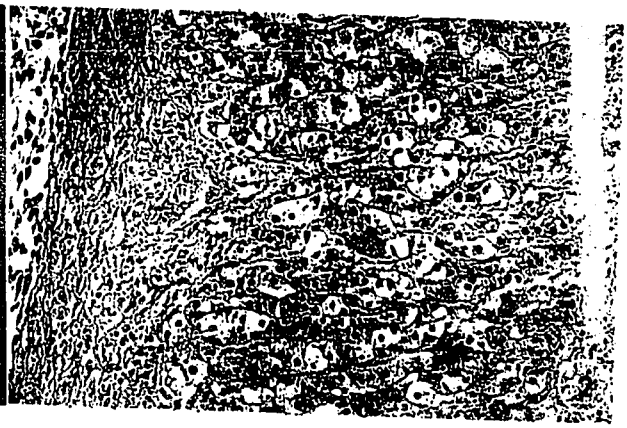
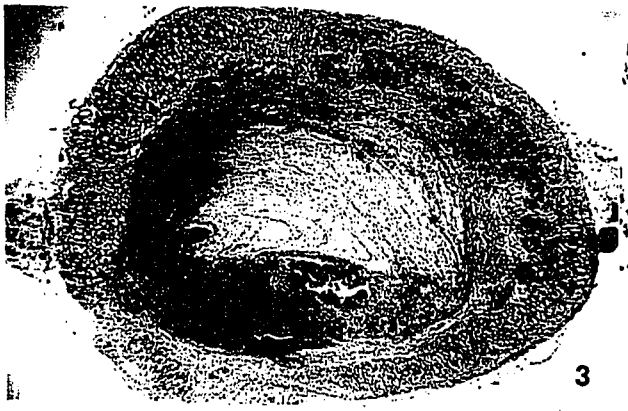

1
2
3
4
5
6
7
8

Figure 5

Regeneration of Enucleated Adrenals in Acclimated ACTH treated, Warm Exposed Animals

1	6 days post operatively, ACTH (4 days)	whole gland	24 x
2		cortex	160 x
3	10 days post operatively, ACTH (8 days)	whole gland	24 x
4		cortex	160 x
5	14 days post operatively, ACTH(12 days)	whole gland	24 x
6		cortex	160 x





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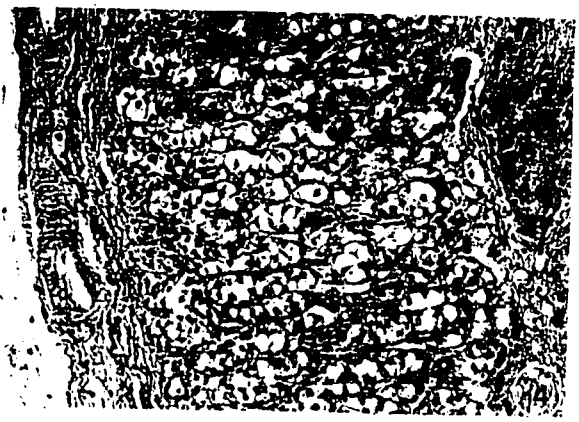

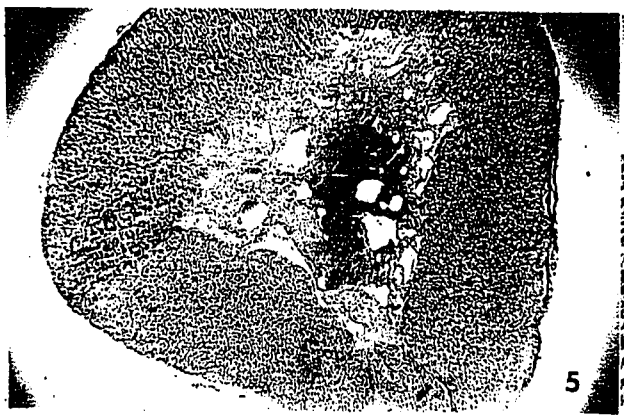

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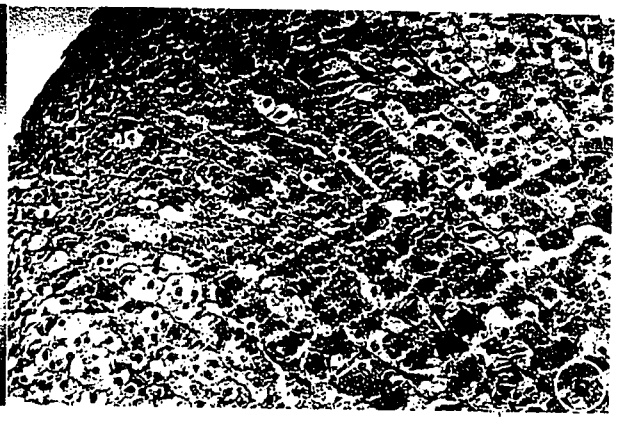
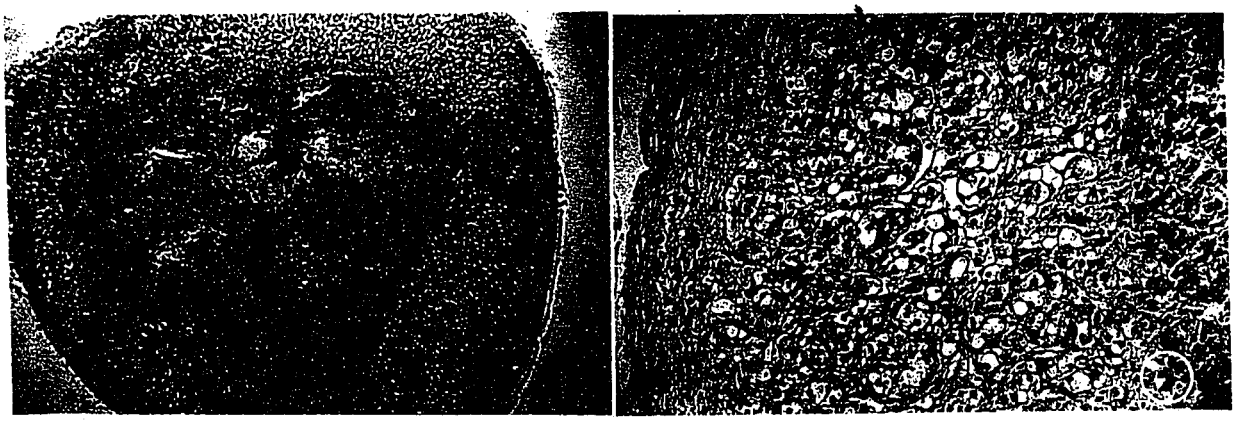


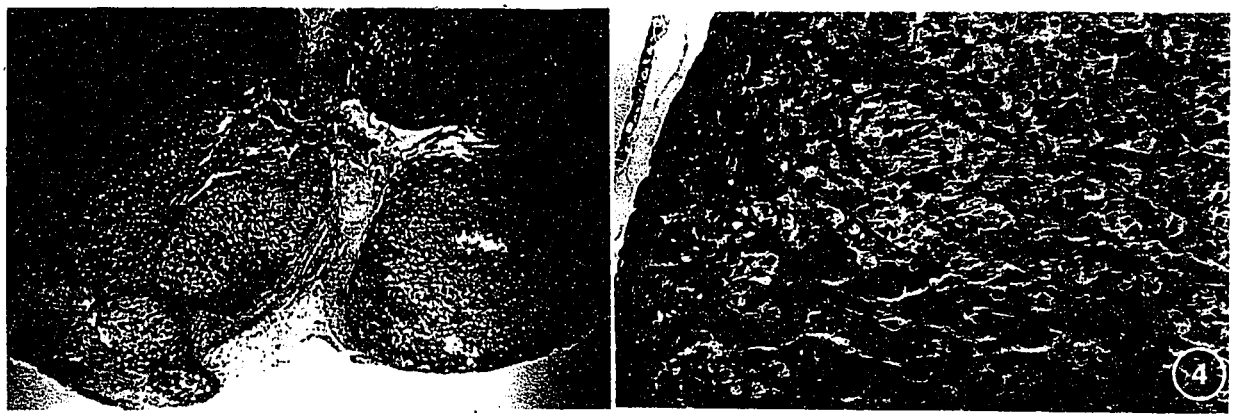
Figure 6

Regeneration of Enucleated Adrenals in Acclimated ACTH treated Cold Exposed Animals

1 10 days post operatively, ACTH (5 days) whole gland x24
2 cortex x160

3 14 days post operatively, ACTH (9 days) whole gland x24
4 cortex x160

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in the cytoplasm. The two inner zones occupy the major portion of the gland (Figures 6-3, 6-4). The medullary portion of all of the regenerated glands is filled with connective tissue and some blood elements.

Thymus Weights (Table II and Plate XI

Changes in thymus weights (and thymic involution) have often been associated with glucocorticoid secretion by the adrenals (51); the weights are therefore included as an indication of the secretory ability of the adrenal glands.

The non-acclimated animals show an increase in their thymus weights, with the exception of the enucleated which undergo some thymolysis on the 12th day (fig. 1).

The acclimated animals all show some thymolysis at the end of the 12th day (fig. 2). The cold exposed animals show a marked and constant thymic involution (fig. 4); the castrated show less thymolysis and presumably less glucocorticoid activity than the other groups which have a thymic weight depression of at least 30%.

The administration of ACTH to the warm exposed animals indicates a differential response (fig. 3); the dual operated and the enucleated show an apparent lack of glucocorticoid secretion, whereas the intacts demonstrate a marked thymolysis. The castrated indicate a reponse intermediate to that of the intacts and the enucleated.

In the cold (fig. 5), treatment with ACTH does not result in thymic involution greater than that observed in the untreated

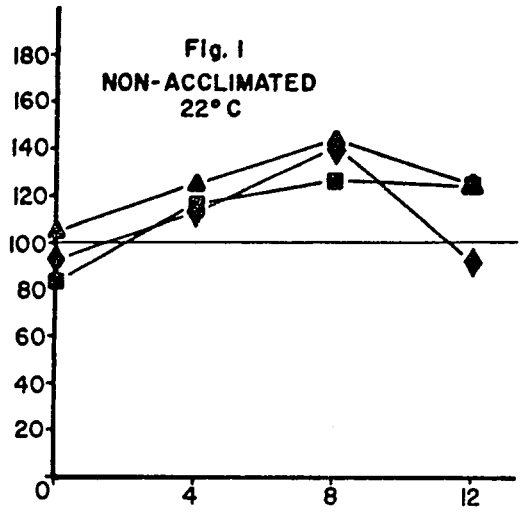
TABLE 11

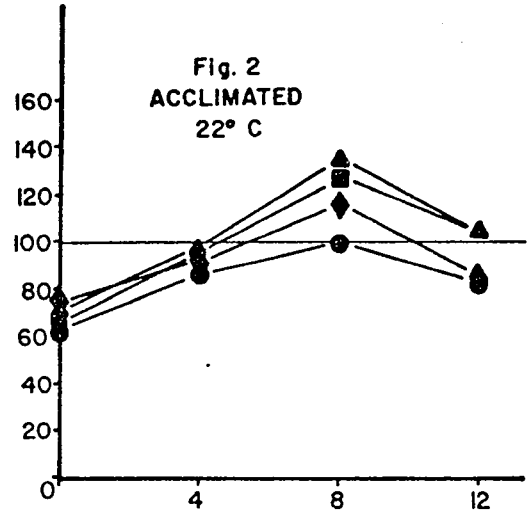
THYMUS - WEIGHT (mgm)

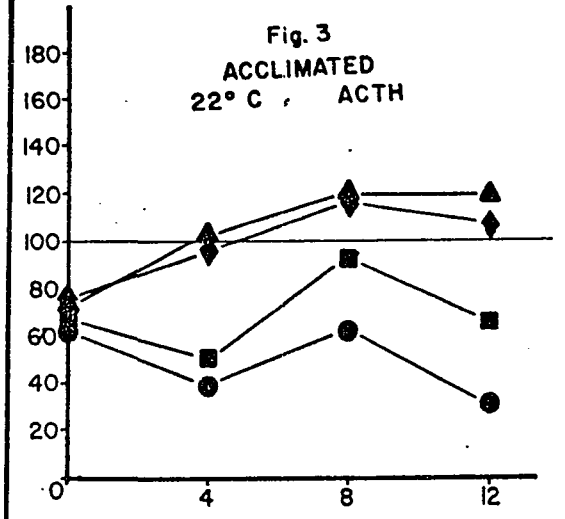
Class of Treatment		Day 0	Day 4	Day 8	Day 12
Absolute	Control[1]	488.4[2] ± 42.9[3] (8)[4]	479.5 ± 26.9 (10)	429.4 ± 35.2 (8)	451.6 ± 25.5 (8)
	Enucleate	456.5 ± 23.1 (10)	546.1 ± 33.5 (9)	600.6 ± 45.3 (10)	412.2 ± 47.4 (10)
	Castrate	407.1 ± 27.8 (10)	552.7 ± 34.7 (10)	542.7 ± 29.8 (10)	558.7 ± 46.8 (9)
	Dual	509.2 ± 17.9 (10)	596.9 ± 51.8 (9)	606.0 ± 45.2 (9)	559.2 ± 84.6 (9)
Preacclimated	Control	303.1 ± 12.6 (11)	412.0 ± 30.0 (6)	429.6 ± 19.8 (6)	368.7 ± 38.5 (7)
	Enucleate	365.6 ± 34.5 (11)	440.1 ± 15.5 (6)	502.1 ± 38.3 (6)	385.0 ± 20.3 (7)
Warm	Castrate	322.1 ± 23.5 (6)	456.4 ± 38.3 (7)	544.4 ± 24.5 (7)	464.3 ± 23.8 (7)
	Dual	357.7 ± 47.3 (6)	458.2 ± 35.3 (8)	575.1 ± 41.7 (8)	467.5 ± 37.0 (6)
Preacclimated	Control	364.0 ± 29.4 (14)	301.8 ± 31.9 (7)	243.5 ± 40.1 (6)	272.8 ± 25.0 (7)
	Enucleate	336.3 ± 25.5 (15)	373.9 ± 36.7 (8)	242.3 ± 28.6 (8)	242.0 ± 57.9 (7)
Cold	Castrate	318.6 ± 25.9 (7)	296.5 ± 37.9 (8)	343.5 ± 40.4 (8)	408.1 ± 47.6 (7)
	Dual	267.4 ± 23.5 (8)	381.2 ± 60.1 (6)	349.7 ± 61.1 (5)	229.4 ± 51.6 (5)
Preacclimated	Control		187.8 ± 24.4 (5)	262.5 ± 41.0 (5)	138.0 ± 11.9 (5)
Warm	Enucleate		461.0 ± 32.4 (3)	496.2 ± 42.8 (7)	479.5 ± 13.6 (4)
ACTH[5]	Castrate		237.0 ± 29.2 (4)	397.0 ± 44.1 (8)	292.0 ± 52.6 (5)
	Dual		487.0 ± 24.8 (7)	503.5 ± 39.1 (7)	538.0 ± 92.1 (4)
Preacclimated	Control		230.5 ± 21.8 (9)	239.4 ± 20.2 (10)	184.0 ± 21.1 (7)
Cold	Enucleate[6]		366.3 ± 17.1 (4)	281.5 ± 65.1 (4)	183.6 ± 22.7 (6)
ACTH[5]	Castrate		164.0 ± 37.6 (4)	327.5 ± 19.9 (7)	265.0 ± 34.2 (6)
	Dual[6]		467.0 ± 99.7 (4)	- - - - - - - -	- - - - - - - -

[1] Absolute Control
[2] Arithmetical mean
[3] Standard error of the mean
[4] Group Population
[5] ACTH - 15 I.U./Day in 16% gelatin I.P.
[6] ACTH began on day +3 of treatment

PLATE XI





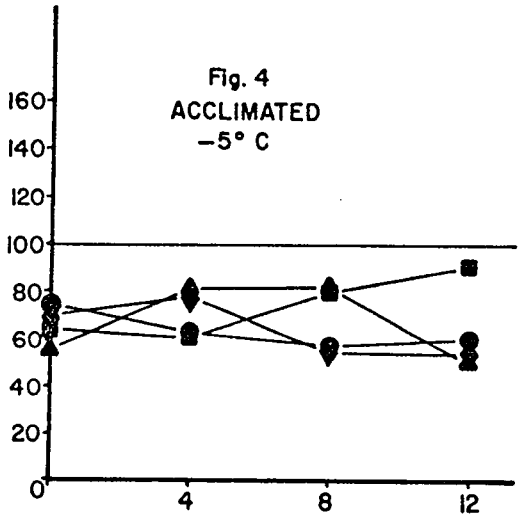


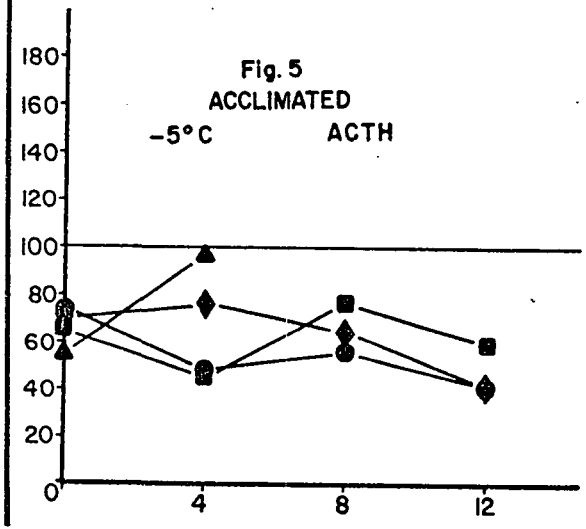
THYMUS

WEIGHT

% OF CONTROL (100%)
VERSUS
DAYS OF TREATMENT

- INTACT
- ENUCLEATED
- CASTRATED
- DUAL-OPERATED





cold exposed animals. The single sampling of the dual operated indicates a lack of thymus involution.

DISCUSSION

Androgenic Expression with Intact Adrenals

The estimation of the adrenal androgenic potential in our experiments used the differential response between the castrate and the intact animal in answer to the stress of severe cold (-5°C). The results indicated that there was an augmentation of adrenal androgens in the intacts but not in the castrated, suggesting a dependence upon the testicular element in the androgenic expression of the adrenal cortex. The small androgenic response in the cold exposed intacts showed that cold stress cannot be considered as the optimum stimulus in obtaining an adrenal-androgenic response. Although cold stress results in an increased ACTH secretion (67), there is also an inhibition of testicular activity; the net effect is probably a masking of the adrenal contribution (11, 12, 13, 15). The use of exogenous ACTH in the warm exposed intacts resulted in the pure adrenal-androgenic stimulation of the sexual accessories and overcame the objections to the use of cold stress.

The stimulation of the sexual accessory organs through ACTH can thus be considered as the model system: ACTH causes production and release of adrenal androgens which affect the target tissues causing an increase in weight of the androgenic indicator organs. The model did not apply in the castrated animals although their adrenals appeared similar in structure and secretory ability to the glands of the intact animals:

thymolysis in both castrated and intact animals was similar but significant androgenic activity was absent in the castrates.

Androgenic Expression with Regenerating Cortices

The determination of the adrenal androgenic potential in enucleates indicated a) that the enucleated glands were capable of affecting the sexual accessory organs, b) that under ACTH stimulation, the enucleated glands secreted androgens at a lower level than that of intact animals treated with ACTH and c) that the androgenic effect appeared transient; the androgenic potential increased with the regeneration of the cortical tissue.

The results of our in vivo study are in contradiction to the in vitro observations of Birmingham et al (44) who were unable to detect androgens in incubated 30 day regenerated glands; their findings may be due to altered metabolism during the extra 16 days of regeneration they allowed in their experiments. The lack of thymolysis in ACTH treated warm exposed enucleated suggests little glucocorticoid activity and corroborates the findings of the same workers (44) who observed an impairment of steroid biogenesis in the zona glomerulosa of the regenerated gland. Hyde and Skelton (42),on the other hand, found an increase in glucocorticoids in enucleated animals, but of a short duration, under stressed and normal conditions. Brudieux and Delost (51) surprisingly, indicate a fall in the corticosteroid content of glands demedullated by electrocoagula-

tion; this may also contribute to the lack of observeable thymolysis. Kolthoff, Macchi and Whyman (43) found similar results in cold stressed enucleates: increased blood volume, increased blood channels, increased retention of hormone in the glands and a decreased steroid level in the blood. Fortier and DeGroot (41) however, observed a supranormal release of corticosterone after 16 days regeneration in situ.

The lack of observeable thymolysis in ACTH treated enucleates and the androgenic stimulation in these animals suggests a dissociation between androgens and glucocorticoids within the enucleated gland and indicates that ACTH may act on more than one site. This is in contradiction to the finding of Singer and Stack - Dunne who found that ACTH acted mainly on the glucocorticoids of the adrenal (52).

It is clear that the enucleated gland cannot be superimposed upon the model of adrenal-androgenic expression formulated for the intact gland; the androgenic activity of the enucleated gland cannot be predicted.

Although the cortical zonation of the regenerating adrenal appeared complete within 10 days after operation, our findings indicated that the enucleated gland does not achieve the androgenic potential of the intact gland in the 14 days post-enucleation. The reduced zona glomerulosa of the enucleated gland corroborates the findings of Birmingham et al (44) that was an impairment of steroid biogenesis within the gland affecting glucocorticoids. The well developed inner zones (reticularis and fasciculata) suggest that

androgenic activity may be present (50, 65); this was verified in the ACTH treated warm exposed enucleates.

While the adrenal contribution to the androgenic pool is minimal under "normal" conditions, a small increase was observeable in cold exposed intacts; the burden of severe cold stress was not sufficient to cause a visible increase in the adrenal androgenic contribution of enucleates indicating the lack of androgenic capability of the enucleated gland under conditions of stress.

Androgenic Expression in Castrates and Dual Operated

One would expect to find a pure adrenal contribution in the absence of the testis. However, neither the cold exposed nor the ACTH treated castrate appeared to display a significant elevation in the circulating androgens. On the other hand, the cold exposed dual operated showed significant androgenic activity at day 4 of treatment; the maintenance of the seminal vesicles at an increased level for a short period could be caused by the transient secretion of androgenic material from the enucleated gland.

As this appeared as the only point where a "pure" androgenic response is suggested after cold stress, it is interesting to note that the administration of ACTH to the cold exposed dual operated also caused significant androgenic activity. However the mortality of these animals after 4 days of treatment precludes extrapolation of these findings.

The treatment of warm exposed castrated and dual operated animals with ACTH failed to elicit significant androgenic activity. Gonbertz (53) suggested that male sex hormone diminished the effect of ACTH on the adrenal cortex, but were this so, the reduced gonadal hormone level in castrated animals would have resulted in an increased ACTH effect. Kitay (54) found that testosterone enhanced adrenal secretion of steroids without moderation by the pituitary and ACTH. He also found (55) that gonadal hormones exerted a multiple effect by inhibiting the pituitary and stimulating the adrenal. Thus, the gonadal hormones may be necessary for the expression of the adrenal hormones. Kitay (55) significantly, found that steroidogenesis in the adrenals of castrated rats remained the same as controls up to 4 weeks after gonadectomy. This supports our contention that the adrenals of intact and castrated animals were similar in our experiments, and that the differential response to exogenous ACTH between castrates and intacts is caused by some other factor than the lack of androgens. Troop and Passanza (56) suggested that a factor is removed in castration which controls the level of steroidogenesis within the animal.

Factors in Androgenic Expression

The depression of the androgenic response in cold exposed enucleates and intacts compared to warm exposed animals is primarily due to the systemic effects of cold upon the organism. This factor is also considered in the responses observed in the

ACTH treated animals and is superimposed upon the endocrine effects resulting in the depression of testicular function in the intacts and enucleated (14).

Since only one level of ACTH was administered (15 I.U./day I.P.), the maximum androgenic potential of the adrenal cortex cannot be determined. The fact that a plateau has not been achieved in the androgenic indices of the intact animals suggests that a submaximal androgenic potential was elicited and is supported by the increase in the adrenal weights.

But enucleated glands show a plateau in their weights and the androgenic secretory ability appeared transient. The androgenic capabilities of enucleated and intact glands thus, cannot be compared.

Our observations on the differences between the intact and enucleated glands are related to those of Chester Jones and Spalding (57) and Chester Jones and Wright (34) who found that ACTH inhibited formation of glomerulosa cells in enucleated glands such that the cells in the zona reticularis and zona fasciculata now predominated. This suggests that there may be a functional zonation of the glands of enucleated animals.

The validity of our findings in the observed differences of thymolysis in enucleated and intact animals is supported by Bergner's and Deane's (58) work on the relationship between increased glucocorticoid output by the adrenal and thymolysis in intact animals.

The Response of the Target Organs to Androgens

There is evidence to indicate that hypertension develops in rats with regenerating adrenal cortices under conditions of high salt intake (59, 60). Grollman (61) ascribed the hypertension to a hyper-responsitivity of the target tissues to corticoids during the initial period of adrenal insufficiency. This may explain the high mortality among ACTH treated cold exposed dual operated animals; the combination of adrenal insufficiency, development of hypertension and cold stress coupled with increased adrenal steroid output consequent to exogenous ACTH may have been sufficient to cause death. This is substantiated by the decreased mortality obtained by withholding the ACTH treatment of cold exposed enucleates until day + 3 of cold exposure; allowing the animals to overcome the initial adrenal insufficiency.

A hyper-responsivity of the androgenic target organs during the post-operative period in the enucleates may have contributed to the transient androgenic response observed in the enucleated and dual operated animals.

The assessment of the circulating androgens by means of the androgenic indicators (sexual accessory organs and zinc 65 uptake by the dorsolateral prostate) must take into account the differential responses of the tissues to the circulating androgens under various physiological states; thus the response from one tissue to another is not always of the same magnitude.

The high androgenic activity of dual operated ACTH treated warm exposed animals at day 12 is not supported in the other androgenic indices and could be considered an artefact.

The possibility that a steroid will visibly affect the sexual accessories is dependent upon i) adsorption, ii) utilization, iii) binding at the active site, iv) conversion and v) excretion. Since some steroids are excreted rather quickly ( ) it is possible that excretion may exceed conversion and hence utilization. This implies that while the assessment of the androgenic potential by weights of the sexual accessories is a relatively reliable method (45), it does not exclude the possibility that the androgens produced and released by the adrenals are not being expressed. This also emphasizes the importance of experimentation at the whole animal level, rather than in vitro studies. Furthermore, the variation from organ to organ indicates the importance of multiple parameters in the assessment of the circulating androgens.

Hypothetical Modes of Adrenal-Androgenic Expression

To account for the difference in response between the ACTH stimulated intact and castrated animals we propose two hypothetical schemes whereby the adrenal androgens are expressed in vivo.

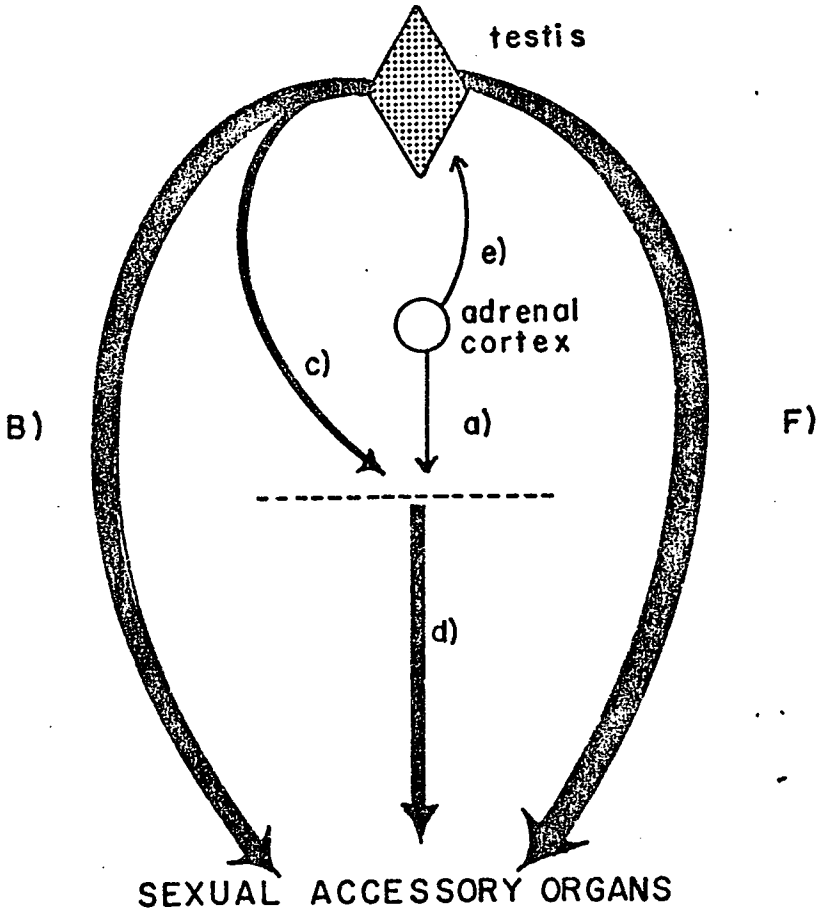
It is obvious from our experiments that we can elicit and observe an adrenal androgenic response in the sexual accessories

FIGURE 7

HYPOTHETICAL MODES OF ADRENAL-ANDROGENIC EXPRESSION

1 PERMISSIVE ACTION of TESTES

2 TESTES as METABOLIC SITE



by using exogenous ACTH in intact animals, but not to the same extent in castrated animals. It is our belief that the differential response may be due to the removal of a factor in the castration procedure which precludes expression of the adrenal androgens.

In the first scheme (Figure 7-1), we propose a permissive role for the testes. The adrenal androgens are represented by (a) and the testicular androgens by (b). The testicular androgens (b) would have a component (c) which is present at threshold levels or higher. At these levels the component (c)can potentiate the adrenal androgens (a) to produce the androgenic vector (d) which is greater than the androgenic component (a) alone.

In the intact animal, the permissive level is provided by vector(c)and the adrenal androgens (a), supplied by the action of ACTH on the adrenal cortex, are potentiated to be expressed in the increased weight of the sexual accessory organs.

Following castration, the testicular androgens (b) disappear and consequently vector(c)is removed; the permissive level is reduced below threshold and the androgenic component (a) becomes relatively expressionless even under high ACTH stimulation.

The vector(c)may take many forms including a binding protein, catalyst, or co-factor. It is not improbable that testosterone, or other steroid peculiar to the testes in small

quantities, may be the potentiating factor, just as corticosterone has been shown to exert an inhibitory effect on the sexual accessories (62, 63).

The second scheme (Figure 7-2), proposes that the testes can exist as a metabolic site for the adrenal androgens. Here, the testicular androgens are represented by (f) and the adrenal androgens by (e). The adrenal androgens (e) could be converted by the testes to the more potent testicular androgens and emerge as a more potent testicular effluent (f).

Thus, in a normal animal, ACTH stimulation causes hypersecretion of adrenal androgens (e), which are transported to the testes and converted in the metabolic machinery of the testes to more potent forms and hence the testicular effluent (f) appears to be increased in quantity of androgens.

In the castrate, however, the testes are removed, and the metabolic site, with the result that a much poorer androgenic display can be evoked with ACTH.

The potency of the main adrenal androgen, Δ4-androstene-3, 17-dione, is about one fifth of the main testicular androgen, testosterone (45). Furthermore the conversion of the adrenal androgen to the testicular androgen has been shown to occur in testicular tissue but not to a great extent in adrenal tissue except in abnormal situations (65, 67).

This scheme does not exclude the possibility that the testes may exist only as a link in a chain of reactions leading to the formation of the expressed adrenal androgens. Thus in a

cascade of step reactions, the chain may start in one tissue (eg. the adrenal), move to another tissue, and lead back to the original tissue for an end result that may be the first ever seen by the observer (66). The removal of one of the links in the chain would thus preclude total expression of the adrenal androgens. The facts of our experiments lead us to believe that the lack of expression of the adrenal androgens is not simply due to the absence of androgens from the gland, but due to the removal of the testes themselves, suggesting the presence of a positive factor from the testes necessary for the full expression of the adrenal androgens. It also indicates the importance of the testes in the maintenance of the pituitary - adrenal - testicular axis; the adrenal cannot maintain the sexual accessory organs in the absence of the testes even when additional ACTH is added.

CONCLUSIONS

1) The androgenic capability of the adrenal cortex of intact animals can be elicited in the presence of the testes by both ACTH and stress (severe cold -5°C).

2) Regenerating adrenal cortices have androgenic activity, demonstrable only under exogenous ACTH administration to enucleated warm exposed animals.

3) The adrenal glands of intact and enucleated animals are incapable of maintaining the sexual accessories in the absence of the testes in adult male rats.

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