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## ABSTRACT

THE PRESENT EXPERIMENTS ATTEMPT TO EVALUATE THE ADRENAL CONTRIBUTION TO THE OVERALL ANDROGENIC ECONOMY IN THE ADULT MALE RAT USING THE STRESS OF SEVERE COLD ( $-5^{\circ}\text{C}$ ) AND PHARMACOLOGICAL REPLACEMENTS.

IN MALE RATS CHRONICALLY EXPOSED TO COLD THE GENITAL ACCESSORY ORGANS SUFFERED A TRANSIENT DEPRESSION BUT WERE RESTORED TO ALMOST NORMAL ALTHOUGH THE TESTES REMAIN ATROPHIC. THE GENITAL ACCESSORIES OF COLD-EXPOSED CASTRATED RATS ALSO SHOWED PARTIAL STIMULATION AND ADRENOCORTICOTROPHIN TREATMENT RESTORED MINIMALLY THE ACCESSORIES OF THE CASTRATES.

THE SECRETION OF LARGE AMOUNTS OF ANDROGENS BY THE TESTIS MASKS THE NORMAL ADRENAL CONTRIBUTION TO THE OVERALL ANDROGENIC SUPPLY. HOWEVER, UNDER STRESS (COLD) THE ADRENAL HYPERTROPHY MAY COEXIST WITH AN INCREMENT OF ANDROMIMETIC FUNCTION. IN THE STRESSED (COLD) CASTRATES, THIS INCREMENT OF ADRENAL FUNCTION WOULD APPEAR AS THE ONLY SUPPORT OF THE ACCESSORY GENITAL ORGANS.

EXPERIMENTS WERE DESIGNED TO SIMULATE THE ACTION OF THE ADRENAL BY PHARMACOLOGICAL USE OF RECOGNIZED ADRENAL ANDROGENS, IN NORMAL, CASTRATED, ADRENALECTOMIZED AND DUAL OPERATED ANIMALS. THE CONTRIBUTION OF THE PHARMACEUTICALS, THE TESTES, THE ADRENALS AND THE DIFFERENCE BETWEEN THE RESPONSES OF THE ANIMALS AT ROOM TEMPERATURE AND IN COLD WERE COMPUTED, WITH THE VIEW OF EVALUATING THE ADRENAL PRODUCTION OF ANDROGENS.

THE RESULTS OBTAINED SHOW THAT:

1. IN COLD-EXPOSED ( $-5^{\circ}\text{C}$ ) ADULT MALE RATS, THE HYPERACTIVE ADRENAL GLAND SECRETES ANDROGENIC MATERIAL, WHICH IN THE CASTRATES IS SUFFICIENT

TO SUPPORT PARTIALLY, THE GENITAL ACCESSORIES AND THUS DISPLAY A  
' REPLACEMENT ' ACTION.

2. ADRENALECTOMIZED ADULT MALE RATS EXPOSED TO COLD ( $-5^{\circ}\text{C}$ ) FOR 12 DAYS,  
HAVE SMALLER ACCESSORIES THAN THOSE OF THE INTACT RATS AT THE SAME  
TEMPERATURE.

3. THE GENITAL ACCESSORIES OF THE COLD-EXPOSED ADRENALECTOMIZED-CASTRATED  
RATS ARE MORE DEPRESSED THAN THOSE OF THE CASTRATES ALONE AT THE SAME  
TEMPERATURE.

4. THE ADRENAL ANDROGENS, ANDROSTENEDIONE AND ADRENOSTERONE ANTAGONIZE  
THE COLD-INDUCED ATROPHY OF THE GENITAL ACCESSORIES IN ADRENALECTOMIZED  
AND ADRENALECTOMIZED-CASTRATED RATS.

5. THE RESPONSE OF THE GENITAL ACCESSORIES TO THE EXOGENOUS ADRENAL  
ANDROGENS IS INCREASED WHEN THE RATS ARE EXPOSED TO SEVERE COLD ( $-5^{\circ}\text{C}$ ).

IT IS CONCLUDED THAT UNDER NORMAL CONDITIONS, THE CONTRIBUTION TO  
THE CIRCULATING ANDROGENS BY THE TESTIS AMOUNTS TO ABOUT 93% AND BY THE  
ADRENALS ABOUT 7%; UNDER STRESS (COLD) THE TESTIS CONTRIBUTES ABOUT 51%  
AND ADRENAL 23%.

## RESUME

LA PRÉSENTE RECHERCHE PROPOSE UNE ESTIMATION DE LA CONTRIBUTION SURRÉNALIENNE À L'ÉCONOMIE DES HORMONES ANDROGÈNES CHEZ LE RAT MÂLE ADULTE; LE STRESS DU FROID RIGOREUX ( $-5^{\circ}\text{C}$ ) ET LE REMPLACEMENT THÉRAPEUTIQUE ÉTANT LES PRINCIPALES SITUATIONS EXPÉRIMENTALES.

CHEZ LE RAT MÂLE EXPOSÉ AU FROID, LES ACCESSOIRES SEXUELS SUBISSENT UNE DÉGÉNÉRESCENCE TEMPORAIRE, MAIS REVIENNENT À UN NIVEAU PRESQUE NORMAL MALGRÉ L'ATROPHIE PERSISTANTE DU TESTICULE. CHEZ LE CASTRÉ, LES ACCESSOIRES SEXUELS DE L'ANIMAL AU FROID DÉMONTRENT UNE RÉPARATION PARTIELLE ET L'ACTH CAUSE UNE STIMULATION MINIMALE, MAIS CARACTÉRISTIQUE.

AUX CONDITIONS NORMALES, LA SÉCRÉTION ABONDANTE PAR LE TESTICULE MASQUE LA CONTRIBUTION SURRÉNALIENNE AUX ANDROGÈNES CIRCULANTS; CEPENDANT, CHEZ L'ANIMAL SOUMIS AU STRESS, L'HYPERTROPHIE CORTICALE POURRAIT S'ALLIER À UNE AUGMENTATION DE LA SÉCRÉTION ANDROMIMÉTIQUE. CHEZ LE CASTRÉ SOUMIS À L'AGRESSION (FROID), CET INCRÉMENT DE LA FONCTION CORTICALE SEMBLE FOURNIR LE SEUL SUPPORT DES ACCESSOIRES SEXUELS.

LE DEVIS EXPÉRIMENTAL POURVOIT À UNE SIMULATION DE L'ACTION CORTICALE, PAR L'USAGE D'ANDROGÈNES SURRÉNALIENS RECONNUS, CHEZ DES ANIMAUX CASTRÉS, SURRÉNALOPRIVES ET SOUMIS À LA DOUBLE OPÉRATION. LES CALCULS 1) DE LA CONTRIBUTION DES MÉDICAMENTS, DU TESTICULE ET DE LA SURRÉNALE, ET 2) DE LA DIFFÉRENCE DES RÉPONSES À LA TEMPÉRATURE NORMALE ET AU FROID, RENDENT POSSIBLE UNE ESTIMATION DE LA PRODUCTION ANDROGÉNIQUE DE LA SURRÉNALE.

LES RÉSULTATS DÉMONTRENT QUE:

- 1) CHEZ LE RAT MÂLE ADULTE EXPOSÉ AU FROID ( $-5^{\circ}\text{C}$ ), LE CORTEX SURACTIVÉ SÉCRÈTE DES ANDROGÈNES QUI, CHEZ LE CASTRÉ, SUFFISENT AU SUPPORT PARTIEL DES ACCESSOIRES SEXUELS ET AINSI, DÉMONTRENT UN "REMPLACEMENT THÉRAPEUTIQUE".
- 2) LE RAT SURRÉNALOPRIVE EXPOSÉ AU FROID ( $-5^{\circ}\text{C}$ ) DURANT 12 JOURS, POSSÈDE DES ACCESSOIRES PLUS PETITS QUE CEUX DE L'ANIMAL INTACT EXPOSÉ AU FROID.
- 3) LES ACCESSOIRES SEXUELS DU CASTRÉ SURRÉNALOPRIVE EXPOSÉ AU FROID SONT PLUS PETITS QUE CEUX DU SIMPLE CASTRÉ EXPOSÉ AU FROID.
- 4) LES ANDROGÈNES SURRÉNALIENS, ANDROSTÈNEDIONE ET ADRÉNOSTÉRONE, COMBATTENT LA DÉPRESSION DUE AU FROID DES ACCESSOIRES SEXUELS CHEZ LES SURRÉNALOPRIVES ET LES CASTRÉS SURRÉNALOPRIVES.
- 5) LA RÉPONSE DES ACCESSOIRES SEXUELS AUX ANDROGÈNES SURRÉNALIENS D'IMPORT EST ACCENTUÉE PAR L'EXPOSITION AU STRESS DU FROID RIGOUREUX ( $-5^{\circ}\text{C}$ ).

NOUS CONCLUONS QUE NORMALEMENT, LES ANDROGÈNES CIRCULANTS PROVIENNENT DU TESTICULE À RAISON D'ENVIRON 93% ET DES SURRÉNALES À RAISON D'ENVIRON 7%; LORS DE L'AGRESSION (FROID), LA CONTRIBUTION DU TESTICULE TOMBE À ENVIRON 51% ET CELLE DE LA SURRÉNALE MONTE À ENVIRON 23%.

## INTRODUCTION

I. REVIEW OF THE LITERATURE

THE PRESENCE OF C-19 STEROIDS IN ADRENAL TISSUE WAS NOTED DURING THE EARLY STUDIES OF REICHSTEIN (1936), WHO ALSO REALIZED THAT THE BIOLOGICALLY ACTIVE STEROIDS OF THE CORTICOID SERIES (C-21) COULD BE EASILY CONVERTED (IN VIVO) TO THE C-19 GROUP. SINCE THEN, SEVERAL C-19 STEROIDS HAVE BEEN ISOLATED FROM ADRENAL TISSUES, UNDER CONDITIONS THAT POINT TO THEIR PHYSIOLOGICAL SIGNIFICANCE (DORFMAN, 1962).

THE ADRENAL-GONAD RELATIONSHIP HAS BEEN EXTENSIVELY REVIEWED BY PARKES (1945). THE MARKED DEVELOPMENT OF THE VENTRAL PROSTATE IN YOUNG CASTRATED RATS (PRICE, 1936) WAS ATTRIBUTED BY HOWARD (1938A, 1938B) TO THE ACTION OF ANDROGENS FROM THE ADRENAL CORTEX. THE SAME EXPLANATION WAS SUGGESTED FOR THE EXTENSIVE DEVELOPMENT OF THE SEMINAL VESICLES AND PROSTATE IN YOUNG CASTRATED MICE (HOWARD, 1939). THE VENTRAL PROSTATE DOES NOT DEVELOP IN IMMATURE CASTRATED-ADRENALECTOMIZED RATS (BURRILL AND GREENE, 1939; HOWARD, 1941). COMPLETE CASTRATION ATROPHY WAS FOUND IN THE VENTRAL PROSTATES OF 26, 31, 36 AND 41 DAY-OLD ADRENALECTOMIZED-CASTRATED RATS. THE VENTRAL PROSTATES WERE PARTIALLY MAINTAINED IN CASTRATED NON-ADRENALECTOMIZED ANIMALS AT 26 AND 31 DAYS, BUT NOT AT 36 AND 41 DAYS. IN THE PRESENCE OF THE TESTIS, ADRENALECTOMY ALONE DID NOT MODIFY THE VENTRAL PROSTATES AT ANY OF THESE AGES. THE ADRENALS HAD AN ANDROGENIC CAPACITY IN THE YOUNGER IMMATURE RATS (26 AND 31 DAYS OLD) BUT NOT IN THE OLDER IMMATURE ANIMALS (36 AND 41 DAYS) OR IN ADULT RATS (BURRILL AND GREENE 1940; GERSH AND GROLLMAN, 1939). THE INABILITY OF THE ADRENAL CORTEX TO MAINTAIN THE ACCESSORY

SEX ORGANS OF THE ADULT CASTRATE MIGHT BE DUE TO A HIGHER THRESHOLD OF RESPONSE OF THE ADULT SEX ACCESSORIES AS COMPARED TO THOSE OF THE IMMATURE ANIMAL (KATSCH ET AL, 1948). GONADECTOMY IN YOUNG MALE MICE OF AN INBRED STRAIN PRODUCED ADRENOCORTICAL CARCINOMA CORRELATED WITH STRONG STIMULATION OF THE PROSTATES AND SEMINAL VESICLES (WOOLLEY AND LITTLE, 1945A, 1945B). AUTOTRANSPLANTATION OF MALE RAT ADRENALS INTO ONE SEMINAL VESICLE OF ADULT CASTRATED RATS PRODUCED A DEFINITE BUT LOCAL STIMULATION OF THE ACCEPTOR GLAND AND ANDROGENIC EFFECTS ON THE OTHER SEMINAL VESICLE AND ON THE VENTRAL PROSTATE (PRICE AND INGLE, 1957). ADRENOCORTICOTROPHIN TREATMENT IN YOUNG CASTRATED MALE RATS CAUSED STIMULATION OF THE VENTRAL PROSTATE (DAVIDSON AND MOON, 1936; DAVIDSON, 1937) AND OF THE SEMINAL VESICLES (NELSON, 1941; DEANESLY, 1960). HOWEVER, OTHER OBSERVERS (MOORE, 1953; VAN DER LAAN, 1953) WERE UNABLE TO CONFIRM THESE RESULTS. LOSTROCH AND LI (1957) OBTAINED SOME GROWTH OF THE VENTRAL PROSTATE AND SEMINAL VESICLES AFTER ADRENOCORTICOTROPHIN TREATMENT IN HYPOPHYSECTOMIZED-CASTRATED RATS AT CERTAIN DOSAGE LEVELS AND THEY EMPHASIZED THAT DOSAGE IS A CRITICAL FACTOR IN DEMONSTRATING THE ANDROGEN-SECRETING ABILITY OF THE ADRENAL CORTEX UNDER ACTH STIMULATION. ADMINISTRATION OF ACTH TO ADRENALECTOMIZED-HYPOPHYSECTOMIZED-CASTRATED RATS DOES NOT AFFECT THE ACCESSORY GLANDS.

THE ADRENALS ARE SUSCEPTIBLE TO ADVERSE ENVIRONMENTAL CONDITIONS; IN RATS EXPOSED TO COLD THE ADRENALS UNDERGO CHANGES IN SECRETORY ACTIVITY AND THE CORTEX INCREASES IN SIZE (INGLE, 1958).

INCREASED ADRENOCORTICAL FUNCTION DURING EXPOSURE TO COLD HAS BEEN INDICATED BY THE HYPERTROPHY OF THE ADRENALS AND THE DEPLETION OF ASCORBIC ACID AND CHOLESTEROL (LEVIN, 1945; SAYERS ET AL, 1944,1946). THE HYPERTROPHY REPRESENTS HYPERPLASIA OF THE ZONA FASCICULATA; THIS ZONE IS CONSIDERED RESPONSIBLE FOR THE PRODUCTION OF MOST OF THE STEROIDS (HÉROUX AND SCHÖNBAUM, 1959).

THE EFFECTS OF LONG, CONTINUOUS COLD ENVIRONMENT ON THE ENDOCRINE TESTIS OF THE RAT HAVE BEEN STUDIED EXTENSIVELY IN RECENT TIME. HÉROUX AND CAMPBELL (1959) REPORTED A SLIGHT BUT SIGNIFICANT REDUCTION IN THE WEIGHT OF THE GENITALS OF MALE RATS EXPOSED TO COLD FOR THREE MONTHS. AFTER PROLONGED EXPOSURE TO COLD (2°C) CRYPTORCHISM WAS PRODUCED IN NORMAL UNTREATED RATS; THE TESTIS AND SEMINAL VESICLES BECAME ATROPHIC (DUGAL ET AL, 1962). IN ACUTE EXPOSURE TO SEVERE COLD (-5°C) ALSO, A CONSIDERABLE DECREASE IN THE WEIGHT OF THE TESTIS, WAS OBSERVED AND THE SEX ACCESSORIES, ESPECIALLY PROSTATES AND SEMINAL VESICLES POSSESSED THE TYPICAL EUNUCHOID CHARACTERISTICS. PHYSIOLOGICAL INFORMATION DERIVED FROM MORPHOLOGICAL DATA REVEALED THAT THE EFFECT OF COLD ON THE MALE GENITAL APPARATUS IS TWO-FOLD: SYSTEMIC AND ENDOCRINE; THE TWO EFFECTS ARE SUPERIMPOSED AND ADDITIVE, BUT DISTINCT (PERRAULT AND DUGAL, 1962 AND 1963 ; OUELLETTE ET AL, 1965). A BIPHASIC RESPONSE OF THE GENITAL ACCESSORIES WAS OBTAINED IN SEQUENTIAL STUDIES OF THE EFFECT OF PROLONGED EXPOSURE TO COLD (2°C). THE SEMINAL VESICLE WAS INITIALLY DEPRESSED ALONG WITH THE TESTIS BUT FINALLY, THE WEIGHT OF THE SEMINAL VESICLES OF ALL ANIMALS, WITHOUT EXCEPTION RETURNED TO NORMAL.

THE SOURCE OF ANDROGENS MAY HAVE BEEN IN PART, OUTSIDE THE TESTIS, POSSIBLY IN THE ADRENALS.

IN THE CASTRATED RATS ALSO, THE SEMINAL VESICLE WEIGHT WAS INITIALLY DEPRESSED AFTER PROLONGED EXPOSURE TO COLD ( $2^{\circ}\text{C}$ ) BUT FINALLY SHOWED A SIGNIFICANT INCREASE OVER THAT OF THE CASTRATES MAINTAINED AT ROOM TEMPERATURE. THE SOURCE OF ANDROGENS IN THIS CASE OBVIOUSLY WAS EXTRATESTICULAR AND MOST PROBABLY IN THE ADRENALS (DUGAL ET AL. 1962)

TREATMENT WITH ADRENOCORTICOTROPHIN PRODUCED SIGNIFICANT STIMULATION OF THE ACCESSORY GENITAL ORGANS IN ADULT CASTRATED MALE RATS, THUS SIMULATING THE ACTION OF COLD ( $-5^{\circ}\text{C}$ ). THIS OBSERVATION AGAIN POINTS TO THE SIGNIFICANCE OF ADRENAL ANDROGENS IN COLD-EXPOSED RAT (PERRAULT AND DUGAL, 1963).

## 2. STATEMENT OF THE PROBLEM

THE AIM OF THE PRESENT EXPERIMENT WAS TO OBTAIN DIRECT EVIDENCE OF THE CONTRIBUTION OF THE ADRENAL TO THE ANDROGENIC POOL.

IN THE CASTRATED MALE RATS EXPOSED TO SEVERE COLD THE HYPERACTIVE ADRENAL CORTEX PROBABLY PRODUCES ENOUGH ANDROGENIC SUBSTANCE TO SUPPORT THE GENITAL ACCESSORIES AT LEAST PARTIALLY AND THUS DISPLAY A 'REPLACEMENT' ACTION.

THE NATURE OF THIS ADRENAL ACTION IS ASCERTAINED BY ADMINISTRATION OF RECOGNIZED ADRENAL ANDROGENS TO COLD-EXPOSED MALE RATS WITH OR WITHOUT CASTRATION AND (OR) ADRENALECTOMY.

## MATERIALS AND METHODS

1. ANIMALS AND DIET

A GROUP OF MALE RATS (WISTAR ALBINO) WAS DISTRIBUTED AS SHOWN IN TABLE 1. THE ANIMALS WERE CAGED INDIVIDUALLY AND MAINTAINED EITHER AT ROOM TEMPERATURE ( $21^{\circ}\text{C}$ ) OR IN THE COLD ROOM (AT  $2^{\circ}\text{C}$  FOR ONE MONTH AND  $-5^{\circ}\text{C}$  FOR NEXT 12 DAYS). THE DIET CONSISTED OF PURINA LAB CHOW AND TAP WATER AD LIBITUM; ADRENALECTOMIZED ANIMALS WERE PROVIDED WITH AN AQUEOUS SOLUTION OF SODIUM CHLORIDE (1%) INSTEAD OF TAP WATER. TO PREVENT NESTING IN THE COLD, A LIMITED BUT SUFFICIENT NUMBER OF CHOW BISCUITS WAS PROVIDED.

2. EXPOSURE TO COLD

AFTER THE USUAL 5-6 DAY QUARANTINE AT ROOM TEMPERATURE, THE EXPERIMENTAL ANIMALS WERE PLACED IN THE COLD AT A TEMPERATURE OF  $2^{\circ} \pm 1^{\circ}\text{C}$  FOR ONE MONTH, FOR ACCLIMATION. THE COLD ACCLIMATED RATS (WITH OR WITHOUT TREATMENT OR SURGERY) WERE THEN EXPOSED TO A TEMPERATURE OF  $-5^{\circ} \pm 1^{\circ}\text{C}$  FOR A FURTHER PERIOD OF 12 DAYS. THE COLD ROOMS WERE VENTILATED, THERMOSTATICALLY CONTROLLED AND ILLUMINATED UNDER AUTOMATIC CONTROL FROM 8 A.M. TO 6 P.M. DAILY.

3. ROOM TEMPERATURE CONTROLS

THESE ANIMALS ARRIVED FROM THE SUPPLIER SIX DAYS PRIOR TO SURGERY. ACCORDING TO A SCHEDULE BASED ON THE AVERAGE GROWTH RATE OF THIS STRAIN OF RATS, THEY WERE SELECTED FROM THE STOCK TO BE ABOUT THE SAME AGE AS THE COLD-ACCLIMATED ONES. THE SURGERY WAS PERFORMED AT ROOM TEMPERATURE AFTER THE USUAL QUARANTINE OF SIX DAYS, IN THE

TABLE I  
DISTRIBUTION OF ANIMALS

GROUPS	21°C	2°C FOR 30 DAYS -5°C FOR 12 DAYS
<u>UNTREATED</u>		
UNOPERATED*	33 <sup>A</sup>	35
ADRENALECTOMIZED	35	45
CASTRATED	29	30
DUAL OPERATED	26	46
<u>ANDROSTENEDIONE<sup>B</sup></u>		
UNOPERATED	11	10
ADRENALECTOMIZED	13	30
CASTRATED	10	10
DUAL OPERATED	11	30
<u>ADRENOSTERONE<sup>C</sup></u>		
UNOPERATED	12	10
ADRENALECTOMIZED	12	30
CASTRATED	10	12
DUAL OPERATED	12	30

A. GROUP POPULATION

B. 0.5 MG PER RAT DAILY FOR 12 DAYS SUBCUTANEOUSLY.

C. 3.0 MG PER RAT DAILY FOR 12 DAYS SUBCUTANEOUSLY.

\* UNTREATED UNOPERATED AT ROOM TEMPERATURE (21°C) = "ABSOLUTE CONTROL".

SAME MANNER AS FOR THE COLD-ACCLIMATED ONES AND THE ANIMALS WERE KEPT AT 21<sup>0</sup>C UNTIL SACRIFICE.

#### 4. SURGERY

SURGERY WAS PERFORMED AT ROOM TEMPERATURE UNDER LIGHT ETHER ANESTHESIA; CASTRATION WAS PERFORMED BY ABDOMINAL APPROACH AND ADRENAL-ECTOMY BY DORSAL APPROACH. THE ANIMALS WERE MAINTAINED AT ROOM TEMPERATURE FOR 24 HOURS AFTER SURGERY, RETURNED AT 2<sup>0</sup>C FOR THE NEXT 24 HOURS AND FINALLY EXPOSED TO -5<sup>0</sup>C FOR 12 DAYS.

#### 5. TREATMENTS

ANDROSTENEDIONE<sup>1</sup> DISSOLVED IN CORN OIL WAS ADMINISTERED SUBCUTANEOUSLY (0.5 MG PER RAT DAILY FOR 12 DAYS) BEGINNING ON THE DAY OF ENTRY TO -5<sup>0</sup>C. ADRENOSTERONE<sup>2</sup> SUSPENDED IN ETHYLENE GLYCOL WAS ADMINISTERED IN THE SAME MANNER AT THE DAILY DOSE OF 3 MG PER RAT. THE DOSE-RESPONSE RELATIONSHIP OF THESE HORMONES IS DETAILED IN THE APPENDIX.

THE CASTRATED, ADRENALECTOMIZED AND THE DUAL OPERATED GROUPS RECEIVING NO HORMONE TREATMENT, ALONG WITH THE ABSOLUTE CONTROLS, WERE DIVIDED INTO THREE SUBGROUPS: ANIMALS OF THE FIRST SUBGROUP RECEIVED CORN OIL (0.2 ML/RAT/DAY FOR 12 DAYS) SUBCUTANEOUSLY, THOSE OF THE SECOND RECEIVED ETHYLENE GLYCOL (0.2 ML/RAT/DAY FOR 12 DAYS) AND THOSE OF THE THIRD WERE LEFT UNTREATED. THE AUTOPSY DATA OF THE THREE SUB-GROUPS WERE IDENTICAL IN EACH GROUP AND HENCE POOLED TOGETHER.

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<sup>1</sup>  $\Delta^4$ -ANDROSTENE-3, 17-DIONE; NUTRITIONAL BIOCHEMICAL CORPORATION, CLEVELAND, OHIO, U.S.A.; CONTROL No. 5665.

<sup>2</sup>  $\Delta^4$ -ANDROSTENE-3, 11, 17-TRIONE; REICHSTEIN'S SUBSTANCE G; SIGMA CHEMICAL COMPANY, ST. LOUIS, MISSOURI, U.S.A.; LOT No.: 94B-0580.

6. OUTLINE OF THE SCHEDULE

DAY  
MINUS 38 - ARRIVAL OF ANIMALS FOR ACCLIMATION, QUARANTINE.

DAY  
MINUS 32 - ACCLIMATION PERIOD BEGINS AT 2<sup>0</sup>C.

DAY  
MINUS 8 - ARRIVAL OF ROOM TEMPERATURE (21<sup>0</sup>C) CONTROL ANIMALS.

DAY  
MINUS 2 - ALL SURGERY AT ROOM TEMPERATURE AND 24 HOURS AT ROOM TEMPERATURE

DAY  
MINUS 1 - ROOM TEMPERATURE CONTROLS REMAIN AT ROOM TEMPERATURE, ACCLIMATED ANIMALS RETURN TO 2<sup>0</sup>C.

DAY 0 - EXPOSURE OF ACCLIMATED ANIMALS TO -5<sup>0</sup>C BEGINS, TREATMENT WITH ANDROSTENEDIONE AND ADRENOSTERONE TO ALL ANIMALS BEGINS.

DAY  
PLUS 12 - AUTOPSY.

7. AUTOPSY DATA

AUTOPSIES WERE PERFORMED ON DAY ZERO (DAY 2 POST-OPERATIVELY) <sup>1)</sup> TO OBTAIN THE "INITIAL VALUES," AND <sup>2)</sup>

WHEN THE EXPERIMENTS WERE TERMINATED ON DAY 12 OF COLD EXPOSURE (DAY 14 POST-OPERATIVELY). THIS PERIOD OF EXPOSURE TO -5<sup>0</sup>C WAS SELECTED BECAUSE <sup>1)</sup> THE COMPLETE CASTRATION EFFECTS WERE PRESENT AND <sup>2)</sup> BECAUSE OF ACCLIMATION, THE SURVIVAL OF THE ACCLIMATED ADRENALECTOMIZED ANIMALS AMOUNTED TO ABOUT 25-28% OF THE INITIAL POPULATION. THE ANIMALS WERE SACRIFICED BY DECAPITATION, THE ORGANS WERE DISSECTED OUT, EXAMINED,

BLOTTED AND WEIGHED WET ON A ROLLER-SMITH BALANCE (UP TO 500 MG TO THE NEAREST 0.2 MG) OR A CENT-O-GRAM BALANCE (FOR HIGHER WEIGHTS).

THE ALKALINE PHOSPHATASE ACTIVITY OF THE SEMINAL VESICLES WAS DETERMINED BY THE METHOD OF HAWK ET AL. (1954). THE ZINC<sup>65</sup> UPTAKE BY THE DORSOLATERAL PROSTATE WAS DETERMINED BY THE METHOD OF GUNN ET AL. (1960); TWENTY-FOUR HOURS PRIOR TO SACRIFICE, THE TRACER<sup>1</sup> WAS ADMINISTERED INTRAVENOUSLY IN THE EXTERNAL JUGULAR (VIA A SMALL SKIN INCISION). AT POST-MORTEM, THE GLAND WAS CAREFULLY DISSECTED OUT, DRIED TO CONSTANT-WEIGHT, COUNTED IN A GAS-FLOW, LOW-BACKGROUND GEIGER-MUELLER DETECTOR (NUCLEAR CHICAGO, MODEL C115); THE RESULTS ARE EXPRESSED IN COUNTS PER MINUTE FOR EACH GLAND.

#### 8. STATISTICS

ALL VALUES WERE SCREENED BY MEANS OF THE DIXON'S TEST FOR EXTREMES (DIXON, 1951). THE TABLES QUOTE THE ARITHMETICAL MEAN WITH THE STANDARD ERROR OF THE MEAN. THE DIFFERENCE BETWEEN THE COLD-EXPOSED AND RESPECTIVE CONTROL IS EXPRESSED IN PERCENT DIFFERENCE FROM THE CONTROL AND SUBJECTED TO THE T TEST. HISTOGRAMS REPRESENTING THE FRACTION OF THE ABSOLUTE CONTROL ARE ALSO SUBMITTED.

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<sup>1</sup> ZINC<sup>65</sup> SUPPLIED BY ATOMIC ENERGY OF CANADA LIMITED. AS NITRATE IN NITRIC ACID; SPECIFIC ACTIVITY: 558 MC PER GRAM; CONCENTRATION: 15.4 MC PER ML; DILUTED WITH ALKALINE SALINE (FINAL PH AROUND 5.7); ADMINISTRATION: 40  $\mu$ C PER 100 GM BODY WEIGHT IN 0.1 ML, INTRAVENOUSLY.

## RESULTS

1. SURVIVAL

THE SURVIVAL OF THE RATS AT ROOM TEMPERATURE WAS NOT MATERIALLY ALTERED BY SURGERY OR TREATMENTS. IN COLD-EXPOSED ( $-5^{\circ}\text{C}$ ) ANIMALS HOWEVER, BECAUSE OF ACCLIMATION ( $2^{\circ}\text{C}$ ), 28% OF THE INITIAL POPULATION OF ADRENALECTOMIZED RATS SURVIVED AND 25% OF THE POPULATION SUBJECTED TO THE DUAL OPERATION SURVIVED 12 DAYS AT  $-5^{\circ}\text{C}$ . TREATMENT WITH EITHER OF THE TWO HORMONES DID NOT MODIFY SIGNIFICANTLY THE SURVIVAL OF THESE TWO OPERATED GROUPS IN THE COLD ( $-5^{\circ}\text{C}$ ). MORTALITY IN OTHER COLD-EXPOSED GROUPS WAS NOT SIGNIFICANT.

2. BODY WEIGHT (TABLE II AND FIGURE 1)

THE ADRENALECTOMIZED ANIMALS AT ROOM TEMPERATURE ( $21^{\circ}\text{C}$ ) WITH SODIUM CHLORIDE SUPPORT, SHOWED A NOTABLE DELAY OF BODY GROWTH; CASTRATION HAD NO EFFECT AND THE DUAL OPERATION WAS NOT MORE TRAUMATIC THAN ADRENALECTOMY ALONE. THE HORMONE TREATMENT DID NOT HAVE ANY SIGNIFICANT INFLUENCE ON THE BODY WEIGHT. THE SYSTEMIC EFFECT OF EXPOSURE TO COLD ( $-5^{\circ}\text{C}$ ) WAS REFLECTED BY AN UNIFORM DEPRESSION OF THE GROWTH RATE; THIS EFFECT WAS MINIMAL IN THE DUAL-OPERATED ANIMALS. THE ADRENALECTOMIZED MAINTAINED A MEDIAN POSITION, BECAUSE OF A LOWER INITIAL BODY WEIGHT.

3. TESTIS WEIGHT (TABLE III AND FIGURE 2)

ADRENALECTOMY HAD A NEGLIGIBLE EFFECT ON TESTIS WEIGHT. TREATMENT WITH ANDROSTENEDIONE CAUSED A DEPRESSION OF TESTIS WEIGHT IN THE

TABLE 11 BODY WEIGHT (GRAMS)  
 PRE-ACCLIMATION BODY WEIGHT:  $182 \pm 2.4^A (430)^B$

GROUPS	INITIAL <sup>C</sup> (DAY 0)		TERMINAL <sup>D</sup>		PERCENT DIFFERENCE (N)(F)
	21°C	2°C	21°C(N)	-5°C(F)	
<u>UNTREATED</u>					
UNOPERATED	280±2.7(16)	272±6.3(22)	319±4.7(16)	256±6.4(22)	-20(38)
ADRENALECTOMIZED	268±6.1(16)	251±3.2(35)	285±8.8(16)	245±8.4(10)	-14(26)
CASTRATED	278±4.8(13)	271±4.8(18)	308±4.4(13)	253±6.4(16)	-18(29)
DUAL OPERATED	270±4.7(13)	261±3.2(36)	275±4.7(13)	239±5.0(9)	-13(22)
<u>ANDROSTENEDIONE</u>					
UNOPERATED	285±5.2(11)	270±4.4(10)	326±6.3(11)	272±5.1(10)	-17(21)
ADRENALECTOMIZED	265±3.8(15)	254±2.9(30)	277±7.8(13)	237±7.7(9)	-15(22)
CASTRATED	274±6.5(10)	268±5.0(10)	307±5.2(10)	267±5.7(10)	-13(20)
DUAL OPERATED	268±8.2(12)	250±6.3(30)	277±6.6(11)	235±6.9(7)	-15(18)
<u>ADRENOSTERONE</u>					
UNOPERATED	283±8.4(12)	269±5.8(10)	325±6.3(12)	272±4.6(10)	-16(22)
ADRENALECTOMIZED	263±4.8(15)	260±3.6(30)	295±4.5(12)	249±6.3(8)	-16(20)
CASTRATED	279±5.1(10)	270±6.4(12)	314±5.6(10)	271±2.7(11)	-14(21)
DUAL OPERATED	263±3.7(14)	256±4.8(30)	282±6.3(12)	231±5.9(7)	-18(19)

NOTE: RESULTS UNDERScoreD BY A SINGLE LINE INDICATE  
 $P < 0.01$ ; THOSE UNDERScoreD BY A DOUBLE LINE  
 INDICATE  $P < 0.001$ .

A. STANDARD ERROR OF THE MEAN.  
 B. POPULATION  
 C. AFTER 32 DAYS AT 2°C (2 DAYS POST-OPERATIVELY)  
 D. AFTER 12 DAYS AT -5°C (14 DAYS POST-OPERATIVELY)

TABLE III  
TESTIS WEIGHT (GRAMS)

GROUPS	DAY 0 <sup>A</sup>		DAY 12 <sup>B</sup>		PERCENT DIFFERENCE (N ~ F)
	21°C(N)	2°C(F)	21°C(N)	-5°C(F)	
<u>UNTREATED</u>					
UNOPERATED	2.96±0.28 <sup>C</sup> (17) <sup>D</sup>	2.71±0.23(13)	3.22±0.01(16)	3.01±0.02(22)	-7(38)
ADRENALECTOMIZED	2.96±0.19(19)	2.90±0.25(10)	3.05±0.07(16)	2.73±0.06(10)	-10(26)
<u>ANDROSTENEDIONE</u>					
UNOPERATED			3.18±0.02(11)	2.97±0.05(10)	-7(21)
ADRENALECTOMIZED			2.79±0.06(13)	2.65±0.04(9)	-4(22)
<u>ADRENOSTERONE</u>					
UNOPERATED			2.80±0.03(10)	2.64±0.04(10)	-6(20)
ADRENALECTOMIZED			2.78±0.04(12)	2.62±0.04(8)	-6(20)

A. AFTER 32 DAYS AT 2°C.

B. AFTER 12 DAYS AT -5°C.

C. STANDARD ERROR OF THE MEAN.

D. POPULATION

NOTE: RESULTS UNDERSCORED BY A SINGLE LINE INDICATE  $P < 0.01$ ;  
THOSE UNDERSCORED BY A DOUBLE LINE INDICATE  $P < 0.001$ .

ADRENALECTOMIZED ANIMALS ONLY. ADRENOSTERONE TREATMENT PRODUCED A DEPRESSION OF TESTIS WEIGHT EVEN IN INTACT ANIMALS. COLD ( $-5^{\circ}\text{C}$ ) HAD A DEPRESSING EFFECT ON TESTIS WEIGHT WHICH WAS HOWEVER WITHIN THE RANGE OF SYSTEMIC LOSS.

#### 4. GENITAL ACCESSORIES

##### A. VENTRAL PROSTATE WEIGHT (TABLE IV AND FIGURE 3)

THE VENTRAL PROSTATES WERE UNIFORMLY AND SIGNIFICANTLY STIMULATED IN ACCLIMATED ANIMALS. AT DAY 12, THE ANIMALS AT ROOM TEMPERATURE DID NOT SHOW CHANGE IN WEIGHT DUE TO ADRENALECTOMY. CASTRATION PRODUCED THE CLASSICAL ATROPHY AND DUAL OPERATED ANIMALS DISPLAYED THE CASTRATION EFFECT.

TREATMENT WITH EITHER OF THE TWO HORMONES SHOWED A STIMULATION OF THE WEIGHT, WHICH WAS MORE MARKED WITH ADRENOSTERONE IN ALL ANIMALS, ESPECIALLY IN CASTRATES WITH OR WITHOUT COLD.

THE COLD-EXPOSED ( $-5^{\circ}\text{C}$ ) INTACT ANIMALS SHOWED A WEIGHT DEPRESSION EMPHASIZED BY ADRENALECTOMY. IN THE CASTRATES, COLD-EXPOSURE PRODUCED A SIGNIFICANT INCREASE IN WEIGHT AS COMPARED TO THE ROOM TEMPERATURE CASTRATES. THE DUAL OPERATED ANIMALS DID NOT SHOW ANY CHANGE DUE TO COLD EXPOSURE. THE EFFECT OF COLD WAS CANCELLED BY THE HORMONE TREATMENTS.

##### B. SEMINAL VESICLES

###### 1) WEIGHT (TABLE V AND FIGURE 4)

THE INTACT AND ADRENALECTOMIZED COLD-ACCLIMATED ANIMALS SHOWED A STIMULATION WHEN COMPARED TO THE ROOM TEMPERATURE ONES AT DAY ZERO.

TABLE IV

VENTRAL PROSTATE WEIGHT (MG)

GROUPS	DAY 0		DAY 12		PERCENT DIFFERENCE (N~F)
	21°C(N)	2°C(F)	21°C(N)	-5°C(F)	
<u>UNTREATED</u>					
UNOPERATED	263.5±7.6 <sup>A</sup> (17) <sup>B</sup>	321.4±9.0(13)	371.3±6.3(16)	298.8±6.6(22)	-19(38)
ADRENALECTOMIZED	266.4±7.9 (19)	341.9±6.4(10)	360.0±6.8(16)	209.9±6.8(10)	-42(26)
CASTRATED	231.0±11.4(16)	295.4±6.4(12)	30.7±2.0(13)	41.5±1.9(16)	+35(29)
DUAL OPERATED	202.5±12.7(13)	300.9±7.8(10)	27.6±0.9(13)	28.2±1.9(9)	+2 (22)
<u>ANDROSTENEDIONE</u>					
UNOPERATED			406.1±3.2(11)	401.3±5.7(10)	- 1(21)
ADRENALECTOMIZED			392.2±6.8(13)	314.5±10.2(9)	-20(22)
CASTRATED			101.0±3.2(10)	153.0±2.9(10)	+51(20)
DUAL OPERATED			151.2±5.5(11)	148.4±9.3(7)	- 2(18)
<u>ADRENOSTERONE</u>					
UNOPERATED			438.3±5.8(12)	418.5±6.6(10)	- 5(22)
ADRENALECTOMIZED			411.0±5.0(12)	375.1±4.5(8)	- 9(20)
CASTRATED			248.8±9.4(10)	314.6±6.3(11)	+26(21)
DUAL OPERATED			263.6±6.4(12)	263.1±12.4(7)	± 0(19)

A. STANDARD ERROR OF THE MEAN

B. POPULATION NOTE: RESULTS UNDERSCORED BY A SINGLE BROKEN LINE INDICATE P < 0.05; THOSE UNDERSCORED BY A DOUBLE LINE INDICATE P < 0.001.

TABLE V

SEMINAL VESICLE WEIGHT (MG)

GROUPS	DAY 0		DAY 12		PERCENT DIFFERENCE (N ~ F)
	21°C(N)	2°C(F)	21°C(N)	-5°C(F)	
<u>UNTREATED</u>					
UNOPERATED	287.6±6.6 <sup>A</sup> (17) <sup>B</sup>	327.0±10.8(13)	401.6±5.1(16)	350.6± 6.6(22)	-13(38)
ADRENALECTOMIZED	283.6±9.0 (19)	342.6± 3.6(10)	404.1±5.8(16)	226.5±12.9(10)	-44(26)
CASTRATED	264.9±8.1 (16)	261.9±10.2(12)	103.9±3.2(13)	111.0± 2.4(16)	+ 7(29)
DUAL OPERATED	260.8±9.9 (13)	273.4± 9.4(10)	107.3±2.4(13)	101.7± 3.9(9)	- 5(22)
<u>ANDROTENEDIONE</u>					
UNOPERATED			435.1±2.9(11)	409.2± 8.0(10)	- 6(21)
ADRENALECTOMIZED			429.6±4.3(13)	313.9±10.6(9)	-27(22)
CASTRATED			144.0±6.9(10)	211.8± 3.4(10)	+52(20)
DUAL OPERATED			173.6±3.9(11)	176.2± 4.4(7)	+ 1(18)
<u>ADRENOSTERONE</u>					
UNOPERATED			484.4±10.3(12)	420.0± 5.6(10)	-13(22)
ADRENALECTOMIZED			473.5± 9.6(12)	345.8± 9.1(8)	-27(20)
CASTRATED			246.0±14.2(10)	363.1± 9.2(11)	+48(21)
DUAL OPERATED			254.4± 8.8(12)	259.2± 8.9(7)	+ 2(19)

A. STANDARD ERROR OF THE MEAN

B. POPULATION

NOTE: RESULTS UNDERScoreD BY A SINGLE LINE INDICATE  $P < 0.01$ ; THOSE UNDERScoreD BY A DOUBLE LINE INDICATE  $P < 0.001$ .

ON DAY 12, THE SURGICAL EFFECTS WERE THE SAME AS OBSERVED WITH VENTRAL PROSTATE WEIGHT.

TREATMENT WITH HORMONES PRODUCED STIMULATION AND ADRENOSTERONE WAS AGAIN MORE EFFECTIVE THAN ANDROSTENEDIONE IN ALL ANIMALS. THERE WAS A DEPRESSION IN WEIGHT IN COLD-EXPOSED INTACT ANIMALS, EMPHASIZED BY ADRENALECTOMY. IN THE CASTRATES AND DUAL OPERATED ANIMALS THE WEIGHT WAS NOT MODIFIED AFTER EXPOSURE TO  $-5^{\circ}\text{C}$ .

11) ALKALINE PHOSPHATASE ACTIVITY (TABLE VI AND FIGURE 5)

ON DAY ZERO, THE INTACT, ADRENALECTOMIZED AND DUAL OPERATED, COLD-ACCLIMATED ANIMALS ( $2^{\circ}\text{C}$ ), SHOWED AN INCREASED ACTIVITY WHEN COMPARED TO THE ROOM TEMPERATURE ANIMALS.

ON DAY 12, ADRENALECTOMY CAUSED A MILD DEPRESSION AT ROOM TEMPERATURE, CASTRATION PRODUCED A SEVERE ATROPHY AND THE DUAL OPERATION REPRESENTED MAINLY THE CASTRATION EFFECT.

THE ACTION OF CASTRATION AND OF THE DUAL OPERATION WAS LESS MARKED ON THE ENZYME ACTIVITY THAN ON THE ORGAN WEIGHT. TREATMENT, ESPECIALLY ADRENOSTERONE, STIMULATED THE ACCESSORIES OF ALL ANIMALS.

COLD EXPOSURE ( $-5^{\circ}\text{C}$ ) PRODUCED A DEPRESSION OF ACTIVITY, EMPHASIZED BY ADRENALECTOMY. A STIMULATION WAS OBSERVED IN THE CASTRATES BUT NOT IN THE DUAL OPERATED ANIMALS. THE DEPRESSION DUE TO COLD AND SURGICAL STATE WAS CANCELLED BY THE ANDROMIMETIC AGENTS, ESPECIALLY BY ADRENOSTERONE WHERE THE OBSERVED VALUES WERE HIGHER THAN THOSE OF THE ABSOLUTE CONTROLS.

TABLE VI  
SEMINAL VESICLE ALKALINE PHOSPHATASE (MG P/G/HOUR)

GROUPS	DAY 0		DAY 12		PERCENT DIFFERENCE (N ~ F)
	21°C(N)	2°C(F)	21°C(N)	-5°C(F)	
<u>UNTREATED</u>					
UNOPERATED	13.6±0.3 <sup>A</sup> (17) <sup>B</sup>	17.3±0.5(13)	14.2±0.3(16)	12.9±0.2(22)	- 9(38)
ADRENALECTOMIZED	12.3±0.2 (19)	16.8±0.3(10)	13.2±0.3(16)	7.8±0.3(10)	-41(26)
CASTRATED	13.6±0.3 (16)	15.8±0.4(12)	6.5±0.3(13)	7.7±0.2(16)	+19(29)
DUAL OPERATED	10.8±0.3 (13)	14.2±0.3(10)	6.1±0.2(13)	6.0±0.2(9)	- 2(22)
<u>ANDROSTENEDIONE</u>					
UNOPERATED			15.6±0.3(11)	14.0±0.3(10)	-10(21)
ADRENALECTOMIZED			16.7±0.2(13)	12.0±0.9(9)	-28(22)
CASTRATED			10.1±0.2(10)	10.9±0.3(10)	+ 8(20)
DUAL OPERATED			8.0±0.3(11)	9.1±0.2(7)	+14(18)
<u>ADRENOSTERONE</u>					
UNOPERATED			17.7±0.4(12)	16.9±0.2(10)	- 5(22)
ADRENALECTOMIZED			15.7±0.3(12)	15.4±0.5(8)	- 2(20)
CASTRATED			16.3±0.6(10)	16.5±0.4(11)	+ 1(21)
DUAL OPERATED			13.3±0.4(12)	12.4±0.2(7)	- 7(19)

A. STANDARD ERROR OF THE MEAN

B. POPULATION

NOTE: RESULTS UNDERSCORED BY A BROKEN LINE INDICATE  $P < 0.05$ ; THOSE UNDERSCORED BY A SINGLE SOLID LINE INDICATE  $P < 0.01$ . RESULTS UNDERSCORED BY A DOUBLE LINE INDICATE  $P < 0.001$ .

C. DORSOLATERAL PROSTATE

i) WEIGHT (TABLE VII AND FIGURE 6)

THE INTACT AND ADRENALECTOMIZED COLD-ACCLIMATED ANIMALS SHOWED AN INCREASE IN WEIGHT WHEN COMPARED TO THE ROOM TEMPERATURE ANIMALS ON DAY ZERO. ON DAY 12, ADRENALECTOMY DID NOT PRODUCE ANY EFFECT ON THE WEIGHT OF THE DORSOLATERAL PROSTATE AT ROOM TEMPERATURE; CASTRATION CAUSED THE CLASSICAL EFFECTS AND THE DUAL OPERATED ANIMALS DISPLAYED THE ATROPHY MAINLY DUE TO CASTRATION.

ANDROSTENEDIONE TREATMENT HAD A STIMULATING EFFECT ON THE WEIGHT OF THE DORSOLATERAL PROSTATE IN ALL ANIMALS AND ADRENOSTERONE TREATMENT STIMULATED THE GLAND TO REACH THE CONTROL LEVEL.

EXPOSURE TO  $-5^{\circ}\text{C}$  PRODUCED A DEPRESSION OF THE WEIGHT OF THE GLAND IN INTACT CONTROLS, EMPHASIZED BY ADRENALECTOMY. AN INCREASE IN WEIGHT WAS PRODUCED IN THE CASTRATES (BUT NOT IN THE DUAL OPERATED ANIMALS) AFTER 12 DAYS COLD EXPOSURE.

ii) ZINC<sup>65</sup> UPTAKE (TABLE VIII AND FIGURE 7)

THE ZINC<sup>65</sup> UPTAKE BY THE DORSOLATERAL PROSTATE FOLLOWED THE SAME PATTERN OF CHANGES DUE TO SURGERY AND COLD EXPOSURE ( $-5^{\circ}\text{C}$ ) AS THE ORGAN WEIGHT.

TREATMENT ESPECIALLY WITH ADRENOSTERONE PRODUCED AN INCREASE IN UPTAKE.

COLD ( $-5^{\circ}\text{C}$ ) HAD A DEPRESSING ACTION ON THE ZINC<sup>65</sup> UPTAKE IN THE UNOPERATED ANIMALS, THIS DEPRESSION WAS MORE MARKED IN THE ADRENALECTOMIZED ANIMALS. THE CASTRATES SHOWED A MARKED STIMULATION IN THE

TABLE VII  
DORSOLATERAL PROSTATE WEIGHT (MG)

GROUPS	DAY 0		DAY 12		PERCENT DIFFERENCE (N ~ F)	PERCENT DIFFERENCE (N ~ F)
	21°C(N)	2°C(F)	21°C(N)	-5°C(F)		
<u>UNTREATED</u>						
UNOPERATED	220.8±5.2 <sup>A</sup> (17) <sup>B</sup>	241.6±6.2(13)	275.2±7.0(16)	218.5±6.0(22)	+10(30)	-21(38)
ADRENALECTOMIZED	200.0±8.0(19)	267.3±6.7(10)	264.5±10.1(16)	153.4±5.5(10)	+33(29)	-42(26)
CASTRATED	174.6±7.1(16)	169.9±7.7(12)	52.2±2.0(13)	74.2±2.6(16)	-3(28)	+42(29)
DUAL OPERATED	160.8±8.5(13)	177.0±4.9(10)	48.8±2.9(13)	41.8±2.5(9)	+9(23)	-14(22)
<u>ANDROSTENEDIONE</u>						
UNOPERATED			306.9±9.5(11)	293.0±6.4(10)		-5(21)
ADRENALECTOMIZED			274.5±9.9(13)	203.2±2.8(9)		-26(22)
CASTRATED			96.8±4.8(10)	131.6±6.9(10)		+36(20)
DUAL OPERATED			106.9±5.0(11)	109.0±4.4(7)		+2(18)
<u>ADRENOSTERONE</u>						
UNOPERATED			343.1±7.2(12)	531.2±8.5(10)		-4(22)
ADRENALECTOMIZED			329.3±8.1(12)	273.6±9.1(8)		-17(20)
CASTRATED			216.5±5.9(10)	284.2±4.9(11)		+31(21)
DUAL OPERATED			275.1±6.2(12)	264.1±8.6(7)		-3(19)

A. STANDARD ERROR OF THE MEAN.

B. POPULATION

NOTE: RESULTS UNDERScoreD BY A BROKEN LINE INDICATE  $P < 0.05$ ; THOSE UNDER-  
SCORED BY A DOUBLE LINE INDICATE  $P < 0.001$ .

TABLE VIII  
ZINC <sup>65</sup> UPTAKE BY THE DORSOLATERAL PROSTATE (C.P.M.)

GROUPS	DAY 0		DAY 12		PERCENT DIFFERENCE (N~F)
	21°C(N)	20°C(F)	21°C(N)	-5°C(F)	
<u>UNTREATED</u>					
UNOPERATED	3253±154.4 <sup>A</sup> (17) <sup>B</sup>	3406±202.0(13)	3889± 58.3(16)	2437±81.2(22)	- <u>37</u> (38)
ADRENALECTOMIZED	3067±153.5 (19)	3174±160.3(10)	3624±109.5(16)	1572±91.6(10)	- <u>57</u> (26)
CASTRATED	1608±109.3 (16)	1718±101.4(12)	276± 8.2(13)	471±14.0(16)	+ <u>71</u> (29)
DUAL OPERATED	1505±120.4 (13)	1781±144.5(10)	213± 7.2(13)	176± 6.8(9)	- <u>17</u> (22)
<u>ANDROSTENEDIONE</u>					
UNOPERATED			5297±128.7(11)	3752± 77 (10)	- <u>29</u> (21)
ADRENALECTOMIZED			4136±58.3(13)	3829±83.2(9)	- <u>7</u> (22)
CASTRATED			1508±70.0(10)	2470±76.3(10)	+ <u>64</u> (20)
DUAL OPERATED			1444±80.2(11)	1448±71.4(7)	± 0(18)
<u>ADRENOSTERONE</u>					
UNOPERATED			5677±139.0(12)	4609±69.4(10)	- <u>19</u> (22)
ADRENALECTOMIZED			4399±103.4(12)	4773±80.2(8)	+ 9(20)
CASTRATED			4027± 84.5(10)	4714±87.0(11)	+ <u>17</u> (21)
DUAL OPERATED			3872±127.0(12)	3739±88.5(7)	- 3(19)

A. STANDARD ERROR OF THE MEAN.

B. POPULATION

NOTE: RESULTS UNDERScoreD BY A SINGLE LINE INDICATE  $P < 0.01$ ; THOSE UNDERScoreD BY A DOUBLE LINE INDICATE  $P < 0.001$ .

COLD WHILE THE DUAL OPERATION DISPLAYED A SLIGHT STIMULATION. THE EFFECT OF COLD AND SURGERY WAS CANCELLED BY THE HORMONE TREATMENTS.

THE GRADATION OF RESPONSES TO SURGERY AND TO HORMONE TREATMENTS WAS MORE PRONOUNCED IN THIS PARAMETER THAN IN ANY OTHER PARAMETER. IT IS EVIDENT, FROM THE ANDROGENIC PARAMETERS THAT IN GENERAL, IN RATS EXPOSED TO SEVERE COLD ( $-5^{\circ}\text{C}$ ), ADENALECTOMY PRODUCES A SIGNIFICANT DEPRESSION IN ADDITION TO THAT PRODUCED BY COLD ITSELF.

A STIMULATION IS OBSERVED IN THE CASTRATES AFTER COLD-EXPOSURE, BUT NOT IN THE DUAL OPERATED ANIMALS.

THE GENITAL ACCESSORIES OF THE COLD EXPOSED ANIMALS ARE STIMULATED BY THE EXOGENOUS ADMINISTRATION OF ADRENAL ANDROGENS AND ADRENOSTERONE HAS A GREATER EFFECT THAN ANDROSTENEDIONE.

THE RESPONSE OF THE GENITAL ACCESSORIES TO THE HORMONES IS MORE PRONOUNCED IN THE COLD EXPOSED ( $-5^{\circ}\text{C}$ ) ANIMALS THAN IN THE CORRESPONDING RATS AT ROOM TEMPERATURE.

#### 5. ADRENAL WEIGHT (TABLE IX AND FIGURE 8)

A SIGNIFICANT STIMULATION OF THE ADRENALS WAS FOUND AFTER 30 DAYS OF COLD ACCLIMATION AT  $2^{\circ}\text{C}$ .

FEMINIZATION OF THE ADRENALS DUE TO CASTRATION WAS CANCELLED BY TREATMENT WITH THE TWO HORMONES. THE HORMONES HAD A DEPRESSING ACTION ON THE ADRENALS OF THE INTACT ANIMALS.

AN EXPOSURE OF 12 DAYS AT  $-5^{\circ}\text{C}$  PRODUCED A SIGNIFICANT INCREASE IN THE ADRENAL WEIGHTS IN THE INTACT AS WELL AS IN THE CASTRATES, OVER THE VALUES OBSERVED AT DAY ZERO IN THE ACCLIMATED ANIMALS. THE HYPERTROPHIED ADRENALS OF THE COLD-EXPOSED WERE TONED DOWN BY THE HORMONE

TABLE IX  
ADRENAL WEIGHT (MG)

GROUPS	DAY 0		DAY 12		PERCENT DIFFERENCE (N~F)
	21°C(N)	2°C(F)	21°C(N)	-5°C(F)	
<u>UNTREATED</u>					
UNOPERATED	44.5±1.0 <sup>A</sup> (17) <sup>B</sup>	58.1±1.0(13)	47.6±1.0(16)	67.9±0.8(22)	+43(38)
CASTRATED	48.5±1.2(16)	59.4±0.8(12)	58.6±1.5(13)	74.7±2.4(16)	+27(29)
<u>ANDROSTENEDIONE</u>					
UNOPERATED			40.9±0.5(11)	57.3±0.6(10)	+40(21)
CASTRATED			46.4±0.5(10)	66.6±1.5(10)	+44(20)
<u>ADRENOSTERONE</u>					
UNOPERATED			41.8±0.9(12)	57.0±0.3(10)	+36(22)
CASTRATED			43.8±0.5(10)	63.3±0.4(11)	+45(21)

A. STANDARD ERROR OF THE MEAN

B. POPULATION

NOTE: RESULTS UNDERSCORED BY A DOUBLE LINE INDICATE  $P < 0.001$ .

TREATMENTS. NO DIFFERENCE WAS OBSERVED BETWEEN THE ADRENAL RESPONSE TO THE TWO HORMONES.

6. THYMUS WEIGHT (TABLE X AND FIGURE 9)

ACCLIMATION AT 2°C FOR 30 DAYS PRODUCED THYMIC INVOLUTION IN ALL ANIMALS. A JUVENILE THYMUS WAS OBSERVED IN BOTH THE ADRENALECTOMIZED AND THE CASTRATED ANIMALS AT DAY 12; CONJOINT ADRENALECTOMY AND CASTRATION CAUSED A GREATER HYPERTROPHY OF THE THYMUS AT ROOM TEMPERATURE.

TREATMENT, ESPECIALLY WITH ADRENOSTERONE, PRODUCED A MILD DEPRESSION OF WEIGHT IN THE UNOPERATED ANIMALS AT ROOM TEMPERATURE. BOTH HORMONES REDUCED THE WEIGHT OF THE JUVENILE THYMUS IN ADRENALECTOMIZED, CASTRATED OR DUAL OPERATED ANIMALS; ADRENOSTERONE WAS MORE EFFECTIVE THAN ANDROSTENEDIONE.

THE SEVERE STRESS OF EXPOSURE TO -5°C FOR 12 DAYS PRODUCED THYMOLYSIS IN ALL ANIMALS, GREATER IN THE JUVENILE THYMUS (ADRENALECTOMIZED, CASTRATED OR DUAL OPERATED) BECAUSE OF ITS GREATER SENSITIVITY TO STRESS.

TREATMENT WITH THE HORMONES CAUSED A PRONOUNCED DEPRESSION OF WEIGHT IN ALL COLD-EXPOSED ANIMALS.

TABLE X

## THYMUS WEIGHT (MG)

GROUPS	DAY 0		DAY 12		PERCENT DIFFERENCE (N~F)
	21°C(N)	2°C(F)	21°C(N)	-5°C(F)	
<u>UNTREATED</u>					
UNOPERATED	384.2±8.6 <sup>A</sup> (17) <sup>B</sup>	250.6±3.1 (13)	392.6±11.5(16)	271.5± 9.1 (22)	-31(38)
ADRENALECTOMIZED	422.4±7.3 (19)	265.4±7.3 (10)	617.4±7.9(16)	437.9±13.3(10)	-29(26)
CASTRATED	389.4±13.6(16)	245.0±5.7 (12)	650.0±16.4(13)	341.1± 8.7(16)	-48(29)
DUAL OPERATED	443.3±11.8(13)	279.2±11.4(10)	692.3±9.4 (13)	402.0± 8.8(9)	-42(22)
<u>ANDROSTENEDIONE</u>					
UNOPERATED			356.1±10.1(11)	184.3±10.1(10)	-48(21)
ADRENALECTOMIZED			553.1±16.3(13)	384.6± 9.3(9)	-30(22)
CASTRATED			545.6±10.6(10)	258.7± 7.2(10)	-47(20)
DUAL OPERATED			575.4±10.6(11)	323.3± 9.7(7)	-44(18)
<u>ADRENOSTERONE</u>					
UNOPERATED			339.2±7.4 (12)	178.3± 7.3(10)	-47(22)
ADRENALECTOMIZED			456.3±10.8(12)	323.4±12.8(8)	-29(20)
CASTRATED			484.5± 7.4(10)	177.9± 7.7(11)	-37(21)
DUAL OPERATED			482.3±10.4(12)	311.0± 9.9(7)	-36(19)

A. STANDARD ERROR OF THE MEAN.

B. POPULATION

NOTE: RESULTS UNDERSCORED BY A DOUBLE LINE INDICATE  $P < 0.001$ .

## DISCUSSION AND CONCLUSION

THE ONLY METHOD OF EVALUATION OF THE ADRENAL ANDROGENS IS THE ESTABLISHMENT OF A BALANCE SHEET OF THE SEPARATE ANDROGENIC SOURCES: TESTES, ADRENALS AND PHARMACEUTICALS. THE VALID INDICATORS OF THE ANDROGENIC FUNCTION ARE THE MORPHOLOGY AND PHYSIOLOGY OF THE SEX ACCESSORIES.

IN THE CASTRATED ANIMALS, THE FEMINOID MORPHOLOGY MAY COEXIST WITH AN INCREMENT OF ADRENAL PRODUCTS, ESPECIALLY ANDROGENS. IN THE ADRENALECTOMIZED ANIMALS, BY FEEDBACK OR COMPENSATION, THE TESTICULAR PRODUCTION OF ANDROGENS MAY INCREASE.

EXPOSURE TO COLD MAY HAVE DIVERGENT ACTIONS, SUCH AS DEPRESSION OF TESTICULAR ACTIVITY AND STIMULATION OF THE ADRENALS BY WAY OF THE PITUITARY RESPONSE TO STRESS; A POSSIBLE PREFERENTIAL REORIENTATION OF THE ANTERIOR PITUITARY SECRETION CANNOT BE DISREGARDED.

THE PHARMACEUTICAL ACTION OF EXOGENOUS ANDROGENS MAY WELL INDUCE A DEPRESSION OF THE PRIMARY ORGAN (TESTES, ADRENAL) BY WAY OF NEGATIVE FEEDBACK MECHANISM ON THE PITUITARY; THE DIRECT STIMULATION OF THE SEX ACCESSORIES MAY BE MODIFIED BY IMPOSITION OF STRESS.

IN THE INTACT UNTREATED ANIMALS AT ROOM TEMPERATURE THE ANDROGENS ARE PRODUCED BY THE TESTES AND THE ADRENALS. UNDER TREATMENT WITH EXOGENOUS ANDROGENS THE PITUITARY GONADOTROPHINS ARE DEPRESSED RESULTING IN A DECREASE OF TESTICULAR AND ADRENAL ANDROGENS (NALBANDOV, 1964), BUT THE GENITAL ORGANS DEMONSTRATE A SIGNIFICANT STIMULATION DUE TO THE DIRECT ACTION OF THE HORMONES. THE ENDOCRINE EFFECT OF COLD IS THE RESULTANT OF A DEPRESSION OF TESTICULAR ANDROGENS BY WAY OF PITUITARY DEFICIENCY AND AN INCREMENT OF ADRENAL ANDROGENS BY WAY OF CORTICAL STIMULATION. THE ADMINISTRATION OF EXOGENOUS ADRENAL ANDROGENS IN

COLD-EXPOSED RATS CAUSED AN OVERALL STIMULATION OF THE GENITAL ACCESSORIES REPRESENTING THE DIRECT ACTION OF THE HORMONES.

IN THE UNTREATED CASTRATES AT ROOM TEMPERATURE THE ATROPHY OF THE ACCESSORIES IS DUE TO THE LOSS OF TESTICULAR ANDROGENS; A COMPENSATORY INCREASE IN ADRENAL ANDROGENS BECOMES POSSIBLE. AFTER TREATMENT WITH THE HORMONES THE GENITAL ACCESSORIES ARE POWERFULLY STIMULATED. ' REPLACEMENT THERAPY ' STIMULATED THE SEX ACCESSORIES AND MASKED ANY COMPENSATORY ACTION OF ADRENAL ANDROGENS. IN THE CASTRATED RATS EXPOSED TO SEVERE COLD ( $-5^{\circ}\text{C}$ ) FOR 12 DAYS, AUTOPSY REVEALS WEIGHT VALUES FOR THE SEXUAL ACCESSORIES GREATER THAN THOSE OBSERVED IN THE CASTRATES AT ROOM TEMPERATURE (PERRAULT AND DUGAL, 1963). A SIMILAR PICTURE WAS OBTAINED IN THE PRESENT EXPERIMENT; IN ADDITION TO THE WEIGHT OF THE GENITAL ACCESSORIES, THE ALKALINE PHOSPHATASE ACTIVITY OF THE SEMINAL VESICLES AND THE ZINC<sup>65</sup> UPTAKE BY THE DORSOLATERAL PROSTATE WERE ALSO INCREASED. THE ADRENAL ANDROGENS ARE THE ONLY CAUSATIVE FACTORS FOR THIS INCREMENT OF THE SEX ACCESSORIES. A PARALLEL RESPONSE WAS OBTAINED WITH ACTH TREATMENT OF CASTRATES AT ROOM TEMPERATURE (DEANESLY, 1960). IN COLD-EXPOSED CASTRATES, ADMINISTRATION OF THE HORMONES PRODUCED STIMULATION OF THE ACCESSORIES; THE POTENTIAL DEPRESSION BY PITUITARY FEEDBACK IS SUPERCEDED BY STRESS-INDUCED STIMULATION OF THE ADRENAL CORTEX. IN ACCORDANCE WITH THE OBSERVATION OF KORENCHEVSKY ET AL (1937). ANDROSTENEDIONE PROVED TO BE OF LOW ANDROGENICITY ALTHOUGH A SIGNIFICANT STIMULATION WAS VISIBLE.

IN THE ADRENALECTOMIZED ANIMALS AT ROOM TEMPERATURE, THE TESTICULAR ANDROGENS ARE SOLELY RESPONSIBLE FOR THE MAINTENANCE OF THE SEX ACCESSORIES; THEIR CHARACTERISTICS WERE NOT DIFFERENT FROM THE INTACT CONTROLS.

THIS CONFIRMS THE FINDINGS OF BURRILL AND GREENE (1939), KATSH (1948) AND MOORE (1953). TREATMENT WITH THE HORMONES CAUSED DIRECT STIMULATION OF THE ACCESSORIES, MASKING THE POSSIBLE PITUITARY FEEDBACK. IN COLD-EXPOSED RATS, THE REMOVAL OF THE ADRENALS PRODUCED A SIGNIFICANT REDUCTION BEYOND THE EFFECT OF COLD IN THE WEIGHT AND ACTIVITY OF THE ACCESSORY GENITAL ORGANS. THIS REVEALED THE IMPORTANCE OF THE ADRENAL CONTRIBUTION TO THE OVERALL ANDROGENIC POOL IN THE STRESSED (COLD) ANIMALS. IN HIS REVIEW ON THE SECRETION OF SEX HORMONES BY THE ADRENALS, SHORT (1960) SHOWED THAT 1) THE GLAND IS CAPABLE OF SECRETING ALL THREE TYPES OF SEX HORMONES (ESTROGENS, PROGESTERONE, ANDROGENS); 2) ONLY A HYPERACTIVE STATE REVEALS THE CORTICAL POTENTIALITIES.

THE DUAL-OPERATED ANIMALS LACKING BOTH SOURCES OF ANDROGENS DISPLAYED DEGENERATED ACCESSORIES UNAFFECTED BY COLD STRESS. THE RESPONSE OF THE ACCESSORIES TO THE EXOGENOUS ANDROGENS THEREFORE, REPRESENTED PURELY THE PHARMACOLOGICAL ACTION OF THE HORMONES.

THE RESPONSE OF THE ACCESSORY GENITAL ORGANS TO EXOGENOUS ANDROGENS WAS GENERALLY GREATER IN THE COLD-EXPOSED RATS, POSSIBLY BECAUSE OF INCREASED SENSITIVITY OF THE ACCESSORIES TO THIS ADVERSE SITUATION.

OUR RESULTS INDICATE THAT IN GENERAL THE WEIGHT ~~AND~~ THE ACTIVITY OF THE GENITAL ACCESSORIES ARE INCREASED AFTER ACCLIMATION (30 DAYS AT 2°C); PROBABLY BY WAY OF A COMPENSATORY RESPONSE OF THE TESTES TO PITUITARY ACTION (OUELLETTE ET AL. 1965).

#### 4 THE BALANCE SHEET

BECAUSE OF ITS GREATER RELIABILITY, ZINC<sup>65</sup> UPTAKE BY THE DORSOLATERAL PROSTATE WAS SELECTED AS THE MOST INFORMATIVE PARAMETER. ADRENOSTERONE WAS CHOSEN BECAUSE OF ITS HIGH ANDROGENIC POTENCY AND ITS EFFECTIVENESS AS A REPLACEMENT AGENT. COMPUTATIONS WERE DONE FROM THE HISTOGRAMS (CONTROL FRACTION), ALTHOUGH THE DIRECT VALUES OF ZINC<sup>65</sup> UPTAKE GAVE SIMILAR INFORMATION.

##### A) SOURCES OF ERRORS

THE EXPECTED DECREASE IN THE TESTICULAR AND THE INCREASE IN THE ADRENAL ANDROGENIC CONTRIBUTION AFTER EXPOSURE TO COLD ARE THEORETICALLY COMPUTED BY SIMPLE ARITHMETICAL SUM. IN THE EXPERIMENTAL CONDITION THE CHANGES MIGHT HAVE BEEN OTHERWISE.

THE FEMINOID ADRENAL OF THE CASTRATED ANIMALS MAY CAUSE AN INCREMENT IN ANDROGEN PRODUCTION, WHICH WAS NOT DETECTED IN THE EXPERIMENTS BUT MAY BE A SOURCE OF ERROR IN THE COMPUTATION. IT MAY BE POSSIBLE THAT BY FEEDBACK OR COMPENSATION, THE TESTICULAR PRODUCTION OF ANDROGENS INCREASES IN THE ADRENALECTOMIZED ANIMALS, ALTHOUGH IT COULD NOT BE DETECTED IN THE EXPERIMENTS. THIS MAY ALSO CAUSE AN ERROR IN THE COMPUTATIONS.

b) ANDROGENIC CONTRIBUTIONS:

	<u>UNSTRESSED</u>	<u>STRESSED</u>
TESTICULAR	A	A <sup>1</sup>
ADRENAL	B	B <sup>1</sup>
EXOGENOUS	C	C

c) EXPERIMENTAL CLASSES:

I. UNOPERATED + INJECTION	A+B+C	A <sup>1</sup> +B <sup>1</sup> +C
II. UNOPERATED	A+B	A <sup>1</sup> +B <sup>1</sup>
III. CASTRATE + INJECTION	B+C	B <sup>1</sup> +C
IV. CASTRATE	B	B <sup>1</sup>
V. ADRENALECTOMIZED + INJECTION	A + C	A <sup>1</sup> + C
VI. ADRENALECTOMIZED	A	A <sup>1</sup>
VII. DUAL OPERATED + INJECTION	C	C
VIII. DUAL OPERATED	NIL	NIL

d) ALGEBRAIC COMPUTATION

		<u>UNSTRESSED</u>	<u>STRESSED</u>
1) <u>TESTICULAR CONTRIBUTION</u>			
I - III = VI	$(A+B+C)-(B+C) =$	A	A <sup>I</sup>
II - IV = VI	$(A+B)-(B) =$	A	A <sup>I</sup>
V - VII = VI	$(A+C)-(C) =$	A	A <sup>I</sup>
2) <u>ADRENAL CONTRIBUTION</u>			
I - V = IV	$(A+B+C)-(A+C) =$	B	B <sup>I</sup>
II - VI = IV	$(A+B)-(A) =$	B	B <sup>I</sup>
III - VII = IV	$(B+C)-(C) =$	B	B <sup>I</sup>
3) <u>EXOGENOUS CONTRIBUTION</u>			
I - II = VII	$(A+B+C)-(A+B) =$	C	C
III - IV = VII	$(B+C)-(B) =$	C	C
V - VI = VII	$(A+C)-(A) =$	C	C
VII - VIII = VII	$(C)-NIL =$	C	C

E) ARITHMETICAL COMPUTATION

<u>EXPERIMENTAL GROUP</u>	<u>CONTROL FRACTION (Zn<sup>65</sup> UPTAKE)</u>		<u>DIFFERENCE (N-F)</u>
	<u>UNSTRESSED(N)</u>	<u>(OBSERVED) STRESSED(F)</u>	
I	146	116	-30
II	100	63	-37
III	104	121	+17
IV	7	12	+ 5
V	113	123	+10
VI	93	40	-53
VII	100	96	- 4
VIII	6	5	- 1

UNSTRESSED1) TESTICULAR CONTRIBUTION

$$I - III = 146 - 104 = 42$$

$$II - IV = 100 - 7 = 93^1 = VI \quad (93)^2$$

$$V - VII = 113 - 100 = 13$$

STRESSED

$$116 - 121 = -5$$

$$63 - 12 = 51 = VI \quad (40)$$

$$123 - 96 = 27$$

2) ADRENAL CONTRIBUTION

$$I - V = 146 - 113 = 33$$

$$II - VI = 100 - 93 = 7 = IV \quad (7)$$

$$III - VII = 104 - 100 = 4$$

$$116 - 123 = -7$$

$$63 - 40 = 23 = IV \quad (12)$$

$$121 - 96 = 25$$

3) THERAPEUTIC CONTRIBUTION

$$I - II = 146 - 100 = 46$$

$$III - IV = 104 - 7 = 97$$

$$V - VI = 113 - 93 = 20$$

$$VII - VIII = 100 - 6 = 94 = VII \quad (100)$$

$$116 - 63 = 53$$

$$121 - 12 = 109$$

$$123 - 40 = 83$$

$$96 - 5 = 91 = VII \quad (96)$$

<sup>1</sup> COMPUTED

<sup>2</sup> OBSERVED

F) SUMMARY OF COMPUTATION

	UNSTRESSED(N)	STRESSED(F)	DIFFERENCE (N-F)
TESTICULAR CONTRIBUTION (V)	93%	51%	-42%
ADRENAL CONTRIBUTION (IV)	7%	23%	+16%
REPLACEMENT THERAPY (VII) (DEGREE OF EFFECTIVENESS)	94%	91%	

G) REMARKS

1) COMPUTATIONS ARE VALID ONLY IF "FUNCTION" (I) AND "ABSENCE OF FUNCTION" (IV OR VI) ARE COMPARED; IN EFFECT THE COMPUTED VALUES COINCIDE WITH THE OBSERVED VALUES.

2) COMPARISON BETWEEN I AND III WAS LEFT OUT BECAUSE THE RESPONSE TO EXOGENOUS HORMONES PROBABLY CAUSES A FEED BACK ON THE PITUITARY AND A COMPENSATORY ATROPHY OF THE SEX ACCESSORIES, RESULTING IN UNRELIABLE COMPUTATION. SAME REASON APPLIES FOR ELIMINATION OF OTHER COMPARISONS.

3) UNDER STRESS (COLD), TESTICULAR ANDROGENIC CONTRIBUTION DECREASES AS EVIDENCED BY N-F DIFFERENCE REPRESENTING THE RESULTANT OF ENDOCRINE AND SYSTEMIC EFFECTS.

4) THE RESULTS SUGGEST AN INCREMENT OF ADRENAL ANDROGENIC FUNCTION UNDER STRESS. THE ADRENAL CONTRIBUTION INCREASES FROM 7% (UNDER NORMAL CONDITIONS) TO 23% (UNDER STRESS).

H) CONCLUSION

1. STRESS (COLD) WAS USED AS A MEAN OF EXPLAINING THE ANDROGENIC FUNCTION OF THE ADRENALS.

2. THE ADRENAL CONTRIBUTION UNDER NORMAL CONDITIONS WOULD AMOUNT TO ABOUT 7% OF THE CIRCULATING ANDROGENS. UNDER STRESS (COLD), THE ADRENAL CONTRIBUTES ABOUT 23% OF THE CIRCULATING ANDROGENS.

## APPENDIX

## PHARMACOLOGICAL EVALUATION OF ADRENAL ANDROGENS

## INTRODUCTION

THE ASSAY OF ANDROGENS IN CASTRATED RATS HAS BEEN PRACTICED FOR MANY YEARS AND A VARIETY OF METHODS HAVE BEEN EMPLOYED. THESE HAVE BEEN REVIEWED IN SOME DETAIL BY DORFMAN (1962). VARIATION IN RESULTS WAS OBSERVED EVEN WHEN THE SAME TECHNIQUE WAS APPLIED BY DIFFERENT WORKERS IN DIFFERENT LABORATORIES.

THE AIMS OF THE PRESENT EXPERIMENT WERE 1) TO FIND OUT THE DOSE RESPONSE RELATION OF THREE RECOGNIZED ANDROGENS OF ADRENAL ORIGIN AND 2) TO SELECT THE PROPER DOSAGES FOR USE IN THE MAIN EXPERIMENT.

THE DOSES USED WERE VALUES BELOW, ABOVE AND WITHIN THE PHARMACOLOGICAL RANGE OF THE THREE ADRENAL ANDROGENS, BASED ON THE DATA OF FARRIS AND GRIFFITH (1949), BARNES AND ELTHERINGTON (1964) AND DORFMAN AND DORFMAN (1963).

## MATERIALS AND METHODS

1. ANIMALS AND DIET

A RANDOM GROUP OF 160 MALE RATS (WISTAR ALBINO) WEIGHING BETWEEN 35 AND 45 GM WAS ARRANGED ACCORDING TO TABLE I AND MAINTAINED UNDER UNIFORM CONDITIONS AS DESCRIBED IN THE MAIN EXPERIMENT.

2. SURGERY

AFTER THE USUAL 6-DAY QUARANTINE THE ANIMALS WERE CASTRATED BY ABDOMINAL APPROACH; THE INJECTIONS WERE STARTED TWO WEEKS POST-OPERATIVELY (DORFMAN, 1962).

TABLE I  
BODY WEIGHT (G)

GROUPS		PRETREATMENT	FINAL
<u>UNTREATED</u>	(20) <sup>A</sup>	150±1.0 <sup>B</sup>	185±1.4
CORN OIL	(15)	155±1.0	178±1.3
ETHYLENE GLYCOL	(15)	162±1.2	193±1.5
<u>ADRENOSTERONE</u>			
1000 µG	(12)	156±1.2	190±1.6
3000 µG	(12)	153±1.1	181±1.5
6000 µG	(12)	158±1.2	189±1.7
<u>ANDROSTENEDIONE</u>			
25 µG	(12)	150±1.3	183±1.8
500 µG	(12)	154±0.9	191±1.7
1000 µG	(12)	157±1.1	187±1.7
<u>DEHYDROISOANDROSTERONE</u>			
100 µG	(12)	151±1.0	178±1.4
500 µG	(12)	155±1.1	188±1.6
1000 µG	(12)	150±1.1	183±1.6

A. POPULATION

B. STANDARD ERROR OF THE MEAN.

NOTE: ALL INJECTIONS WERE ADMINISTERED IN A VOLUME OF 0.2 ML PER RAT  
SUBCUTANEOUSLY, DAILY FOR 10 DAYS.

### 3. TREATMENT

THE INJECTIONS WERE GIVEN SUBCUTANEOUSLY IN AN UNIFORM VOLUME OF 0.2 ML EACH DAY FOR TEN DAYS.

ADRENOSTERONE: SUSPENSION IN ETHYLENE GLYCOL; DAILY DOSES: 1000, 3000 AND 6000  $\mu$ G.

ANDROSTENEDIONE: DISSOLVED IN CORN OIL; DAILY DOSES: 25,500 AND 1000  $\mu$ G.

DEHYDROISOANDROSTERONE: DISSOLVED IN CORN OIL; DAILY DOSES: 100, 500 AND 1000  $\mu$ G.

### 4. AUTOPSY DATA

TWENTY-FOUR HOURS AFTER THE LAST INJECTION THE ANIMALS WERE SACRIFICED WITH ETHER; THE SEMINAL VESICLES, VENTRAL AND DORSOLATERAL PROSTATES WERE DISSECTED OUT, EXAMINED, BLOTTED AND WEIGHED WET TO THE NEAREST 0.2 MG ON A ROLLER-SMITH BALANCE. THE TABLES QUOTE THE ARITHMETICAL MEAN WITH THE STANDARD ERROR OF THE MEAN. DOSE RESPONSE CURVES AS LOG OF ORGAN WEIGHT VERSUS LOG OF DOSE ARE ALSO SUBMITTED.

## RESULTS

### 1. BODY WEIGHT (TABLE I)

NO EFFECT OF DRUGS WAS FOUND ON THE GROWTH RATE.

### 2. WEIGHT OF THE GENITAL ACCESSORIES (TABLE II AND FIGURE 10)

#### A) ADRENOSTERONE

(DOSE RANGE, 1000  $\mu$ G - 6000  $\mu$ G)

ALL THREE SEX ACCESSORIES SHOWED UNIFORM RESPONSE DEMONSTRATED BY IDEAL DOSE-RESPONSE CURVES AND HAD HIGH ANDROGENIC ACTION.

TABLE II

WEIGHT OF THE GENITAL ACCESSORIES (MG)

GROUPS	SEMANAL VESICLE	VENTRAL PROSTATE	DORSOLATERAL PROSTATE
<u>UNTREATED</u>	16.8±0.57 <sup>A</sup>	9.5±0.68	10.8±0.47
CORN OIL	15.4±0.52	10.8±0.71	9.6±0.52
ETHYLENE GLYCOL	17.9±0.58	9.4±0.64	11.4±0.36
<u>ADRENOSTERONE</u>			
1000 µg	21.5±0.86	12.3±0.52	21.2±0.83
3000 µg	208.0±4.25	145.9±3.16	120.8±2.88
6000 µg	246.5±4.89	152.3±4.10	131.5±3.12
<u>ANDROSTENEDIONE</u>			
25 µg	20.8±0.95	13.1±0.48	13.8±0.60
500 µg	45.7±1.78	37.4±2.51	36.5±1.42
1000 µg	54.7±1.43	45.3±1.50	43.8±1.71
<u>DEHYDROISOANDROSTERONE</u>			
100 µg	17.0±0.98	11.2±0.70	12.9±0.87
500 µg	17.4±1.12	10.7±0.63	14.3±0.92
1000 µg	21.1±0.80	11.5±0.54	16.1±0.85

A. STANDARD ERROR OF THE MEAN.

b) ANDROSTENEDIONE

(DOSE RANGE, 25  $\mu\text{g}$  - 1000  $\mu\text{g}$ )

DEMONSTRATED LOW BUT TYPICAL ANDROGENIC ACTION, VENTRAL PROSTATE ONLY GAVE RECOGNIZABLE SIGMOID CURVE.

c) DEHYDROISOANDROSTERONE

(DOSE RANGE, 100  $\mu\text{g}$  - 1000  $\mu\text{g}$ )

DEMONSTRATED A MINIMAL ANDROGENIC ACTION, THUS REJECTED FROM USE IN THE MAIN EXPERIMENT.

## DISCUSSION

DORFMAN AND DORFMAN (1963) DEMONSTRATED THAT THE POTENCY OF ADRENOSTERONE WAS 8% OF THAT OF TESTOSTERONE. MELI (1963) EMPLOYED A DOSE OF 0.4 MG OF TESTOSTERONE, ADMINISTERED DAILY FOR 7 DAYS BY THE SUBCUTANEOUS ROUTE TO PRODUCE A HYPERTROPHY OF THE SEMINAL VESICLES IN RATS. CONSIDERING THE POTENCY OF ADRENOSTERONE AS 8% OF TESTOSTERONE THE CALCULATED DOSE OF ADRENOSTERONE BECOMES 3500  $\mu\text{g}$  PER RAT DAILY FOR TEN DAYS. ADRENOSTERONE WAS THEREFORE ADMINISTERED AT DOSES OF 1000  $\mu\text{g}$ , 3000  $\mu\text{g}$  OR 6000  $\mu\text{g}$  TO OBTAIN A LINEAR RELATION BETWEEN THE DOSES AND THE WEIGHT RESPONSE OF THE GENITAL ORGANS.

THE DOSES OF ANDROSTENEDIONE WERE BASED ON THE DESCRIPTION IN FARRIS AND GRIFFITH (1949), WHERE A DOSE RANGE BETWEEN 25-500  $\mu\text{g}$  WAS RECOMMENDED FOR THE DEMONSTRATION OF A SIGNIFICANT INCREASE IN THE SEMINAL VESICLE WEIGHT. THE HIGHER DOSE OF 1000  $\mu\text{g}$  WAS SELECTED TO MAKE SURE THAT, AT THAT DOSE THE LINEAR RELATIONSHIP BETWEEN THE DOSE AND RESPONSE WAS PLATEAUED.

THE DOSES OF DEHYDROISOANDROSTERONE WERE BASED ON THE DESCRIPTION IN FARRIS AND GRIFFITH (1949), WHERE A DOSE RANGE BETWEEN 100 -

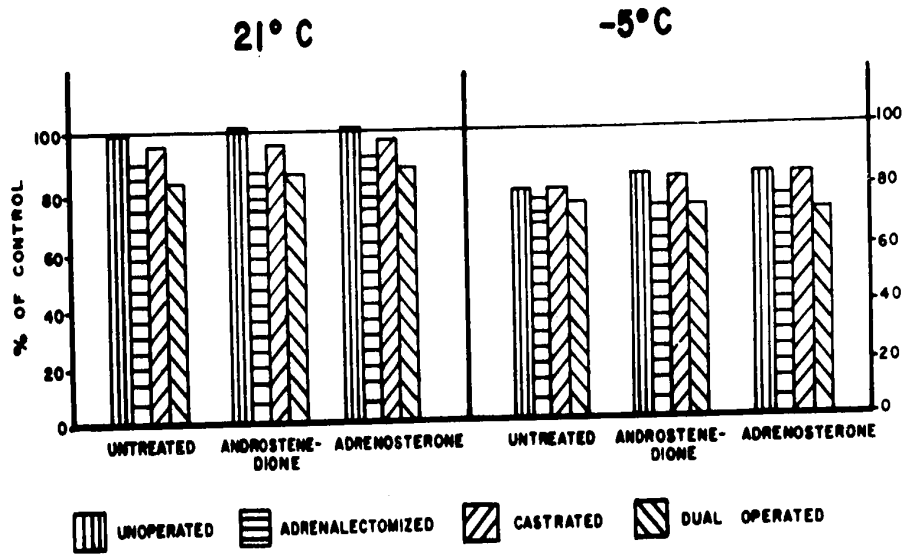
500  $\mu\text{G}$  WAS RECOMMENDED FOR THE DEMONSTRATION OF SIGNIFICANT INCREASE IN THE SEMINAL VESICLE WEIGHT. BUT EVEN WITH THE HIGHEST DOSE OF 1000  $\mu\text{G}$  USED IN THIS EXPERIMENT NO SIGNIFICANT RESPONSE OF THE GENITAL ACCESSORIES WAS OBTAINED, PROBABLY DUE TO EXTREMELY SHORT-BIOLOGICAL HALF LIFE OF THE HORMONE. DEHYDROISOANDROSTERONE WAS THEREFORE, NOT USED IN THE MAIN EXPERIMENT.

THE SELECTION OF THE DOSAGES FOR USE IN THE MAIN EXPERIMENT WAS BASED ON DOSE-RESPONSE CURVES AND WERE TAKEN IN THE UPPER DOSAGE RANGE.

- A) ADRENOSTERONE - 3000  $\mu\text{G}$ .
- B) ANDROSTENEDIONE - 500  $\mu\text{G}$ .

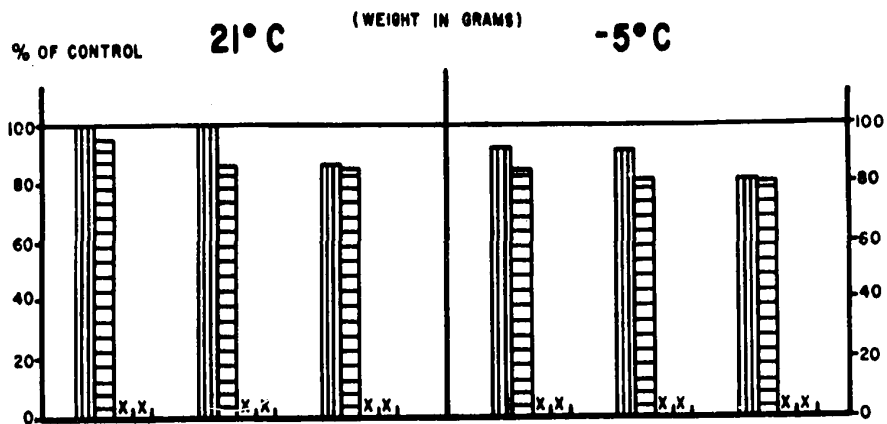
FIGURE 1. TERMINAL BODY WEIGHTS (FRACTION OF ABSOLUTE CONTROL).

### BODY WEIGHT





**TESTES**



**VENTRAL PROSTATE**

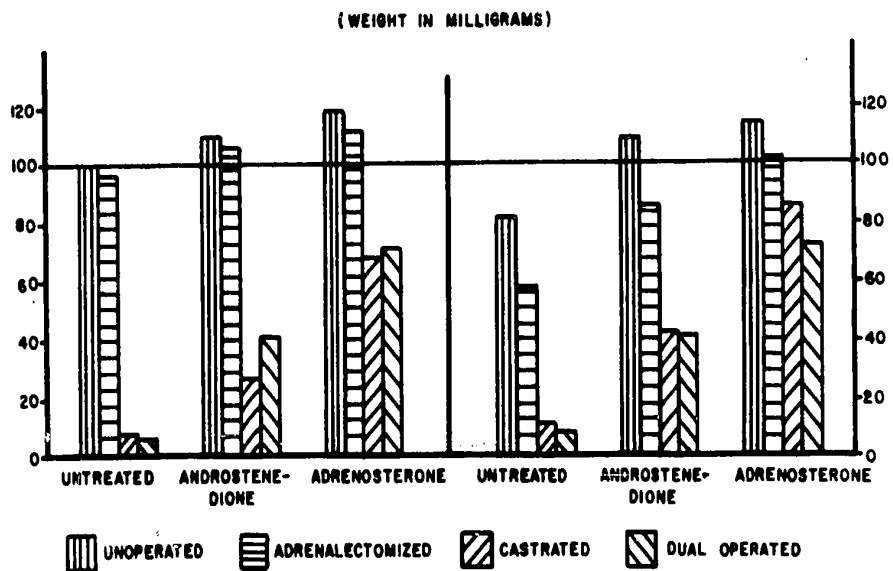
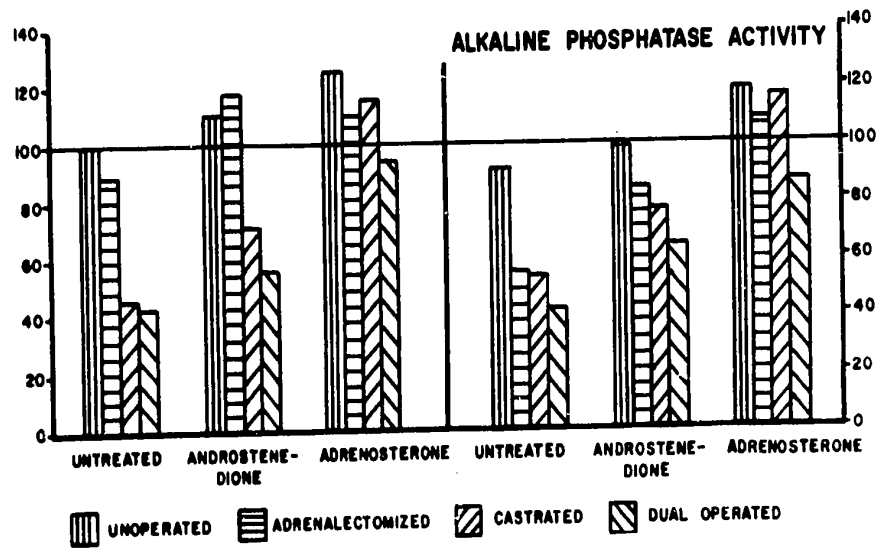
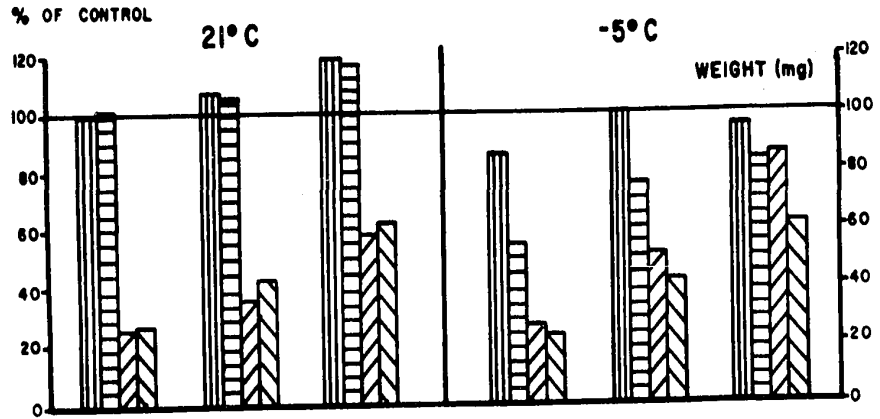


FIGURE 4. SEMINAL VESICLE (WEIGHT - FRACTION OF ABSOLUTE CONTROL)

FIGURE 5. SEMINAL VESICLE (ALKALINE PHOSPHATASE ACTIVITY -  
FRACTION OF ABSOLUTE CONTROL ).

SEMINAL VESICLES



UNOPERATED
  ADRENALECTOMIZED
  CASTRATED
  DUAL OPERATED

FIGURE 6. DORSOLATERAL PROSTATE

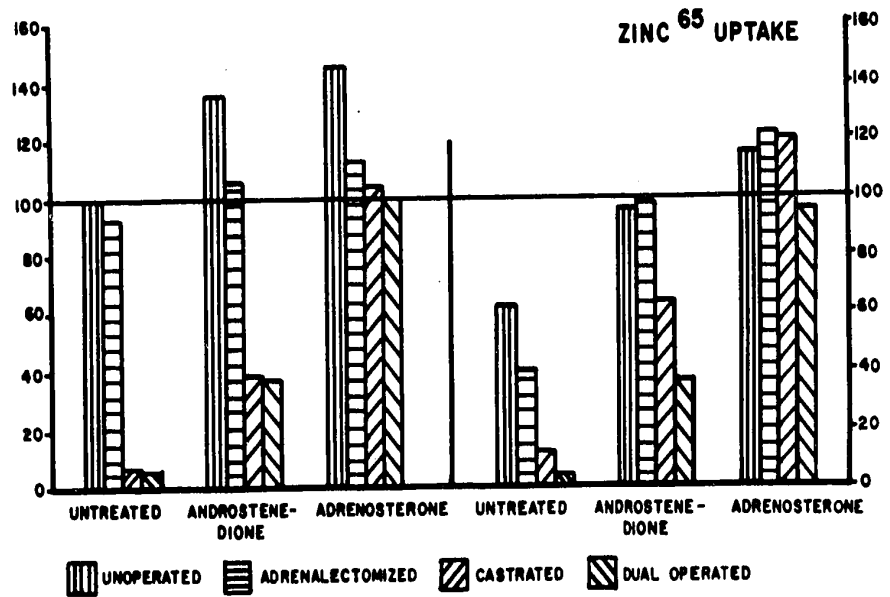
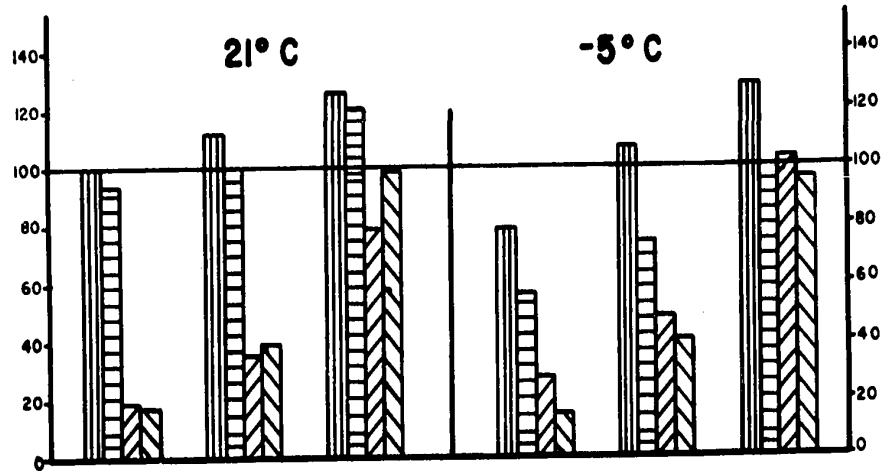
(WEIGHT - FRACTION OF ABSOLUTE CONTROL)

FIGURE 7. DORSOLATERAL PROSTATE

(ZINC<sup>65</sup> UPTAKE - FRACTION OF ABSOLUTE CONTROL)

DORSOLATERAL PROSTATE

WEIGHT (mg)



UNOPERATED
  ADRENALECTOMIZED
  CASTRATED
  DUAL OPERATED

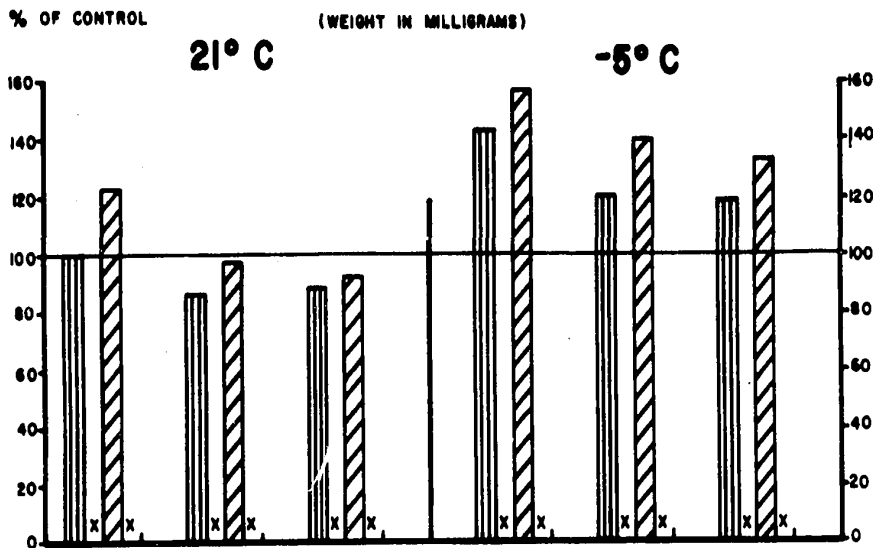
FIGURE 8. ADRENAL WEIGHT

(WEIGHT OF ABSOLUTE CONTROL)

FIGURE 9. THYMUS WEIGHT

(FRACTION OF ABSOLUTE CONTROL)

**ADRENALS**



**THYMUS**

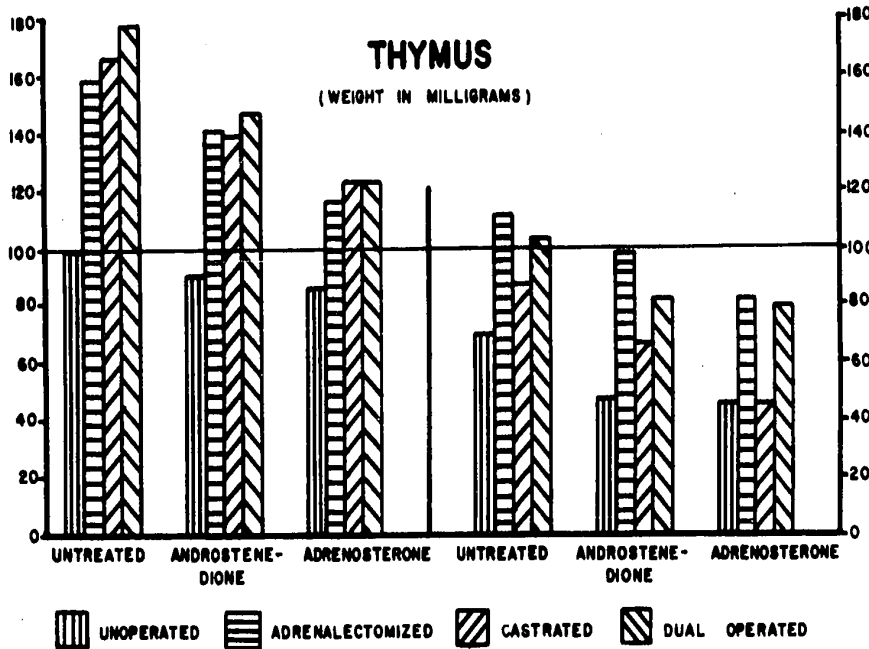
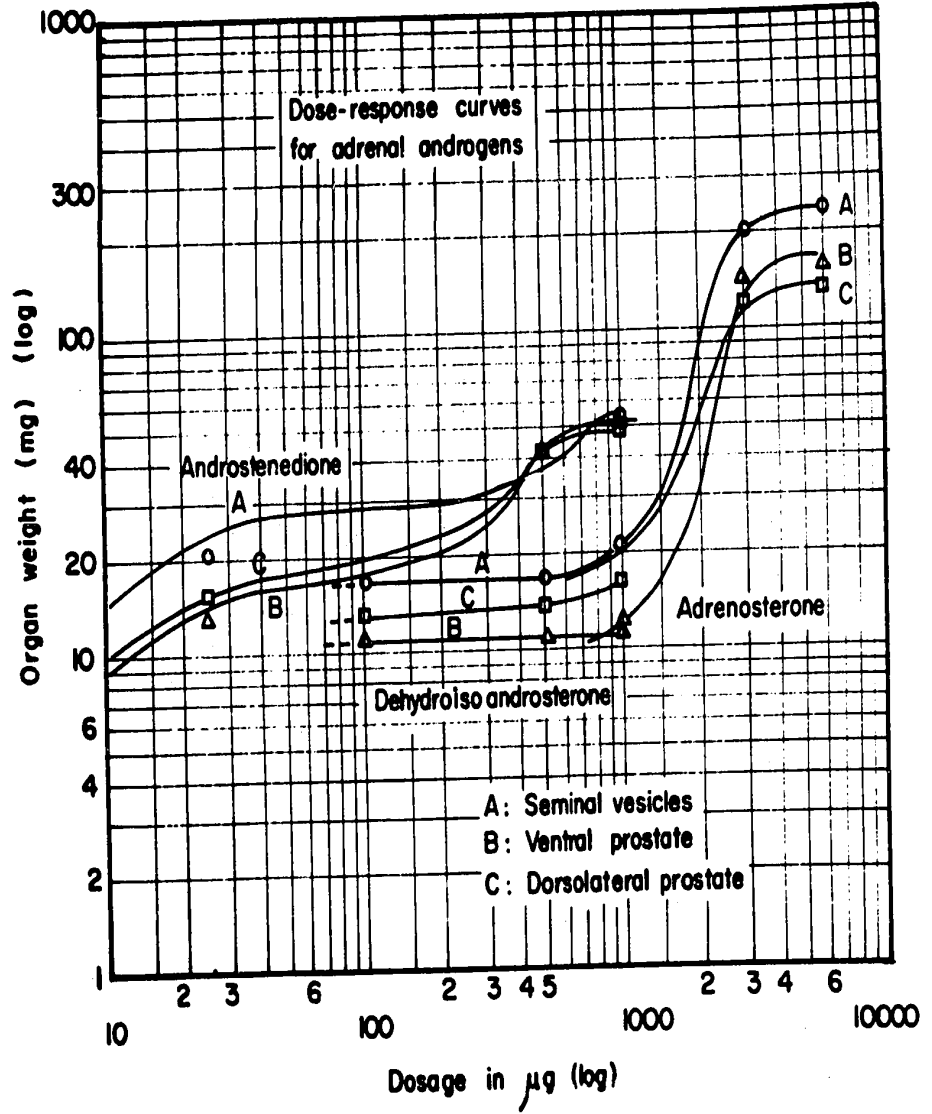


FIGURE 10. DOSE-RESPONSE CURVES OF THREE ADRENAL ANDROGENS.





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