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UNIVERSITY OF LOUISVILLE

THE INFLUENCE OF TEMPERATURE ON THE INTERNAL SECRETORY
ACTIVITY OF TRANSPLANTED OVARIES IN THE RAT.

A DISSERTATION

SUBMITTED TO THE FACULTY
OF THE GRADUATE SCHOOL OF THE UNIVERSITY OF LOUISVILLE
IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE

DEPARTMENT OF PATHOLOGY

BY

ARTHUR K. LAMPTON

1939

DATE 5-27-39

APPROVED BY

COMMITTEE:

ENGLISH DEPARTMENT _____

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FOR THEIR TIMELY ADVICE AND ENCOURAGEMENT, I ALSO WISH TO THANK DOCTORS HAROLD GORDON, H. C. LAWSON, AND L. A. GRAY.

ARTHUR K. LAMPTON

THE INFLUENCE OF TEMPERATURE ON THE INTERNAL
SECRETORY ACTIVITY OF TRANSPLANTED
OVARIES IN THE RAT

RAT No. 11-B-1

BOTH EARS CONTAIN LARGE OVARIAN TRANSPLANTS.

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INTRODUCTION

THE INFLUENCE OF TEMPERATURE ON THE INTERNAL SECRETORY ACTIVITY OF TRANSPLANTED OVARIES IN THE RAT

INTEREST IN THE INTERNAL SECRETORY ACTIVITY OF THE GONADS HAS BEEN ENHANCED RECENTLY BY THE DEVELOPMENT OF METHODS OF ASSAY. PEZARD, IN 1911, FIRST USED THE COCK'S COMB METHOD OF BIOASSAY OF MALE SEX HORMONE. A FEW YEARS LATER, LOEWE AND FRANK MADE THE USE OF CASTRATE MICE FOR THE IDENTIFICATION OF THE FEMALE SEX HORMONE PRACTICAL. SINCE THAT TIME, COLORIMETRIC METHODS FOR THE IDENTIFICATION AND MEASUREMENT OF BOTH THE MALE AND FEMALE SEX HORMONES HAVE BEEN INTRODUCED.

IN THE LAST TWENTY YEARS, OVARIAN TRANSPLANTATION HAS BECOME A VERY POPULAR METHOD FOR STUDYING THE INTERNAL SECRETORY ACTIVITY OF THAT ORGAN. MOST EXPERIMENTERS AGREE THAT INTRA-ABDOMINAL TRANSPLANTATION OF THE OVARY RESULTS IN INTERNAL SECRETORY ACTIVITY, WHICH, AS MANIFESTED BY CHANGES IN OTHER ORGANS (UTERUS, VAGINA, CLITORIS, MAMMAE) IS NOT GREATLY ALTERED.

GARDNER¹ FOUND THAT OVARIES, WHEN TRANSPLANTED INTO THE TESTES OF MICE CONTINUE TO SECRETE OESTROGENIC HORMONES, BUT PRODUCE NO MARKED EFFECT ON THE SEMINAL VESICLES AND PROSTATE. SEVERAL WORKERS, ON THE OTHER HAND, FOUND THAT INJECTIONS OF LARGE DOSES OF OESTROGENIC HORMONE PRODUCED MUSCULAR AND CONNECTIVE TISSUE HYPERTROPHY AS WELL AS INVOLUTION OF THE EPITHELIUM OF THE ACCESSORY TRACT.

INJECTIONS OF ANDROGENIC SUBSTANCES INTO CASTRATE MALE RATS AND MICE CAUSE THE EPITHELIUM OF THE ACCESSORY GLANDS TO RETURN TO NORMAL. RECENTLY, THIS PROCEDURE HAS BEEN ADOPTED AS A MEANS OF IDENTIFYING ANDROGENIC SUBSTANCES. THIS EFFECT CANNOT BE SIMULATED BY THE INJECTION OF ANY QUANTITY OF OESTROGENIC HORMONE.

HILL² BY TRANSPLANTING OVARIES INTO THE EARS OF CASTRATE MALE MICE, AND SUBSEQUENTLY KEEPING THESE MICE IN THE PROPER ENVIRONMENTAL TEMPERATURE, COULD BRING ABOUT A RESTORATION OF THE CASTRATE TYPE OF SEMINAL VESICLES AND PROSTATE GLANDS TO NORMAL. THIS EFFECT WAS OBSERVED IN HIS GROUP OF MICE WHICH WERE KEPT IN A COOL ENVIRONMENT (22 DEGREES C.), BUT NOT IN THE GROUP OF ANIMALS IN A WARM ENVIRONMENT (33 DEGREES C.). THIS, HE TENTATIVELY EXPLAINED, IS DUE TO THE FACT THAT THE TEMPERATURE OF THE EAR OF ANIMALS IN A COOL ENVIRONMENT IS NEARLY THAT OF THE SCROTUM, WHEREAS THE EAR TEMPERATURE OF THE ANIMALS IN A WARM ENVIRONMENT IS MORE NEARLY THAT OF THE ABDOMEN. THIS, HE CONCLUDED, SUGGESTS THAT TEMPERATURE PARTLY CONTROLS THE INTERNAL SECRETORY ACTIVITY OF THE OVARY.

IN A LATER PAPER, THE SAME AUTHOR³ PRESENTED DATA WHICH SUGGESTED THAT THE ANDROGEN PRODUCED BY THE OVARY IS PERHAPS CHEMICALLY DIFFERENT FROM THAT SECRETED BY THE TESTES, BUT THAT THE TWO ARE CLOSELY RELATED IF NOT PHYSIOLOGICALLY IDENTICAL.

DEANSELY⁴ REPEATED THE WORK OF HILL USING RATS INSTEAD OF MICE. FROM HER WORK, SHE CONCLUDED THAT THE "ANDROGENIC ACTIVITY DOES NOT DEPEND ON THE TEMPERATURE AT WHICH THE RAT IS KEPT; IT IS ASSOCIATED WITH THE LUTEINIZATION OF THE THECA INTERNA OF THE FOLLICLES WHICH OCCURS COMMONLY IN ESTABLISHED GRAFTS. THIS THECA LUTEINIZATION SEEMS TO OCCUR INDEPENDENTLY OF THE TEMPERATURE AT WHICH THE ANIMAL IS KEPT. THE FACT THAT HILL WAS ABLE TO CONTROL THE ANDROGENIC SECRETION OF OVARIAN GRAFTS IN MICE BY TEMPERATURE REGULATION SUGGESTS THAT HE THEREBY INFLUENCED THE HISTOLOGICAL DEVELOPMENT OF THE GRAFT."

THE INTRODUCTION OF SYNTHETIC MALE SEX HORMONES INTO THIS

FIELD OF STUDY HAS FURTHER ADDED TO THE COMPLEXITY OF THE SITUATION. NELSON AND MERCKEL⁵ FOUND THAT DAILY INJECTIONS OF ANDOSTERONE INTO SPAYED FEMALE RATS PREVENTED THE USUAL CASTRATION CHANGES.

GREENE, BURRIL, AND IVY⁶ FOUND THAT THE ADMINISTRATION OF A SUFFICIENT QUANTITY OF TESTOSTERONE PROPIONATE TO NEW BORN FEMALE RATS WOULD CAUSE A MASCULINIZATION OF THE EXTERNAL GENITALIA.

PREVIOUSLY, GREENE AND IVY⁷ HAD SHOWN THAT INTERSEXUALITY, AS MANIFESTED BY A RUDIMENTARY VAGINA AND ENLARGED CLITORIS, COULD BE PRODUCED BY INJECTIONS OF TESTOSTERONE INTO PREGNANT FEMALES DURING THE LATTER PART OF PREGNANCY.

SALMON⁸ OBSERVED THE EFFECT OF TESTOSTERONE PROPIONATE ON THE GENITAL TRACT OF IMMATURE FEMALE RATS AND FOUND: (1) THAT SINGLE INJECTIONS CAUSE A PREMATURE OPENING OF THE VAGINA, (2) THAT CONTINUOUS INJECTIONS CAUSE AT FIRST A PROLONGATION OF THE CORNIFIED CELL STAGE OF THE CYCLE (5-8 DAYS) WITH A SUBSEQUENT SUPPRESSION OF THE OESTRUS CYCLE AS LONG AS THE INJECTIONS WERE CONTINUED, (3) THAT THE UTERI OF ANIMALS RECEIVING INJECTIONS SHOW ENLARGEMENT WITH HYPERTROPHY OF MUSCLE AND PROLIFERATION OF MUCOSA AS EARLY AS SEVENTY-TWO HOURS AFTER THE FIRST INJECTION.

LATER, MAZER AND MAZER⁹ BROUGHT OUT THE FACT THAT PROLONGED ADMINISTRATION OF TESTOSTERONE PROPIONATE TO BOTH IMMATURE AND MATURE FEMALE RATS PRODUCES ATROPHY OF THE OVARIES AND UTERI. THEY ALSO POINT OUT THAT THE OVARIAN ATROPHY PRODUCED BY PROLONGED TREATMENT WITH TESTOSTERONE IS IN CONTRAST TO THE OVARIAN STIMULATION FOLLOWING RELATIVELY SHORT TREATMENT,

AND STRESS THE IMPORTANCE OF THE TIME ELEMENT IN THE ADMINISTRATION OF THE SUBSTANCE.

MORE RECENTLY, GREENE, BURRIL, AND IVY¹⁰ HAVE DEMONSTRATED THAT PROGESTERONE IS ANDROGENIC, AND THAT THE USUAL CASTRATION CHANGES IN THE PROSTATE AND SEMINAL VESICLES CAN BE PREVENTED, AND EVEN RESTORED TO NORMAL BY THE ADMINISTRATION OF RELATIVELY LARGE DOSES OF PROGESTIN AND SYNTHETIC PROGESTERONE TO CASTRATE MALE RATS.

FROM THE ABOVE DATA IT IS NOT ENTIRELY CLEAR WHETHER OR NOT THE INTERNAL SECRETORY ACTIVITY OF THE OVARY IS CHANGED BY TRANSPLANTATION AND TEMPERATURE. THE FOLLOWING EXPERIMENTS WERE DEVISED FOR THE PURPOSE OF FURNISHING ADDITIONAL DATA.

MATERIALS

AND

METHODS

MATERIALS AND METHODS

ANIMALS.

WHITE RATS OF ORDINARY LABORATORY STOCK AND OF YOUNG AND SUBADULT AGE WERE DIVIDED INTO FIVE GROUPS AS FOLLOWS:

GROUP I. FEMALES, CASTRATED AND OVARIES TRANSPLANTED SUBCUTANEOUSLY INTO EACH EAR AND KEPT IN THE INCUBATOR AT 33 DEGREES C.

GROUP II. FEMALES, CASTRATED AND OVARIES TRANSPLANTED SUBCUTANEOUSLY INTO EACH EAR AND KEPT AT ROOM TEMPERATURE (22 DEGREES C.)

GROUP III. MALES, CASTRATED AND OVARIES TRANSPLANTED SUBCUTANEOUSLY INTO EACH EAR AND KEPT IN THE INCUBATOR AT 33 DEGREES C.

GROUP IV. MALES, CASTRATED AND OVARIES TRANSPLANTED SUBCUTANEOUSLY INTO EACH EAR AND KEPT AT ROOM TEMPERATURE (22 DEGREES C.)

GROUP V. CONTROLS. NORMAL AND CASTRATE MALES AND FEMALES PLACED IN THE SAME ENVIRONMENT AS THOSE OF THE ABOVE FOUR GROUPS.

SURGERY.

THE OVARIES WERE REMOVED UNDER ETHER ANESTHESIA THROUGH A LOW MIDLINE ABDOMINAL INCISION AND PLACED IMMEDIATELY IN NORMAL SALINE. THE TRANSPLANTATION SITE (EAR) WAS SHAVED AND WASHED WITH 70% ALCOHOL, FOLLOWED BY NORMAL SALINE. A SMALL INCISION 1-2 MM. IN LENGTH WAS THEN MADE THROUGH THE SKIN ON THE DORSAL SIDE OF THE EAR NEAR THE BASE. WITH THE AID OF CURVED FORCEPS, THE SKIN WAS SEPARATED FROM THE SUBCUTANEOUS TISSUES TO FORM A RECEPTIVE POUCH INTO WHICH THE OVARY WAS PLACED AND THE INCISION CLOSED WITH A SINGLE SUTURE.

THE MALES WERE CASTRATED IN THE USUAL WAY.

TEMPERATURE.

HIGH ENVIRONMENTAL TEMPERATURES (33 DEGREES C.) WERE EFFECTED BY PLACING THE ANIMALS IN A THERMOSTATICALLY CONTROLLED INCUBATOR WHICH MAINTAINED A CONSTANT TEMPERATURE OF 33 DEGREES C.

LOW ENVIRONMENTAL TEMPERATURES WERE EFFECTED BY PLACING THE ANIMALS IN A WELL VENTILATED ROOM, IN WHICH THE TEMPERATURE WAS APPROXIMATELY 22 DEGREES C.

TYPES AND METHODS OF OBSERVATION.

DETERMINATION OF URINARY ANDROGENS.

TWENTY-FOUR HOUR URINE SAMPLES WERE COLLECTED AND ANALYZED FOR ANDROGENIC SUBSTANCES BEFORE THE ANIMALS WERE SUBMITTED TO SURGERY. POSTOPERATIVE ANDROGENIC DETERMINATIONS WERE DONE AT VARIOUS INTERVALS.

THE URINE WAS OBTAINED BY PLACING EACH ANIMAL IN AN EIGHT INCH GLASS FUNNEL, TO WHICH WAS ATTACHED A CLEAN, WELL STOPPERED COLLECTING BOTTLE. WIRE GAUZE WAS PLACED IN THE APEX OF THE FUNNEL TO PREVENT FECAL CONTAMINATION.

FOR DETERMINING THE URINARY ANDROGENS, THE COLORIMETRIC TECHNIQUE DEVELOPED BY OESTING¹¹ WAS USED. THIS METHOD IS BASED UPON THE FACT THAT THE RCH_2 GROUP OF THE SEX HORMONES WHEN REACTED WITH VARIOUS SUBSTANCES (E.G. META DINITRO BENZENE) PRODUCES A CHROMAGEN WHICH CAN BE MEASURED COLORIMETRICALLY. IN THE CASE OF THE MALE HORMONES, A VIOLET COLOR IS FORMED.

THIS TECHNIQUE BRIEFLY IS AS FOLLOWS. THE 24 HOUR URINE SAMPLE IS ACIDIFIED WITH CONCENTRATED H_2SO_4 TO A PH BELOW ONE, AND AUTOCLAVED AT 15 LBS. PRESSURE FOR 15 MINUTES.

THE URINE IS THEN EXTRACTED WITH BENZENE AND ETHER. THE ETHER EXTRACT IS SAPONIFIED WITH 10% SODIUM HYDROXIDE TO REMOVE THE FEMALE HORMONES. THE ETHER IS EVAPORATED AND THE HORMONE RESIDUE DISSOLVED IN 60% ALCOHOL. THIS ALCOHOLIC EXTRACT IS REACTED WITH META DINITRO BENZENE AND MEASURED IN A SPECIAL TYPE OF COLORIMETER DESIGNED BY DR. OESTING.

UP TO THE PRESENT TIME, BUT VERY LITTLE DATA HAS APPEARED IN THE LITERATURE CONCERNING THE STANDARDIZATION OF THE COLORIMETRIC TECHNIQUE. THE QUESTION AROSE AS TO WHETHER THESE URINE EXTRACTS CONTAINED ACTIVE HORMONE, OR WHETHER THE COLOR PRODUCED WAS DUE TO SUBSTANCES OTHER THAN THE MALE HORMONES. IT WAS ALSO DESIRED THAT THE EQUIVALENT OF THE COLOR UNIT IN TERMS OF ACTUAL MILLIGRAMS OF PURE MALE HORMONE BE KNOWN. TO ANSWER THESE QUESTIONS, THE FOLLOWING EXPERIMENTS WERE DONE.

WHITE LEGHORN CAPONS WERE INJECTED WITH CONCENTRATED URINE EXTRACTS AND OBSERVED FOR CHANGES IN COMB SIZE AND COLOR. IN ONE CASE, FORTY COLOR UNITS WERE GIVEN OVER A PERIOD OF FIVE DAYS. BY THE SIXTH DAY, THE COMB SHOWED AN INCREASE IN SIZE OF 22 MM., MEASURED IN LENGTH PLUS HEIGHT.

THE STANDARD UNIT OF MALE SEX HORMONE IS THE CAPON UNIT, AND IS DEFINED AS THE AMOUNT OF MATERIAL WHICH WHEN DIVIDED INTO FIVE EQUAL DOSES AND INJECTED DAILY INTO WHITE LEGHORN CAPONS, WILL PRODUCE BY THE SIXTH DAY AN INCREASE IN COMB SIZE OF 3-4 MM., MEASURED IN LENGTH PLUS HEIGHT.

THUS BY DEFINITION, FORTY COLOR UNITS ARE EQUIVALENT TO APPROXIMATELY 5-7 CAPON UNITS, OR ONE CAPON UNIT IS EQUIVALENT TO ABOUT SIX COLOR UNITS. THIS IS CONSISTENT WITH OTHER WORK WHICH HAS BEEN DONE.

IN ORDER TO DETERMINE THE HORMONE EQUIVALENT OF THE COLOR UNIT, PURE CRYSTALLINE TESTOSTERONE, ANDROSTERONE, AND TRANS-DEHYDRO ANDROSTERONE* WERE DISSOLVED IN 60% ALCOHOL AND MEASURED COLORIMETRICALLY. THE FOLLOWING FIGURES WERE OBTAINED:

ONE COLOR UNIT IS EQUIVALENT TO 0.265 MGM. OF TESTOSTERONE.

ONE COLOR UNIT IS EQUIVALENT TO 0.093 MGM. OF ANDROSTERONE.

ONE COLOR UNIT IS EQUIVALENT TO 0.058 MGM. OF TRANS-DEHYDRO-ANDROSTERONE.

TO RULE OUT THE POSSIBILITY OF THE PRESENCE OF FEMALE HORMONES IN THE EXTRACTS, CASTRATE FEMALE RATS WERE INJECTED WITH THESE URINE EXTRACTS AND VAGINAL SMEAR STUDIES CARRIED OUT. ALL OF THESE TESTS WERE NEGATIVE.

THESE DATA INDICATE THAT THERE IS A RATHER CLOSE CORRELATION BETWEEN THE COLORIMETRIC AND OTHER METHODS OF ASSAY FOR THE MALE SEX HORMONES.

VAGINAL SMEAR STUDIES.

THESE WERE DONE ON ALL FEMALES AND RESULTS WILL BE GIVEN IN THE PROPER PLACE.

OTHER ANTE MORTEM OBSERVATIONS.

OTHER ANTE MORTEM OBSERVATIONS SUCH AS BODY WEIGHT, ENLARGEMENT OF THE CLITORIS, ETC., WERE MADE AND DATA CONCERNING THESE WILL BE GIVEN LATER.

POSTMORTEM EXAMINATION.

AFTER THE DESIRED TIME HAD ELAPSED, THE ANIMALS WERE SACRIFICED. THE EARS OF ALL ANIMALS WERE SECTIONED AND THE TRANSPLANTS STUDIED MICROSCOPICALLY. THE UTERI, SEMINAL VESICLES AND PROSTATES WERE EXAMINED BOTH GROSSLY AND MICROSCOPICALLY.

* SUPPLIED BY THE CIBA PHARMACEUTICAL CO.

RESULTS

A.

B.

FIG. 1

- A. PHOTOMICROGRAPH. CROSS SECTION OF UTERINE HORN OF ANIMAL 11-A-11, CASTRATED AND OVARIES TRANSPLANTED INTO THE EARS, AND KEPT IN A HIGH ENVIRONMENTAL TEMPERATURE (33 DEGRESS C.).
- B. SAME OF A SIMILARLY TREATED FEMALE, 11-B-4, KEPT IN A LOW ENVIRONMENTAL TEMPERATURE (22 DEGRESS C.).

CHART No. 1

SERIES 11-A

FEMALES

33 DEGREES C.

RAT. No.	TYPE OF ANIMAL	DAYS OF OBSER- VATION	UTERUS (MICROSCOPY)	TRANSPLANTS		URINARY ANDROGENS	
				FOLLICULAR	LUTEINIZED	Ave. PRE- OPERATIVE	Ave. POST OPERATIVE
11-A-2	C&T	65	NOT ATROPHIC	++	++++	0.4 C.U.	0.0 C.U.
11-A-3	C&T	113	NOT ATROPHIC	++	++++	2.0 C.U.	0.6 C.U.
11-A-4	C&T	103	NOT ATROPHIC	++++	+	0.0 C.U.	1.04 C.U.
11-A-11	C&T	116	NOT ATROPHIC	++	++++	0.6 C.U.	0.87 C.U.
11-A-12	C&T	122	NOT ATROPHIC	++	++	0.8 C.U.	2.16 C.U.
11-A-15	C&T	78	NOT ATROPHIC	+	+++	0.0 C.U.	1.6 C.U.
Ave.						0.63 C.U.	1.02 C.U.
11-A-20	C	82	ATROPHIC			1.6 C.U.	1.0 C.U.
11-A-9	N	64	NORMAL			0.8 C.U.	
11-A-10	N	107	NORMAL			1.2 C.U.	
11-A-22	N	82	NORMAL			2.3 C.U.	

CODE: C INDICATES CASTRATION
 T INDICATES TRANSPLANTATION
 N NORMAL
 + INDICATES TYPE AND DEGREE OF DEVELOPMENT
 C.U. COLOR UNIT

RESULTS

GROUP I. FEMALES

THIS GROUP CONSISTED OF TEN ANIMALS, OF WHICH SIX WERE CASTRATED AND TRANSPLANTED, THREE WERE NORMAL AND ONE WAS CASTRATED. THE LATTER FOUR ANIMALS SERVED AS CONTROLS. THESE ANIMALS WERE PLACED IN A THERMOSTATICALLY CONTROLLED INCUBATOR AT 33 DEGREES C. SEE CHART No.1.

ANDROGENIC FINDINGS:

THE POSTOPERATIVE AVERAGE ANDROGENIC OUTPUT OF THIS GROUP WAS SLIGHTLY INCREASED OVER THE PREOPERATIVE AVERAGE. (CHART No.1)

VAGINAL SMEAR STUDIES:

THESE FAILED TO SHOW ANYTHING IN THE WAY OF A NORMAL OESTRUS CYCLE. THERE WAS, HOWEVER, EVIDENCE OF SLIGHT OESTROGENIC STIMULATION, AS MANIFESTED BY A SUDDEN DECREASE IN LEUCOCYTES AND A SHARP RISE IN CORNIFIED AND NUCLEATED EPITHELIAL CELLS. THIS EFFECT WAS TRANSIENT, HOWEVER, LASTING ONLY A FEW HOURS.

POSTMORTEM EXAMINATION:

AT THE TIME THE ANIMALS WERE KILLED, THE UTERI OF ALL SIX OF THE EXPERIMENTAL ANIMALS WERE FOUND TO BE LARGE, WELL VASCULARIZED AND NORMAL IN APPEARANCE.

MICROSCOPIC EXAMINATION OF THE UTERI REVEALED NO EVIDENCE OF ATROPHY.(FIG.1-A). IN ALL CASES THERE WAS EVIDENCE OF OVARIAN INFLUENCE. THE UTERI OF THREE OF THE SIX EXPERIMENTAL ANIMALS SHOWED EVIDENCE OF BOTH FOLLICULAR AND CORPUS LUTEUM HORMONES, WHEREAS THE REMAINING THREE SHOWED EVIDENCE OF FOLLICULAR HORMONE STIMULATION ONLY.

A.

B.

FIG. 11

A. PHOTOMICROGRAPH. CROSS SECTION THROUGH PORTION OF EAR CONTAINING OVARIAN TRANSPLANT OF ANIMAL 11-A-11, A CASTRATED FEMALE, KEPT IN A HIGH ENVIRONMENTAL TEMPERATURE (33 DEGREES C.).

B. SAME OF A SIMILARLY TREATED ANIMAL, 11-B-4, KEPT IN A LOW ENVIRONMENTAL TEMPERATURE (22 DEGREES C.).

CHART No. 11

SERIES 11-B
FEMALES
22 DEGREES C.

RAT No.	TYPE OF ANIMAL	DAYS OF OBSER- VATION	UTERUS (MICROSCOPY)	TRANSPLANTS		URINARY ANDROGENS	
				FOLLICULAR	LUTEINIZED	Ave. PRE- OPERATIVE	Ave. POST OPERATIVE
11-B-1	C&T	70	ATROPHIC	++	++	1.6 C.U.	0.0 C.U.
11-B-2	C&T	70	ATROPHIC	++		3.2 C.U.	0.9 C.U.
11-B-3	C&T	70	ATROPHIC	++	++	1.2 C.U.	0.9 C.U.
11-B-4	C&T	70	ATROPHIC	++	++	1.2 C.U.	0.8 C.U.
11-B-5	C&T	70	ATROPHIC	+		0.0 C.U.	0.6 C.U.
11-B-6	C&T	66	ATROPHIC	+		0.8 C.U.	0.9 C.U.
11-B-7	C&T	69	NOT ATROPHIC	++	+	1.2 C.U.	1.2 C.U.
11-B-8	C&T	69	ATROPHIC	++	++	0.8 C.U.	0.8 C.U.
11-B-9	C&T	69	ATROPHIC	++	++	1.2 C.U.	1.3 C.U.
Ave.						1.2 C.U.	0.83 C.U.
11-B-12	C	69	ATROPHIC			0.8 C.U.	0.2 C.U.
11-B-13	C	69	ATROPHIC			1.2 C.U.	0.3 C.U.
11-B-14	N	69	NOT ATROPHIC			0.2 C.U.	
11-B-15	N	69	NOT ATROPHIC			0.56 C.U.	

CODE: C INDICATES CASTRATION
T INDICATES TRANSPLANTATION
N NORMAL
+ INDICATES TYPE AND DEGREE OF DEVELOPMENT
C.U. COLOR UNIT

MICROSCOPICALLY, THE OVARIAN TRANSPLANTS OF THIS GROUP WERE WELL DEVELOPED, WELL VASCULARIZED AND SHOWED A PREPONDERANCE OF CORPUS LUTEUM TISSUE. (FIG. 11-A).

GROUP 11. FEMALES.

THIS GROUP CONSISTED OF THIRTEEN ANIMALS, OF WHICH NINE WERE CASTRATED AND TRANSPLANTED, TWO WERE CASTRATE ONLY, AND TWO WERE NORMAL ANIMALS. THE LATTER FOUR ANIMALS SERVED AS CONTROLS. (CHART NO. 11). THIS GROUP WAS KEPT IN A LOW ENVIRONMENTAL TEMPERATURE (22 DEGREES C.).

ANDROGENIC FINDINGS:

AS SHOWN BY CHART NO. 11, THE AVERAGE POSTOPERATIVE LEVEL OF THIS GROUP AS A WHOLE WAS SLIGHTLY LOWER THAN THE AVERAGE PREOPERATIVE LEVEL.

VAGINAL SMEAR STUDIES:

VAGINAL SMEAR STUDIES ON THIS GROUP OF ANIMALS FAILED TO REVEAL ANY EVIDENCE OF OESTRUS EXCEPT IN ONE CASE, ANIMAL 11-B-7, WHICH SHOWED EVIDENCE OF SLIGHT OESTROGENIC STIMULATION.

POSTMORTEM EXAMINATION:

AT THE TIME OF AUTOPSY, THE CLITORA OF ALL ANIMALS OF THIS GROUP WERE ENLARGED. THE UTERI OF EIGHT OF THE EXPERIMENTAL ANIMALS WERE AVASCULAR, THREAD-LIKE AND ATROPHIC. THIS ATROPHY WAS EVEN MORE MARKED MICROSCOPICALLY. (FIG. 1-B).

THE EXCEPTION IN THIS GROUP WAS ANIMAL 11-B-7, WHICH SHOWED A NORMAL UTERUS. THE EXPLANATION FOR THIS IS NOT EVIDENT. IT IS BELIEVED, HOWEVER, THAT THE OVARIES WERE NOT COMPLETELY REMOVED AND THAT SOME OVARIAN TISSUE WAS LEFT IN THE ABDOMINAL CAVITY.

A.

B.

FIG. III

- A. PHOTOMICROGRAPH. PROSTATE OF A NORMAL ANIMAL I-B-14.
- B. PHOTOMICROGRAPH. PROSTATE OF A CASTRATED ANIMAL I-A-7.

CHART No. 111

SERIES I-A

MALES

33 DEGREES C.

RAT. No.	TYPE OF ANIMAL	DAYS OF OBSER- VATION	SEMINAL VESICLE	PROST- TATE	TRANSPLANTS		URINARY ANDROGENS	
					FOLLICULAR	LUTEINIZED	Ave. PRE- OPERATIVE	Ave. POST OPERATIVE
I-A-2	C&T	42	++++*	-	++	++++	1.2 C.U.	1.4 C.U.
I-A-4	C&T	52	++++*	+*		++	1.2 C.U.	0.0 C.U.
I-A-5	C&T	52	++++*	++*	++	+	1.2	1.2
I-A-21	C&T	75	++*	+	+++	+	0.8	1.3
I-A-22	C&T	73	++++*	++++*	TRANSPLANTS NECROTIC	LARGE, BUT NECROTIC	1.2	0.5
I-A-23	C&T	75	+*	+	++	+++	1.6	0.85
I-A-25	C&T	76	++*	++*	++	++	0.8	0.92
Ave.							1.0 C.U.	0.8 C.U.
I-A-7	C	90	++++*	++++*			1.6	0.46
I-A-10	N	40	-	-			1.4 C.U.	
I-A-20	N	74	-	-			0.91 C.U.	

CODE: T INDICATES TRANSPLANTATION

C INDICATES CASTRATION

N NORMAL

+* INDICATES DEGREE OF ATROPHY

+ INDICATES DEGREE AND TYPE OF DEVELOPMENT

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THE OVARIAN TRANSPLANTS OF THIS GROUP WERE NOT AS WELL DEVELOPED AND DID NOT CONTAIN AS MUCH LUTEINIZED TISSUE AS THOSE OF THE ANIMALS WHICH WERE KEPT AT A HIGH TEMPERATURE. (FIG. II-B).
GROUP III. MALES.

THIS GROUP CONSISTED OF TEN ANIMALS OF WHICH SEVEN WERE CASTRATED AND TRANSPLANTED, TWO WERE NORMAL AND ONE WAS CASTRATED. THE LATTER THREE SERVED AS CONTROLS. THESE ANIMALS WERE KEPT IN THE INCUBATOR AT 33 DEGRESS C. (CHART No. III).

ANRODGENIC FINDINGS:

THE AVERAGE POSTOPERATIVE ANDROGENIC OUTPUT OF THIS GROUP AS A WHOLE WAS LOWER THAN THE PREOPERATIVE AVERAGE. (CHART No. III).

POSTMORTEM FINDINGS:

THE PROSTATES AND SEMINAL VESICLES WERE NOT REMARKABLE GROSSLY. MICROSCOPIC EXAMINATION OF THE ACCESSORY TRACT REVEALED ATROPHY, BUT IT WAS MUCH LESS THAN THAT FOUND IN CASTRATE ANIMALS. (FIGS. III, IV). THE ATROPHY WAS MORE SEVERE IN THE SEMINAL VESICLES THAN THE PROSTATES.

THE OVARIAN TRANSPLANTS OF ALL ANIMALS WERE LARGE, WELL DEVELOPED, WELL VASCULARIZED AND CONTAINED MANY LARGE CORPORA LUTEA AND A FEW SMALL FOLLICLES. (FIG. V-A).
GROUP IV. MALES.

THIS GROUP CONSISTED OF FOURTEEN ANIMALS, TEN OF WHICH WERE CASTRATED AND TRANSPLANTED, TWO WERE NORMAL AND TWO WERE CASTRATED. THE LATTER FOUR SERVED AS CONTROLS. (CHART No. IV)
THIS GROUP WAS KEPT IN A LOW ENVIRONMENTAL TEMPERATURE (22 DEGRESS C.).

ANDROGENIC FINDINGS:

THE PROSTATES AND SEMINAL VESICLES WERE NOT

A.

B.

FIG. IV

- A. PHOTOMICROGRAPH. PROSTATE OF ANIMAL I-B-7, A MALE WHICH WAS CASTRATED, OVARIES TRANSPLANTED INTO THE EARS AND KEPT IN A LOW ENVIRONMENTAL TEMPERATURE (22 DEGRESS C.).
- B. PROSTATE OF ANIMAL I-A-5, A MALE WHICH WAS CASTRATED, OVARIES TRANSPLANTED INTO THE EARS AND KEPT IN A HIGH ENVIRONMENTAL TEMPERATURE (33 DEGRESS C.).

CHART No. IV

SERIES I-B MALES 22 DEGREES C.

RAT. No.	TYPE OF ANIMAL	DAYS OF OBSER- VATION	SEMINAL VESICLE	PROST- TATE	TRANSPLANTS		URINARY ANDROGENS	
					FOLLICULAR	LUTEINIZED	Ave. PRE- OPERATIVE	Ave. POST OPERATIVE
I-B-1	C&T	71	+++*	++*	+	+	0.8 C.U.	1.7
I-B-2	C&T	71	±*	±*	++		0.8	1.0
I-B-3	C&T	71	±*	±*	+++	+	1.2	1.04
I-B-4	C&T	72	±*	±*	++	++	1.2	0.6
I-B-5	C&T	52	++++*	++++*	±		2.4	0.6
I-B-6	C&T	72	++*	±*	+	++	1.2	1.12
I-B-7	C&T	71	±*	±*	+++	+	1.2	0.56
I-B-8	C&T	71	+++*	++*		++	0.8	0.8
I-B-9	C&T	71	±*	±*	++		1.2	0.72
I-B-10	C&T	71	+++*	±*	++++	++	1.2	0.40
Ave.							1.2	0.85
I-B-12	C	71	++++*	++++*			3.2	0.24
I-B-13	C	71	++++*	++++*			4.0	0.30
I-B-14	N	71	-	-			1.3 C.U.	
I-B-15	N	71	-	-			1.6 C.U.	

CODES: T INDICATES TRANSPLANTATION
C INDICATES CASTRATION
N NORMAL
+ INDICATES DEGREE OF ATROPHY
+ INDICATES DEGREE AND TYPE OF DEVELOPMENT
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REMARKABLE GROSSLY. MICROSCOPICALLY, THERE WAS SOME ATROPHY IN THE PROSTATES AND SEMINAL VESICLES, BUT IT DID NOT APPROACH CASTRATE PROPORTIONS NOR WAS IT AS SEVERE AS OBSERVED IN THE GROUP OF MALES WHICH WERE KEPT IN A HIGH ENVIRONMENTAL TEMPERATURE.(FIG.IV). HERE AGAIN, THE SEMINAL VESICLES WERE MORE ATROPHIC THAN THE PROSTATES.

EXAMINATION OF THE TRANSPLANTS OF THIS GROUP SHOWS SOME DEVELOPMENT AND VIABILITY. THE GRAFTS WERE PREDOMINANTLY FOLLICULAR IN TYPE AND AS A WHOLE DID NOT SHOW THE DEVELOPMENT FOUND IN THOSE OF THE ANIMALS WHICH WERE KEPT AT A HIGH TEMPERATURE.(FIG.V).

A.

B.

FIG. V

- A. PHOTOMICROGRAPH. CROSS SECTION THROUGH PORTION OF EAR CONTAINING OVARIAN TRANSPLANT OF ANIMAL 1-A-5, A MALE WHICH WAS CASTRATED, OVARIES TRANSPLANTED INTO THE EARS AND KEPT IN A HIGH ENVIRONMENTAL TEMPERATURE (33 DEGRESS C.).
- B. SAME OF A SIMILARLY TREATED MALE, 1-B-7, KEPT IN A LOW ENVIRONMENTAL TEMPERATURE (22 DEGRESS C.).

DISCUSSION

DISCUSSION

FROM THE DATA CONCERNING THE TWO GROUPS OF FEMALES, IT AT ONCE BECOMES OBVIOUS THAT TO SOME EXTENT, AT LEAST, THE OESTROGENIC ACTIVITY OF THE OVARY CAN BE CONTROLLED BY TRANSPLANTATION AND TEMPERATURE REGULATION. IT SEEMS THAT OVARIES IN THE EARS OF ANIMALS WHICH ARE KEPT AT A HIGH TEMPERATURE HAVE THE POWER OF PREVENTING THE USUAL CASTRATION CHANGES IN THE UTERUS, WHEREAS THOSE IN THE EARS OF ANIMALS WHICH ARE KEPT AT A LOW TEMPERATURE DO NOT HAVE THIS POWER.

THERE ARE TWO FACTORS WHICH MUST BE CONSIDERED IF AN ATTEMPT IS MADE TO EXPLAIN THIS PHENOMENON. THE FIRST OF THESE FACTORS IS TEMPERATURE, WHICH, IT SEEMS MAY INFLUENCE OVARIAN FUNCTION IN TWO WAYS: (1) BY DIRECTLY INHIBITING THE INTERNAL SECRETORY ACTIVITY, (2) BY INTERFERING WITH THE DEVELOPMENT OF THE OVARY AND THUS INDIRECTLY INFLUENCING THE INTERNAL SECRETORY ACTIVITY. SECTIONS OF THE TRANSPLANTS OF THE LOW TEMPERATURE ANIMALS SHOW THAT THE TRANSPLANTS DO NOT TAKE NOR DEVELOP AS WELL AS THOSE OF ANIMALS WHICH ARE KEPT IN A HIGH ENVIRONMENTAL TEMPERATURES. (FIG. 11). THEREFORE, THE CASTRATION CHANGES WHICH DEVELOP MAY NOT BE DUE TO A COMPLETE ABSENCE OF OVARIAN HORMONE, BUT RATHER TO A SUB-THRESHOLD AMOUNT OF THE HORMONE.

THE SECOND FACTOR TO BE CONSIDERED INVOLVES THE POSSIBILITY OF THE PRODUCTION OF AN ANDROGEN BY TRANSPLANTED OVARIES WHICH ARE KEPT COOL BY A LOW ENVIRONMENTAL TEMPERATURE.

THE LITERATURE GIVEN IN THE FIRST PART OF THIS PAPER SHOWS THAT ANDROGENIC SUBSTANCES SUCH AS TESTOSTERONE PROPIONATE, WHEN INJECTED INTO INTACT FEMALE RATS OVER A LONG PERIOD OF TIME WILL PRODUCE ATROPHY OF THE GENITAL TRACT, WITH A SUPPRESSION OF THE OESTRUS CYCLE AS LONG AS THE INJECTIONS ARE CONTINUED. SINCE THE CHANGES PRODUCED IN THE UTERUS BY THIS

METHOD CANNOT BE DISTINGUISHED FROM THE USUAL CASTRATION CHANGES, IT IS IMPOSSIBLE TO DETERMINE WHETHER THE ATROPHY OBSERVED IN OUR SERIES IS DUE TO THE ACTION OF ANDROGENS OR TO THE LACK OF OVARIAN FUNCTION.

IT IS IMPORTANT TO NOTE, HOWEVER, THAT THE CLITORA OF ALL OF THE LOW TEMPERATURE FEMALES WERE ENLARGED. THIS HAS BEEN A CONSTANT FINDING BY WORKERS WHO HAVE INJECTED MALE HORMONES OVER A LONG PERIOD OF TIME.

THE URINARY ANDROGENIC FINDINGS IN THESE ANIMALS ARE DIFFICULT TO EVALUATE. THE SLIGHT INCREASE IN THE AVERAGE POST-OPERATIVE LEVELS OF THE FEMALES KEPT IN A HIGH ENVIRONMENTAL TEMPERATURE CAN BE EXPLAINED IN PART BY THE INCREASE IN METABOLIC RATE WHICH EVIDENTLY OCCURS IN THESE ANIMALS.

THE FACT THAT THE URINARY ANDROGENS OF THE FEMALES KEPT AT A LOW TEMPERATURE WERE DECREASED POSTOPERATIVELY MAY BE DUE IN PART TO THE RATHER POOR DEVELOPMENT OF THEIR TRANSPLANTS. THIS DECREASE IS NOT AS MARKED, HOWEVER, AS THAT OBSERVED IN CASTRATE ANIMALS. THIS POINTS TOWARD THE POSSIBILITY THAT THESE TRANSPLANTED OVARIES ARE PRODUCING ANDROGENIC SUBSTANCES.

IT SEEMS AT PRESENT THAT PERHAPS THE ATROPHY OF THE UTERI OF THE FEMALES WHICH WERE KEPT AT A LOW TEMPERATURE IS DUE TO BOTH FACTORS DISCUSSED ABOVE, AND THAT PERHAPS THE EFFECT OF A DECREASED OUTPUT OF OESTROGENIC HORMONE BY THE OVARIES IS POTENTIATED BY THE ACTION OF AN ANDROGENIC SUBSTANCE COMING FROM THE SAME ORGAN.

OBSERVATIONS ON THE GROUPS OF MALE ANIMALS INDICATE THAT TO SOME EXTENT, THE USUAL CASTRATION CHANGES CAN BE PREVENTED

BY TRANSPLANTING OVARIES INTO THE EARS OF CASTRATE MALES. THE EXPLANATION FOR THIS IS VAGUE. THE WORK OF GREENE, BURRIL, AND IVY¹⁰ ON THE ANDROGENIC ACTIVITY OF PROGESTERONE MAY OFFER A POSSIBLE EXPLANATION, NAMELY, THAT THESE TRANSPLANTED OVARIES PRODUCE ENOUGH PROGESTERONE TO PREVENT THE CASTRATION CHANGES. THERE IS MUCH EVIDENCE AGAINST THIS. FIRST, THOSE MALES WHICH WERE KEPT IN THE INCUBATOR AT A HIGH TEMPERATURE SHOW MORE ATROPHY IN THE PROSTATES AND SEMINAL VESICLES THAN DO ANIMALS WHICH ARE MAINTAINED AT ROOM TEMPERATURE. (FIG. IV). THE TRANSPLANTS OF ANIMALS KEPT AT A HIGH TEMPERATURE SHOW BETTER DEVELOPMENT AND MORE LUTEINIZATION THAN DO THOSE OF ANIMALS WHICH ARE KEPT AT A LOW TEMPERATURE. IF THE PREVENTION OF THE CASTRATION CHANGES IS DUE TO THE ACTIVITY OF PROGESTERONE, ONE WOULD EXPECT LESS ATROPHY IN THE PROSTATES AND SEMINAL VESICLES OF THE ANIMALS KEPT IN THE INCUBATOR, SINCE THEIR TRANSPLANTS ARE LARGER AND SHOW MORE LUTEINIZATION THAN THOSE OF ANIMALS WHICH ARE KEPT AT A LOW TEMPERATURE.

OTHER EVIDENCE AGAINST THIS IDEA IS THE FACT THAT THE FEMALES WHICH WERE TRANSPLANTED AND KEPT AT A LOW TEMPERATURE SHOWED UTERI WHICH WERE ATROPHIC. (FIG. I-B). FROM THIS IT IS POSSIBLE TO AT LEAST PARTLY RULE OUT THE ACTION OF ESTRIN OR PORGESTIN IN THE ACTIVATION OF THE ACCESSORY TRACT.

ANOTHER EXPLANATION IS THAT THE CASTRATION CHANGES HAVE NOT HAD TIME TO DEVELOP. THIS POSSIBILITY CAN BE RULED OUT BY EXAMINING A CASTRATE CONTROL ANIMAL, WHICH IN THE SAME LENGTH OF TIME HAD DEVELOPED FULL BLOWN CASTRATION CHANGES. (FIG. III).

A FINAL EXPLANATION LIES IN THE POSSIBILITY THAT OVARIES, WHEN TRANSPLANTED TO THE EARS AND KEPT COOL BY A LOW ENVIRON-

MENTAL TEMPERATURE MAY PRODUCE ANDROGENIC SUBSTANCES. THE URINARY ANDROGENIC FINDINGS ON THESE ANIMALS OFFER SOME EVIDENCE FOR THIS. IT IS TRUE THAT THE AVERAGE POSTOPERATIVE LEVEL OF BOTH GROUPS OF MALES WAS LOWER THAN THE PREOPERATIVE AVERAGE. THIS IS TO BE EXPECTED, FOR EVEN IF THESE OVARIES ARE PRODUCING MALE HORMONES, THEY WOULD NOT BE EXPECTED TO PRODUCE AS MUCH HORMONE AS DO THE TESTES. IT IS ALSO TRUE THAT THE POSTOPERATIVE LEVELS IN THESE GROUPS WERE NOT AS LOW AS THOSE OF THE CASTRATE CONTROLS. THIS SEEMS TO INDICATE THAT THESE TRANSPLANTED OVARIES ARE PRODUCING A MALE HORMONE, OR SOME SUBSTANCE WHICH IS PHYSIOLOGICALLY AND CHEMICALLY SIMILAR TO THE MALE HORMONES.

THE ANDROGENIC FINDINGS ARE SOMEWHAT HARD TO EVALUATE. DUE TO THE SMALL AMOUNT OF URINE WHICH IS AVAILABLE AND TO VARIOUS FLAWS IN THE COLORIMETRIC TECHNIQUE WHICH MIGHT LEAD TO ERRORS, THEY WOULD BE OF LITTLE VALUE WITHIN THEMSELVES. HOWEVER, SINCE THEY CAN BE CORRELATED WITH THE HISTOLOGICAL CHANGES, IT IS BELIEVED THAT THEY ARE OF SOME SIGNIFICANCE. THE POSITIVE CORRELATION BETWEEN THE ANDROGENIC AND HISTOLOGICAL FINDINGS MERELY ADDS TO THE CONCLUSIVENESS OF THE LATTER.

CONCLUSIONS

CONCLUSIONS

1. THE OESTROGENIC ACTIVITY OF THE OVARIES IS INHIBITED IF THEY ARE TRANSPLANTED TO THE EARS, AND KEPT COOL BY A LOW ENVIRONMENTAL TEMPERATURE.
2. THE OESTROGENIC ACTIVITY OF OVARIES TRANSPLANTED TO THE EARS IS NOT COMPLETELY INHIBITED, BUT ALTERED QUANTITATIVELY (AS MANIFESTED BY VAGINAL SMEAR STUDIES) IF THE ANIMALS ARE KEPT IN A HIGH ENVIRONMENTAL TEMPERATURE.
3. THE CASTRATION CHANGES CAN BE PREVENTED IN MALE RATS BY TRANSPLANTING OVARIES INTO THE EARS. THIS EFFECT IS MORE MARKED IN THE ANIMALS WHICH ARE KEPT IN A LOW ENVIRONMENTAL TEMPERATURE (22 DEGREES C.) THAN IN THOSE KEPT IN A HIGH ENVIRONMENTAL TEMPERATURE (33 DEGREES C.).
4. TRANSPLANTED OVARIES KEPT WARM BY A HIGH ENVIRONMENTAL TEMPERATURE DEVELOP BETTER AND SHOW MORE LUTEINIZATION THAN DO THOSE WHICH ARE KEPT COOL BY A LOW ENVIRONMENTAL TEMPERATURE.

SUMMARY

SUMMARY

MALE AND FEMALE RATS WERE CASTRATED, OVARIES TRANSPLANTED SUBCUTANEOUSLY INTO EACH EAR AND DIVIDED INTO TWO GROUPS, ONE CONSISTING OF MALES AND FEMALES KEPT AT A CONSTANT TEMPERATURE OF 33 DEGREES C. IN AN INCUBATOR, THE OTHER CONSISTING OF MALES AND FEMALES KEPT AT ROOM TEMPERATURE (22 DEGREES C.).

THESE ANIMALS WERE OBSERVED FOR PERIODS OF 52-122 DAYS, DURING WHICH TIME THE URINE OF BOTH MALES AND FEMALES WAS ANALYZED COLORIMETRICALLY FOR ANDROGENIC SUBSTANCES, AND THE VAGINAL EPITHELIUM STUDIED BY MEANS OF SMEARS.

AT THE TIME THE ANIMALS WERE KILLED, THE OVARIAN TRANSPLANTS, UTERI, SEMINAL VESICLES AND PROSTATES WERE STUDIED MICROSCOPICALLY.

THE DATA OBTAINED FROM THESE EXPERIMENTS SEEM TO INDICATE THAT THE INTERNAL SECRETORY ACTIVITY OF THE OVARY CAN BE INFLUENCED BY TRANSPLANTATION AND TEMPERATURE. THE UTERI OF THE ANIMALS KEPT AT ROOM TEMPERATURE WERE ATROPHIC INDICATING A LACK OF OVARIAN FUNCTION, WHEREAS THE PROSTATES AND SEMINAL VESICLES OF THE MALES KEPT AT ROOM TEMPERATURE SHOWED EVIDENCE OF ANDROGENIC STIMULATION.

THE ANIMALS KEPT AT A HIGH TEMPERATURE SHOWED UTERI WHICH WERE NOT ATROPHIC, INDICATING OVARIAN FUNCTION. THE SEMINAL VESICLES AND PROSTATES WERE SOMEWHAT ATROPHIC, BUT SHOWED DEFINITE EVIDENCE OF ANDROGENIC STIMULATION.

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