AMERICAN SOCIETY
OF
ANDROLOGY

PROGRAM

FIRST ANNUAL MEETING

MARCH 31 – APRIL 2,

1976

WORCESTER

Massachusetts

UNIVERSITY OF MASSACHUSETTS

MEDICAL SCHOOL

American Society of Andrology

PROGRAM COMMITTEE

1975 - 1976

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	Leo E. Reichert, Jr., Ph.D.
	Richard J. Sherins, M.D.
	Anna Steinberger, Ph.D.
	Philip Troen, M.D.

SCIENTIFIC PROGRAM

Wednesday, March 31
Morning

SESSION I

Amphitheater 1

8:30 a.m. **OPENING REMARKS**

Eugenia Rosemberg, M.D.
Program Chairman

Roger J. Bulger, M.D.
Dean
University of Massachusetts Medical School

Edward Budnitz, M.D.
President, Serono Research Foundation, USA, Inc.

Rune Eliasson, M.D.
President, CIDA

SESSION II: SYMPOSIUM PAPER

Amphitheater 1 - 8:50 a.m.

Hormonal and Genetic Factors Affecting the Development of the Male Genital System

Alfred Jost, M.D.
Professeur au Collège de France et de
l'Université P. et M. Curie, Paris, France

9:50 a.m. Coffee Break

SESSION SCHEDULE

UNIVERSITY OF MASSACHUSETTS
SCHOOL OF MEDICINE

AMPHITHEATER I.		
DAY	MORNING	AFTERNOON
March 30		Registration 2:00 – 7:00 1. Salisbury Room Sheraton – Lincoln Inn 2. Main Lobby Medical School
March 31	Registration 8:00 – 8:30 Medical School Lobby 8:30 – Opening Remarks 8:50 – Symposium Paper 9:50 – Coffee Break 10:05 – Session III. Short Communications	2:00 – State of the Art Lecture 3:00 – Coffee Break 3:15 – Session V Short Communications 7:00 – Reception
April 1	Registration 8:00 – 8:30 Medical School Lobby 8:30 – Symposium Paper 9:30 – Coffee Break 9:45 – Session VII. Short Communications	2:00 – Session VIII. Short Communications 4:00 – Coffee Break 4:15 – Business Meeting 7:30 – Annual Banquet Presidential Address
April 2	8:30 – Clinical Session 10:30 – Coffee Break 10:45 – Panel Discussion 12:00 – Closing Remarks	

American Society of Andrology

PROCEEDINGS

FIRST ANNUAL MEETING

SPONSORS

THE MEDICAL SCHOOL, UNIVERSITY of MASSACHUSETTS; THE MEDICAL RESEARCH INSTITUTE of WORCESTER INC; and THE SERONO RESEARCH FOUNDATION, U.S.A. INC.

EDITOR

Eugenia Rosemberg, M.D.
PROGRAM CHAIRMAN
American Society of Andrology

American Society of Andrology

March 1976

The American Society of Andrology was founded in Detroit, Michigan, on April 25, 1975, in response to a growing need for closer interaction among American scientists and clinicians specializing in the study of the male reproductive tract.

At this, the first Annual Meeting of the Society, we are bringing together the basic scientific and clinical disciplines which comprise the study of andrology. The program promises to be outstanding with presentations by three guest lecturers, a well-rounded selection of short communications to be given by members and guests of the Society, as well as a didactic clinical session. A panel discussion is also scheduled.

We are indebted to those dedicated scientists and individuals who have served on the Local Committee on Arrangements and on the Ladies' Committee. Their combined efforts are responsible for the efficient execution of our meeting. They are named in the following pages. We wish to also thank the members of the Society who will chair the various sessions at this meeting and ensure its success.

This meeting could not have taken place without the support of the University of Massachusetts Medical School which will host the scientific sessions, nor without the cooperation of the staff of the Medical Research Institute of Worcester, Inc., which was operative in the preparation and execution of this meeting. We are particularly grateful to the Serono Research Foundation, U.S.A., Inc., an educational and scientific non-profit institution established under the laws of the Commonwealth of Massachusetts, for the generous financial support of this, the first Annual Meeting of our Society.

Eugenia Rosemberg, M.D.

Program Chairman

American Society of Andrology

1975-1976

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Vice-President	S. Jan Behrman, M.D.
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NOMINATING	Donald W. Fawcett, M.D.
PUBLICATION	Eugenia Rosemberg, M.D.
FINANCE	S. Jan Behrman, M.D.

INVITATION TO MEMBERSHIP

The Society invites clinicians and scientists interested in research, diagnosis, and treatment of disorders of the male reproductive and associated systems to participate in its activities. Applications for Membership may be obtained at this Meeting at the Registration Desk, or by writing to E.S.E. Hafez, Ph.D., Secretary of the American Society of Andrology, C. S. Mott Center, 275 East Hancock Avenue, Detroit, Michigan 48201.

GENERAL INFORMATION

Headquarters for the 1976 Meeting of the American Society of Andrology will be at the University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, where all meetings will be held.

On March 30, 1976, registration for the Annual Meeting for participants who have not registered in advance will be from 2:00 to 7:00 p.m. at the University of Massachusetts Medical School. Participants who have registered in advance will obtain their badges and Program Books at the Sheraton-Lincoln Inn, Salisbury Room, 500 Lincoln Street, Worcester, from 2:00 to 7:00 p.m.

After March 30, participants will be able to register at the University of Massachusetts Medical School. Tickets for the Annual Banquet and Applications for Membership will be available at the Registration Desk.

CONDUCT OF SCIENTIFIC PROGRAM

The Scientific Program of the 1976 Meeting will open at 8:30 a.m. on March 31. There will be a Symposium, a State of the Art Lecture, and two Short Communication Sessions. On April 1, there will be one Symposium Lecture and two Short Communication Sessions. On April 2, there will be a Clinical Session and a Panel Discussion.

ANNUAL BUSINESS MEETING

The Annual Business Meeting of the American Society of Andrology will be convened immediately following the Short Communication Session on Thursday, April 1, in the same amphitheater. Committee reports, unfinished and new business will be presented. Attendance is limited to members of the Society; they are urged to attend.

COCKTAIL RECEPTION

All registrants, guests, and their spouses are welcomed to the Cocktail Reception (open cash bar) from 7:00 to 8:30 p.m., Wednesday, March 31, at the Sheraton-Lincoln Inn. This event affords an opportunity for speakers and registrants to mingle and get acquainted.

ANNUAL BANQUET

The Annual Banquet will take place on Thursday, April 1, at 7:30 p.m. at the Sheraton-Lincoln Inn. The Presidential Address will be given at the Annual Banquet. Tickets may be purchased at the Registration Desk.

GUEST SPEAKERS

Alfred Jost, M.D. - Professeur au Collège de France et de l'Université P. et M. Curie, Paris, France

Jean D. Wilson, M.D. - Professor of Internal Medicine, University of Texas Health Science Center at Dallas, Dallas, Texas

Mortimer B. Lipsett, M.D. - Director of the Cancer Center for Northeast Ohio and Professor, Case Western Reserve University, Cleveland, Ohio

C. Alvin Paulsen, M.D. - Professor of Medicine, University of Washington, Seattle, Washington

S. Jan Behrman, M.D., M.S., F.R.C.O.G. - Professor of Obstetrics and Gynecology and Director, Center Research Reproductive Biology, University of Michigan, Ann Arbor, Michigan

Emil Steinberger, M.D. - Professor and Chairman, Department of Reproductive Medicine and Biology, University of Texas Medical School at Houston, Houston, Texas

LOCAL COMMITTEE ON ARRANGEMENTS

Chairpersons: Dr. H. Maurice Goodman
Dr. Andrzej Bartke

Members:

Dr. Lewis E. Braverman
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Dr. Joel Feinblatt
Dr. Mary Harris
Dr. T. W. Honeyman
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Dr. M. S. Smith
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Mrs. Ruth Stern
Mrs. Simone Weinert
Mrs. Betty Wheeler

SESSION SCHEDULE

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l'Université P. et M. Curie, Paris, France

9:50 a.m. Coffee Break

Wednesday Morning

SESSION III: METABOLIC PATHWAYS - RECEPTORS - ANTIFERTILITY AGENTS

Amphitheater 1

Chairpersons: Nancy J. Alexander, Ph.D.
C. Alvin Paulsen, M.D.

10:05 1. Steroid Metabolism in Isolated Epithelium of Guinea Pig Seminal Vesicle. Randolph C. Steer* and Carlo M. Veneziale, Mayo Medical School, Rochester, MN

10:20 2. Steroid Metabolites of the Marmoset Testis. James P. Preslock*, Univ. of Texas Med. Sch. at Houston, Houston, TX

10:35 3. Synthesis and Metabolism of Prostaglandin F_{2alpha} By The Human Prostate. Alice H. Cavanaugh*, SUNY at Buffalo and VA Hospital, Buffalo, NY

10:50 4. The Effects of Sympathectomy and Testosterone Propionate On Prostatic Cytosol Receptors. M. James Cosentino* and P. Jones-Witters, Ohio University, Athens, OH

11:05 5. Receptor Sites on Human Prostate Tissue for Prostaglandin F_{2alpha}. Wells E. Farnsworth* and Alice H. Cavanaugh, SUNY at Buffalo and VA Hospital, Buffalo, NY

11:20 6. Properties of Specific Androgen Receptors in the Hypothalamus and Pituitary Gland of Adult Male Rats. W. W. Leavitt* and L. J. Sholiton, Univ. of Cincinnati Coll. of Med., Cincinnati, OH

11:35 7. The Antispermatogenic Effects of o-Nitro-benzenesulfonamide (ORF 11, 133) in Male Rats. Larry A. Kraft* and Allen F. Hirsch, Ortho Pharmaceutical Corp., Raritan, NJ

11:50 8. Partial Characterization of the Antispermatogenic Effects of 5-Aminoindazole. Thomas J. Lobl* and S. E. Porteus, The Upjohn Co., Kalamazoo, MI

* Presenting Author

12:05 9. Studies on the Antitesticular Action of Dl-6-(N-2-Pipecolinomethyl)-5-Hydroxy-Indane In the Rat. Victor S. Fang* and Winston A. Anderson, Univ. of Chicago, Chicago, IL

12:20 10. Mechanism of Suppression of Rat Ventral Prostate Weights By Methandrostenolone. R. E. Steele*, F. Didato, and B. G. Steinetz, CIBA-GEIGY Corp., Ardsley, NY

EXHIBIT 10a. The Molecular Conformation of Androgens and Anti-Androgens. Jane F. Griffin and William L. Duax, Med. Fdn. of Buffalo, Buffalo, NY

Wednesday Afternoon

SESSION IV: STATE OF THE ART LECTURE

Amphitheater 1 - 2:00 p.m.

Genetic Disorders and Sexual Development

Jean D. Wilson, M.D.
Professor of Internal Medicine
University of Texas Health Science
Center at Dallas, Dallas, Texas

3:00 Coffee Break

SESSION V: PHYSIO-ANATOMIC ANDROLOGY - SPERM

Wed. PM - Amphitheater 1

Chairpersons: Donald Fawcett, M.D.
Stuart S. Howards, M.D.

3:15 11. Prepubertal Histochemical and Ultrastructural Changes in the Epididymis of the Rat. Frank E. Snydle*, C. S. Mott Center, Detroit, MI

3:30 12. Ultrastructural Changes in Human Fetal Leydig Cells at Mid-Gestation. Bernard Gondos* and Mitchell S. Golbus, Univ. of California, San Francisco, CA

3:45 13. Inhibition of the Motility and Metabolism of Human Spermatozoa by Cytochalasin B. Rudolph N. Peterson*, JoAnn Gram, and Matthew Freund, New York Med. Coll., Valhalla, NY

4:00 14. Micropuncture Studies of the Effect of Caffeine and Cyclic Nucleotides on the Motility of Rat Epididymal Sperm. Stuart S. Howards* and Robert Wyker, Univ. of Va. Sch. of Med., Charlottesville, VA

4:15 15. Effect of Glycerol and Orvus ES Paste on Sperm Cell Acrosin During Freezing and Storage at -196°C. Lawrence A. Johnson* and V. G. Pursel, Animal Physiol. and Genetics Inst., A.R.S., U.S.D.A., Beltsville, MD

4:30 16. Kinetic Studies on the Interaction and Specificity of Synthetic Proteinase Inhibitors Towards Human Acrosin. A. K. Bhattacharyya* and L. J. D. Zaneveld, Univ. of Ill. at the Med. Ctr., Chicago, IL

4:45 17. Importance of Seminal Plasma Components on the Structural Stability of Human Spermatozoa. Rune Eliasson* and U. Kvist, Karolinska Institutet, Stockholm, Sweden

RECEPTION

7:00 Sheraton-Lincoln Inn

Thursday, April 1
Morning

SESSION VI: SYMPOSIUM PAPER

Amphitheater 1 - 8:30 a.m.

Physiological Regulation of Male Reproductive Function

Mortimer B. Lipsett, M.D.
Director of the Cancer Center for Northeast Ohio
and Professor, Case Western Reserve University,
Cleveland, Ohio

9:30 Coffee Break

SESSION VII: CLINICAL ANDROLOGY

Thur. AM - Amphitheater 1

Chairpersons: Richard J. Sherins, M.D.
Joseph N. Corriere, M.D.

10:00 ~~9:45~~ 18. Endocrine Evaluation of Infertile Men. Gerald S. Bernstein*, Oscar A. Kletzky, Alfredo Ortiz, and Uwe T. Goebelsmann, Univ. So. Calif. Sch. of Med., Los Angeles, CA

10:00 19. Study of FSH, LH, and Prolactin Before and After LH-RH and TRH in Infertile Men. R. Roulier*, A. Mattei, and P. Franchimont, Ctr. des Problèmes de la Réprod. Humaine, Marseilles, France, and Radioimmunoassay Lab., Liège, Belgium

10:15 20. The GnRH Stimulation Test in the Evaluation of Unilateral Cryptorchidism: A Twenty-Year Retrospective Study. Larry I. Lipshultz* and Peter J. Snyder, Hosp. of the Univ. of Pennsylvania, Philadelphia, PA

Thursday Morning

10:30 21. Gonadal Function in Patients with Chronic Renal Failure Maintained with Haemodialysis. Relationship Between Length of Treatment and Patient's Age. G. Bucciante*, A. DaTos, R. DeToni, V. Frizzi, A. Bernardi, and G. Toscano, Univ. of Padua, Hosp. of Pistoia, Padua, Italy

10:45 22. The Seminiferous Tubule Wall in Human Hypogonadism. V. Santiemma*, S. Francavilla, F. Francavilla, G. Poccia, G. DiCarlo, A. Celli, C. DeMartino, and A. Fabbrini, Istituto Univ. Med. e Chirurgia, L'Aquila, Italy.

11:00 23. Semen Analysis: Apparent Biological Breaks in Semen Quality When Related to Sperm Concentration. K. A. Dougherty*, A. T. K. Cockett, and R. L. Urry, Univ. of Rochester, Sch. of Med. and Dent., Rochester, NY

11:15 24. Treating the "Subfertile" Male: Improvement in Semen Characteristics After Low Dose Androgen Therapy. R. L. Urry* and A. T. K. Cockett, Univ. of Rochester, Sch. of Med. and Dent., Rochester, NY

11:30 25. Clomiphene Test and Clomiphene Therapy in the Hypofertile Male. J. C. Emperaire*, A. Ruffie, P. Roger, and J. Riviere, USN Haut-Leveque, Bordeaux-Pessac, France

11:45 26. Effect of Long-Term Estrogen Therapy on the Human Testes. L. Rodriguez*, R. K. Tcholakian, K. D. Smith, and E. Steinberger, Univ. of Texas Med. Sch. at Houston, Houston, TX

12:00 27. Seasonal Changes in Body Weight, Testicular Volume, and Semen Parameters of Rhesus Monkeys Following Sham, Unilateral and Bilateral Vasectomy. Richard M. Harrison*, G. Domingue, P. Heidger, J. Roberts, and J. U. Schlegel, Tulane Univ. Med. Sch., Covington, LA

12:15 28. The Absence of Sperm-Agglutinating Activity in Human Seminal Fluid After Vasectomy. Aarne I. Koskimies*, Univ. of Helsinki, Helsinki, Finland

Farnsworth Prostatic Growth Cockett.
in Biochemical
Eliasson 2 Slides

SESSION VIII: HYPOTHALAMIC - PITUITARY - GONADAL AXIS

Thur. PM - Amphitheater 1

Chairpersons: Philip Troen, M.D.
Fletcher C. Derrick, M.D.

2:00 29. A New Procedure For the Clinical Determination of Urinary LH: Application to Testicular Disorders. Alexander Albert*, A. Nureddin, and R. E. Emslander, Mayo Clinic and Mayo Med. Sch., Rochester, MN

2:15 30. Existence of a Follicle-Stimulating Hormone-Inhibiting Factor in Ram Rete Testis Fluid. P. Franchimont*, S. Chari, M. T. Hagelstein, M. L. Debruche, S. Duraiswami, J. Walton, and G. M. H. Waites, Radioimmunoassay Lab., Liège, Belgium, and Univ. of Reading, Reading, England

2:30 31. Recovery of Pituitary-Testicular Axis After Acute or Chronic Suppression With Estradiol. R. K. Tcholakian*, M. Chowdhury, and E. Steinberger, Univ. of Texas Med. Sch. at Houston, Houston, TX

2:45 32. Mechanism of Luteinizing Hormone (LH) and Testosterone (T) Suppression By Fluoxymesterone (Halotestin). R.A. Vigersky* and D. L. Loriaux, N.I.H., NICHHD, Bethesda, MD

3:00 33. Effect of Human Chorionic Gonadotropin (HCG) on Interstitial Cells and Androgen Production in the Immature Rat Testis. H. E. Chemes*, M. A. Rivarola, and C. Bergada, Buenos Aires Childrens Hospital, Buenos Aires, Argentina

3:15 34. Advanced Puberty in Males. FSH and LH Studies. Salvatore Raiti*, Noel K. Maclaren, and F. Akesode, Univ. of Md. Sch. of Med., Baltimore, MD

3:30 35. Hypothalamic, Pituitary, and Gonadal Hormones in Sexual Maturation of the Male Rat. Anita H. Payne*, R. P. Kelch, E. P. Murono, and J. T. Kerlan, Univ. of Mich., Ann Arbor, MI

3:45 36. Differences in the Testosterone-Aggression Relationship Between Men and Women. Harold Persky*, C. P. O'Brien, K.D. Smith, G. K. Basu, and M. A. Khan, Univ. of Penn. and Philadelphia Gen. Hosp., Philadelphia, PA

4:00 Coffee Break

BUSINESS MEETING 4:15 - 5:30 p.m.

Amphitheater 1

7:30 **ANNUAL BANQUET** Sheraton-Lincoln Inn

Presidential Address:

Emil Steinberger, M.D.
President

Friday, April 2
Morning

SESSION IX: CLINICAL SESSION

Fri. AM - Amphitheater 1

8:30 C. Alvin Paulsen
Semen Analysis: Importance in the Evaluation of Male Infertility

9:00 Discussion

9:10 S. Jan Behrman
Immunological Role of the Female Reproductive Tract

9:40 Discussion

9:50 E. Steinberger
Medical Treatment of Male Infertility

10:20 Discussion

Friday Morning

10:30 Coffee Break

SESSION X: PANEL DISCUSSION

Fri. AM - Amphitheater 1

Chairperson: Eugenia Rosemberg, M.D.

10:45 Panelists:

A. Albert	C. A. Paulsen
N. Alexander	L. E. Reichert, Jr.
S. J. Behrman	G. T. Ross
R. Eliasson	R. Sherins
E. S. E. Hafez	A. Steinberger
A. Jost	E. Steinberger
M. Lipsett	P. Troen

12:00 **CLOSING REMARKS**

Eugenia Rosemberg, M.D.
Program Chairman

4th floor Dr Goodman's Ofc
Physiology for luggage.

1

STEROID METABOLISM IN ISOLATED EPITHELIUM
OF GUINEA PIG SEMINAL VESICLE

Randolph C. Steer and Carlo M. Veneziale
Mayo Med. School, Rochester, MN 55901

The metabolism of nine radioactively labelled steroids in the epithelium of the seminal vesicle of the mature guinea pig has been studied. The rapid assimilation and metabolism of these steroids demonstrate the very active biochemical nature of this tissue. Based on the use of several thin-layer chromatography systems and comparison to the locations of known standards, the following was observed: testosterone was rapidly converted to dihydrotestosterone and androstandiol. The latter was the major metabolite of dihydrotestosterone and of androsterone. Androstenedione was readily converted to androstanedione, testosterone, and dihydrotestosterone, although it formed little androstandiol. Dehydroepiandrosterone was converted to small amounts of androstenediol and androstandiol. Pregnenolone was rapidly converted to an unidentified highly polar compound only. Progesterone was converted to 5α-pregnane-3β-ol-20-one and its 3α-isomer. Dehydroepiandrosterone and progesterone were also significantly converted to unidentified highly polar compounds.

The major metabolites of 17-hydroxyprogesterone were co-chromatographed with standard androstandiol, testosterone, and an unidentified metabolite possessing intermediate chromatographic mobility. In addition, 17-hydroxyprogesterone was converted to small amounts of compounds possessing R_f values identical to standard androstenedione and dihydrotestosterone. The identification of the products of 17-hydroxyprogesterone metabolism and their physiologic significance must await critical evaluation.

Because of its homogeneity, isolated epithelium of guinea pig seminal vesicle shows promise as a tissue preparation for use in future studies that might elucidate the role(s) of individual androgens in secretory tissues of the male accessory sex organs. Our demonstration of extensive steroid interconversions in this tissue is a logical prerequisite to such studies.

2

STEROID METABOLITES OF THE MARMOSET TESTIS

James P. Preslock
Univ. of Texas Med. School at Houston, Houston, TX 77025

Comparatively little information is available regarding the biosynthesis of steroids by the testis of non-human primates. Marmosets are New World primates of the family Callithricidae, and the following studies were to determine the major steroid metabolites formed from selected androgen precursors by the testis of this primate species. The left testis was removed from an adult marmoset and cut into 50 mg fragments. The fragments were teased, placed into flasks containing Krebs-Ringer bicarbonate buffer, pH 7.4, and incubated at 37°C. Fragments were incubated in duplicate for 3 hours with pregnenolone-7-^{3}H(2μCi) or progesterone-7-^{3}H(2μCi), or for 5 hours with acetate-1-^{14}C(5μCi). Reactions were terminated, incubation media extracted with cold diethyl ether:chloroform (4:1), and metabolites separated by paper chromatography in hexane:formamide (1:1), and hexane:benzene:formamide (1:1). Metabolites were identified by comparison of mobilities in selected thin-layer chromatography systems with that of authentic standards, formation of acetylated derivatives, and recrystallization to constant specific activities. 17α-Hydroxyprogesterone was the predominant metabolite formed from incubation of marmoset testicular fragments with radiolabelled pregnenolone, with 42.7% of the pregnenolone con-

verted into this metabolite. Testosterone was the next predominant metabolite formed, with 20.5% of the pregnenolone converted into it, while androstenedione and progesterone contained 11.4% and 9.2% of the original radioactivity, respectively. Major metabolites of progesterone were 17α-hydroxyprogesterone (49.0%), testosterone (21.2%), and androstenedione (10.7%). Radiolabelled acetate was converted into progesterone (14.8%), testosterone (17.3%), 17α-hydroxyprogesterone (20.1%), pregnenolone (12.4%), and androstenedione (18.5%). These results demonstrate that the marmoset testis can convert selected precursors into androgens and androgen intermediates similar to that of other vertebrate species. The relatively high levels of 17α-hydroxyprogesterone resulting from incubation of marmoset testicular fragments with pregnenolone and progesterone are similar to that reported for incubation of progesterone with human testicular biopsies from patients with Klinefelter's Syndrome.

3

SYNTHESIS AND METABOLISM OF PROSTAGLANDIN F_{2alpha} BY THE HUMAN PROSTATE

Alice H. Cavanaugh
SUNY at Buffalo and VA Hospital, Buffalo, NY 14215

We have found that the human prostate possesses the ability to both synthesize and metabolize prostaglandin F_{2alpha}. After incubating prostate tissue with arachidonic acid, we have been able to isolate and quantitate PGF_{2alpha} by radioimmunoassay. Quantitation of the PGF metabolites, 15-keto-PGF_{2alpha} and 13,14-dihydro-15-keto-PGF_{2alpha}, by the Upjohn Company, also revealed significant metabolism of the prostaglandin. We were able to show prostaglandin synthesis and metabolism using both prostate minces and microsomal preparations; however, microsomes showed less activity, indicating the need for an intact membrane.

Since prostate is an androgen-dependent tissue, we incubated prostate tissue in the presence of 10^{-9} to 10^{-7}M testosterone. Since lactogens are known to favor prostate reception to androgens, human placental lactogen (HPL) was also added to some incubations. Little effect on assayable prostaglandin F was observed; however, significant influences on metabolism were evident in the androgen- and lactogen-treated tissue. Metabolism was followed by incubating ^{3}H-PGF_{2alpha} with prostate minces in the presence and absence of testosterone and HPL. Radioactivity in the metabolites was significantly increased when testosterone and HPL were added together.

These results lead us to believe that the human prostate is an adequate site for prostaglandin synthesis. Furthermore, the metabolism of the prostaglandin seems to be enhanced by androgen. That we saw no apparent increase in assayable PGF_{2alpha} may be a reflection of both accelerated synthesis and metabolism. The significance of this will be clarified when the biological activity of prostaglandin metabolites has been elucidated.

This work was performed at the VA Hospital, Buffalo, New York, in the laboratory of Dr. W. Farnsworth. Thanks is given to the Upjohn Company, Kalamazoo, Michigan, for their generous donation of prostaglandin antisera, prostaglandin standards, and helpful suggestions. Assays of prostaglandin metabolites were done through the courtesy of Dr. John Wilks of the Upjohn Company.

4

THE EFFECTS OF SYMPATHECTOMY AND TESTOSTERONE PROPIONATE ON PROSTATIC CYTOSOL RECEPTORS

M. James Cosentino and P. Jones-Witters
Ohio University, Athens, OH 45701

The effects of bilateral sympathectomy (SYM), testosterone propionate (TP) at 1 mg/day, or both (SYM-TP) on cytosol receptor binding of dihydrotestosterone (DHT) were studied in ventral prostate tissue obtained from adult Holtzman rats. Prostate tissue from rats of each group was perfused with isotonic saline, homogenized in tris-HCl buffer, and the 105,000x g supernatant obtained. Incubation with ^{3}H-DHT was done *in vitro* for 16 hours at 4°C. Cytosol receptor binding of DHT was quantified using the dextran-coated charcoal method. Specific binding of DHT was represented by the calculated difference between values obtained after incubation of ^{3}H-DHT alone (total binding) and those obtained after incubation with ^{3}H-DHT plus unlabelled DHT. Assessment of total binding indicated 7.4×10^{-15} moles of DHT bound/mg of cytosol protein for untreated control animals. Binding assays showed a marked decrease of total binding in SYM animals (4.0×10^{-15} moles/mg protein) and for SYM-TP animals (4.1×10^{-15} moles/mg protein) as compared to the untreated controls. The TP animals showed an increase in DHT-receptor interaction (9.8×10^{-15} moles/mg protein). The prostate weights (mg%) of the SYM animals showed no significant variation from the controls, while the SYM-TP group and the TP animals showed a marked increase in gland weight. This suggests that the sympathectomies did not alter the androgen secretion of the testis. Addition of unlabelled steroid showed no specificity for binding DHT in the sympathectomized group and greater specificity for binding in the group receiving TP alone. Even though the total binding in SYM-TP animals was markedly decreased, the specificity for DHT was maintained. The results indicate that both hormonal and neural influences are involved in regulation of steroid receptor specificity and receptor concentrations. The effects of a sympathetic blocking agent on prostatic cytosol receptors are currently being studied.

5

RECEPTOR SITES ON HUMAN PROSTATE TISSUE FOR PROSTAGLANDIN F_{2alpha}

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We have found specific binding sites on prostatic tissue membranes for prostaglandin F_{2alpha}.

Prostate tissue obtained from suprapubic prostatectomy was frozen until ready for use. Tissue was thawed, and membranes were isolated by the procedure of Medolesi. After suspending the membranes in .01 Tris-HCl buffer, aliquots were incubated 1 hour at room temperature with high specific activity (approximately 100 μC/mmole) PGF_{2alpha}. Unbound PGF_{2alpha} was separated from bound by filtration on a Millipore filter followed by extensive washing of the filter with the Tris buffer. Filters were then cut in half and counted in a liquid scintillation counter. Significant amounts of activity were found bound to the membranes caught on the filter. Activity could be displaced by adding cold PGF_{2alpha} to the incubation mixture. The influence of lactogen on prostaglandin binding is now under investigation.

6

PROPERTIES OF SPECIFIC ANDROGEN RECEPTORS IN THE HYPOTHALAMUS AND PITUITARY GLAND OF ADULT MALE RATS

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Different brain regions and the anterior pituitary gland were examined for specific binding components (receptors) for 5α-dihydrotestosterone (DHT). Cytosol fractions were prepared in 50 mM Tris buffer pH 7.5 containing 1 mM EDTA, 12 mM thioglycerol, and 10% glycerol (buffer A). The sedimentation properties of specific ^{3}H-DHT binding components in cytosol and serum samples were studied by density gradient centrifugation using 5-20% sucrose gradients prepared in buffer A. The binding affinity (K_A) and quantity of receptors were measured by Scatchard plot analysis of specific ^{3}H-DHT binding data. Binding specificity was evaluated by a competitive binding assay and by competition studies done using density gradient centrifugation procedures. Cytosol fractions from ventral prostate, anterior pituitary, and hypothalamus contained a high affinity, limited capacity ^{3}H-DHT binding component with an 8S sedimentation coefficient in low ionic strength medium. This component was not detected in serum nor in cytosol fractions prepared from cerebral cortex, hippocampus, and olfactory lobe. This substance possessed the properties expected of a hormone receptor, i.e., high binding affinity ($K_A \sim 10^9 M^{-1}$) and hormonal binding specificity (DHT > testosterone > estradiol = progesterone > cortisol). In adult male rats castrated for 24 hours, the receptor concentration in ventral prostate was 18 pmole/gm fresh tissue (0.5 pmole/mg protein). Receptor concentration in anterior pituitary and hypothalamus was about one-half and one-tenth that of ventral prostate, respectively. After one week of castration, the prostate receptor titer dropped to low levels, whereas the receptor concentration in pituitary and hypothalamus remained at levels comparable to those present 24 hours after castration. These results demonstrate that hypothalamic and pituitary DHT receptors are maintained after castration which is in contrast to the rapid decline observed in prostate receptor levels. This observation suggests that hypothalamic and pituitary DHT receptor levels may be regulated in a different manner than are prostate receptor titers. Since the physicochemical properties of DHT receptors in hypothalamus, pituitary, and prostate were found to be similar, i.e., 8S sedimentation coefficient and a high binding specificity for DHT, our results support the hypothesis that similar DHT receptor molecules are present in these target tissues, but the control of DHT receptor levels in hypothalamus and pituitary is different from that operative in ventral prostate. (Supported by VA grant MRIS 7877.)

7

THE ANTISPERMATOGENIC EFFECTS OF o-NITROBENZENESULFONAMIDE (ORF 11,133) IN MALE RATS

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The inhibition of male fertility by o-nitro-benzenesulfonamide (ORF 11,133) was investigated in adult Wistar rats following oral (i.g.) administration at 1, 5, and 10 mg/kg. Fertility of the males was determined at weekly intervals by cohabitation with proestrus females which were autopsied on day 14 of gestation for the examination of implantation sites. Twenty-eight days of treatment with ORF 11,133 at 1 mg/kg did not reduce fertility nor alter the histological morphology of the testes. At treatments of both 5 and 10 mg/kg, fertility was normal after 14 days, markedly decreased after 21 days, and showed complete inhibition by 28 days. Fertility returned 4-5

weeks after cessation of treatment, but did not reach normal rates for an additional 3-4 weeks. A dose-related inhibition of spermatogenesis was observed in histological preparations of the testes. When administered at 5 mg/kg for 14 days, ORF 11,133 induced partial germinal epithelial atrophy and loss of spermatozoa; however, testes weights were only slightly reduced, and some tubules were still undergoing spermatogenesis. In contrast, 14 days of treatment at 10 mg/kg caused approximately a 50% decrease in testes weights and nearly complete aspermatogenesis in most of the seminiferous tubules. The results after 28 days of treatment were similar at both 5 and 10 mg/kg; spermatozoa and spermatids were completely eliminated, and a partial disappearance of primary and secondary spermatocytes was observed. Spermatogonia were present in all tubules, but appeared to be reduced in quantity in some areas. Sertoli cells and Leydig cells appeared to be unaffected. Neither libido nor accessory sex organ weights were decreased in these ORF 11,133 treated male rats. The antispermatogenic activity of various nitro-heterocyclic compounds is well-known. These data indicate that a nitro-aromatic compound also has antifertility effects in male rats.

8

PARTIAL CHARACTERIZATION OF THE ANTISPERMATOGENIC EFFECTS OF 5-AMINOINDAZOLE

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Interruption of the normal spermatogenic process in rats has been achieved with 5-aminoindazole, a compound not previously known to have this property. 5-Aminoindazole, or a molar equivalent of its water soluble hydrochloride salt, administered orally 4 days a week for 2 weeks at 200 mg/kg daily, was found to inhibit spermatogenesis and fertility without significantly changing seminal vesicle or ventral prostate weights. The compound causes the formation of giant and multinucleated cells and the exfoliation of immature germ cells of all developmental stages into the lumen of the seminiferous tubule. An effective dose causes the seminiferous epithelium in many tubules to be reduced to a basal layer of primary spermatogonia and Sertoli cells. The maximal effect appears about 10 days subsequent to the last dose. The epididymis empties of germ cells rapidly after an effective antispermatogenic treatment and is largely void of sperm by day 21 of the experiment. In an experiment where a group of rats were dosed as described above and serially mated starting on day 21 until day 132 of the experiment, sperm were seen in the caput epididymis of 3/3 rats by day 62, and fertility was demonstrated in 3/4 rats by day 132. Histological examination of the rat testes taken on day 132 found some inactive tubules where there was little recovery of the germinal epithelium. We conclude from these experiments that 5-aminoindazole is an orally active and rapidly effective antispermatogenic compound in the rat, and that short-term administration of the compound causes reversible infertility as judged by mating.

9

STUDIES ON THE ANTITESTICULAR ACTION OF DL-6-(N-2-PIPECOLINOMETHYL)-5-HYDROXY-INDANE IN THE RAT

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The antitesticular action of DL-6-(N-2-pipecolinomethyl)-5-hydroxy-indane maleate (PMHI) was reported by Boris et al. The effects of PMHI on the male reproductive endocrine functions and the precise mechanism of action have not been investigated. We treated both prepubertal and adult rats with a single oral, submaximal dose of either 60 mg or 120 mg of PMHI per kg of body weight. Their testicular weight was drastically reduced. A follow-up, beginning on the 3rd day post-treatment and continuing for a period of 50 days, showed normal growth of PMHI-treated rats. The hormonal profile indicated that none of the serum levels of LH, FSH, estrogen, or testosterone were abnormal. Testicular histology revealed that the spermatogenic process in PMHI-treated rats recovered at a dose-related rate. Electron microscopic sections of testes of adult rats treated with PMHI similarly showed cytoplasmic vacuolation in the Sertoli cells 5 h post-treatment. The subsequent cascade of arrested spermiogenesis included abnormal acrosomal condensation of spermatids and sloughing of mono- and polynucleated spermatids. Some spermatocytes also seemed to be affected, but spermatogonia and Leydig cells remained intact. These hormonal and histological results suggest that PMHI acts primarily on Sertoli cells and causes arrest in the spermiogenetic stage of the spermatids. In rats treated with a higher and toxic dose of PMHI (180 mg/kg), however, spermatocytes and even spermatogonia were also affected, probably due to the extensive damage of the supporting Sertoli cells by the compound. This work was partially supported by NIH grant HD-07110.

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MECHANISM OF SUPPRESSION OF RAT VENTRAL PROSTATE WEIGHTS BY METHANDROSTENOLONE

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Intact adult rats received seven daily s.c. injections of 1.25 mg methandrostenolone (M)/100 g/day in oil which reduced the organ/body wt (bwt) ratio of the testis to 87%, seminal vesicles to 62%, and ventral prostate (VP) to 67% of control values. Body and kidney wts were not affected, while the levator ani was increased to 133% of control values. To determine if the reduction in VP wt resulted from an anti-androgenic effect of M, the ability of M to inhibit DNA synthesis by VP tissue was assessed. Rats (350-400g) were castrated and 1 week later injected s.c. daily for 3 days with either 450 μg testosterone (T)/100 g, 900 μg M/100 g, the combination of M + T, or oil (vehicle). Twenty-four hrs after the last injection, the rats were killed, and the VPs excised, weighed, and minced. Aliquots (300 mg) were incubated for 20 min at 37°C in 5 ml of Eagle's Basal Medium containing 15 μCi of (Me-^{3}H) thymidine/ml (50-55 Ci/mmole). The nuclear fractions were isolated, and total radioactivity and DNA determined (Burton, Biochim.J., 62: 315, 1956). VPs were 3 times heavier, and DNA synthesis was more than 30-fold greater in T-treated rats than in oil-treated controls (>3000 DPM/μg DNA/20 min vs. <100 DPM/μg DNA/20 min). When administered concomitantly with T, M failed to antagonize the effects of T on VP wt or DNA synthesis. Thus, M did not act as an anti-androgen. To assess the possible effects of M on endogenous secretion of T, intact rats (350-400 g) were fitted with catheters and injected daily s.c. with either 4 mg M, or oil (vehicle). At

16 and 24 hrs after each injection, a blood sample was obtained for radioimmunoassay of plasma T. T values for controls followed a diurnal variation with morning values (3346 ng/ml) being significantly ($p<0.05$) higher than afternoon values (1585 ng/ml). Within 48 hrs after administration of M, plasma T was reduced to <500 ng/ml. Similarly, M reduced the elevated plasma LH of castrated rats (240-560 ng/ml) to values subnormal for intact rats (4-11 ng/ml). These findings suggest that M reduces VP wts of intact rats by suppressing LH and thereby lowering plasma T.

10a
(Exhibit)

THE MOLECULAR CONFORMATIONS OF ANDROGENS AND ANTI-ANDROGENS

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The crystal structure determinations of over 280 natural and synthetic steroid molecules reported from 1956 to 1976 constitute the most detailed block of structural data on any class of biologically active molecules in existence and provide a wealth of raw material concerning molecular conformation and intermolecular interactions ideally suited to the analysis of structure-function correlations. The utilization of these data for purposes of exploring biochemical reactions at the molecular level has been inhibited by lack of communication among structural chemists, biochemists, and clinicians. In order to facilitate such a utilization of structural data in the exploration of molecular mechanisms of steroid hormone action, all crystallographic data concerning estranes, androstanes, and pregnanes has been assembled in the Atlas of Steroid Structure, Volume I, William L. Duax and Dorita A. Norton, Editors, Plenum Press, New York, New York, 1975.

The Atlas of Steroid Structure contains twelve androgen, anti-androgen, or known inactive compounds. In addition, six other androgen or anti-androgens have already been analyzed for inclusion in Volume II of the Atlas. Of particular interest are the structures of the anti-androgens cyproterone acetate (PR54) and BOMT (AN09) which are known to selectively block the high affinity binding of 5α-DHT without impairing 5α-reductase activity; this means they do not compete with testosterone. By analyzing the structural data together with specific binding data, it is possible to gain some insight into the nature of the active sites of the androgen receptor and the 5α-reductase.

Copies of the Atlas of Steroid Structure are available for inspection at this meeting, and charts and diagrams illustrating comparative conformational analysis of the androgen and anti-androgens have been prepared based upon the data presented in the Atlas.

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11 PREPUBERTAL HISTOCHEMICAL AND ULTRASTRUCTURAL CHANGES IN THE EPIDIDYMIS OF THE RAT

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Spermatozoa are transported to the epididymis of the rat in about 45-50 days. These spermatozoa may mature and acquire fertilizing ability due to interactions with epididymal epithelial secretions. Epididymides of prepubertal rats of ages 7, 14, 21, 30, and 45 days were used in histochemical and/or TEM investigations to determine maturational changes in the epididymis prior to the entry of testicular spermatozoa. Metachromatic staining of nucleoprotein by azure B indicated the presence of ribonucleoprotein (RNP) in the proximal segments of the caput epididymides. RNP was present as large accumulations of rough endoplasmic reticulum, indicating that proteins may be elaborated long before epididymal spermatozoa are present. In the distal segments of the caput, the basal regions of the epididymal epithelium showed invaginations of the plasma membrane with enclosed mitochondria, indicating absorptive function and possible transfer of luminar materials to the blood supply underlying the basal lamina in these areas. This supposition is based on the striking subcellular structural similarities between this tissue and renal tissue of known absorptive function. Histochemical monitoring of changes taking place in sections of whole epididymides included acid mucopolysaccharide, alkaline phosphatase, and neutral mucopolysaccharide localization utilizing Spicer's alcian blue techniques, Takamatsu's cobalt nitrate technique, and McManus's periodic acid-Schiff technique, respectively.
Acknowledgements: Thanks to Dr. Stu Swihart for his generosity and Mr. Phil Sherman for his expert technical assistance.

12 ULTRASTRUCTURAL CHANGES IN HUMAN FETAL LEYDIG CELLS AT MID-GESTATION

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The extensive development of human fetal Leydig cells during the 3rd and 4th months of gestation is well-known, but some question exists as to the fate of these cells after the early developmental period. The present study was undertaken to evaluate the fine structure of Leydig cells in their period of active proliferation and in the regressive phase beginning at 18 weeks. Electron microscopic examination was performed on testicular specimens obtained from 22 fetuses following abortion by prostaglandin induction or hysterotomy. The crown-rump lengths ranged from 5.2 to 19.0 cm., corresponding to fetal ages of 10 to 20 weeks. During the 10- to 18-week period, Leydig cells occupied a major portion of the testicular parenchyma, and, in 0.5 to 1 µm, sections of plastic-embedded tissue were seen to be arranged in dense groups of large, round to oval cells with abundant cytoplasm containing large numbers of mitochondria and other organelles. By electron microscopy, the cells were filled with smooth endoplasmic reticulum and large pleomorphic mitochondria, some of which contained osmiophilic lipid-like inclusions. The cell membranes of adjacent cells were closely aligned, with many gap junctions evident. In contrast, after 18 weeks, the cells were less closely aggregated, often occurring as single cells. Some retained the morphologic appearance of fully differentiated Leydig cells, while others had a more oval to elongated shape, with fewer mitochondria, more lipid, and lesser amounts of smooth endoplasmic reticulum than the fully differentiated

cells, and mitochondria generally lacked lipid-like inclusions. The nuclei and general architecture of these cells remained intact, and no degenerating forms were seen. This last observation indicates that the regressive changes are not associated with cell death, but rather represent a reversion to a less well-differentiated state. The findings suggest that the cells which undergo regressive changes at mid-gestation remain to redifferentiate at a later time, possibly contributing to the stock of adult Leydig cells which appear at the time of puberty. Supported by grants from the U.S. Public Health Service (HD 08202) and the National Foundation-March of Dimes (CRBS-321).

13 INHIBITION OF THE MOTILITY AND METABOLISM OF HUMAN SPERMATOZOA BY CYTOCHALASIN B

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Cytochalasin B, an agent which interacts with filamentous proteins in cells, inhibits the motility and metabolism of washed human spermatozoa at low concentrations (20-200uM). Glycolysis is inhibited by 50% at a cytochalasin B concentration of 100uM and by more than 70% at 200uM. The motility of spermatozoa declines slowly upon addition of 100uM cytochalasin B to 20-40% of control values after 45 minutes, but is not abolished even after prolonged treatment (2 hours). The addition of caffeine to washed sperm suspensions increases the percentage of motile cells 10-20%, increases the rate of flagellar contraction, and markedly stimulates the rate of glycolysis. However, if cytochalasin B is given simultaneously with caffeine, there is no change in the inhibition of motility nor in the inhibition of metabolism caused by the presence of cytochalasin B. In view of recent evidence suggesting a relation between cyclic AMP and the function of filamentous proteins (Olsen, R.W., J. Ther. Biol., 49:263, 1975), it is proposed that cytochalasin B may interfere with the interaction of cyclic AMP and microfilaments in human spermatozoa. Supported by Grant HD-0934-01, NICHD, USPHS.

14 MICROPUNCTURE STUDIES OF THE EFFECT OF CAFFEINE AND CYCLIC NUCLEOTIDES ON THE MOTILITY OF RAT EPIDIDYMAL SPERM

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These studies were conducted to determine the effects of dilution, dextrose, caffeine, 3'5' cyclic AMP (CAMP), and dibutyryl CAMP (diCAMP) on the motility of sperm obtained *in vivo* by micropuncture from the rete testis, caput epididymides, and cauda epididymides of the rat. The testicles and epididymis of mature, anesthetized rats were exposed for micropuncture. Samples were aspirated in micropipettes from the rete testis, caput, and cauda. The samples were divided into the following six groups: I, no dilution; II, dilution with a physiologic buffer; III, as Group II plus 10mM dextrose; IV, as Group II plus 10μM caffeine; V, as Group III plus 5μM CAMP; VI, as Group III plus 5μM diCAMP. The specimens were placed on a slide warmer, viewed with a microscope, and the motility was evaluated. Samples from the rete testis did not demonstrate significant motility under any experimental

conditions, and epididymal samples were not motile in their native fluid. Addition of a physiologic buffer induced motility in caput and caudal sperm. The addition of dextrose further increased motility of sperm from the proximal and distal epididymis ($p < .02$ and $p < .003$). Caffeine, CAMP, and diCAMP significantly increased the motility of caput sperm ($p < .004, < .003, < .003$), but did not induce progressive motility in these sperm. DiCAMP was very slightly more effective than CAMP ($p = .04$). Caffeine slightly but significantly increased the motility of caudal sperm ($p = .01$). CAMP and diCAMP did not affect the motility of caudal sperm. This work confirms in epididymal sperm obtained in vivo by micropuncture the previously observed effects of dilution and dextrose on motility. We have also confirmed Hoskins's observation of stimulation of caput sperm by caffeine and cyclic nucleotides. The absence of response of caudal sperm to CAMP and diCAMP may be explained by the high base line motility (58.8 ± 4.4 per cent) in these sperm under our experimental conditions.

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EFFECT OF GLYCEROL AND ORVUS ES PASTE ON SPERM CELL ACROSIN DURING FREEZING AND STORAGE AT -196°C

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A study was conducted to determine the effect of 0, 0.5, or 1% Orvus ES Paste (OEP) with or without 1% glycerol (G), as components of the Beltsville freezing extender (BF-5), on the acrosin (EC 3.4.21.10) content of porcine spermatozoa. The extender and the freezing and thawing procedures used to process the semen have been described (Pursel, V.G. and Johnson, L.A., J. Anim. Sci., 40:99-102, 1975). Semen collected as the sperm-rich fraction from three boars was pooled and split into aliquots containing 3×10^9 sperm. Six billion sperm were frozen and stored at -196° C for each of the six combinations. Three billion sperm were thawed and extracted for acrosin, 2 to 4 days after freezing (T1); the remaining 3×10^9 sperm were thawed and extracted 2 months later (T2). Morphological evaluation of the sperm acrosomes and sperm motility estimates were made after thawing. Extracts were assayed for enzyme activity using Benzoyl-arginine ethyl ester (BAEE) and fractionated using acrylamide gel electrophoresis (pH 4.3). Gels were stained for acrosin activity by the hydrolysis of Benzoyl-arginine-β-napthylamide (BANA) coupled to a Fast Black K salt (FBK). Specific enzyme activity was not altered by any treatment at either T1 or T2 ($P > .05$); average values (units/mg protein; $n = 6$) were 5.58 and 5.29 for T1 and T2, respectively. Four molecular fractions of acrosin activity were visualized by BANA-FBK. The amount of protein extracted from the sperm cells did not differ ($P > .05$) among the treatments; mean protein was $3.27 \mu g/10^6$ sperm at T1 and 4.21 at T2. A higher percentage of sperm acrosomes was morphologically damaged in 0% OEP - 0% G and 0% OEP - 1% G than for the other treatments ($P < .05$). Sperm motility was higher for 0.5% OEP - 1% G and 1% OEP - 1% G than for the other treatments ($P < .05$). At the levels tested, OEP and G had no effect on boar sperm cell acrosin concentration whether stored at -196° C for 4 days or 2 months.

16 KINETIC STUDIES ON THE INTERACTION AND SPECIFICITY OF SYNTHETIC PROTEINASE INHIBITORS TOWARDS HUMAN ACROSIN

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The sperm acrosome contains a neutral proteinase (acrosin, EC 3.4.21.10) which aids the spermatozoon in penetrating zona pellucida of the ovum during fertilization. The addition of natural and synthetic inhibitors of acrosin to capacitated spermatozoa prevents fertilization. Synthetic inhibitors also prevent conception when placed vaginally before coitus. Such inhibitors have the potential to be practical contraceptive agents, and a search was therefore performed to find the most active synthetic acrosin inhibitor(s) that shows the greatest specificity for acrosin. Sixty-four synthetic proteinase inhibitors were obtained from commercial sources and tested for their inhibitory activity towards human acrosin and, if active, towards human pancreatic trypsin. The Michaelis constant for human sperm acrosin, using BAEE concentrations ranging from 0.0125 mM to 0.4 mM, was 4.25×10^{-5}M. The most active inhibitor ($K_i = 1.5 \times 10^{-8}$M) was p-nitrophenyl p'-guanidino benzoate (NPGB), which inhibited acrosin instantaneously, even at a concentration of 1×10^{-7}M. The compounds M and B 4596, p-amino benzamidine, p-[m-(m-fluorosulphonyl phenylureido) phenoxy elhoxy]benzamidine, n-α-p-tosyl-L-lysine chloromethyl ketone, 2-2' dibromopropamidine isethionate, pentamidine isethionate, and propamidine isethionate were also effective inhibitors of human acrosin (K_i values ranged from 10^{-3}M to 10^{-6}M), but were at least 200-3000 times less active than NPGB. All other inhibitors were less active. The K_i values of the active inhibitors towards pancreatic trypsin were approximately the same as those towards acrosin, although occasionally 5-fold differences were observed. Dixon plots showed that all active inhibitors, with the exception of NPGB, possessed a competitive type of inhibition towards acrosin. NPGB showed a mixed or non-competitive type of inhibition. Recent studies showed that NPGB is also the most active antifertility agent of all the inhibitors tested to date when evaluated *in vitro* using mouse gametes or *in vivo* as a vaginal depository using the primate (*Maccaca arctoides*) as the experimental animal.

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17 IMPORTANCE OF SEMINAL PLASMA COMPONENTS ON THE STRUCTURAL STABILITY OF HUMAN SPERMATOZOA

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Spermatozoa undergo maturation in the epididymis resulting in the establishment of structural stability. Ejaculated spermatozoa from sub-human mammalian species have a homogenous degree of stability when exposed to sodium dodecyl sulphate (SDS), with or without dithiothreitol (DTT). In contrast, the nuclei of ejaculated human spermatozoa reveal considerable variation in the degree of lysis when exposed to these agents. Bedford (J.Repr. Fert. 1973, 33, 19-29) noted that SDS alone caused moderate to gross swelling in 16% of the nuclei in ejaculates from normal volunteers, but up to 60% in semen from some infertile men. With the same technique, we noted that spermatozoa have a lower resistance to SDS a few minutes after ejaculation than later. In semen with biochemical evidence of normal secretory function of the prostate and seminal vesicles, full stability was reached within 15

minutes, but significantly slower development was seen in semen with low concentrations of zinc and magnesium and low acid phosphatase activity. Spermatozoa removed from the first part of split-ejaculates (i.e., "prostatic" fluid) were much more resistant to SDS than spermatozoa recovered from the "vesicular" fluid. Spermatozoa from semen with indication of decreased prostatic function were therefore transferred to prostatic fluid from "normal" men. With this approach, a significant acceleration in the development of structural stability was noted. Stability could also be obtained by adding zinc to the seminal plasma; however, magnesium and calcium had no effect. Our observations confirm that human spermatozoa display heterogeneity in structural stability. Factors in the seminal plasma -- particularly in the prostatic fluid -- influence the development of this stability. Therefore, the secretory function of the human male accessory genital glands is necessary for the functional properties of the spermatozoa.

SHERINS

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ENDOCRINE EVALUATION OF INFERTILE MEN

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FSH, LH, testosterone (T), and DHT were measured by radioimmunoassay in the sera of 91 men attending the U. S. C. infertility clinic. A group of men with normal semen analyses attending the vasectomy clinic served as controls. The control group had normal hormone levels with a log-normal distribution. Patients with normal sperm counts whose infertility was caused by an immunological factor or infection also had normal hormone levels. All of the infertility patients had normal thyroid and adrenal function.

There were two subgroups of 18 men with azoospermia, and 37 men with oligospermia for whom there were sufficient data to arrive at a diagnosis. Six of the patients with azoospermia had a 47XXY karyotype and elevated gonadotrophins, although four had normal levels of T. Four patients had Sertoli-cell only syndrome. Two of them had elevated gonadotrophins, but two had normal gonadotrophins, contrary to what was expected for this condition. Patients with obstructive azoospermia secondary to vasectomy or an anatomical abnormality had normal hormone levels.

Patients with oligospermia were divided into three groups depending on whether their gonadotrophin levels were elevated (4 men), normal (31 men), or low (1 man), and these groups were further divided on the basis of a normal or low T. (DHT levels were closely related to T values.) Patients with a varicocele had normal gonadotrophins and T levels, but fourteen of the other patients with normal gonadotrophins had reduced levels of T. There was no correlation between testicular histology and T levels in those cases where the patient had a testicular biopsy.

The division of oligospermic patients into subgroups on the basis of gonadotrophin and T levels may be of value in selecting a method of therapy. Some of the patients included in this evaluation and new patients entering the clinic are being treated with some of the otherwise non-specific therapeutic agents in order to test this hypothesis.

Prol and 2 Brom Ergo bryptine

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STUDY OF FSH, LH, AND PROLACTIN BEFORE AND AFTER LH-RH AND TRH IN INFERTILE MEN

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LH-RH (100μg) and TRH (200μg) tests were performed on 150 infertile male subjects.

FSH, LH, and prolactin were assayed by radioimmunological methods, and from this study we can conclude the following:

1. In primary testicular disorders (Klinefelter's syndrome, post-orchitis, etc.), the FSH basal levels are elevated, and the LH-RH response is proportional to basal levels. When the LH basal levels are normal or elevated, the LH-RH response is augmented.
2. A good correlation between FSH and LH response is found. Basal levels of prolactin are normal, but, in 20% of the subjects, the response to TRH injection is elevated.
3. In idiopathic azoospermia and oligospermia
   a. The basal levels and the response to stimulation tests are variable;
   b. In 40% of the subjects, the response is comparable to those described in primary testicular disorders;
   c. 40% of the subjects elicited a normal response;
   d. In 10% of the subjects, a diminution of basal levels of FSH or LH and decreased response to the LH-RH test is seen;
   e. In 10% of the subjects, excluding hypophysial tumors and iatrogenic causes, an increase of prolactin basal levels is noted;
   f. TRH response in these subjects is normal or elevated.

On the basis of this study, treatment with 2 bromo-α-ergocryptine is being undertaken for those subjects with increased prolactin levels. Gonadotropins are being administered to subjects with diminished basal levels or diminished LH-RH response. Supported by INSERM, and, at the moment, R. Roulier is spending a year with Professor P. Franchimont.

20

THE GnRH STIMULATION TEST IN THE EVALUATION OF UNILATERAL CRYPTORCHIDISM: A TWENTY-YEAR RETROSPECTIVE STUDY

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The ultimate fertility following orchidopexy at any age is often questioned. This study examines clinically and endocrinologically a group of 29 patients with unilateral cryptorchidism at least twenty years after orchidopexy.

111 Patients born between 1935-54 were randomly selected from a review of hospital charts coded for cryptorchidism. 63 patients could be located; nine were then rejected when found to be bilaterally cryptorchid. Of the remaining 54, 23 would submit to histories only. 31 patients had, in addition to a complete history, a physical examination including testicular measurements and two semen analyses. Assessment of their hypothalamic-pituitary-gonadal axis was performed using baseline gonadotrophins (LH,FSH), and plasma testosterone, as well as the LH and FSH response to a GnRH stimulation test. Two patients, found to have gross endocrinopathies, were excluded. An age-matched control group was obtained from healthy volunteers (N = 30).

There was no significant difference in age, marital status, or infertility rate in the study (N = 29) or "histories-only" (N = 23) groups. In the study group, 85% of patients had a smaller operated than non-operated testis,

and their sperm density was significantly lower ($p < .001$) than the controls.

The cryptorchid patients demonstrated definite differences in their endocrine evaluation. Basal LH and FSH were significantly greater ($p < .01$) than the controls; basal testosterone was not. After 250 μgm of intravenous GnRH, the LH response area was not significantly different than controls, whereas the FSH response was significantly greater ($p < .001$).

The cryptorchid patients in this study, irrespective of age at surgery, demonstrated a higher incidence of poor semen quality and gross testicular damage than previously reported. In addition, both gonadotrophins were elevated, and the FSH response to GnRH was hyperreactive. Whether this response indicated a basic abnormality in the gonadal axis or a sudden physiologic release of gonadotrophins due to prolonged ineffective feedback from a poorly functioning end-organ remains to be evaluated.

21

GONADAL FUNCTION IN PATIENTS WITH CHRONIC RENAL FAILURE MAINTAINED WITH HAEMODIALYSIS. RELATIONSHIP BETWEEN LENGTH OF TREATMENT AND PATIENT'S AGE

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Twenty patients with chronic renal failure maintained with haemodialysis (HD) for periods ranging from six (6) months to ten (10) years received 200 mg of testosterone given once weekly. Circulating levels of plasma testosterone, FSH, and LH were determined by radioimmunoassay before the initiation of HD and after discontinuation of treatment. Pituitary stimulation and suppression tests with GnRH and testosterone, respectively, were carried out during the treatment period. Examination of the seminal fluid was performed at regular intervals. A testicular biopsy was obtained from one of the patients so treated.

In patients under 40 years of age, FSH and LH levels increased after three (3) years of treatment. Above age 40, the increase in FSH and LH levels occurred at a much shorter interval. The suppression and stimulation tests were normal in young patients during the first year of treatment. Oligospermia and reduction in sperm motility were observed during the first years of treatment. With prolonged periods of treatment, abnormal forms and azoospermia were seen. A testicular biopsy, obtained from a 40-year old patient who had received treatment for four (4) years, demonstrated germinal cell damage, peri-tubular fibrosis, and a diminished number of Leydig cells.

These data indicate that, as a consequence of chronic renal failure, derangement of the function of the hypothalamic-pituitary-testicular axis occurs.

22 THE SEMINIFEROUS TUBULE WALL IN HUMAN HYPOGONADISM

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The structure and function of the seminiferous tubule wall in the mammalian testis has been the subject of several recent studies. In the human, gonadal disorders often present alterations of the tubular wall. Few detailed descriptions of these alterations and their possible pathophysiologic importance have been reported; therefore, we conducted a study on 202 testicular biopsy specimens taken from patients with primary hypogonadism, hypogonadotropic hypogonadism, cryptorchidism, and spermatidic arrest of the germinal epithelium. These specimens were studied by light and electron microscopy.

The most frequent alteration was hyalin thickening of the tubular wall, of which three types could be distinguished. In the first type, frequently found in cryptorchidism, PAS-positive material accumulated on the basement membrane. The second type was due to accumulation of hyalin PAS-negative, and sometimes RO-positive material, and to an increased number of irregularly oriented collagen fibrils in the internal acellular layer between the basement membrane and the myoid cell layer. This was the most frequent form of thickening and could be observed in all testicular disorders. In the third type, similar material accumulated in the external acellular layer, and was usually associated with thickening of the internal acellular layer.

The frequent finding of alterations of the tubular wall's internal cellular layers in both primary and secondary hypogonadism suggests that a similar response is elicited by different pathogenetic factors. It can be speculated that the myoid cells, like other cells of mesenchymal origin, react to stress by increasing their fibrillogenetic activity at the expense of their contractile activity, and that the fibroblasts also increase their fibrillogenetic activity only under more severe conditions.

23 SEMEN ANALYSIS: APPARENT BIOLOGICAL BREAKS IN SEMEN QUALITY WHEN RELATED TO SPERM CONCENTRATION

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Multiple semen analyses were performed on 225 men attending an andrology clinic. The percentage of oval, viable, and active sperm cells, as well as the motility scores, were lower in samples with sperm counts of less than 10×10^6/ml, increased in counts of $10\text{-}40 \times 10^6$/ml, and again increased in counts more than 40×10^6/ml. The percentage of semen samples with abnormalities in the measured parameters dramatically increased as the sperm count decreased. The percentage of samples with significant numbers of white blood cells and problems with agglutination was higher in the samples with sperm counts less than 10×10^6/ml and in the azoospermic patients. The results indicate that two biological breaks seem to occur in semen quality which relate to sperm cell concentration. The first break seems to be in those samples with counts of less than 10×10^6/ml when compared to those with counts above 10×10^6/ml. The parameters then seem to remain constant in samples with counts up to 40×10^6/ml and again change in those with counts more than 40×10^6/ml. It is important to note that samples with counts of $10\text{-}40 \times 10^6$ spermatozoa/ml do not seem to be significantly different in the several parameters of semen quality examined. The data does not give support to the current practice of considering samples with less than 20×10^5 spermatozoa/ml

as those with problems. Perhaps samples with counts of less than 40×10^6 spermatozoa/ml but greater than 10×10^6/ml should be grouped together, while those samples with counts of less than 10×10^6 spermatozoa/ml should be placed in another group.

Single Sample Control Data!

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TREATING THE "SUBFERTILE" MALE: IMPROVEMENT IN SEMEN CHARACTERISTICS AFTER LOW DOSE ANDROGEN THERAPY

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Semen samples from patients undergoing fertility evaluations were analyzed 15 minutes to one hour after collection for volume, sperm concentration, percentage of active spermatozoa, sperm motility, percentage of live spermatozoa, and sperm morphology. Patients with consistent sperm counts close to or above normal (greater than 20×10^6 sperm per ml), but with asthenospermia or low volume, were treated with 2 mg of halotestin (fluoxymesterone) daily. At 4- to 6-week intervals thereafter, each patient returned, and another semen sample was evaluated. Preliminary results suggest that, of the patients so treated to date, 85% have improved in at least four or more of the six categories of semen quality. At least 60% of the patients had an increase in semen volume, sperm count, motility score (quantitative expression of motility), percentage of live spermatozoa, or percentage of oval spermatozoa. The therapeutic regimen may, therefore, be most beneficial for individuals with reduced sperm motility, poor sperm morphology, and/or low semen volume. Additional studies are underway to evaluate other dosages to determine the usefulness of halotestin for the treatment of semen problems in the male with fertility difficulties.

25

CLOMIPHENE TEST AND CLOMIPHENE THERAPY IN THE HYPOFERTILE MALE

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A clomiphene test was performed in 100 cases of oligoasthenospermia and secretory azoospermia, and in 10 normal fertile men. Clomiphene citrate was given at 50 mg per day for 15 days (day 1 to day 15). Plasma testosterone, FSH, and LH were evaluated by radioimmunoassay on days 0 and 15.

As a group, the hypofertile males showed an apparently normal function of the hypothalamo-hypophyseal-testicular axis in comparison with the fertile group: clomiphene induced a significant elevation of the plasma levels of the three hormones. However, individual responses could be divided into three types: complete (elevation of all 3 hormones); dissociated (lack of elevation of 1 or 2 hormones); and no response at all. These distinctions were made with reference to a group of 10 hypofertile patients assayed under the same conditions, twice at 15-day intervals, but without clomiphene administration.

Forty hypofertile patients were treated with clomiphene citrate, 50 mg per day for 100 days, with spermiograms taken before and at the end of treatment. Monthly control assays showed that the hormonal response, when present, was maintained throughout the therapy. Our results indicate that the spermatogenic response, as evaluated by the spermiogram, occurs much less frequently

than the hormonal response.

From our studies, we conclude that there is no correlation between the results of the clomiphene test and of clomiphene therapy: a positive clomiphene test cannot predict a therapeutic result; on the other hand, there will be no improvement of the spermiogram in the absence of a hormonal response.

26

EFFECT OF LONG-TERM ESTROGEN THERAPY ON THE HUMAN TESTES

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It has been suggested in the past that certain pathologic conditions of the human testes associated with abnormal steroidogenesis are also associated with an increase in 20α-hydroxylase activity. The purpose of the present study was to determine the effect of long-term estrogen therapy in young adult males on *in vitro* steroid biogenesis in the testes with particular emphasis on formation of 20α-hydroxyprogesterone. Four male transsexuals were treated with estrogen for at least one year prior to sex reversal. At surgery, testicular tissue was obtained for morphologic and metabolic studies. In one patient, testicular biopsies were obtained prior to commencement of estrogen treatment. The tissue was incubated under appropriate conditions with [7(n)-^{3}H]progesterone. Throughout treatment all four patients exhibited complete suppression of circulating testosterone and FSH and LH levels, while plasma estradiol levels were consistently high. The *in vitro* studies demonstrated marked suppression of testicular steroidogenesis as evidenced by highly significant decrease in total substrate conversion when compared to pretreatment steroid metabolism. The specific effects were associated with a significant decrease in 17α-hydroxyprogesterone and testosterone formation. This was associated with a significant increase in 20α-hydroxylase activity. More than 50% of the total substrate conversion resulted in formation of 20α-hydroxyprogesterone. These results suggest that the effect of estradiol on androgen production in the testis is related either to an increase in 20α-hydroxylase activity, or to suppression of 17α-hydroxylase activity. It remains to be determined whether this is the result of decreased gonadotropic stimulation secondary to estrogen effect on the hypothalamic pituitary axis, or to a direct effect of estrogen on testicular steroidogenesis.

27

SEASONAL CHANGES IN BODY WEIGHT, TESTICULAR VOLUME, AND SEMEN PARAMETERS OF RHESUS MONKEYS FOLLOWING SHAM, UNILATERAL, AND BILATERAL VASECTOMY

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Forty-seven mature male rhesus monkeys (Macaca mulatta) were subjected to sham, unilateral, or bilateral vasectomy. These animals were studied for periods up to 72 weeks in order to ascertain the morphologic and immunologic effects of the procedures. Records were kept of body weights, testicular volumes, and semen evaluations. Analysis of these data were made to determine if there were seasonal effects on these parameters, and if these effects were modified by the surgical procedures.

Control monkeys had an average sperm concentration of 415 x 10^{6} sperm/ml

with 58% of the sperm showing progressive motility. Motility varied less than concentration over a long interval of time. Concentration was lowest from June through November. The unilaterally occluded monkeys showed less variation, but the same lower concentrations during summer and fall. Sperm concentrations in the bilaterally vasectomized monkeys were zero in all cases by 10 weeks post-vasectomy.

Body weight changes in all groups reflected seasonal variability. Decreases in weights were noted in the winter and early spring followed by increases until mid-summer and then fairly constant weights throughout the fall and early winter months.

Testicular volumes were estimated using the formula and measurements for a prolate spheroid. Volumes ranged from highs around 35 cm^3 in January to lows around 15 cm^3 in July and August. The seasonal changes in testicular volumes generally paralleled the body weight changes, but were not directly correlative.

No changes in body weights or testicular volumes could be contributed to the vasectomy or sampling procedures. Changes in these parameters did indicate that the monkeys remained responsive to environmental stimuli throughout the study period. This work was sponsored by NIH Contract N01-HD-3-2758.

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THE ABSENCE OF SPERM-AGGLUTINATING ACTIVITY IN HUMAN SEMINAL FLUID AFTER VASECTOMY

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Sperm-agglutinating antibodies were studied in the serum and seminal fluid of thirteen (13) men 4 and 8 weeks after vasectomy. Determination of sperm agglutinin titer was performed by a gelatine-agglutination test (Kibrick et al., 1952) and by a microagglutination technique (Friberg, 1974). The type of agglutination could be revealed by the latter test (head-to-head or head-to-tail sperm-agglutination activity). Sperm-agglutinating antibodies were present in 8 sera 4 weeks after vasectomy. The serum sperm antibody titers ranged from 1:8 to 1:4096. One of these men had a sperm agglutination titer of 1:8 prior to the operation. Agglutinating antibodies could not be found in the seminal fluid samples, although five (5) men had high serum titers of sperm-agglutinating activity ($\geq$ 1:32). The incidence of sperm antibodies 8 weeks after the operation was the same as 4 weeks post-vasectomy, and no significant rise in the antibody titers could be demonstrated. By the microagglutination test, it was found that the sperm-agglutinating activity was of head-to-tail type. Head-to-tail agglutination in the male sera is known to be caused by IgG antibodies against human spermatozoa and may also occasionally be caused by IgA or IgM antibodies (Friberg, 1974). In man, IgG and IgA are found in seminal fluid, but not IgM. The failure of the agglutinins to enter the seminal fluid deserves further characterization of the sperm-agglutinin antibody in sera from vasectomized men.

29 A NEW PROCEDURE FOR THE CLINICAL DETERMINATION OF URINARY LH: APPLICATION TO TESTICULAR DISORDERS

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Clinical assay of urinary LH involves extraction of urine and estimation by 5 day ventral prostate weight (VPW) bioassay as estimator system or 5 day radioimmuno estimator system. We report a new procedure combining a new extraction method (2-hours) with a 3-hour radioligand receptor estimator system, thus performing the clinical assay in one day. A new extraction method is needed because neat urine cannot be used with RIA or RRA; nor can the standard extraction procedure (Albert-Kaolin Acetone Method) be employed. The new method involves two (2) successive iso-electric precipitations at pH 5.0 and 9.2, after which the urine is processed by the standard procedure. The soluble extract obtained contains one-third of one-sixth the solids, but all of the biologic potency of the standard method. The extract was assayed simultaneously by three estimator systems: standard VPW or rat uterine weight, standard double antibody RIA with NIH reagents, and RRA using rat ovarian homogenate -- I^{125}hCG reagents. Standard (2nd IRP) was included in every assay run, thus achieving homologous unknown -- standard conditions. The extinction points which can be experimentally varied were 3, 1.2, and 0.2 IU LH per 24-hours for bioassay, RRA and RIA. Normal men excreted 3-10 IU per day, all three systems in agreement. Klinefelter's Syndrome had elevated values (20-60) by all three methods (RRA being twice bioassay and RIA). The urine of a fertile eunuch contained no LH by all three methods; urinary FSH was present. Use of RRA estimator with a rapid suitable chemical extraction has certain advantages not shared by bioassay, RIA, or by using blood serum: reduction of labor, high precision, low cost, reduced time, nondependence on NIH reagents restricted to research purposes.

Aided by grants from the National Institutes of Health (AM 01738), Bethesda, Maryland, and from the Ortho Research Foundation, Raritan, New Jersey.

30 EXISTENCE OF A FOLLICLE-STIMULATING HORMONE-INHIBITING FACTOR IN RAM RETE TESTIS FLUID

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Ram rete testis fluid (RTF), centrifuged at 4°C to remove spermatozoa and protein from the supernatant, was precipitated by addition of alcohol to a concentration of 86%. The precipate was recovered by centrifugation, washed twice with acetone at -20°C, dissolved in distilled water, and lyophyilised.

Batches of 250 mg were subjected to gel chromatography on Sephadex G200 in columns with a packed dimension of 15/90 cm using 0.06 M ammonium acetate buffer pH 7.0 for equilibration as well as elution.

This yielded 4 peaks: RTF_{I}, RTF_{11}, RTF_{111}, and RTF_{1V}. RTF_{1}, RTF_{11}, RTF RTF_{1V} caused no significant decrease in serum FSH and LH of the castrated adult male rat and normal immature female rats, whereas 50-400 μg of RTF_{111}, whether administered intravenously or intraperitoneally, caused a consistent and significant decrease in serum FSH without affecting LH in either bioassay.

No steroid-binding capacity for testosterone, dihydrotestosterone, and 17β-oestradiol could be detected for RTF_{111}. Moreover, the possibility that

the biological effects of the active material were due to contamination with steroids or their conjugates was excluded after appropriate organic solvent extraction, by radioimmunoassay of testosterone, progesterone, and 17β-oestradiol. Furthermore, the observed action of RTF_{111} is not related to the presence of gonadotropins, their fragments, or metabolites.

To ascertain the polypeptide nature of the RTF_{111} fraction isolated by gel filtration, it was subjected to pepsine and trypsine digestion. These enzymatic digestions destroyed the FSH-inhibiting activity of RTF_{111}. RTF_{111} contains more than one component which are currently separated by ion exchange chromatography. Supported by Grant No. 74039 from WHO and No. 20305 from the Belgian F. R. S. M.

? Rats

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RECOVERY OF PITUITARY-TESTICULAR AXIS AFTER ACUTE OR CHRONIC SUPPRESSION WITH ESTRADIOL

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Changes in testicular (TT) and plasma (PT) levels of testosterone, pituitary (PLH), and serum (SLH) levels of LH were examined at intervals after cessation of estrogen treatment. One hour after a single injection of 50 µg EB, TT decreased to 25% and PT to 48% of the pretreatment levels, and, after 8 h, to 7% and 19%, respectively. Subsequently, a progressive increase in the testosterone levels was noted. By 4 d, TT returned to 66% and PT to 100% of pretreatment levels and then remained unchanged for 11 d. PLH and SLH remained unchanged during the first 4 d after injection. During the subsequent 11 d, PLH decreased slightly while SLH rose significantly. TT and PT levels were markedly depressed (7% and 29%, respectively) after 14 daily injections of 50 µg EB, returned to 50% of pretreatment levels by 4 d after the last injection where they remained until termination of the experiment (7 weeks). SLH decreased slightly after 14 d EB treatment, but increased significantly between 30-60 d after cessation of treatment. PLH significantly diminished after 14 d of treatment, returned to normal 20 d later, but significantly decreased by the 7th week. Apparently a single EB injection rapidly blocks testosterone synthesis without concomitant changes in SLH or PLH, but the gonado-pituitary axis must be disturbed in these animals because of the marked decrease in PLH 15 d after EB injection. After chronic (14 d) EB treatment, testosterone synthesis recovers rapidly although SLH remains slightly and PLH significantly depressed for approximately 20 d. The data demonstrate a direct effect of EB on testosterone synthesis and an alteration of LH production patterns during the post-treatment recovery period.

T clearance ~ 1 hr.
rhLH clearance ~ 40'
r-?LH 140'

50 µg EB. pharmacologic
is ester cleaved?
E_2 plasma levels?

Testis T after chronic EB
remains low and LH ↑ demonstrate
Direct effect of E → T synthesis

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MECHANISM OF LUTEINIZING HORMONE (LH) AND TESTOSTERONE (T) SUPPRESSION BY FLUOXYMESTERONE (HALOTESTIN)

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Chronic fluoxymesterone (Halotestin) administration results in a suppression of plasma T, presumably through pituitary LH suppression. However, recently it has been shown that Halotestin suppresses plasma T without suppressing LH. The ability of Halotestin to bind to Testosterone-Estradiol Binding Globulin (TeBG) and to modify the pulsatile release of LH was investigated to elucidate the mechanism of its complex effects. Equilibrium dialysis was performed at 22° and 37°C. Halotestin binds to TeBG with an apparent K = 1.0×10^8 and 1.9 x 10> at 22°, and K = 5.2 x 10> and 8.0×10^6 at 37°, in female and male plasma, respectively. In polyacrylamide gel electrophoresis, 1000-fold molar excess of fluoxymesterone decreased the peak of TeBG-bound T by 45.

Blood was obtained from four (4) normal men, ages 18-21, every 30 minutes for 48 hours. Halotestin 10 mg every 6 hours was given during the second 24 hours. Halotestin decreased the mean number of LH spikes and integrated 24-hour LH level by 47% and 27%, respectively. Mean T level was decreased by 62% after 24 hours of Halotestin treatment. Despite the overall LH suppression, isolated LH spikes occurred with a subsequent rise in T within 30 minutes. In an orchiectomized and adrenalectomized man, and, in a genotypic and phenotypic man with congential anorchia, there was no LH suppression after 72 hours of Halotestin administration.

We conclude that Halotestin displaces T from TeBG and causes a fall in both T and LH. Since there was no LH suppression in a patient without endogenous T, the mechanism of the Halotestin induced fall of LH and T in normals appears to be via displacement of T from TeBG leading to greater free T available to cross the blood-brain barrier and to be metabolized.

Alternative No LH supp in Castrate!

33

EFFECT OF HUMAN CHORIONIC GONADOTROPIN (HCG) ON INTERSTITIAL CELLS AND ANDROGEN PRODUCTION IN THE IMMATURE RAT TESTIS

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The administration of HCG induces an increased testosterone secretion by the adult human testis that can also be elicited in prepubertal boys to various degrees according to age, with the greatest response at puberty. The effects of administration of HCG on the testicular interstitial cells of immature rats were determined by studying the sequence of histological events and their correlation with testosterone levels. Immature rats were injected with HCG for different periods of time. The number of Leydig cells, their mitosis and precursor fibroblasts, as well as plasma testosterone levels, were determined and statistically analysed.

A progressive stimulation of Leydig cell mitosis was observed after 3 days of HCG treatment, but stabilization occurred after 5 days. Leydig cell numbers were significantly greater at 5 and 10 days. The number of precursor fibroblasts had increased at 5 days and was still increasing at 10 days. Plasma testosterone showed a progressive and continuous increase in all treated groups with a parallel increase in the weight of the seminal vesicles and prostate.

The Leydig cell hyperplasia is considered to be due to a combination of stimulation of Leydig cell mitosis and differentiation of precursor fibro-

blasts. Leydig cell mitosis seems to precede fibroblastic differentiation, but the latter continued when the mitotic rate had stabilized. The elevation of plasma testosterone concentrations is probably due firstly to the stimulation of the existing Leydig cells, and then to the increase in the number of hormone-secreting cells. The parallelism between the cellular and humoral changes induced by HCG administration indicates that the interstitium of the immature rat testis is able to respond to gonadotropic stimulation in a way similar to the adult testis. It also shows a close correlation between the morphological and functional parameters of Leydig cell maturation in the immature rat.

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ADVANCED PUBERTY IN MALES. FSH AND LH STUDIES

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We measured FSH and LH excretions (1) (by radioimmunoassay) and production rates (P.R.) (2,3) in boys with idiopathic precocious puberty (P.P.) and with untreated congenital adrenal hyperplasia (C.A.H.).

Patient	Chron.Age (years)	Puberty Stage	LH(IU/24 hours)		FSH(IU/24 hours)	
			Excretion	PR	Excretion	PR
Adult Males	21 - 50		22.3-45.1	250-465	8.5±3.6	22-48
P.P.						
J.A.	4 0/12	2	5.0	122	2.2	17.9
	4 6/12	3	9.2	398	-	-
C.D.	3 0/12	3	5.6	459	3.2	21.8
	4 6/12	5	11.8	197	2.2	21.8
C.A.H.						
J.W.	3 weeks	1	0.35	-	0.31	-
T.W.	4 weeks	1	1.1	-	0.21	-
P.M.	1 0/12	2	5.9	166	6.2	41.5
M.B.	6 6/12	3	11.8	141	6.7	40.2
J.M.	81	Adult	16.4	-	13.6	-

We conclude that in P.P., the increases in FSH and LH excretion and production follow the pattern expected for normal puberty. In untreated C.A.H., after the neonatal period, there is marked increase in FSH excretion and production. The possible mechanisms for these observations will be discussed.

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HYPOTHALAMIC, PITUITARY, AND GONADAL HORMONES IN SEXUAL MATURATION OF THE MALE RAT

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Observations were made on groups of rats at 5-day (d) intervals from birth to d 64, and at d 74 and d 89. Hypothalamic content of gonadotropin-releasing hormone (GnRH) was determined by radioimmunoassay with Niswender antisera R-42. LH, FSH, and testosterone were measured by double-antibody radioimmunoassays. 3β-hydroxysteroid dehydrogenase (3βHSD) and 17β-hydroxysteroid reductase (17βHSR) activities were assayed in the 10,000xg supernatant fractions of testicular homogenates. Total activity of 3βHSD was quantitated by conversion of [^{3}H]pregnenolone to progesterone and of 17βHSR by conversion of [^{3}H]androstenedione to testosterone. The *in vitro* capacity of the testis to synthesize testosterone was measured in the presence of a saturating dose of rat LH. Hypothalamic GnRH, serum LH, and FSH concentrations and enzyme activities were low at birth. Hypothalamic content of GnRH increased linearly with age up to d 47 and then plateaued. LH concentrations were highly variable and often exceeded adult values between d 10 and d 32. Between d 32 and d 47, there was a steady rise followed by a decline to stable adult values after d 52. Serum FSH increased from 220ng/ml at d 10 to a peak value of 1000ng/ml at d 32. Subsequently, there was a steady decline in FSH until d 89 when it was again 220ng/ml. 3βHSD exhibited a rapid increase between d 19 and d 37. 17βHSR increased in a similar fashion approximately 15 days later. The increase in capacity to synthesize testosterone occurred at the same time as the increase in 17βHSR activity and followed a comparable time course. This study demonstrates that, during sexual maturation in the male rat, changes in serum LH and FSH do not reflect changes in hypothalamic GnRH. The appearance of Leydig cells as monitored by 3βHSD activity precedes by 15 or more days the increase in *in vitro* testicular capacity to synthesize testosterone. The latter coincides with the increase in 17βHSR activity. This suggests that 17βHSR is a limiting factor in the ability of the testis to respond to LH stimulation. Supported by NIH Grants HD-08358, HD-04064, and HD-08333.

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DIFFERENCES IN THE TESTOSTERONE-AGGRESSION RELATIONSHIP BETWEEN MEN AND WOMEN

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Plasma testosterone level (T) and 2 measures of hostility/aggression [Zuckerman & Lubin's Multiple Affect Adjective Check List Hostility Scale (MAACL-H), and Buss & Durkee's Hostility Inventory (B-D-ΣH)] were determined for a group of 18 young men and 21 young women. The young women were assessed during the early follicular phase, ovulatory peak, and late luteal stage of their menstrual cycle. Since no significant differences were obtained among the hostility/aggression test scores across the menstrual cycle between the multiple correlation coefficients between T and the two hostility/aggresion scores nor between the regression coefficients obtained on each of the 3 female testing occasions, the first occasion of testing was used for comparison with the values obtained for males. Means and standard errors for the 3 variables, multiple correlation coefficients (R), and regression coefficients (β) for the men and women were:

SEX	T (ng%)	MAACL-H	B-D-ΣH	R	β MAACL-H	β B-D-ΣH
Men	686 ±46	3.2 ± 0.6	22.3 ± 2.0	0.58*	25.28	10.21*
Women	23 ± 3	7.8 ± 0.5	22.2 ± 1.4	0.46*	- 2.53*	- 0.34

T, MAACL, and B-D-ΣH all fell within normal limits. T was significantly greater for the men than for the women while MAACL-H was greater for the female subjects.

The relationship, $T = \beta_1$ MAACL-H + β_2B-D-ΣH + α, was subjected to multivariate regression analysis, separately for men and women. Multiple correlation coefficients (R) of 0.58 and 0.46 were obtained, both significant at the 5% level. The regression coefficient for MAACL-H for women and for B-D-ΣH for men were both significant at the 5% level also; i.e., the predominant proportion of the variance in T was due to MAACL-H for the women and to B-D-ΣH for the men. While the hostility/aggression indicators loaded positively on T for the men, they were negatively related to T for the women.

Comparison of the two regression equations indicated that the variances about the two lines were highly heterogeneous ($F = 66.48$, $p < .001$). B-D-ΣH is a trait measure of hostility detecting the more consistent aspects of the mood while MAACL-H responds to momentary changes. The combination of positive loading of this trait measure on T in the males and negative loading of the state measure in the females suggests that different regulatory pathways occur between men and women with respect to T and central nervous system expression of aggression.

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IMPORTANCE OF ANAMNESIS
IN THE EVALUATION OF INFERTILE PATIENTS

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It is known that various factors such as infection, injuries, and exposure to radiation can cause disturbances of testicular function leading to infertility. Therefore, it was thought advisable to study 1,187 males exhibiting this condition. The patients were divided into fertile and sub- or infertile groups according to their sperm count and characteristics of the seminal fluid. These data were correlated with 11 anamnestic factors which could have been the cause of infertility as shown in Table 1.

TABLE 1
Order of 11 Anamnestic Group Factors According to Their Significance For Sub-Fertility in an Andrological Case Aggregate of 1,187 Patients.

ANAMNESTIC FACTORS	INCIDENCE RATE n = 1187		NORMO-FERTILITY	SUB-FERTILITY	SIGNI-FICANCE
	(#)	(%)	n=442/37.2%	n=745/62.8%	
Constitutional Defects	95	8.00	19.6%	80.4%	0.001
Occupational Injuries	79	6.7	30.0%	70.0%	0.01
Iatrogenic Injuries	322	27.1	42.4%	57.6%	0.01
Infectious Diseases	349	29.4	43.0%	57.0%	0.01
Accidents	93	7.8	38.3%	61.8%	0.05
Urogenital Infections	162	13.7	40.8%	59.2%	0.05
Physical Thermal Defects	53	4.5	35.4%	64.6%	
Focal Infections	245	20.6	45.6%	56.4%	
Miscellaneous	104	9.2	45.0%	55.0%	
Psychic Traumata	123	10.4	49.3%	50.7%	
Inconspicuous Case History	262	22.07	68.6%	31.37%	0.001

Both the frequency order of the 11 group factors in the total case aggregate and their order of significance were evaluated statistically. The study demonstrates that infectious diseases predominate in the frequency order, whereas constitutional defects are prominent in the order of significance.

Therefore, it can be stated that careful anamnesis will greatly facilitate the work-up of patients attending an infertility clinic.

Behrman – Immunology of Female Reprod.
7 cases of IHE c̄ post coital symptoms.

Reviewers Phyllis 1) Cytol 2) Bx 3) Rx

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