Ciba Foundation Colloquia on Endocrinology Volume 16

ENDOCRINOLOGY OF THE TESTIS

Edited by G. E. W. WOLSTENHOLME, O.B.E., M.A., F.R.C.P., F.I.Biol.

and

MAEVE O'CONNOR, B.A.

With 93 illustrations

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Ciba Foundation
Colloquia on Endocrinology:

Volume 16

ENDOCRINOLOGY OF THE TESTIS

This volume is dedicated to the memory of
WARREN O. NELSON
1906–1964

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The Ciba Foundation

for the Promotion of International Co-operation in Medical and Chemical Research

41 PORTLAND PLACE, LONDON, W.1

Preface

THIS volume marks the end of the Ciba Foundation's series of Colloquia on Endocrinology. The occasional conference on an endocrinological subject will in future appear as an independent publication in a changed format. It is fitting that Mrs. Dorfman (Peggy Cameron), who was a skilled and meticulous editor and dear colleague in the publication of most of our endocrinological colloquia, should help in the organization of this programme and, to our delight, could be present with her husband, who proved a highly expert and enthusiastic Chairman of the meeting.

In the previous 21 Colloquia on Endocrinology—published in 15 volumes and on one occasion (*Nomenclature of Steroids*) in an appropriate journal—little attention was paid to the problems of hormonal function in the male. This omission reflected a general lack of interest in the subject, but by 1966 an awakening concern about many aspects of male endocrinology seemed to make it opportune to hold the small international conference which is reported in this volume. I think that every reader will appreciate and value the range and depth of information which members were able to contribute on this occasion.

It was the wish of members of this colloquium, and of all of us at the Ciba Foundation, that these proceedings should be dedicated to our good friend and colleague, the late Dr. Warren O. Nelson. Although he ploughed a rather lonely furrow in the field of reproductive physiology in the male, his work makes an enduring contribution to the harvest of knowledge in relation to human fertility, and we hope these papers and discussions can be worthy of his example.

Members of the colloquium and the Ciba Foundation's staff were very shocked to hear of the death of Dr. A. H. Baillie during this meeting. He had had a very tiring overnight journey but was eager to join in the first day's discussions, instead of taking the rest he needed. It was not known until after the meeting why he had failed to turn up for the second and third days, having collapsed and died at the home of his sister. It is hoped that his comments in this volume, and the references to his work, can be a source of comfort to his family and strengthen their pride in his early achievements and his exceptional promise.

G.E.W.W.

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Membership

Colloquium on Endocrinology of the Testis held 18th–20th May, 1966

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B. Tamaoki	National Institute of Radiological Sciences, Chiba-shi, Japan
J.-P. Weniger	Laboratoire de Zoologie et d'Embryologie expérimentale, Université de Strasbourg, France

CHAIRMAN'S OPENING REMARKS

R. I. DORFMAN

By the kind invitation of Dr. Gordon Wolstenholme and the fine staff of the Ciba Foundation, we are assembled to discuss the recent advances in the field of "Endocrinology of the Testis". In addition to this task and pleasure we take this opportunity to honour the memory of Dr. Warren Otto Nelson. Our late colleague made imaginative and timely contributions to the knowledge of testicular endocrinology and a number of papers to be presented in this meeting will, directly or indirectly, be extensions of Dr. Nelson's studies.

Dr. Nelson was particularly interested in the possible direct effect of testicular hormones, especially testosterone, on spermatogenesis. He worked on this problem in the hypophysectomized rat and elucidated some of the mechanisms related to this important phenomenon. During the course of this conference Dr. Yves Clermont will deal with recent research in this area.

Dr. Warren Nelson developed methods for the evaluation of the status of both spermatogenic and androgenic function of the testis by testicular biopsies. He made important contributions to the use of this technique and its implications in clinical medicine. During the course of this meeting the use of this technique will be mentioned many times. Newer methods for the evaluation of testicular function will also be considered by Dr. Mann and Dr. Hudson.

Dr. Nelson was particularly interested in genetic aspects of testicular abnormalities. This rapidly developing field of inquiry will be discussed on many occasions in the next few days but in particular when Dr. F. A. de la Balze discusses "Mixed Testicular

Dysgenesis" and Dr. J. K. Grant presents his paper on "Steroid Biosyntheses in Abnormal Testes".

Dr. Nelson and his associates were very much concerned with oestrogen production by the testis, particularly under the influence of gonadotropic hormone stimulation. Their pioneer studies indicated that the changes in oestrogen production and excretion by the testis which result from tropic hormone stimulation were more intense than changes in testicular androgen secretion. Oestrogen production by the testis will be considered in detail at this meeting by Dr. M. B. Lipsett.

This colloquium brings together workers representing many disciplines, all directing their efforts towards solving the problem of the endocrinology of the testis. I believe that this event will not take place unnoticed.

THE RELATIONSHIP BETWEEN THE STRUCTURE OF THE TESTIS AND DIFFERENTIATION OF THE EXTERNAL GENITALIA AND PHENOTYPE IN MAN

J. E. JIRÁSEK

Institute for the Care of Mother and Child, Prague, Czechoslovakia

THE influence of the testis on the phenotype depends upon testicular hormone production and on the ability of the target tissues to respond adequately to hormonal stimuli. Experiments on mammals (Jost, 1958; Burns, 1961) support the view that in the foetal period of life the testicle controls regression of the Müllerian ducts and masculinization of the external genitalia. After birth and especially at puberty the correlation between phenotypical signs and testicular development is well known.

We have studied the relationship between structure of the testicles and differentiation of the genital ducts and external genitalia, and some other phenotypical signs, in 60 normal human embryos and 22 male hermaphrodites.

As regards the morphological problems of the development of the testicles, genital ducts and external genitalia, the following studies should be mentioned: Felix (1912), Spaulding (1921), Wilson (1926), Stieve (1930), Vilas (1933), Koff (1933), Witschi (1948), Gillman (1948), Gruenwald (1942), Tonutti (1960), and Watzka (1963). Some interesting cytological and histochemical descriptions have been given by McKay and co-workers (1953), Rossi, Pescetto and Reale (1957), Jirásek (1962), Jirásek and Raboch (1963), Baillie, Niemi and Ikonen (1965), and Mancini and co-workers (1965). The problems of hermaphroditism are summarized in the books edited by Jones and Scott (1958) and Overzier

(1963), and in the papers of Jones and Zourlas (1965) and Zourlas and Jones (1965).

MATERIAL AND METHODS

The development of the gonads, genital ducts and external genitalia was studied in 60 human foetuses from therapeutic abortions. Their crown-rump (CR) lengths varied from 12 to 73 mm. External genitalia were photographed.

Data from patients whose testicular tissue was obtained through exploratory excision are given in Tables I and II. Sex chromatin in the buccal smears was negative in all of them. The following histochemical techniques were employed: NAD diaphorase (NADd) and lactic dehydrogenase (LDH) were demonstrated in frozen sections after a short fixation in cold calcium-formol. For alcohol dehydrogenase (ADH), succinic dehydrogenase (SDH), glucose-6-phosphate dehydrogenase (G-6-PDH) and 3β-ol steroid dehydrogenase (3β-ol SDH), fresh-frozen sections were used. All dehydrogenases were stained by means of nitro blue tetrazolium (nitro-BT) according to the methods of Hess, Scarpelli and Pearse (1958) and Allen (1960). Hydrolytic enzymes and periodic acid-Schiff (PAS)-positive substances were determined in paraffin sections. These tissue specimens were fixed in calcium-formol (1 hr.), washed in saline (1 hr.) and dehydrated in acetone (22 hr.). All steps were performed at 2–4° C. The specimens were cleared in benzene and had two paraffin baths (15 min. at 54°) before being embedded.

Acid phosphatase was followed by the Gomori method (see Pearse, 1960, p. 881), alkaline phosphatase and non-specific esterases by means of azo-linking. Naphthol AS-phosphate, Fast Red TR Salt and Fast Blue RR were used for the alkaline phosphatase. For the non-specific esterases sections were incubated with α-naphthylacetate and Fast Blue B Salt at pH 7·4. Reticular fibres were impregnated by Gomori's silver impregnation method

Table I

DATA FROM PATIENTS WITH FEMALE EXTERNAL GENITALIA (COMPLETE MALE HERMAPHRODITISM)

Case no.	*Patient and age*	*Testis*		*Vagina (cm.)*	*Breasts*	*Hormonal findings*	*Karyotype*
		Left	*Right*				
1	H.N. 10	Inguinal	Abdominal	3	0	$G < 4$	46/XY
2	L.N. 12	Abdominal	Inguinal	2	0	n	n
3	A.H. 12	Inguinal	Inguinal	5	0	$G < 4$	46/XY
4	A.K. 17	Absent	Inguinal	0	0	17-KS: 8·3 $G < 4$ 17-OHC: 5·6	n
5	J.H. 16	Abdominal	Abdominal	3	Female	n	n
6	E.M. 16	Abdominal	Abdominal	2	Female	17-KS: 10·2 oestrogens 30·4 μg./24hr. G: 10	46/XY
7	J.P. 17	Abdominal	Abdominal	3	Female	n	n
8	V.S. 18	Inguinal	Inguinal	2	Female	17-KS: 7·6	46/XY
9	B.L. 18	Abdominal	Abdominal	5	Female	n	n
10	L.M. 22	Inguinal	Inguinal	10	Female	17-KS: 9·2 G: 16	n
11	A.B. 24	Abdominal	Abdominal	5	Female	n	n
12	J.P. 31	Abdominal	Absent	7	Female	17-KS: 8·2	46/XY

No cases had axillary hair. Pubic hair was sparse or absent. Müllerian structures were absent. (See Table II for explanation of abbreviations, etc.).

TABLE II

DATA FROM PATIENTS WITH MALFORMED EXTERNAL GENITALIA (INCOMPLETE MALE HERMAPHRODITISM), AND NO FEMALE BREAST DEVELOPMENT

Case no.	Patient and age	Gonads		Tubes, uterus	Vagina (cm.)	Hair		Hormonal findings	Karyotype
		Left	Right			Pubic	Axillary		
13	J.D. 3	Streak	Inguinal testis	++	o	o	o	17-KS: 0·22	45/XO 46/XY
14	M.V. 16	Streak	Abdominal testis	+	5	+	o	17-KS: 6·5 G:8	45/XO 46/XY
15	M.R. 16	Abdominal testis	Abdominal testis	+	o	+−	o	17-KS: 7·8 G<64	n
16	A.K. 16	Abdominal testis	Abdominal testis	+	5	+	o	n	n
17	V.J. 13	Gonado-blastoma	Abdominal testis	−+	o	+	+	17-KS: 4·4 G<64	n
18	J.H. 20	Abdominal testis	Dysgermi-noma	−+	o	+	+	n	n
19	C.H. 13	Abdominal testis	Abdominal testis	o	o	+−	+−	n	n
20	M.P. 15	Inguinal testis	Inguinal testis	o	o	+	+	n	n
21	A.K. 23	Abdominal testis	Abdominal testis	o	o	+	+	17-KS: 10·8 G<64	n
22	A.Z. 25	Inguinal testis	Inguinal testis	o	o	+	+	n	n

Abbreviations used: 17-KS: 17-ketosteroids in mg./24 hr.
17-OHC: 17-hydroxycorticosteroids in mg./24 hr.
G: gonadotropins in mouse uterus units/24 hr.
n: not examined.
o: Absent.
+−: sparse hair.
−+: on the side of the tumour Müllerian structures were present, whereas on the side of the testicle there were none.

(Gomori, 1937). Glycogen was distinguished from the other PAS-positive substances by saliva digestion.

The lipids were stained in frozen sections after calcium formol fixation with Sudan Black B.

RELATIONSHIP BETWEEN THE DIFFERENTIATION OF THE TESTICLES, GENITAL DUCTS AND EXTERNAL GENITALIA IN THE FOETAL PERIOD

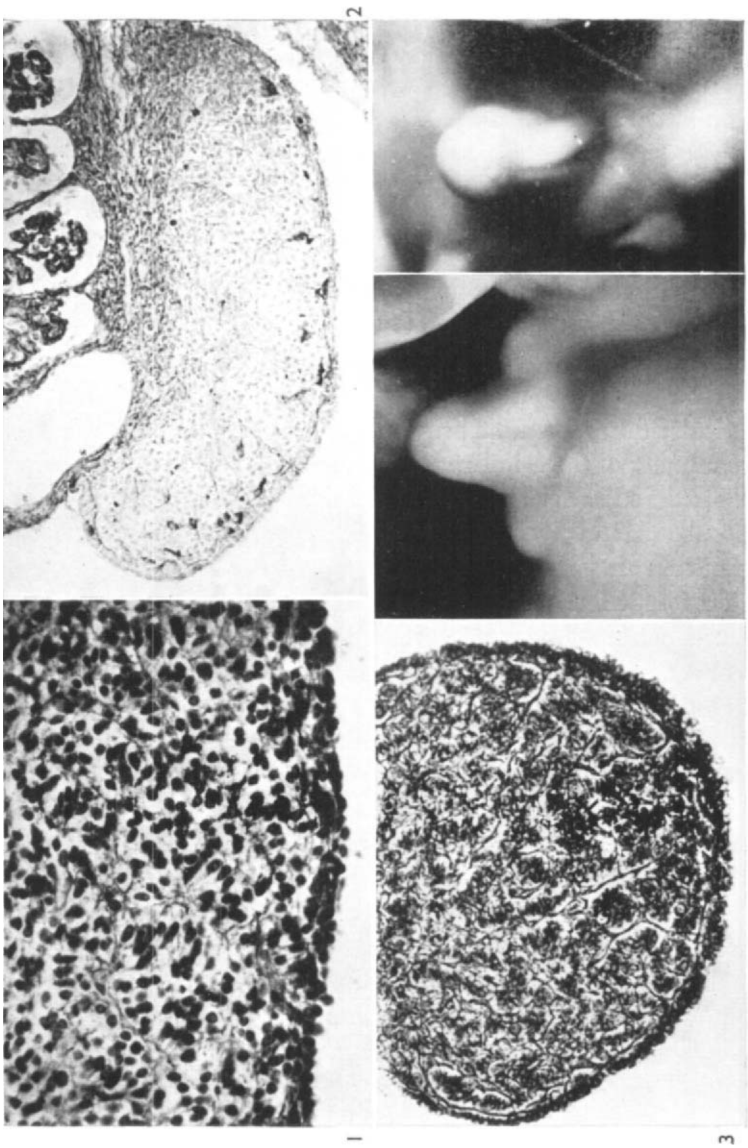
Formation of the testicular cords (Figs. 1–3) was observed in embryos of 15 to 20 mm. CR length (XVIII–XIX Streeter's horizon; Streeter, 1951) and it consists of the differentiation of reticular fibres in the blastemic anlage of the gonads (Fig. 1). Reticular fibres form the primitive tunica albuginea and the basal membranes of the cellular cords (Fig. 3). At this time only single cells in the superficial layers of the testicular anlage contain alkaline phosphatase and glycogen (Fig. 2). Characteristic round primordial germ cells are very rare.

Testicles in this period are the only organs showing sexual differentiation. External genitalia (Figs. 4, 5) are indifferent and have the same appearance in both male and female embryos.

Testicles in embryos of 20 to 30 mm. CR length (XX–XXIII Streeter's horizon) are characterized by the well-differentiated testicular cords containing numerous round germ cells with alkaline phosphatase and glycogen. Undifferentiated cells of the testicular cords (primitive cells of Sertoli) show high NADd, LDH and G-6-PDH activity and in this developmental phase also acid phosphatase activity. The oxidative enzymes show less activity in those parts of cords which are anlage of the rete (Fig. 6).

The interstitial spaces between the cords and under the surface epithelium are filled with mesenchymatous connective tissue. No epithelioid interstitial cells are present. No 3β-ol SDH activity was proved in this period.

External genitalia of these embryos (Figs. 7, 8) are characterized by a prominent large phallus, the underside of which has a urethral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groove. On both sides of the phallus there are labioscrotal swellings. No sexual differences were noticed.

Testicles in the foetuses of more than 30 mm. CR length (30 to 73 mm.) contain epithelioid interstitial cells. Histochemically they are characterized by the presence of acid phosphatase in the paranuclear zone (Fig. 9). Acid phosphatase, a lysosomal enzyme whose role in general is not exactly known, appears during the period when the fibroblast-like mesenchymal cell is being transformed into the foetal epithelioid interstitial cell of Leydig. All foetal interstitial epithelioid cells (even in the foetuses of 33 mm.) were rich in NADd, ADH, LDH, SDH and G-6-PDH (Fig. 10). They also contained 3β-ol SDH (Fig. 11). Their cytoplasm stains with Sudan dyes, suggesting the presence of lipids. Some of them showed activity of organophosphate-sensitive non-specific esterases and alkaline phosphatase; alkaline phosphatase was present in their cell membranes and in the paranuclear zone.

In foetuses of 30 to 73 mm. CR length the number of Leydig cells increases with age. Masculinization of the external genitalia was observed in foetuses of more than 40 mm. CR length. This consists of the lengthening of the anogenital distance and in the formation of the raphe of the scrotum and penis (Figs. 12–14). In normal human foetuses masculinization of the external genitalia is preceded by differentiation of the epithelioid interstitial cells, which have enzymic equipment which enables them to take part in the metabolism of steroids. No masculinization occurs until epithelioid interstitial cells are present.

FIGS. 1–5. DIFFERENTIATION OF THE TESTICULAR CORDS.

FIG. 1. Reticular fibres in the gonadal anlage of an embryo of 15 mm. Gomori's silver impregnation. × 133.

FIG. 2. Glycogen-containing cells (dark) in the testicle of an embryo of 15 mm. Typical germ cells are completely absent. × 66.

FIG. 3. Reticular fibres in the basal membranes of the testicular cords and primitive tunica albuginea. Embryo of 19 mm. Gomori's silver impregnation. × 66.

FIG. 4. External genitalia of male embryo (16 mm.) in the period of differentiation of the testicular cords.

FIG. 5. As for Fig. 4; embryo of 19 mm.

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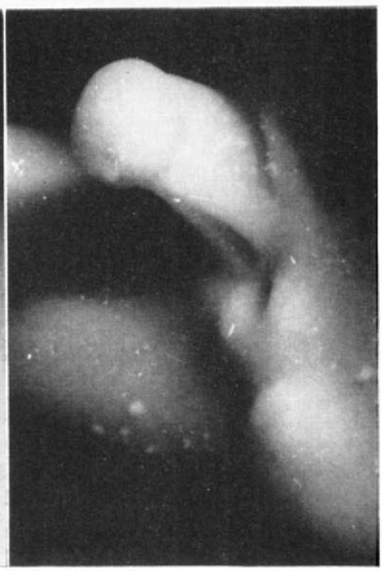

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We found regressive changes of Müllerian ducts in the embryos of 31 to 35 mm. CR length (Fig. 15). This is during the period when Leydig cells start to appear in the testicles with well-formed germinal cords, and no signs of masculinization are yet to be seen in the external genitalia. The lumen of the Müllerian ducts disappears first in the middle parts immediately before the ducts fuse. Both Müllerian ducts had completely disappeared in the foetuses of 43 mm. and more.

The Wolffian ducts (Fig. 16) had disappeared in the female foetuses of 51 to 55 mm. CR length and more, that is, at the time when distinct masculinization of the external genitalia takes place in the male foetuses. This suggests that regression of the Wolffian ducts takes place in every case, provided development has not been influenced by foetal androgens up to this time (about 70 days).

Following the above study, attention was paid to the testicles of persons with no or insufficient foetal masculinization, i.e. to patients with feminine or malformed external genitalia (the malformation consisted of the presence of a phallus or hypertrophied clitoris, and scrotiform labia; neither raphe of the scrotum nor penis was present).

TESTICLES IN PATIENTS WITH NORMAL FEMALE EXTERNAL GENITALIA (COMPLETE MALE HERMAPHRODITISM)

(a) *In children (prepuberal form of complete male hermaphroditism—Fig. 17)*

In the testicles of two of our cases (Nos. 1 and 2) we found no pathological changes, except absence of gonocytes in most non-

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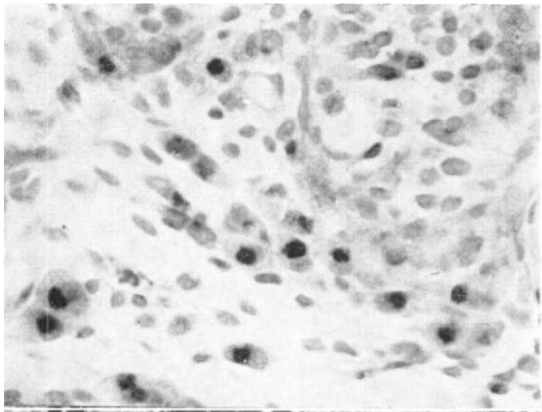
FIG. 6. Differentiation of the mesenchymatous interstitial tissue and primitive tunica albuginea in an embryo of 26 mm. High G-6-PDH activity in the testicular cords. × 100.

FIG. 7. External genitalia of male embryo (26 mm.) in the period of differentiation of the testicular interstitial spaces and primitive tunica albuginea.

FIG. 8. As for Fig. 7; embryo of 28 mm.



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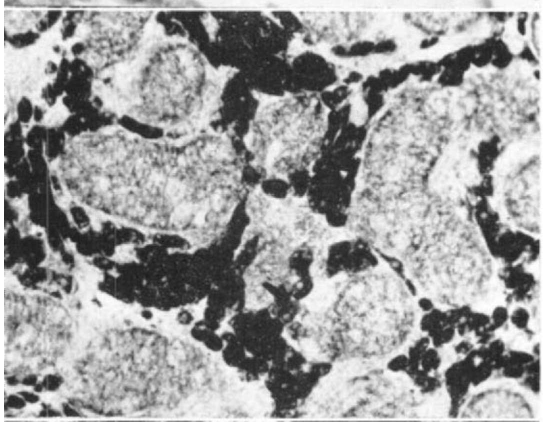

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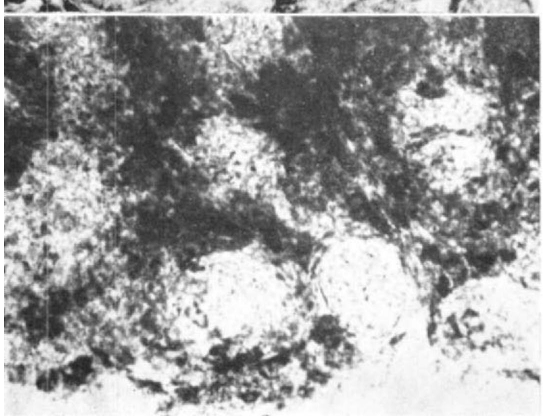

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canalized seminiferous tubules (Fig. 18). The third case had some degenerating seminiferous tubules. No epithelioid interstitial cells were observed in any of these testicles. No 3β-ol SDH was detected. Gonocytes, if present, did not show any alkaline phosphatase activity.

Phenotypically these children did not differ from normal girls of the same age. The testicles were occasionally found during medical examinations for other reasons than hermaphroditism. Genital ducts in all these patients were represented by a blind vagina. Müllerian structures were absent.

(b) *A testicle with a characteristic structure (corresponding to the testicles of hypogonadotropic eunuchoids)*

Such a testicle was found in the right inguinal channel of a 17-year-old eunuchoid patient with female external genitalia and total vaginal aplasia (case 4, Figs. 19–21). The seminiferous tubules were lined with immature Sertoli cells and well-differentiated spermatogonia. Basal membranes of the tubules were somewhat thickened. Interstitial connective tissue was loose and had no epithelioid Leydig cells (Fig. 20). The immature Sertoli cells showed NADd, LDH (Fig. 21) and G-6-PDH activity, some glycogen, and small lipid droplets. In the spermatogonia weak non-specific esterase and alkaline phosphatase activity was found. No 3β-ol SDH was detected in any component of this testicle.

At laparotomy no genital ducts were observed. The case was described (Jirásek, 1966) as a hypogonadotropic eunuchoid form of testicular feminization (eunuchoid form of complete male hermaphroditism). This observation confirms that the absence of the Leydig cells, unless correlated with adrenal hyperfunction or

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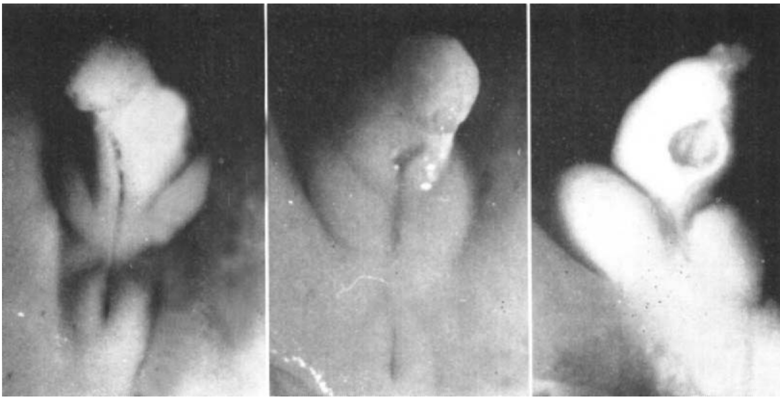
FIG. 9. Granules with acid phosphatase in the paranuclear zone of the foetal Leydig cells. Foetus of 35 mm. × 200.

FIG. 10. Distribution of G-6-PDH (a similar picture is shown by NADd and LDH) in the testicle of a foetus of 55 mm. (about 67 days). × 66.

FIG. 11. 3β-ol SDH in the interstitial cells of the testicle. Foetus of 55 mm. × 66.



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FIGS. 12–14. MASCULINIZATION OF THE EXTERNAL GENITALIA IN MALE FOETUSES.

FIG. 12. Lengthening of the anogenital distance in a foetus of 40 mm.

FIG. 13. Fusion of the labioscrotal swellings in a foetus of 45 mm.

FIG. 14. Scrotum and a "physiological penile hypospadia" in a foetus of 53 mm.

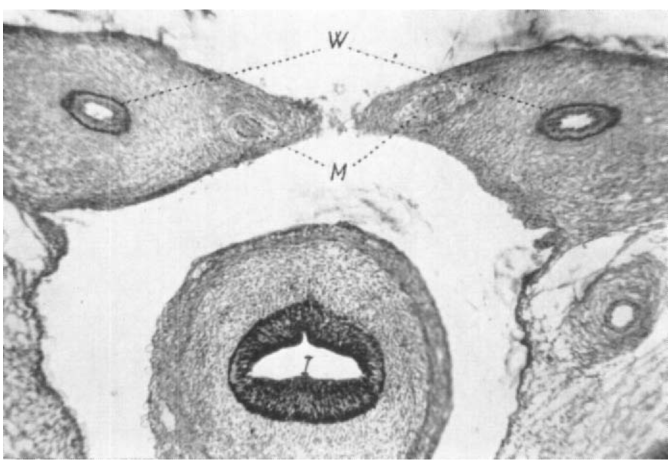

FIG. 15. Genital ducts in a male foetus of 31 mm. Regressive changes of the Müllerian ducts (M). Dark staining of the epithelium of the Wolffian ducts (W) and intestine (I) is caused by glycogen. PAS reaction. ×22·5.