PREGNANCY DISTURBANCES IN RATS BY THE ADMINISTRATION OF LARGE DOSES OF SEX HORMONES

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While adequate amounts of sex hormones are necessary for conception and maintenance of a normal course of pregnancy, many investigators have reported that sex hormones administered in large doses caused two kinds of pregnancy disturbances; the first, preventing conception by suppressing ovulation and interrupting the processes of fertilization, tubal transport and nidation of fertilized ovum (Smith, 1926; Parkes and Bellerby, 1927; Burdick *et al.*, 1940), the second, disturbance in the course of pregnancy by abortion, fetal death (Hugget and Pritchard, 1945; Jost, 1945), fetal intersexuality and prolonged period of pregnancy.

Large doses of progesterone which has recently been widely used in gestation occasionally produced female hermaphrodites. However, the correct dosage, term, and period of administration of sex hormones, as well as the mechanism of such pregnancy disturbances, have not yet been fully established. The present study was therefore undertaken to contribute to the elucidation of these questions with particular emphasis on the action of sex hormones in interrupting pregnancy and influencing the fetus, using rats as experimental animals.

MATERIALS AND METHODS

Adult female albino rats, weighing 170~230 g, were used. They were impregnated and divided into 5 experimental groups and 1 control group as shown in Table 1. The coital date was known for all animals. Several kinds of steroids; progesterone*, testosterone*, androsterone*, Δ^4 -androstenedione* and dehydroepiandrosterone*, one dose being dissolved in 0.1 cc olive oil, were given daily subcutaneously. The controls were injected subcutaneously with 1 cc of olive oil alone. These steroids were given to the pregnant rats for varying periods, in the various doses, and in various stages of pregnancy. During the period of injections, the animals were weighed and vaginal bleeding checked. In cases of unsuccessful pregnancy as indicated by vaginal bleeding or failure of body weight to increase, the pregnant animals were sacrificed by decapitation to study their fetus and placenta grossly and histologically.

One or two newborns from rats which showed no sign of abortion and completed normal delivery were sacrificed to study the presence of any deformity grossly and histologically. The remaining living animals were given to the foster mother, to be examined similarly after maturity (age 70~100 days or older).

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RESULTS

1) *The Action of Interrupting Pregnancy*

Administration of several sex hormones; progesterone, testosterone, androsterone, Δ^4 -androstenedione, dehydroepiandrosterone, in various doses in various periods of pregnancy, caused abortion as indicated in Table 1 (A).

A) *Progesterone* Percentage of normal delivery in animals given 0.5 mg of progesterone was similar to that in the control group. When the dose was increased, however, signs of interrupted pregnancy such as vaginal bleeding and decrease of body weight were observed in many rats, until interruption occurred in practically all animals with doses above 3 mg.

Table 1. Abortion and production of female intersexuality of the offspring after the administration of progesterone, testosterone, androsterone, Δ^4 -androstenedione and dehydroepiandrosterone in large dosage to pregnant rats

(A) Abortion of pregnant rats

Substance	Single dose (mg)	Total dose (mg)	No. of animals injected	Days treated	No. of abortion	No. of livebirth
Progesterone	0.5	5~ 7	4	12~16	0	4
	2.0	16~ 26	3	8~13	2	1
	3.0	18~ 27	4	6~ 9	3	1
	3.0	36	2	12	2	0
	5.0	30	1	6	1	0
	5.0	60~ 85	4	12~17	4	0
	5.0	105~115	4	21~23	4	0
	10.0	110	1	11	1	0
Testosterone	0.3	2.4~ 2.7	3	7~ 9	0	3
	1.0	5~ 9	3	5~ 9	0	3
	2.0	12	2	6	1	1
	3.0	12~ 33	3	4~11	3	0
	3.0	57~ 72	5	19~24	5	0
	5.0	15~ 40	3	3~ 8	1	2
	10.0	30~ 70	2	3~ 7	2	0
	20.0	60~200	8	3~10	7	1
Androsterone	0.5	2~ 9	3	4~19	1	2
	2.0	12~ 18	3	6~10	0	3
	10.0	70~110	5	7~11	2	3
	20.0	160~240	2	8~12	0	2
Δ^4 -Androstenedione	0.5	6~ 9	4	14~18	2	2
	10.0	40~80	4	4~ 8	2	2
	20.0	80	1	4	0	1
Dehydroepiandrosterone	0.5	6.5~ 7.5	4	13~15	2	2
	10.5	40~130	4	5~14	3	1
Olive oil	0.1 cc	0.7~1.7 cc	3	7~14	0	3
	1.0 cc	9.0 cc	1	9	0	1

(B) Production of female intersexuality of the offspring of pregnant rats

Substance	Single dose (mg)	Total dose (mg)	Malformation							
			Slight degree of masculinization	Moderate degree of masculinization					High degree of masculinization	
				Prostate	Atresia vaginae	Hypospadias urethrae	Vagino-urethral fissula	Seminal vesicle present	Vas deferens present	Epididymis present
Progesterone	0.5	5 ~ 7	0	0	0	0	0	0	0	0
	2.0	16 ~ 26	+	0	0	0	0	0	0	0
	3.0	18 ~ 27	+	+	+	+	+	+	+	+
	3.0	36	No livebirth							
	5.0	30								
	5.0	60 ~ 85								
	5.0	105 ~ 115								
	10.0	110								
Testosterone	0.3	2.4 ~ 2.7	0	0	0	0	0	0	0	0
	1.0	5 ~ 9	+	0	0	0	0	0	0	0
	2.0	12	+	+	+	+	+	0	0	0
	3.0	12 ~ 33	No livebirth							
	3.0	57 ~ 72								
	5.0	15 ~ 40	+	+	+	+	+	+	+	+
	10.0	30 ~ 70	No livebirth							
	20.0	60 ~ 200	+	+						
Androsterone	0.5	2 ~ 9	0	0	0	0	0	0	0	0
	2.0	12 ~ 18	+	0	0	0	0	0	0	0
	10.0	70 ~ 110	+	+	+	+	+	+	+	+
	20.0	60 ~ 240	+	+	+	+	+	+	+	+
Δ^4 -Androstenedione	0.5	6 ~ 9	0	0	0	0	0	0	0	0
	10.0	40 ~ 80	+	+	+	+	+	0	0	0
	20.0	80	+	+	+	+	+	+	+	+
Dehydroepiandrosterone	0.5	6.5 ~ 7.5	0	0	0	0	0	0	0	0
	10.5	40 ~ 130	0	0	0	0	0	0	0	0
Olive oil	0.1 cc	0.7 ~ 1.7 cc	0	0	0	0	0	0	0	0
	1.0 cc	9.0 cc	0	0	0	0	0	0	0	0

"+" indicates presence of the structure and "0" indicates absence.

On dissection, the animals showed signs of interrupted pregnancy at various stages such as placental hemorrhage, ablatio placentae, or sanguinous fetus of

various sizes, depending upon the time after abortion. Correlating the doses and the term of pregnancy, it was found that the earlier the stage of pregnancy was, the smaller was the dose of progesterone necessary to cause abortion.

B) *Testosterone* No abortion was found in the group given testosterone in doses below 1.0 mg, but as the dose was increased, the rate of abortion increased in number. Similar correlation was found between the dose and the term of pregnancy as in the group of progesterone.

C) *Androsterone, Δ^4 -androstenedione, dehydroepiandrosterone* The action of interrupting pregnancy was also observed in these weak androgenic steroids. However, comparing with other groups, the aborting action was so weak that even with large doses some of the animals completed a normal course of pregnancy.

2) *Production of Experimental Intersexuality*

The administration of progesterone, testosterone, androsterone, and Δ^4 -androstenedione in doses too small to interrupt the pregnancy in pregnant rats showed a marked effect on the sexual development of the genetic female embryo and intersexual or masculinized female offsprings were produced under the condition as shown in Table 1 (B). The urogenital apparatus of the newborn and young intersex was subjected to gross and microscopic observations 50 days after birth, in comparison with normal newborn and the young as control.

A) *Presentation of findings*

a) *The urogenital apparatus of normal rat* (Figs. 1, 2, 3 and 4)

One or two females were killed immediately after birth, fixed in 10% formalin solution, and examined under dissecting microscope. Representative newborns were serially cut into transverse sections of 10 μ , and subjected to histological examination.

i) Findings of the genital apparatus in the normal newborn female rat:

At birth the phallus of the normal female is hypospadiac. Later, closure of the cleft in the phallus results in the clitorine urethra which characterizes the adult female rat, which has certain homologues of male structures less well developed than in the normal male.

The ovaries of the normal newborn are semi-lobulated, oval and situated just lateral to the caudal pole of the kidney and are almost completely surrounded by the ovarian capsule.

The oviducts, at birth, are not greatly convoluted but enlarge to form the uteri which follow an oblique course medial- and caudalwards. At about the level where the ureters join the bladder, the uteri fuse to form the vagina, which descends dorsally to utethra.

ii) Findings in the normal adult female rat:

In the normal adult the genital organs have essentially the same relation as in the previously described normal newborn. The oviducts become highly convoluted and partially cover the ovary; the cervixes develop at the junctions of the uteri with the vagina. A complete separation of the vaginal epithelium from the dorsal urethral epithelium takes place, the vagina becomes canalized between 40 and 50 days after birth, and opens to the exterior.

The normal clitoris is composed of a small glans completely enclosed by a preputium which is fused to it. The urethra extends through the clitoris to the

PLATE 1

EXPLANATION OF THE PLATES

(PLATE 1)

Schematic drawing of urogenital system of rat

- Fig. 1. Normal female. Frontal view of urogenital system
Fig. 2. Normal female. Sagittal section of cephalic end of normal female urethra
Fig. 3. Normal male. Frontal view of urogenital system
Fig. 4. Normal male. Sagittal section of cephalic end of normal male urethra
Fig. 5. Intersex. Frontal view of urogenital system
Fig. 6. Intersex. Sagittal section of cephalic end of intersexual urethra

end of glans. The distal portion of the functional urethra is a canal formed by the preputium which continues beyond the end of glans.

b) The urogenital apparatus of the intersexual rat (Figs. 5 and 6)

The changes obtained in the intersexual animals differ in degree according to the dosage of sex hormones given to the pregnant mother. The same kind of changes were obtained in the cases of all kinds of hormonal substances used. The following description applies to the most highly modified animals obtained with high dosages of the hormones.

i) Findings in the newborn intersexual rat :

1) Microscopic observations: The ovaries of the normal newborn are completely surrounded by the ovarian capsule (Fig. 7), but the development of those of the intersexual rat is inhibited; consequently the gonads are bare (Fig. 8). In the sections, the vasa deferentia are found to be reserved along their entire course. Cranially, in the region of the gonad, the vasa become convoluted, forming a fairly well developed epididymis which leads to the vasa deferentia. A pair of seminal vesicles, comparable in structure with those of the normal male except being shorter, join the vasa to form the ejaculatory ducts which empty into the urethra on the lateral sides of a median dorsal projection into the lumen of the urethra (Figs. 9, 10 and 11). This projection is identical with Müllerian tubercle found in the male newborn. Dorsally to the urethra, the uteri join to form the upper vagina as in the normal female (Figs. 1 and 2). Caudally, the upper vagina bifurcates in the Müllerian tubercle and connects with the urethra or urogenital sinus (Figs. 10 and 11). The urogenital sinus in these animals is not constricted dorso-ventrally in the intersexual animals. The upper vagina represents the fused Müllerian ducts; the bifurcation at the end of the upper vagina is a retention of embryonic terminations of the Müllerian ducts.

The urethra presents a typically male picture. Numerous prostatic diverticula arise from the urethral epithelium and extend out into the surrounding mesenchyme. All the divisions of the prostate found in the normal male, the coagulating glands, the dorsal and the ventral prostatic lobes are represented in the intersexual animals (Fig. 9, 10 and 11).

2) Gross observations: Externally the intersexual animals are indistinguishable from their male litter mates: They differ from normal females in that the primary urogenital ostium is absent and the phallic urethra is almost completely formed. The ano-phallic distance is as long as in the male.

The gonads are displaced caudally. With the highest dose they lie halfway between the lower pole of the kidney and the top of the bladder.

PLATE 2

EXPLANATION OF THE PLATES

(PLATE 2)

- Fig. 7. Normal newborn female. Cross section through gonad region. $\times 100$
 Fig. 8. Newborn intersex. Cross section through gonad region. Treatment given to the mother: 27 mg progesterone from the 11th to 19th day of pregnancy. $\times 100$
 Fig. 9. Newborn intersex. Cross section from region below fusion of uterus to form upper vagina. Treatment given to the mother; the same as Fig. 8. $\times 400$
 Fig. 10. Newborn intersex. Cross section from region at the end of upper vagina. Treatment given to the mother; the same as Fig. 8. $\times 400$
 Fig. 11. Newborn intersex. Cross section from region where ejaculatory ducts join the urethra, at a level slightly caudal to that shown in Fig. 10. $\times 400$

Abbreviations

BL.: bladder	OV.: oviduct
C.: ovarian capsule	P.PR.: posterior prostate
C.G.: coagulating gland	R.: rectum
E.P.: epididymis	S.V.: seminal vesicle
E.D.: ejaculatory duct	U.: urethra
E.F.: efferent tubules	U.R.: ureter
M.D.: Müllerian duct	UT.: uterus
MES.: mesentery	V.: vagina
O.: ovary	V.D.: vas deferens
OS.: ostium	V.PR.: ventral prostate

Normally, the gonad is semi-lobulated at birth, but in animals treated with the highest dose, it has the simple, elongated kidney-bean shape which is characteristic of the gonad of an 18 day fetus.

The oviducts and the uteri appear to be normal except for some shortening of the latter. Accompanying each uterus and lying in the same mesentery is a grossly visible vas deferens which joins the urethra just below the neck of the bladder. When the bladder is pulled forward and downward the seminal vesicles may be seen lying dorso-lateral to the vasa deferentia. When the ventral abdominal wall is dissected away from the base of the bladder and the upper urethra, prostatic lobes are found closely connected with the urethra at the bladder.

ii) Findings in the intersexual adult rat:

1) Microscopic observation: As in the newborn intersexual rat, development of the ovarian capsule is inhibited and the gonads are bare. In serial sections of the ovary, many primary follicles and corpora lutea were observed, without showing testis-like structure (Fig. 12). The prostates and seminal vesicles of the intersexual animal are smaller than in normal male (Fig. 13), with essentially the same structure (Fig. 14). The ventral prostates which were studied in detail showed no evidence of low to medium cuboidal cells without clear areas. The seminal vesicle appears normal in showing compound formation of tubular glands. The villi, which are highly developed in the functioning gland, are poorly developed or not high-columnar as in the normal.

The lower vagina is absent, but the existing portion of the vagina bifurcates at its caudal end and joins the urethra (Fig. 15), as in the newborn intersexual rat.

PLATE 3

EXPLANATION OF THE PLATES

(PLATE 3)

- Fig. 12. Adult intersex. Section through gonad. Treatment given to the mother: 40 mg testosterone propionate from the 12th to 19th day of pregnancy. $\times 100$
- Fig. 13. Normal adult male. Section through ventral prostate and seminal vesicle. $\times 100$
- Fig. 14. Adult intersex. Section through upper vagina. Treatment given to the mother: the same as Fig. 12. $\times 100$
- Fig. 15. Adult intersex. Section through vagino-urethral junction where the caudal end of bifurcated upper vagina and ejaculatory duct join the urethra. Treatment given to the mother: the same as Fig. 12. $\times 400$

2) Gross observations: Externally the adult intersexual rat appears masculine through the presence of a fairly well developed, evertable penis and the absence of vaginal introitus in comparison with normal adult female (Fig. 16) except for the absence of scrotum (Fig. 17).

On dissection, the gonads of the intersexual animal are displaced caudally from the base of the kidney by about one-fourth to one-third of the distance between the base of the kidney and the base of the bladder. The gonad itself is bare because of the inhibition in the ovarian capsule formation. The uteri, as a consequence of the gonadal displacement, are shorter than in the normal female. Lateral to the cranial end of the uterus is a mass of highly convoluted fine tubules which forms the cauda epididymidis. From the cauda epididymidis, the vas deferens of smaller caliber than the uterus, extends caudally, lateral to the uterus. The uteri meet in the midline, dorsally to the bladder. Each uterus terminates in a cervix and opens into the single, median upper vagina. The lower vagina is absent.

Ventral to the base of the bladder is the bilobed ventral prostate, dorsolateral to the upper vagina, the seminal vesicles and coagulating glands (Figs. 18 and 19), and dorsolateral to the urethra are the posterior lobes of the prostate found.

The structure of the penis resembles that of the normal male, being well developed in the rat given a large dose of sex hormone.

B) Variation in the degree of masculinization

The description of the intersexual rat in the preceding section refers only to the highest degree of abnormality obtained with large doses of hormones given at appropriate periods.

The degree of masculinization and the effect of treatment are shown in Table 1 (B).

Rats were divided into 3 groups according to the degree of masculinization.

a) Slightly masculinized group

With lower doses of progesterone (2 mg), testosterone (1 mg) or androsterone (2 mg), the Wolffian ducts and their derivatives are less well developed, showing unilateral or rudimentary growth. In these animals, only the ventral prostatic lobes are well developed, whereas the seminal vesicle and posterior prostatic lobe are formed unilaterally or absent.

b) Moderately masculinized group

In the group given 2 mg of testosterone or 10 mg of Δ^4 -androstenedione moderately masculinized offsprings with well-developed ventral end of the posterior prostatic lobe and the caudal end of the bifurcated upper vagina joining the urethra were produced. In animals treated with lower doses, however, the communications were non-patent, so that the upper vagina ended blindly. This non-patent state of the vagino-urethral junction has been observed in serial section in newborn animals. As a consequence, in the adult litter mates of these animals, there is a retention of uterine and vaginal secretions so that the uteri and the upper vagina are greatly distended (Fig. 20). In a few animals of this group the perineal region was bulged outward as the result of the pressure exerted by the tremendously distended vagina.

PLATE 4

EXPLANATION OF THE PLATES
(PLATE 4)

Fig. 16. Normal adult female. External genitalia

Fig. 17. Adult intersex. External genitalia. Treatment given to the mother: 40 mg testosterone propionate from the 12th to 19th day of pregnancy

Fig. 18. Normal adult female. Frontal view of genital tract

Fig. 19. Adult intersex. Frontal view of genital tract. Treatment given to the mother: the same as that in Fig. 17

Fig. 20. Adult intersex. Frontal view of genital tract, particularly, showing tremendously distended uterus and vagina as a result of retention of uterine and vaginal excretion.

Treatment given to the mother: 100 mg androsterone from the 11th to 20th day of pregnancy

c) The most highly masculinized group

In the group given 3 mg of progesterone, more than 5 mg of testosterone, more than 10 mg of androsterone, or 20 mg of Δ^4 -androstenedione, the Wolffian ducts and their derivatives were most highly developed, showing the genital sinus, such as the bilateral ductus deferens, a male-like prostate and a hypertrophied clitoris with penis-like structure. (Figs. 1 and 19).

C) Correlation between treatment and degree of masculinization

The variations in the degree of masculinization, when arranged in order of increasing magnitude, show that there is a definite correlation between the degree of change and the treatment which involves 3 factors; 1) quality, 2) dosage and 3) period of pregnancy during which the treatment is given, as shown in Table 1.

In general, hormones less powerful on androgenic action such as progesterone and androsterone and Δ^4 -androstenedione showed lesser degree of masculinization. A higher degree of masculinization was found in the offsprings of mothers given higher doses of the same hormone, provided that pregnancy disturbances, as abortion or fetal death did not occur. The highest degree of masculinization was obtained when the administration of hormone started after the 11th to 13th day of pregnancy, while the same treatment caused a lesser degree of modification if begun later in pregnancy. On the other hand, when the administration of hormone started before the 11th day, abortion or fatal death was the result in most cases.

In the developing female, both duct systems (Müllerian and Wolffian) are present on the 16th day, after which the Wolffian ducts gradually regress. Adequate hormone treatment started on the 11th to 13th day of pregnancy apparently holds this degenerative process in abeyance so that the entire Wolffian duct is retained and even stimulated to develop structures which would normally occur only in the male (epididymis and seminal vesicle). If, however, the treatment is not begun until the 16th or 17th day, the hormonal effect does not prevent the regression of the Wolffian duct, although the degenerative process may be somewhat arrested. Since involution begins near the cranial end of the duct and progresses caudally, the more caudal portions of the Wolffian duct are retained with treatment begun on the 16th or 17th day. This explains the presence of the seminal vesicle, derived from the most caudal portion of the Wolffian duct in the absence of other Wolffian derivatives.

Sexual differentiation of the urogenital sinus occurs relatively late in development. In the male, the prostatic complex begins to form between the 19th and the 20th day. In the female, the longitudinal fusion of the urogenital sinus into the lower vagina and urethra begins on the 20th day.

Hormonal intervention, if of sufficient intensity, can therefore alter some differentiations of the urogenital sinus in the genetic female even if introduced at a very late stage in pregnancy. The female tendency, that is, the formation of the lower vagina, is inhibited and the development of the prostatic anlage is stimulated so that the urogenital sinus assumes male characteristics.

Table 1(B) shows the correlation between the degree of masculinization and various treatment. This table presents the results obtained with a specific hormonal substance, to give a somewhat graphic representation of the findings in the intersexual animals. Under the heading for each structure the 2 symbols of "+" and "0" represent the conditions of the structures on the 2 sides of the body; + indicates presence and 0 indicates absence.

DISCUSSION

It has been reported that sex hormone administration to pregnant animals in large dosage caused following pregnancy disturbances. 1) Abortion or fetal death followed by absorption of fetus (Hugget and Pritchard, 1945), 2) fetal intersexuality (Greene *et al.*, 1938 a, b, c and d; Green *et al.*, 1939 a and b; Jost, 1954; May *et al.*, 1959; Suchowsky, 1960; Revesz *et al.*, 1960).

Concerning the abortion or fetal death by a large dose of sex hormones, Scipiades (1937) observed the fetal death after administration of 5 mg of testosterone a day in pregnant rats at the end of pregnancy. On the other hand, Hamilton and Wolfe (1938) proved disturbance of fetal growth following administration of androgen for 10 days in the first half period of pregnancy to mice. Courier and Jost (1953) observed fetal death when 20 mg of testosterone propionate was injected in pregnant rat from the 16th to 20th day of pregnancy. Dreisbach (1959) observed variable effect of sex hormones in rat fetus according to the period of pregnancy selected for administration.

Concerning the abortion or fetal death, the results of the present experiment confirmed these reports. In this experiment, however, progesterone, Δ^4 -androstenedione and dehydroepiandrosterone also produced masculinization of the fetus. Whether the masculinizing action is in proportion to the androgenic action yet remains to be determined.

The mechanism of abortion or fetal death in the animal given various sex hormones in large doses is not yet clear. D'Amour *et al.* (1934) considered that pregnancy disturbance by estrogen administration during pregnancy is due to the direct effect of estrogen to the fetus. In this experiment, living placenta was sometimes observed while the fetus was dead. This would suggest, if not prove, the direct effect of sex hormone on fetus.

Concerning the masculinizing effect of various hormones given to pregnant animals in large doses, many experiments have been reported indicating an inhibition of the development of the Wolffian duct by testosterone. Many experiments were also conducted attempting sex reversal by the administration of sex hormone to pregnant animal or fetus since Dantchakoff's (1937) experiment on the guinea pig. Opossum (Burns, 1942), guinea pig (Dantchakoff, 1937), rat (Greene, 1938a), field-mouse (Raynaud, 1947), golden-hamster (Bruner and

Witschi, 1946), hedgehog (Mombaerts, 1944), mole (Godet, 1949), rhesus monkey (Dantchakoff, 1950) were also used as experimental animals. In the experiments to produce masculinized female animals, the androgenic substance administered to the pregnant animal reach the developing embryo via the maternal and fetal blood streams. The Wolffian duct which normally regresses and disappears in the female not only persists but is stimulated to differentiate into typically male structures, epididymis, vas deferens and seminal vesicle. The urogenital sinus and genital tubercle, under the influence of the administered androgen, develop in a masculine manner. These structures, which normally give rise to the female urethra, lower vagina and clitoris develop in the masculine type of urethra, prostatic glands and external genitalia. However, androgenic substances have little effect on the development of the ovary or the Müllerian duct. In these experiments, as androgenic substance, testosterone, testosterone propionate, methyltestosterone, ethinyltestosterone, dehydroandrosterone, androstenedione, methylandrostenedione dipropionate, adrenosterone and progestational compounds such as progesterone, 17-ethinyl-19-nortestosterone and 6-methyl-17-acetoxy-progesterone have been used and all these substances except progesterone showed masculinizing effect of different potencies on fetus. Progesterone caused no abnormalities whereas both synthetic progestational compounds produced pseudohermaphroditism (Revesz *et al.* 1960).

In this experiments progesterone also showed similar masculinizing effect on the fetus to that of other androgenic substances. This would indicate the possibility of the presence of human female hermaphrodites produced by the administration of progesterone in large dosage in human gestation.

SUMMARY

Large doses of progesterone, testosterone, androsterone, Δ^4 -androstenedione and dehydroepiandrosterone were administered in pregnant rats to elucidate the action of these sex steroids disturbing pregnancy. Absorption or death of the fetus was observed by the administration of a relatively larger doses of these steroids, while various degrees of masculinization of the genetic female offspring was produced by the administration of smaller doses of these steroids except dehydroepiandrosterone. The degree of masculinization was found to be related with the dose of these sex hormones and also with the period of pregnancy during which they were administered. The highly modified fetus and offspring have oviduct, upper vagina combined with epididymis, vasa deferentia, seminal vesicle, prostate and penis.

REFERENCES

- Bruner, J. A. and E. Witschi (1946). *Anat. Record* **94**, 367.
Burdick, H. O., B. Emerson and R. Whitney (1940). *Endocrinology* **26**, 1081.
Burns, R. K. (1942). *Cold Spring Harbor Symposia Quant. Biol.* **10**, 27.
Courier, R. and A. Jost (1953). *Compt. rend. soc. biol.* **237**, 949.
D'Amour, F. E., M. D. D'Amour and R. G. Gustafson (1934). *J. Pharmacol.* **51**, 353.

- Dantchakoff, V. (1937). *Bull. biol. France et Belg.* **71**, 269.
- Dantchakoff, V. (1950). *Ibid.* **34**, 311.
- Dreisbach, R. H. (1959). *J. Endocrinol.* **18**, 271.
- Godet, R. (1949). *Bull. biol. France et Belg.* **83**, 25.
- Greene, R. R., M. W. Burrill and A. C. Ivy (1938a). *Proc. Soc. Exptl. Biol. Med.* **38**, 1.
- Greene, R. R., M. W. Burrill and A. C. Ivy (1938b). *Ibid.* **38**, 4.
- Greene, R. R., M. W. Burrill and A. C. Ivy (1938c). *Science* **87**, 396.
- Greene, R. R., M. W. Burrill and A. C. Ivy (1938d). *Ibid.* **88**, 130.
- Greene, R. R., M. W. Burrill and A. C. Ivy (1939a). *Endocrinology* **24**, 351.
- Greene, R. R., M. W. Burrill and A. C. Ivy (1939b). *Am. J. Anat.* **65**, 415.
- Hamilton, J. B. and J. M. Wolfe (1938). *Anat. Record* **70**, 433.
- Hugget, A. St. G. and J. J. Pritchard (1945). *Proc. Roy. Soc. Med.* **38**, 261.
- Jost, A. (1945). *Compt. rend. soc. biol.* **139**, 483.
- Jost, A. (1954). *Geburtsh. u. Frauenhk.* **14**, 687.
- May, R. and H. Scheid (1959). *Ibid.* **19**, 783.
- Mombaerts, J. (1944). *Arch. Biol.* **55**, 393.
- Parkes, A. S. and C. W. Bellerby (1927). *J. Physiol.* **62**, 145.
- Raynaud, A. (1947). *Ann. endocrinol. Paris* **8**, 96.
- Revesz, R., I. C. Chappel and R. Gaudry (1960). *Endocrinology* **66**, 140.
- Scipiades, E. (1937). *Proc. Soc. Exptl. Biol. Med.* **37**, 242.
- Smith, M. G. (1926). *Bull. Johns Hopkins Hosp.* **39**, 203.
- Suchowsky, G. (1960). *Geburtsh. u. Frauenhk.* **20**, 1019.