Estrogen and Androgen Production *in Vitro* from 7-^{3}H-Progesterone by Normal and Polycystic Rat Ovaries[1]

JUDITH WEISZ[2] AND CHARLES W. LLOYD
The Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts

ABSTRACT. *In vitro* conversion of 7-^{3}H-progesterone to estrogens and androgens by ovaries of normal and testosterone-sterilized rats (TSR) was studied. Ovaries were obtained from 21- to 350-day-old rats. In incubations of ovaries from normal rats, modest estrogen formation, but no conversion to androgens, could be demonstrated. A marked change in steroidogenesis was demonstrable in the polycystic ovary of the adult TSR; conversion to estradiol and estrone was increased, and testosterone and androstenedione accumulated in appreciable amounts. A pattern of ovarian steroidogenesis similar to that of the TSR was found in older control rats which spontaneously developed prolonged vaginal cornification. At 21 and 28 days of age, no significant difference between the 2 groups was detected. The primary derangement induced by the neonatal administration of testosterone is most probably an alteration in central nervous system regulation of gonadotropin secretion. The findings in the present study are therefore interpreted as evidence that polycystic ovaries with an abnormal pattern of steroidogenesis may be established as a result of stimulation by an abnormal pattern of gonadotropin. (*Endocrinology* **77**: 735, 1965)

FEMALE rats given testosterone in neonatal life develop, after sexual maturation, a syndrome characterized by ovulatory failure, follicular cystic ovaries and persistent vaginal cornification (1–4). The testosterone presumably affects primarily hypothalamic regulation of gonadotropin secretion, and the ovarian pathology then results from an abnormal pattern of gonadotropin stimulation. The ovaries *per se* are normal (3, 5).

Histologically, the ovaries of testosterone-sterilized rats (TSR) resemble in many respects those of patients with the so-called Stein-Leventhal syndrome. An abnormal pattern of steroidogenesis by the polycystic ovaries of the human has been amply documented. These data, reviewed recently (6, 7), have been interpreted by some investigators as evidence for a deficiency of ovarian enzymes. An alternative view, that the ovarian manifestations of the syndrome may be secondary to an alteration in gonadotropin regulation, has also been proposed (8).

Since the structural changes of the ovaries of TSR are not the result of intrinsic ovarian abnormalities and are reversible, it seemed worthwhile to examine these ovaries for the presence of an alteration in steroidogenesis. It was felt that, if the ovaries have a change in steroid biosynthesis similar to that found in human polycystic ovaries, this would support the proposition that the defects in the human ovary could be secondary to changes in gonadotropin regulation.

Received May 12, 1965.

[1] Presented in part at the 46th Annual Meeting of The Endocrine Society, San Francisco, June, 1964. This work was supported in part by Grant AM 08184-2, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, and the Ford Foundation through the Training Program in the Physiology of Reproduction.

[2] Fellow (1962–1963), Training Program for Steroid Biochemistry, supported by Grant 5001 of the United States National Cancer Institute.

A comparison of the *in vitro* conversion of 7-^{3}H-progesterone to androgens and estrogens by ovaries of normal and testosterone-sterilized rats was made. Ovaries were obtained at different times throughout the life cycle of the animals. Parallel studies of the pituitary LH, total gonadotropin and FSH and of pituitary cytology of normal and testosterone-sterilized rats of the same ages have been carried out and will be reported separately (Matsuyama, E., J. Weisz, and C. W. Lloyd, in preparation).

Materials and Methods

First generation hybrid rats resulting from mating of Wistar females with Sprague-Dawley males were used. A single injection of 100 μg testosterone propionate in oil was administered subcutaneously at 5 days of age, the day of birth being counted as day zero. Uninjected litter mates served as controls. The animals were housed in air-conditioned rooms at 74 F with 10 hr of darkness and 14 hr of artificial illumination, and were given water and Purina Chow *ad lib.*

Vaginal smears were taken for a minimum of 10 consecutive days from TSR[3] and for at least 3 normal cycles from the controls. Cycling control rats were sacrificed on the day of proestrus. Preliminary data have indicated that estrogen production is maximal at this stage of the cycle.

In the strain used, it is around 200 days of age that some of the control rats begin to have irregular cycles with prolonged periods of vaginal cornification. By 300 days of age, the majority of control rats are acyclic, with persistent vaginal cornification [spontaneous constant vaginal estrus of old age (9, 10)]. Ovaries of 300-day-old rats with estrous-type smears for at least 4 weeks were used in one incubation.

Rats were killed by decapitation between 10:30 AM and 12:00 noon. The ovaries were placed on filter paper moistened with Hanks medium and kept on ice until enough ovarian tissue had been accumulated. A portion of the ovary from a number of animals was reserved for histological examination.

Incubation conditions. One hundred mg of tissue was used for each incubation in experiments with adult animals. The ovaries were minced with scissors and added to 3 ml of medium of the following composition:

1.2 μM TPN (Calbiochem) in 0.35 ml Hanks medium; pH adjusted to 7.4 with 0.1N NaOH.

12.4 μM glucose-6-phosphate (Calbiochem) in 0.35 ml Hanks medium.

0.27 ml 1.3% $NaHCO_3$ to raise the molarity of the bicarbonate buffer in the Hanks medium to 0.18×10^{-3} to give a pH of 7.4–7.5 under the conditions of the incubation.

90×10^{-4} μM 7-^{3}H-progesterone (25 μc) in 50 μl ethanol. SA 2.78 μc/mμM was obtained by adding inert progesterone to the radioactive steroid (SA 9.5 μc/mμM) purchased from New England Nuclear Corp.

The incubations of ovaries from immature rats were carried out on 30 mg of tissue using 25 μc of the tritiated progesterone without addition of inert steroid to maintain a constant tissue:substrate ratio. All other components of the incubation medium were reduced proportionately.

The incubations were carried out at 37 C for 3 hr in an atmosphere of 95% O_2 and 5% CO_2 in a Dubnoff metabolic shaking incubator. The incubation was terminated by the addition of 20 ml ethyl acetate and freezing at −20 C. Samples were stored at this temperature until extracted.

Chemicals. Solvents were of reagent grade and were distilled before use. Radioactive steroids were obtained from New England Nuclear Corporation. Inert steroids were supplied by Steraloids, Inc.

Measurement of radioactivity. Dried samples were dissolved in 10 ml of scintillation fluid [Liquifluor (Pilot) 42 ml/1 liter toluene: ethanol 98:2, v/v] in low potassium glass vials (Wheaton Glass Co.). No significant quenching occurred as determined by use of internal ^{14}C-toluene and ^{3}H-toluene standards. To dissolve the samples of estrone and estradiol obtained from crystallization, the addition of 0.2 ml methanol to the 10 ml scintillation fluid was required. In these, quenching was less than 2%, and for this no correction was made.

[3] In our experience, vaginal smears of TSR as well as of rats with "constant vaginal estrus" due to other causes such as old age or continuous exposure to light tend to fluctuate between proestrous and estrous type. Relatively few rats exhibit classic estrous-type smears for any length of time.

^{14}Carbon and tritium were counted simultaneously using a Nuclear-Chicago Liquid Scintillation Counter, Model No. 725. Efficiency of the counter was approximately 40% for ^{14}C and 13% for ^{3}H. Sufficient counts were accumulated to give a standard error of less than 2%. Paper chromatograms were scanned by a windowless flow counter (Vanguard Model No. 880).

Tracer amounts of ^{14}C-labeled estrone, estradiol, testosterone, androstenedione (Δ^4-androstene-3,17-dione) and progesterone were added to the incubation before extraction to permit correction for losses during the purification procedures. The amounts added were estimated on the basis of anticipated conversion and were of the order of 2,000–20,000 cpm. Identical quantities were placed in scintillation vials, dried and stored for counting with final samples, as was an aliquot from each batch of 7-^{3}H-progesterone.

The purity of both tracers and precursors was checked periodically by paper chromatography after addition of the corresponding inert steroid.

Extraction and purification. The procedures were adapted to minimize the handling of the initial large amounts of radioactivity. The incubation medium was extracted 5 times with 20 ml ethyl acetate (saturated with water) by horizontal shaking in the original incubation flask. The tissue was homogenized only after the third extraction when the amount of radioactivity remaining was minimal. The combined extracts were dried in a water bath at 40 C under vacuum, dissolved in 70% methanol and partitioned with hexane. After evaporation of the methanol, the watery residue was extracted with chloroform. The dried chloroform extract was separated into neutral and phenolic fractions according to the method of Engel (11). All partitions and extractions were carried out by horizontal shaking.

In the more recent experiments, it was found that an initial paper chromatography [heptane:benzene (2:1):formamide for 9–12 hr at 30 C] of the chloroform extract effected partial separation of neutral and phenolic fractions. The bulk of the radioactivity, including that associated with androstenedione and progesterone, could be recovered from the overflow. Testosterone localized in the middle third of the paper strip. Phenolic steroids, with a small admixture of neutral steroids, were eluted from the upper third. Partition between 1N sodium hydroxide and toluene could then be carried out on this fraction with the handling of smaller quantities of radioactivity.

One hundred μg of authentic steroids (estrone, estradiol, testosterone, androstenedione and progesterone) was added before the first paper chromatography. The following paper chromatographic systems and sequences were used for the isolation of the 5 steroids:

Phenolic (fraction) steroids
Estrone and estradiol:
1. Toluene/75% aqueous methanol.
2. Toluene:petroleum ether (Skellysolve C) (1:1)/70% aqueous methanol.

Neutral steroids
Testosterone:
1. Heptane:benzene (2:1)/formamide overrun for 9–12 hr, or hexane/80% aqueous methanol overrun for 6 hr.
2. Toluene:petroleum ether (Skellysolve C) (1:1)/70% aqueous methanol.
3. Acetylation (2:1 mixture pyridine/acetic anhydride overnight at room temperature).
4. Iso-octane/90% methanol.

Androstenedione:
1. Hexane/80% aqueous methanol overrun for 6 hr.
2. Petroleum ether (Skellysolve C):benzene (33:17)/85% aqueous methanol.
3. Acetylation of contaminating radiometabolites as above.
4. Iso-octane/90% aqueous methanol overrun for 8 hr.

Progesterone:
1. Hexane/80% aqueous methanol overrun for 6 hr.
2. Hexane/formamide.
3. Cyclohexane/propylene glycol.

Radiochemical purity of the above steroids was established by crystallization of all samples with 15 mg reference steroid until in 3 successive crystallizations the ratio of ^{3}H:^{14}C did not deviate more than $\pm$5% from the mean in both the crystals and mother liquor.[4]

[4] In the interest of accurate counting, ratios below 1:1 were considered undesirable, as were very high ratios. A minimum of about 2000 cpm of ^{14}C had to be added to give sufficient counts at crystallization. In samples in which very high ratios (more than 50:1) were found on crystallization, additional ^{14}C tracer could be added after determination of ^{14}C losses.

TABLE 1. $^{3}H/^{14}C$ ratios on crystallization and derivative formation (duplicate incubations A and B)

	Estrone				Estradiol			
	A		B		A		B	
	Crystal	Mother liquor	Crystal	Mother liquor	Crystal	Mother liquor	Crystal	Mother liquor
3rd crystallization	10.33	10.48	10.02	10.31	15.08	14.98	14.35	14.93
4th crystallization	10.71	10.29	9.77	9.87	15.15	14.46	14.73	14.72
5th crystallization	10.70	10.80	10.15	10.00	14.62	15.62	14.33	14.20
Acetate	11.02				15.10			

Additional confirmation of identity and radiochemical purity was obtained by formation of derivatives of 2 representative samples of each of the 4 metabolites studied. Mono- and diacetates of estrone and estradiol, respectively, were formed and isolated by thin-layer chromatography (cyclohexane :ethyl acetate 3:1). Testosterone acetate was converted to androstenedione and isolated by paper chromatography. The thiosemicarbazone derivative of androstenedione was made (12) and isolated by paper chromatography (toluene/propylene glycol run for 9 hr at 30 C). No change in $^{3}H:^{14}C$ ratio occurred through derivative formation in any of the samples.

Table 1 shows the $^{3}H:^{14}C$ ratios of 3 final crystallizations of typical samples of estrone and estradiol, as well as of their acetates from an incubation of TSR ovaries.

The quantities of the 4 steroids produced from the added progesterone as well as the amount of unmetabolized precursor were calculated on the basis of the final ratio of $^{3}H:^{14}C$ on crystallization. The final $^{3}H:^{14}C$ ratio was multiplied by the number of counts of the corresponding ^{14}C tracer that had been added to give the number of counts of tritiated metabolite present at the time of addition of the ^{14}C tracer.

P^{A} = μM precursor
P = cpm precursor
^{3}H = cpm of ^{3}H in final crystals
^{14}C = cpm of ^{14}C in final crystals
T = cpm of ^{14}C tracer added

$$\frac{P^{A}}{P} \times \frac{^{3}H}{^{14}C} \times T = \mu M \text{ metabolite formed}$$

Counting of the final samples from crystallization and of the ^{14}C standards was carried out at the same time.

Results

The stability of the 7-^{3}H-progesterone was established by incubation with the medium alone, as well as with boiled ovarian tissue. After extraction, purification, partition into neutral and phenolic fractions, only a single radioactive peak that corresponded to authentic inert progesterone was found in two consecutive paper chromatographic systems. Of the radioactivity added, 72 and 75% could be eluted from the zone on the second chromatogram corresponding to added inert progesterone. These recoveries were similar to those of steroids from ovarian incubations at the corresponding stage of purification.

The results of duplicate incubations are presented in Table 2. Two halves of each ovary from a pool of ovaries were incubated separately. There was a close agreement in these duplicates of the calculated radioactive conversions, irrespective of the type of ovary used, indicating relative homogeneity of the tissue as well as the reproducibility of the method.

Amounts of estrogens and androgens produced and their ratios are listed in Tables 3 and 4. The production of estrogens by the ovaries of the adult TSR was uniformly several times greater than that of the controls. The amount of added precursor converted to estrone and estradiol by the polycystic ovaries ranged from 0.60 to 3.62×10^{-4} μM and

TABLE 2. Duplicate incubations of three ovarian preparations, steroid production: $\times 10^{-4}$ μM/100 mg of tissue

Type	Age (days)	No. of rats*	Estrone	Estradiol	Andro-stenedione	Testosterone
TSR†	150	6	1.41	3.44	1.28	0.80
			1.09	3.34	1.07	0.75
Control (proestrus)	200	4	0.14	0.30	—	—
			0.16	0.27	—	—
Control (acyclic)	300	6	0.78	1.50	0.25	0.28
			0.68	1.21	0.33	0.33

—= Undetectable.
* Polycystic ovaries of TSR are smaller and weigh about 50% less than those of controls.
† TSR: testosterone-sterilized rats.

1.92 to 13.38 $\times 10^{-4}$ μM, respectively, as compared to 0.15 to 0.28 $\times 10^{-4}$ μM estrone and 0.29 to 0.85 $\times 10^{-4}$ μM estradiol converted by normal ovaries obtained during proestrus.

Testosterone and androstenedione were not produced in identifiable amounts by normal ovaries. On crystallization of androstenedione from some incubations of normal ovaries, a constant $^{3}H:^{14}C$ ratio was achieved. However, since this ratio was less than 0.5 and the radioactivity was very low, it was not considered that this constituted

TABLE 3. Conversion of 7-^{3}H-progesterone (90 $\times 10^{-4}$ μM) to estrogens and androgens by 100 mg ovarian tissue of control and testosterone-sterilized rats (TSR)

Age (days)	Type	Estrone ($\times 10^{-4}$ μM)	Estradiol ($\times 10^{-4}$ μM)	Andro-stenedione ($\times 10^{-4}$ μM)	Testosterone ($\times 10^{-4}$ μM)	Progesterone (residual) ($\times 10^{-4}$ μM)
21	Control[a]	—	0.064	—[b]	—	1.62
	TSR	—	0.068	—	—	1.48
28	Control	0.027	0.084	—	—	1.37
	TSR	0.017	0.096	—	—	1.76
110	Control	0.28	0.85	—	—	1.82
	TSR	3.62	13.38	3.81	3.22	5.58
	TSR	2.70	7.92	9.05	4.60	5.40
	TSR	0.68	1.92	3.64	2.39	6.29
	TSR	sample lost		3.29	0.35	6.85
150	Control	0.21	0.58	—	—	6.15
	TSR[c]	1.25	3.39	1.17	0.77	5.82
200	Control[c]	0.15	0.29	—	—	3.45
	TSR	2.10	3.09	12.58	2.60	4.84
300	Control	0.17	0.32	—	—	3.92
	Control[c,d] (acyclic)	0.73	1.35	0.30	0.25	8.20
	TSR	3.03	9.25	5.70	4.43	5.04
350	TSR	0.60	2.63	4.55	2.56	6.29

[a] All controls sacrificed on day of proestrus.
[b] — = Undetectable.
[c] Average of duplicate incubations.
[d] Spontaneous, constant vaginal estrus of old age.

TABLE 4. Ratios of estrogens and androgens produced from 7-^{3}H-progesterone by 100 mg ovarian tissue of control and testosterone-sterilized rats (TSR)

Type	Age (days)	Estradiol / Estrone	Androstenedione / Testosterone	Estrogens / Androgens
TSR	28	5.65	—	—
	110	3.69	1.18	2.42
		2.93	1.97	0.78
		2.62	1.52	0.43
		- - - -	9.40	- - - -
	150	2.71	1.52	2.39
	200	1.47	4.84	0.34
	300	3.05	1.29	1.21
	350	4.38	1.78	0.45
Control	28	3.10	—	—
	110	3.04	—	—
	150	2.76	—	—
	200	1.90	—	—
	300	1.88	—	—
Constant, vaginal estrus of old age	300	1.85	1.20	3.78

— = Androgens undetectable.
- - - - = Samples of estrogens lost.

positive proof of identity. In contrast, considerable amounts of radioactive testosterone and androstenedione were formed by the ovaries of TSR. Conversion of progesterone to testosterone ranged from 0.35 to 4.6×10^{-4} μM and to androstenedione from 1.17 to 12.58 $\times 10^{-4}$ μM.

The pattern of steroids produced by ovaries of older control rats with spontaneous, constant vaginal estrus of old age resembled that of TSR; the conversion to estrogens was increased and androgens accumulated in identifiable amounts. Histologically, ovaries from these rats resembled those of TSR.

The ratios of quantities of testosterone to androstenedione, of estradiol to estrone, or of the total androgens to estrogens formed by the polycystic rat ovary did not fall into any obvious pattern in relationship to duration of the syndrome. In the normal rats, there appeared to be a gradual decline with age in the ratio of estradiol to estrone formed.

Before vaginal opening, no significant differences were found between the steroids formed by ovaries of normal and testosterone-sterilized rats. At 21 and 28 days of age, estradiol could be isolated in detectable amounts. Estrone could only be identified unquestionably in incubations of ovaries of 28-day-old animals, but not in incubations of ovaries from the 21-day-old rats.

In the phenolic fractions from a number of incubations, two radioactive products in addition to estrone and estradiol were noted. One, with chromatographic mobility similar to that of estriol, could be separated from the estriol standard on chromatography in a benzene/80% aqueous methanol system. The second remained partly associated with estrone on the chromatographic systems used but separated from it readily on crystallization. No further characterization of these two radiometabolites was made.

Since phenolic partition would have destroyed any 16α-hydroxyestrone and 16-oxo-17β-estradiol that might have

formed, 20% of the original chloroform extract from the duplicate incubation of the 200-day-old rats was chromatographed on paper in chloroform/formamide. No significant amounts of radioactivity were associated with the zones corresponding to the appropriate standards of these two estrogens.

No radioactivity was associated with added inert 17α-hydroxyprogesterone.

Discussion

The physiological derangement induced by testosterone in newborn female rats probably lies in those areas of the central nervous system on which cyclic release of gonadotropins depends (13). The ovaries can be made to ovulate and to luteinize by exogenous or endogenous gonadotropin and cycle normally when they are transplanted in normal female rats (3, 13).

The present study was undertaken in an attempt to define the changes in ovarian steroidogenesis that may result from a primary derangement in the regulation of gonadotropin secretion.

Under the conditions we have used, ovaries of adult TSR consistently and reproducibly form several times more labeled estrogens from radioactive progesterone than do ovaries of normal rats. In addition, in contrast to the normal, substantial conversion to labeled androgens also takes place.

The amount of estrogen formed by normal proestrous ovaries is remarkably uniform. From the limited data obtained from incubations of normal rat ovaries, it would appear that there is a decrease in ratio of estradiol to estrone with increasing age. However, a number of additional incubations at the different ages would be required to determine if this is consistent.

The amounts and relative ratios of estrogens and androgens produced by ovaries of adult TSR vary widely. These differences do not appear to be related to the age of the animals nor to reflect the duration of the disease.

In the majority of the TSR in our colony, vaginal opening took place between 30 and 35 days of age, several days earlier than in the controls. It is of interest that at 28 days of age, just preceding this precocious vaginal opening, the total ovarian estrogen production *in vitro* in the TSR is not detectably different from that of the controls. The ratio of estradiol to estrone, however, is greater than in the controls. To determine whether this difference is related in any way to the onset of sexual maturation in the TSR would require a number of additional incubations around the time of vaginal opening. By this age, marked differences in the content of gonadotropins in the pituitaries are already present. Both total gonadotropins and LH are significantly lower in TSR than in the controls (Matsuyama, E., J. Weisz, and C. W. Lloyd, in preparation).

Follicular cystic ovaries of the rat that have resulted from causes other than testosterone administration, such as old age or exposure to constant light, have similar abnormalities in their pattern of *in vitro* steroidogenesis (Spindler, I. J., J. Weisz, and C. W. Lloyd, unpublished data). In addition, the structural changes of the ovaries were found to be essentially similar. These characteristics do not appear to be specific or attributable to a specific etiology.

Following vaginal opening, most of our TSR have at least one or two normal estrous cycles before entering a state of constant vaginal estrus. By using smaller doses of testosterone, this interim phase of normal cyclicity can be substantially prolonged (14). Steroidogenesis during this period when the ovaries are histologically indistinguishable from the nor-

mal is currently being investigated. Preliminary observations indicate that the *in vitro* conversion of progesterone to androgens and estrogens by ovaries of testosterone-treated animals at this stage does not differ from the normal.

The interpretation of these data requires the following qualifications: a) In the present study, only the conversion of added radioactive precursor was estimated. The specific activity of the end products was not measured. Consequently, the degree to which the added radioactivity was diluted by nonisotopic steroids already present or formed during incubation cannot be estimated. An increase in conversion of radioactive steroids does not necessarily signify a parallel increase in over-all steroid production (15, 16). For any given rate of conversion, a significant decrease in the size of the pool of precursor or of any of the steroid intermediates in the ovaries would cause increased conversion of radioactive steroid with end products of high specific activity. Facilitation of an alternative pathway, bypassing a larger intermediate pool, would have a similar effect. It is improbable that a substantial difference in the sizes of progesterone pools in the two groups would account for the differences in the amounts of radioactive steroids produced because the amount of residual labeled progesterone was not greater in the control than in the TSR. b) Extrapolation of the *in vitro* findings to the intact animal is not warranted. The presence of a similar abnormality in steroid production *in vivo* can only be determined by direct studies of secretion rates. However, judging from the constant vaginal cornification that characterizes the adult TSR, it seems probable that estrogens are produced continuously and in significant amounts by these ovaries.

The only study of steroidogenesis by the ovaries of the TSR was that mentioned by Goldzieher and Axelrod (17). Ovaries from TSR were incubated with pregnenolone (but not progesterone) under the conditions used by these investigators in their studies of human polycystic ovaries.[5] They reported a high level of conversion to androgens, but were unable to detect conversion to estrogens. The lack of production of estrogens by the ovaries of TSR in the system employed is unlikely to be due to a difference in animals since the TSR (200-day-old) were supplied from our colony. The difference in findings is probably due to differences in incubation conditions.

There are many common features in the natural history and pathology of the ovaries of the TSR and the human with polycystic ovary disease. Although the ovaries of the TSR are not enlarged and do not have a thickened capsule, both human and rat polycystic ovaries are characterized by multiple follicular cysts, variable degrees of theca luteinization and stromal hyperplasia and virtual absence of corpora lutea. A cardinal feature in the fully established syndrome in both the rat and the human is a loss of the normal rhythmic changes in both ovarian structure and function. This implies, in turn, a loss of normal cyclic gonadotropin stimulation. Preceding this loss of rhythmicity, patients often menstruate more or less regularly and may be fertile. Similarly, the TSR may pass through a phase, between puberty and the onset of constant vaginal estrus, during which vaginal smears show regular cyclic changes and the ovaries appear histologically and functionally normal (14).

[5] In incubations of human polycystic ovaries, the same investigators found the pattern of steroidogenesis to be similar with either progesterone or pregnenolone as precursor.

Studies of the steroidogenesis of ovaries of women with the polycystic ovary syndrome have been carried out, by necessity, only when the syndrome was fully developed. Abnormal patterns of hormone production at this stage have been confirmed by a number of studies which have recently been reviewed (6, 7). Increased production of androgens has been a consistent finding. This has been demonstrated by studies of urinary metabolites, blood testosterone levels, analysis of ovarian cystic fluid and ovarian tissue as well as by *in vitro* incubation of ovaries.

On the other hand, reports concerning estrogen production have been contradictory. Examination of vaginal smears and endometria of patients with polycystic ovaries gives evidence of at least normal levels of estrogen. Urinary estrogen output of most of these women is in the normal range and production rates of estrogen have been found to be normal or elevated (18, 19). Fluid from the cystic follicles characteristically contains little or no estrogen, whereas fluid from normal follicles contains estrogen. However, the estrogen content of the polycystic ovarian tissue was found to be at least as great as that of the normal.

A decrease or absence of *in vitro* conversion by human polycystic ovaries of radioactive precursors to estrogens with increased incorporation into androgens has been reported. These results have been interpreted as supporting the concept of enzymatic defects in the polycystic ovary preventing aromatization. However, some workers (20, 21) have reported *in vitro* conversion of androstenedione and testosterone to estrogen by polycystic ovaries to be essentially normal, and conversion of progesterone to androstenedione to be increased. In incubations of ovaries from patients with the Stein-Leventhal syndrome using the methods identical with those described in this report, we have found increased conversion to both androgen and estrogen (Weisz, J., and C. W. Lloyd, unpublished data).

The pattern of enzymes in micropolycystic ovaries may well be different from that in the normal. These differences are not, however, necessarily the expression of a basic abnormality in the capacity of the ovary to produce certain enzymes under the appropriate stimulation. The alternative interpretation supported by the evidence presented is as follows.

As a result of an inappropriate pattern of gonadotropins, certain cell types (stromal and follicular) are preferentially stimulated. Because of this, the enzymes normally associated with these cell types will dominate; conversely, certain other cell types, such as those of the corpora lutea, and their associated enzymes will be deficient. Similarly, the changes in gonadotropin stimulation may modify steroid production within a particular cell type by enhancing some enzyme systems relative to others. The alteration of ratio of structures and their enzymes will then be reflected in the differences in the pattern of steroidogenesis.

This alternative hypothesis proposes that the changes in the ovary are acquired and not inborn. Micropolycystic ovaries, then, represent an end stage which can be caused by a number of different etiologies that initiate a modification of gonadotropin secretion. Once the polycystic ovaries are established, their abnormal steroid production may be responsible for perpetuating the condition and obscure the basic underlying abnormality. Therefore, the most profitable stage of the syndrome to study to obtain information on the primary physiological defect would seem to be the phase before the polycystic ovary becomes established. In the case of the

human, the cyclic ovulatory ovarian function that follows successful wedge resection may well reflect a more or less temporary reversion to this stage. The investigation of the events that lead to the recurrence of the anovulatory polycystic ovary might be especially rewarding.

Acknowledgment

The authors wish to express their gratitude for the advice so generously given by Drs. D. S. Layne and E. B. Romanoff.

References

1. Greene, R. R., M. W. Burrell, and A. C. Ivy, *Amer J Anat* **65:** 415, 1939.
2. Takasugi, N., *Annot Zool Jap* **25:** 120, 1952.
3. Segal, S. J., and D. C. Johnson, *Arch Anat Micr Morph Exp* **48:** 261, 1959.
4. Barraclough, C. A., *Endocrinology* **68:** 62, 1961.
5. Bradbury, J. T., *Endocrinology* **28:** 101, 1941.
6. Axelrod, L. R., and J. W. Goldzieher, *J Clin Endocr* **22:** 431, 1962.
7. Mahesh, V. B., and R. B. Greenblatt, *Recent Progr Hormone Res* **20:** 341, 1964.
8. Lloyd, C. W., Human Reproduction and Sexual Behavior, Lea & Febiger, Philadelphia, 1964, p. 280.
9. Everett, J. W., *Endocrinology* **25:** 123, 1939.
10. Turner, C. D., *Endocrinology* **28:** 729, 1941.
11. Engel, L. L., *Recent Progr Hormone Res* **5:** 343, 1950.
12. Pearlman, W. H., and E. Cerceo, *J Biol Chem* **203:** 127, 1953.
13. Barraclough, C. A., and A. R. Gorski, *Endocrinology* **68:** 68, 1961.
14. Swanson, H. E., and J. J. Van der Werff ten Bosch, *Acta Endocr (Kobenhavn)* **47:** 37, 1964.
15. Savard, K., J. M. Marsh, and B. F. Rice, *Recent Progr Hormone Res* **21:** 285, 1965.
16. Armstrong, D. T., J. O'Brien, and R. Greep, *Endocrinology* **75:** 488, 1964.
17. Goldzieher, J. W., and L. R. Axelrod, *Fertil Steril* **14:** 631, 1963.
18. Goering, R. W., and W. L. Herrmann, The Endocrine Society, 46th Meeting, 1964, p. 37 (Abstract).
19. Puebla, R. A., V. B. Mahesh, and R. B. Greenblatt, The Endocrine Society, 46th Meeting, 1964, p. 134 (Abstract).
20. Chapdelaine, A., T. Sandor, and A. Lanthier, *Canad J Biochem* **41:** 635, 1963.
21. Lanthier, A., and T. Sandor, *Acta Endocr (Kobenhavn)* **39:** 145, 1962.