Clinical and Biochemical Features of Polycystic Ovarian Disease

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THE EXISTENCE of bilateral polycystic ovarian disease and its occasional improvement following cuneiform ovarian resection was known before the turn of the century.[1–4] Additional clinical and surgical observations were reported sporadically in the ensuing years,[5–8] but a broad interest in this disorder did not develop until 1935 when an associated syndrome consisting of "menstrual irregularity featuring amenorrhea, a history of sterility, masculine type hirsutism, and less consistently retarded breast development and obesity" was described.[9] Excellent therapeutic results following wedge resection were reported by these authors over a span of many years; their results, however, have never been duplicated in any other substantial series of cases. In the eyes of many investigators, the clinical features of this syndrome were neither definitive nor satisfactory, and additional criteria such as a normal urinary 17-ketosteroid excretion[10] were employed in an effort to improve diagnostic accuracy and the consistency of surgical results. With time, increasing numbers of patients with polycystic ovarian disease and atypical clinical findings were observed,[11] and in a significant number of even these cases, a normalization of function followed wedge resection. Netter *et al.*[12] in typical Gallic style, remarked that: "the syndrome of Stein is a fugitive syndrome, with limits less well defined than those of the Sahara or the Sudan." In England, Roberts and Haines[13] studied 14 cases and entitled their report, "Is there a Stein-Leventhal syndrome?" It is noteworthy that these questions were raised from a clinical point of view and not because of the superficial resemblance of polycystic ovaries to the ovarian

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sclerosis and other pathology which is known to accompany androgenic[14, 15] or Cushingoid[16] adrenocortical hyperfunction.

Since there appeared to be growing doubt as to the existence of a clinical syndrome associated with sclerocystic ovaries, we undertook a survey of the world literature in a search for the incidence of certain signs and symptoms in patients who were found at operation to have polycystic ovarian disease. A total of 187 pertinent references yielded acceptable information from 1097 cases, as shown in Table 1. Amenorrhea was observed in only half of the patients, and the per cent of frequency with which this symptom occurred in different series varied tremendously—from 15 to 77%. On the other hand, cyclic menses were noted in a significant number of patients (12%), and functional bleeding was exceedingly common (65%) in some series. The incidence of infertility also varied widely, ranging from 35 to 94% with an over-all average of 74%. However, since infertility is one of the major reasons why patients consult a physician in the first place, the figures probably have a positive bias. By contrast, there appeared to be unequivocal evidence of ovulation in patients with polycystic ovaries as shown by reports of primary dysmenorrhea, by biphasic basal temperature curves in 12–40% of subjects, and finally by the finding of corpora lutea in an average of 22% of the cases where the presence or absence of this feature was specifically mentioned. Hirsutism was observed in an average of 69% of the subjects, but again the incidence varied widely. Virilization appeared with some frequency. Taken together, these statistics make it most difficult to accept as a distinct entity a group of symptoms of which only two nonspecific ones (hirsutism, infertility) are present much more than half the time, where cyclic menses or functional uterine bleeding occur nearly as often as the supposedly important amenorrhea, and where positive evidence contradicts

TABLE 1. Signs and Symptoms Associated with Polycystic Ovarian Disease

Symptom	*Usable No. cases*	*Incidence (%)*	
		Mean	*Range*
Obesity	600	41	16–49
Hirsutism	819	69	17–83
Virilization	431	21	0–28
Cyclic menses	395	12	7–28
Functional bleeding	547	29	6–65
Amenorrhea	640	51	15–77
Dysmenorrhea	75	23	—
Biphasic basal temperature	238	15	12–40
Corpus luteum at operation	391	22	0–71
Infertility	596	74	35–94

Data tabulated from 187 references with a total of 1079 cases.

the postulated anovulation which is thought to cause the infertility. As a matter of fact, in a study of the gross appearance of the ovary in a series of 12,160 gynecological laparotomies, 170 instances (1.4%) of polycystic ovaries were observed.[17] It is difficult to avoid the conclusion that Stein and Leventhal's symptomatology calls attention to a small and perhaps not especially unique fraction of polycystic ovarian disease. It has been claimed in rebuttal that these clinical variations are simply different stages of an evolving disease process. We have indicated elsewhere[15] that this explanation is unsupported by positive findings, and that there is no detectable association between the duration of the disease and the "completeness" of the alleged syndrome.

The success of wedge resection in correcting the infertility and menstrual disorders associated with polycystic ovaries has been in large measure responsible for the wide and continued interest in this disease. Examination of the literature, however, indicates that success is by no means uniform. Where published data are adequate for evaluation, as seen in Table 2, regular cycles are re-established in an average of 80% of operated patients, but in some hands, the rate of success is as low as 6%; results in terms of pregnancies are also most unpredictable, ranging from 13 to 89%. It is quite evident that the clinical material which forms the basis of these studies is inhomogeneous, and that criteria for the selection of candidates for wedge resection are neither uniform nor adequate. In our experience, there has been no difference in the results of wedge resection whether the urinary 17-ketosteroids were normal or elevated; as a matter of fact, the elevated steroid excretion was unaltered by surgery in two-thirds of the patients. The corticosteroid suppression test (see below) has not been helpful, nor has ovarian size, since normal-sized ovaries have responded at about the same rate as enlarged ovaries. One correlation, apparently valid but extremely difficult to evaluate quantitatively, was suggested to us by Southam:[18] the more estrogenic activity and the less androgenic activity there is, the better the results of wedge resection are likely to be.

Efforts to find additional clinical signs and symptoms which would im-

TABLE 2. Results of Wedge Resection in Patients with Polycystic Ovarian Disease

Result	*Usable No. cases*	*Frequency (%)*	
		Mean	*Range*
Regular cycle	447	80	6–95
Pregnancy	640	63	13–89
Decreased hirsutism	205	16	0–18

Data tabulated from 187 references with a total of 1079 cases.

prove the diagnostic and therapeutic "score" have not met with much success, and investigators have turned to cytological and biochemical studies for help. There have been a number of recent reports[19–21] of chromosomal anomalies associated with polycystic ovarian disease. This unexpected finding awaits confirmation and correlation. The occurrence of polycystic ovaries in sisters[15, 22, 23] may be mere coincidence or another intimation of genetic involvement.

Criteria of classic morphology have not provided much assistance, and indeed there is controversy at the present time whether any of the histological changes observed in polycystic ovaries are at all specific[15, 24] or whether they can be observed with frequency in other clinically unrelated conditions. From the practical point of view, Plate[25] has shown that the presence or absence of hyperthecosis was not significantly correlated with the results of wedge resection.

Biochemical studies until very recently were confined to analysis of urinary steroid metabolites. The extensive literature dealing with this aspect of the problem has been reviewed elsewhere.[26] For the enormous amount of effort expended in this direction, the results have been disappointingly meager. Our improved understanding of ovarian and adrenal steroidogenesis goes a long way to explain some of these difficulties. Elevated excretion of urinary 17-ketosteroids has been observed by many investigators, while others rule out the diagnosis of "Stein-Leventhal syndrome" in the face of elevated excretion. Figure 1 shows the ranges of values of total urinary 17-ketosteroids and 17-hydroxy corticoids observed by us in a series of patients with polycystic ovaries. It is evident that there is a diffuse spread of values which does not permit the categorization of these patients into distinct sub-

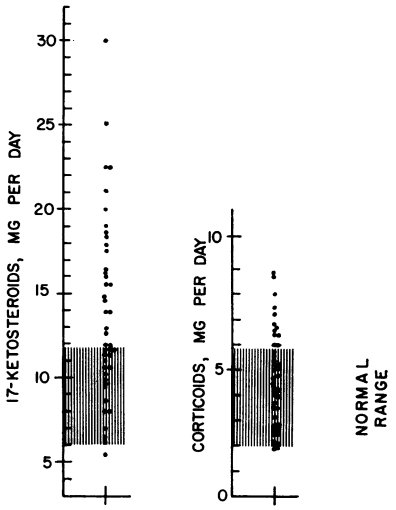
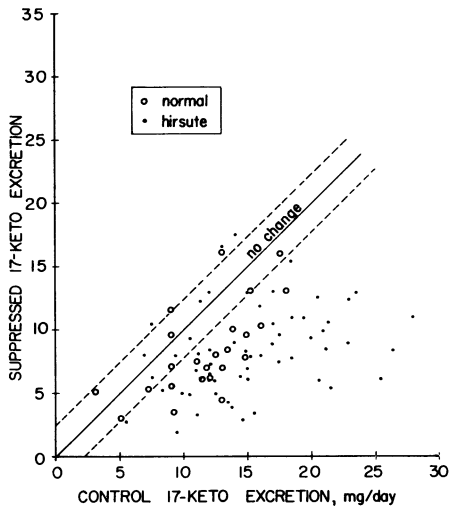


Fig. 1. Urinary excretion of total 17-ketosteroids and 17-hydroxycorticoids in patients with polycystic ovarian disease.

groups. Some of the 17-hydroxycorticoid values significantly exceed our normal range and suggest that certain patients may have had adrenocortical hyperfunction. It should be noted that these elevated values were not associated with extreme obesity, which would tend to increase the level of urinary excretion.[27]

Important methodological difficulties[28] stand in the way of correct interpretation of "total 17-ketosteroid" excretion. Chromatographic separation and measurement of the individual 17-ketosteroids has not been particularly helpful, since both ovarian and adrenal ketosteroids are metabolized to the same excretory products (androsterone and etiocholanolone), and since dehydroepiandrosterone, the major "adrenal" ketosteroid, is now known to be produced by the ovary as well.[29–31] As a consequence, dynamic tests of steroid metabolism have been developed in an attempt to distinguish the ovarian contribution from that of the adrenal. In an early study, we examined the effects of adrenal suppression by a small dose of prednisolone (2.5 mg., t.i.d.) given over a period of 10 days. There appeared to be a significant difference in the response of normal women vs. women with hirsutism but without menstrual disorders (Fig. 2). The higher the initial



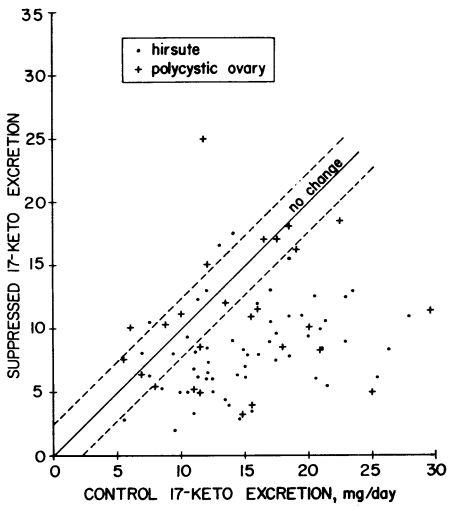


Fig. 2 (*left*). Urinary 17-ketosteroid excretion before and after minimal adrenal suppression. No change in excretion would yield points lying along diagonal line ± the error of measurement. Values below diagonal line indicate degree of suppression. Results were obtained in normal vs. cyclic, but hirsute, women. **Fig. 3** (*right*). Urinary 17-ketosteroid excretion before and after minimal adrenal suppression. No change in excretion would yield points lying along diagonal line ± the error of measurement. Values below diagonal line indicate degree of suppression. Results are from hirsute cyclic women vs. patients with polycystic ovaries.

17-ketosteroid excretion in the hirsutism cases, the greater was the decrease following adrenal suppression. We interpreted these findings as indicating a hyperresponsiveness of the pituitary-adrenal axis in certain cases of hirsutism.[32] When the situation was examined in women with polycystic ovaries, Fig. 3, some were found to show the minimal response seen in normals or certain hypertrichotic women, while others showed the pattern which we interpreted as pituitary-adrenal hyperresponsiveness. This might possibly be an indication of a role of the adrenal cortex in certain cases, but the evidence was equivocal and at any rate offered no clearcut, useful diagnostic index. Netter and others[33–35] have developed dynamic tests involving the use of dexamethasone for adrenal suppression and an estimation of urinary steroid changes following the superimposed administration of large doses of chorionic gonadotropin. Some interesting differences in the excretion of neutral and phenolic steroids have been noted, but the acid test of broad clinical application, especially in diagnostically difficult situations, remains to be carried out.

On the basis of so much work and so little yield in the attempt to distinguish ovarian from adrenal factors, one might well begin to wonder if there is not indeed an adrenocortical component in some cases of polycystic ovarian disease. Sherman[36] has found increased excretion of 11-ketopregnanetriol, undoubtedly an adrenal metabolite, in 16 cases. Wedge resection of the ovaries has frequently failed to alter an elevated excretion of urinary 17-ketosteroids,[26, 37, 38] which could be decreased promptly upon adrenal suppression with corticosteroids. Moreover, in some therapeutic failures of wedge resection, satisfactory results have been achieved subsequently with corticosteroid treatment. On the other hand, there is no reason to believe that adrenal hyperfunction is inevitably associated with polycystic ovarian disease, hence the finding of histologically normal adrenal tissue in a few instances[39] comes as no surprise. It is known, furthermore, that histologic normalcy is no indication of normal function: the adrenals may appear quite unremarkable in full-blown cases of Cushing's syndrome.

There are many indications that the pituitary-adrenal and pituitary-ovarian circuits are not completely isolated from each other, but that there is a good deal of functional "crosstalk" which can be demonstrated in a variety of ways. Some years ago Sohval and Soffer[40] demonstrated that the administration of corticosteroids or ACTH can cause an increase in urinary gonadotropin excretion. In chickens, even ovulation can be triggered by this procedure.[41] Clinically, the occasional appearance of uterine bleeding after administration of ACTH is well known. Looking at the other side of the relationship, it has been shown that estrogenic compounds which are potent gonadotropin inhibitors significantly alter cortisol and aldosterone

secretion rates.[42] Gemzell *et al.*[43] noted in one unusual patient with hirsutism and elevated 17-ketosteroids that the administration of gonadotropin not only increased urinary estrogens but also tripled the 17-ketosteroids and corticoids, suggesting an action of pituitary gonadotropin on the adrenal. Finally, in rats the production of persistent estrus and polycystic ovaries by hypothalamic injury is associated with adrenal hypertrophy,[44] an observation we feel to be of considerable theoretical importance.

The co-existence of adrenal and ovarian disturbances is therefore not entirely unexpected. This complicates the question of the pathogenesis of polycystic ovarian disease, and it becomes necessary to delve more deeply into ovarian endocrine function in an effort to understand the physiologic problems involved.

OVARIAN STEROIDOGENESIS

While the main elements of steroid biosynthesis in adrenal tissue were fairly well clarified 10 years ago, it has been only recently that an equivalent understanding of ovarian steroidogenesis has been achieved. The cyclic nature of ovarian function clearly makes the problem more difficult than with the adrenal. The ovary stores very little steroid material, thus increasing the problems of detecting steroids in its tissue. Even the secreted quantities of ovarian estrogens and androgens are so small that actual isolation (to say nothing of definitive identification) of the compounds strains the reliability of the best methods available. New technics involving the determination of secretion rates by isotope dilution are beginning to yield more precise and meaningful information.

In view of these difficulties, the majority of efforts to establish the steroid biosynthetic pathways have relied on the incubation of precursor substances with ovarian tissue and the isolation and identification of the resulting products. Many of these investigations yielded only tentative identification of the metabolites and were therefore of limited value, but definitive identification of all the steroid intermediates between cholesterol and the estrogens has finally been achieved.[29] In 1962 definitive identification of the complete series of steps from pregnenolone to estrogen in single samples of ovarian tissue was accomplished.[29]

A diagrammatic outline of the ovarian biosynthetic mechanism is shown in Fig. 4. Using readily available cholesterol, the ovarian enzymes first cleave the side chain, yielding $\triangle^5$-pregnenolone. This compound then undergoes hydroxylation to 17-hydroxypregnenolone. Another side-chain-cleaving enzyme system removes the rest of the side chain, leaving the 17-ketosteroid dehydroepiandrosterone. During all this time, an enzyme system

called 3β-ol dehydrogenase has been actively switching the position of a certain double bond (Fig. 5), converting pregnenolone to progesterone, dehydroepiandrosterone to androstenedione, and probably acting at all the intermediate stages as well. Progesterone itself also undergoes the same trans-

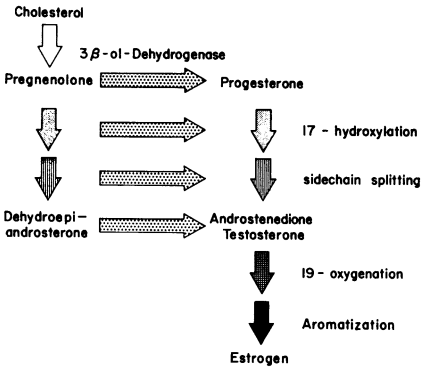
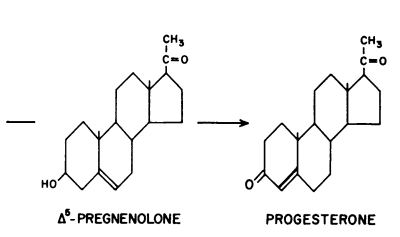


Fig. 4. Schematic representation of steroid biosynthesis in human ovarian tissue.



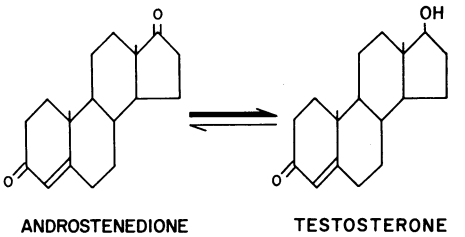


Fig. 5 (*left*). Conversion of Δ^5-pregnenolone to progesterone by 3β-ol dehydrogenase system. **Fig. 6** (*right*). Conversion of androstenedione to testosterone by 17-reductase enzyme system.

formations of 17-hydroxylation and side-chain cleavage to form androstenedione. Thus parallel biosynthetic pathways, with interactions at each stage, are formed. It is important to recognize at this point what the ovarian biosynthetic mechanism has accomplished: *pregnenolone and progesterone formed from still earlier precursors, have been transformed into 17-ketosteroids (dehydroepiandrosterone and androstenedione).* Both are relatively weak androgens, but androstenedione is readily convertible to testosterone (Fig. 6) by a reductase which is present in ovarian tissue and blood and in peripheral tissues as well. Thus, the formation of testosterone by the ovary, long suspected on the basis of circumstantial evidence,* was finally proved in definitive fashion.[46] Biosynthesis of these 17-ketosteroids has been amply confirmed in both normal and polycystic ovaries.[31, 47–51]

*See Parkes[45] for a masterful review.

Studies of ovarian venous blood[52, 53] have demonstrated the presence of 17-ketosteroids, and elevated levels of blood testosterone have been observed in virilized patients with polycystic ovaries. [54, 55] Thus, there is no longer any doubt as to the existence and identity of the ovarian androgens. These

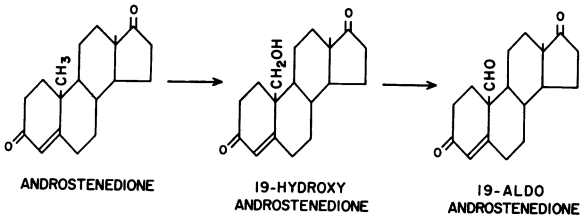


Fig. 7. Progressive oxidation of 19-methyl group of androstenedione.

findings explain the different levels of urinary 17-ketosteroid excretion associated with adrenal vs. ovarian virilization. Since the major adrenal ketosteroid is dehydroepiandrosterone (apparently little or no testosterone is produced by the adrenal), it requires large quantities of this weak androgen to bring about pronounced clinical manifestations, and this elevated output is reflected in a high level of urinary 17-ketosteroids. The ovary, on the other hand, produces testosterone, a much more potent androgen, and consequently extreme degrees of virilization (as with arrhenoblastomas) may be the result of quantitatively much smaller production of steroid, causing little or no elevation of urinary 17-ketosteroids.

Before turning to a consideration of abnormalities of ovarian androgen production, it is necessary to complete the discussion of ovarian steroidogenesis by examining the mechanism which transforms these various androgens into the natural estrogens. This transformation takes place in two major stages. The first stage consists of an oxidative attack on the C-19 methyl group, which sticks out at right angles from the front face of the steroid molecule. This methyl group is first oxidized to an alcohol (forming 19-hydroxyandrostenedione or testosterone), then to an aldehyde (19-aldoandrostenedione or testosterone), Fig. 7. These intermediates have been identified in biosynthetic experiments with normal and polycystic ovarian tissue.[29] The second stage which involves aromatization of ring A begins by an enzymatic removal of the oxidized methyl group together with a nearby hydrogen atom. This results in an unstable intermediate which spontaneously rearranges to form the phenolic ring-A characteristic of the natural estrogens (Fig. 8). Evidence in support of this mechanism has been presented elsewhere.[56] By this route, therefore, androstenedione is converted to estrone, testosterone into estradiol. It is interesting to note that in our biosynthetic studies with normal or polycystic ovarian tissue, estradiol has always been the predominant estrogen formed.

Biosynthetic experiments in vitro reveal the potentialities of tissue under the given conditions, and presumably this reflects the activity of the organ in vivo; however, the relationship between in vitro and in vivo conditions is in need of further study. This question involves some surprising complications. Falck[57] in a technical *tour de force* transplanted vaginal epithelium and small nests of ovarian cells side by side in the anterior chamber of the eye of experimental animals, and showed that no single cell type produced cornification of the adjacent vaginal epithelium. When two types of ovarian cells in various combinations (theca interna or interstitial cells combined with granulosa or corpus luteum cells) were transplanted, however, cornification (i.e., estrogen production) occurred. In rabbits, it has been found that the interstitial tissue can produce as much progestagen as control ovaries with intact follicles,[58] and in humans, the medullary portion of polycystic ovaries is as active as the cortical zone in steroidogenesis.[59] Some workers have chosen to examine the steroid content of ovarian tissue in an effort to find correlations with the functional state of the ovary. Some of the data on tissue steroid concentration are summarized in Table 3.[30, 60–65] It is

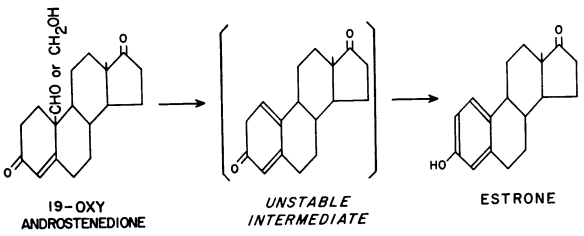


Fig. 8. Conversion of 19-oxygenated 17-ketosteroid into estrogen ("aromatization").

TABLE 3. Concentration of Steroids in Ovarian Tissue

	Concentration (μg./gm.)	
Steroid	*Normal*	*Polycystic*
Progesterone	0–90	
17-hydroxypregnenolone	0	0–4.5
17-hydroxyprogesterone	0–1.7	3–6
"20-hydroxyprogesterone"	0–19	
Δ^4-androstenedione	0.6	0–15
Testosterone	<0.3	
Dehydroepiandrosterone	0–1	0–7
Estrone	0.07–2	0.3–1.5
Estradiol	0.01–0.9	1.1–2.5
Estriol	0–0.6	0–0.9

Summary of results by various authors. Since different technics were used, the results are not necessarily comparable.

evident that ovarian tissue does not store appreciable quantities of steroids; indeed, the levels are so low in most instances as to be at the limit of detectability (to say nothing of reliability) of current technics. This approach to the problem therefore presents formidable difficulties. Moreover, since little is known of the relationship of tissue storage to the level of hormonal activity of the organ, the results are difficult to relate to ovarian function.

The escape of some of the intermediate steroid compounds from the ovarian biosynthetic "factory" into the peripheral circulation and hence into the urine might theoretically prove of value as an index of ovarian activity. Indeed, urinary excretion of Δ^5-pregnenetriol,[66, 67] pregnanetriol,[68] and other metabolites has been examined with this aspect in mind, but as with the urinary 17-ketosteroids, the contribution from the adrenal confuses the findings.

The technic of determining steroid secretory rates by isotope dilution has been widely employed in studies of adrenal function where determinations of cortisol or aldosterone secretory rates are fairly routine research procedures. It is only recently, however, that this methodology has been applied to the androgens,[69] estrogens,[70] and progesterone. Dominguez *et al.*[71] have found a progesterone secretory rate of 2.5–5.4 mg./day in the preovulatory phase, rising to 22–43 mg./day during the postovulatory phase. At the ovulatory peak, estradiol secretory rates of 0.2–0.5 mg./day have been observed.[70] Important studies in normal and hirsute patients have been carried out by MacDonald *et al.*[31] using dual-label technics to overcome the difficulty presented by the fact that both dehydroepiandrosterone and testosterone (or androstenedione) are metabolized to the same urinary excretory products. Normal ovaries were estimated to produce 1–2 mg. dehydroepiandrosterone and up to 1.6 mg. testosterone+androstenedione per day. By comparison, the adult testis produces 4–5 mg. dehydroepiandrosterone and 2.8–4.5 mg. testosterone+androstenedione per day. In hirsute women with normal 17-ketosteroid excretion and in some instances with polycystic ovaries, secretory rates were determined with the adrenals suppressed by dexamethasone. Under these conditions, increased secretion of dehydroepiandrosterone and/or testosterone+androstenedione was demonstrated. In one instance the ovaries produced 16 mg. dehydroepiandrosterone (but no testosterone+androstenedione) per day, while in another instance 4.5 mg. dehydroepiandrosterone and 5.6 mg. testosterone+androstenedione were produced—a rate comparable to that of the normal testis. Suppression of the pituitary gonadotropic stimulus with norethindrone acetate invariably resulted in a prompt drop of ketosteroid secretion, thus further confirming their ovarian origin. Studies such as these are time-consuming and require highly sophisticated technics, and when complications such as the secretion

of conjugated dehydroepiandrosterone by the adrenal are taken into consideration the mathematics become quite complex. There is also the important possibility that the state of the pituitary-ovarian axis may be altered by the dexamethasone used for adrenal suppression during these studies. Nevertheless, it will require methods such as these to provide fundamental information about the in vivo activity of the ovaries.

To explore these and other areas of endocrine abnormality in polycystic ovaries, many investigators, including ourselves, have concentrated on the information to be gained by examining the in vitro biosynthetic potential of polycystic ovarian tissue. Table 4 shows a comparison of the definitively identified metabolites from incubations of normal and polycystic ovarian tissue with progesterone and pregnenolone. The normal ovarian tissue is seen to metabolize all but 10.7% of the starting material at the end of 2 hr., the majority of the metabolites being in the form of 17- and/or 20-hydroxyprogesterones. Formation of androstenedione and testosterone is found; there is of course no dehydroepiandrosterone since the precursor was progesterone. There are considerable quantities of 19-oxygenated steroids and 4.8% conversion of the starting material to estradiol. No estrone or estriol was detected. The polycystic ovaries A and B also incubated with radioprogesterone were, if anything, even more active than the normal ovary, since they metabolized 99 and 100% of the starting material, and since much less 17- and/or 20-hydroxylated progesterone was left. However, a

TABLE 4. Steroids Produced by the Incubation of Normal and Polycystic Ovarian Tissue with Various Precursors

	% conversion			
		Polycystic ovary		
		With progesterone		
Steroid	*Normal ovary with progesterone*	*A*	*B*	*With pregnenolone*
Unconverted substrate	10.7	0.9	0.0	63.9
17- and/or 20-hydroxylated metabolites	78.2	43.1	46.8	6.1
Androstenedione	1.1	22.6	25.1	1.9
Testosterone	0.4	2.2	3.4	9.1
Dehydroepiandrosterone				15.1
19-oxygenated androstenedione or testosterone	3.9	31.3	24.4	3.5
Estrone	0.0	0.0	0.0	0.0
Estradiol	4.8	0.0	0.0	0.0

tremendous quantity of androstenedione (22.6 and 25.1%) and testosterone (2.2 and 3.4%) accumulated. Equally impressive is the conversion to 19-oxygenated steroids, being 6 to 10 times as great as that seen in the normal. However, no detectable estrogen was formed by these particular ovaries, immediately suggesting that the aromatizing mechanism which converts 19-oxygenated compounds to estrogens was defective and that the accumulation of androgens and 19-oxy compounds represents a "backing up" of steroid material behind the roadblock.

In the polycystic ovary incubated with pregnenolone, there is a very poor conversion of the starting material and also an accumulation of dehydroepiandrosterone, in addition to the abnormalities already seen in polycystic ovaries A and B. This would suggest an inadequacy of the 3β-ol dehydrogenase system in this particular ovary, as well as the aromatizing defect, which is clearly present in view of the 3.5% conversion to 19-oxygenated steroids with no detectable conversion to estrogen. These findings are entirely consistent with secretion rate studies of MacDonald *et al.*[31] described above.

Such observations do not imply the uniform existence of invariable, all-or-none enzyme defects in polycystic ovarian tissue. The very fact that measurable urinary estrogen excretion along with more or less cornification of the vaginal epithelium does occur proves that in certain cases and at certain times biosynthesis can go on to aromatization. (In one polycystic ovary incubated with radiotestosterone, we have observed 10.1% conversion to estradiol and 3.2% conversion to estrone.) It appears, however, that there is still some obstruction to aromatization in these cases, as shown by the disproportionately large ratio of androstenedione and testosterone to estrogen formed. To put it another way, in the process of producing a particular amount of estrogen, the ovary is forced to synthesize and therefore secrete excessive amounts of androgen. Short and London[72] have examined cyst fluid from human ovaries, and in "Stein-Leventhal" instances found an increased content of androstenedione and an absence of estrogens. On this basis, they also concluded that there was a defect in estrogen synthesis of polycystic ovaries. Confirmatory results by means of tissue incubation have been reported although unfortunately the necessary technical details were lacking.[53]

It would appear incontrovertible then, that the androgenic manifestations associated with polycystic ovaries are in part due to an overproduction of ovarian androgens as a consequence of variable enzymatic defects in the aromatizing mechanism, the 3β-ol dehydrogenase system, and possibly other enzyme functions as well. That these defects do *not* define a clinical entity is shown by consideration of the cases which provided incubations A and

B in Table 4. The biosynthetic pattern of these two tissues is virtually identical. Clinically, however, "polycystic ovary A" presented a typical picture of androgenic adrenal hyperfunction (adrenogenital syndrome) without obesity or amenorrhea. Virilization and infertility were present; the 17-ketosteroid excretion ranged from 19 to 23 mg/day and was readily suppressed by minimal corticosteroid therapy, and a corpus luteum was found at wedge resection of the slightly enlarged, polycystic ovaries. "Polycystic ovary B" had all the symptoms associated with the "Stein-Leventhal syndrome." The urinary 17-ketosteroids ranged from 18 to 27 mg. per day and were not diminished by the standard corticosteroid suppression test. No corpus luteum was found on wedging the polycystic ovaries. It is paradoxical that the patient with the adrenal disorder became pregnant after wedging, whereas the "Stein-Leventhal" did not. Findings such as these, together with the established fact that surgical damage of the ovaries normalizes their function in a significant per cent of cases, indicate that the enzymatic defects found in polycystic ovaries are not irreversible, and that they may be the consequence of still more recondite influences which initiated the ovarian pathology. A search for these factors leads to the hypothalamic-pituitary mechanism which largely governs ovarian activity.

HYPOTHALAMIC-PITUITARY-OVARIAN RELATIONSHIPS

Our knowledge of hypothalamic regulation of the menstrual cycle in primates and especially in humans is far less advanced than one might suppose. Most of the information available has been derived from studies of rodents, particularly those having an estrus cycle.[73] It is usually assumed that the basic features of the estrus cycle are comparable to those of the primate menstrual cycle, but there is reason to believe that future research will show important fallacies in this assumption. Nevertheless, one must deal with the information at hand, recognizing its tentative and very possibly species-specific character.

Our current concept of the neural-pituitary-ovarian circuit is diagrammed in Fig. 9. A region in the preoptic area of the hypothalamus, possibly the suprachiasmatic nucleus, appears to act as a biological "clock," imparting a cyclic character to the rest of the mechanism and thus accounting for the estrus cycle and the menstrual rhythm. These neural impulses are registered in the region of the median eminence of the hypothalamus, probably the ventromedial and arcuate nuclei.[74] These centers produce a neurohormone[75] which has been called the gonadotropin-releasing factor (GRF). GRF is transported by means of the hypophyseal portal veins (note the similarity to the role of corticotrophin-releasing polypeptide in the pituitary-adrenal axis)

to the adenohypophysis, where it causes a synthesis and release of pituitary gonadotropin. Thus, the median eminence area of the hypothalamus exerts a tonic stimulation on the pituitary, and the preoptic area superimposes a cyclic variation on this mechanism. The released gonadotropin (whatever its

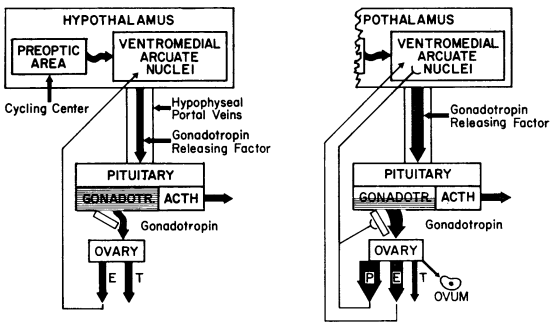


Fig. 9. Hypothalamic-pituitary-ovarian mechanism. At left, preovulatory relationships; At right, post-ovulatory relationships. E = estrogens; T = testosterone (androgens); P = progesterone.

true qualitative character may be) stimulates the ovary to secrete androgens and estrogens approximately in the ratio of 7:1. The estrogens released into the circulation have a stimulatory effect on the nuclei near the median eminence (a "positive feedback" effect), causing increased secretion of GRF, which in turn increases gonadotropin secretion, and so on. At a certain point, the rising estrogen output triggers ovulation.[76] With the occurrence of ovulation and the production of an egg, dramatic changes in the ovarian steroid output occur. In addition to estrogen, there is now a tremendous output of progesterone (milligrams of progesterone as compared to micrograms of estrogens). Progesterone inhibits the release (but not the formation) of pituitary gonadotropins[77, 78] (Fig. 9), and also has an inhibitory effect on the hypothalamic centers. This "negative feedback" mechanism serves to diminish the gonadotropic stimulus of the ovary, and together with the waning phase of the preoptic "cycling" center brings about the termination of the cycle.

The mechanism permits an interpretation of some remarkable experiments based on the rediscovery of certain simple methods for producing persistent estrus in rats.[79] Electrolytic lesions or single massive injections of steroids in the first few days of life apparently damage the cycling center of the rat hypothalamus; the results are shown in Fig. 10. There is now a *tonic* discharge of GRF from the ventromedial and arcuate nuclei, causing a continuous release of pituitary gonadotropin. The resulting tonic secretion of estrogen and positive feedback turn this into a situation where the pituitary never gets a chance to pause and store up gonadotropin for the large burst needed in the process of ovulation. Thus an anovulatory mechanism is set up, with a continuous low-level stimulation of the ovary. As these

rats grow to maturity, *the ovaries become enlarged and polycystic, and there also occurs a hypertrophy of the adrenals.*

Under these conditions of continuous, noncyclic ovarian stimulation it is conceivable that changes in the ovarian steroid biosynthetic mechanism might occur. For this reason, we incubated a number of such enlarged, polycystic rat ovaries in the same manner as used for the studies on human ovaries. The results of one such experiment are shown in Table 5. Keeping in mind the possibility of species-specific differences in ovarian steroidogenesis,[80, 81] one fact emerges with great clarity: *although there is ample conversion of the pregnenolone to androgens, no aromatization to estrogens is detectable.* The similarity to one of the enzymatic abnormalities observed in human polycystic ovaries is apparent. It is possible that experimental modifications which take into account the fact that rat tissue metabolizes much faster than human tissue (a cycle is complete in 4 days vs. 28 days for man) might have demonstrated the presence of other intermediates such as 19-oxygenated compounds. It is also evident from the fact that these animals are in persistent estrus that *some* estrogen is being formed (although other steroids are known to be able to produce vaginal cornification),[82–84] but the important fact, as seen with human tissue also, is that the ratio of androgen to estrogen formed must be extremely high. This overproduction of androgen, now observed in both rat and man, might have some bearing on the formation of capsular fibrosis in human polycystic ovarian disease, since the

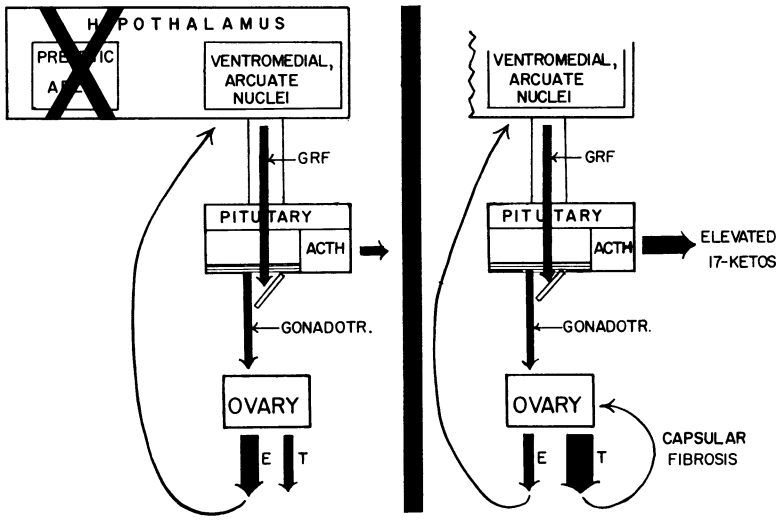


Fig. 10. Genesis of polycystic ovaries. Disruption of hypothalamic-pituitary-ovarian mechanism in persistent-estrus rat. At left, early stage; At right, breakdown of enzyme systems and possible cause of ovarian fibrosis seen in human disease.

TABLE 5. Steroidogenesis in Ovarian Tissue of Persistent-Estrus Rats

Substrate: pregnenolone-4-C-14	
Metabolite	*Total DPM*
Unconverted pregnenolone	391,540
Δ^5-pregnen-3β, 20β-diol	10,870
17α-hydroxyprogesterone	6,890
Dehydroepiandrosterone	8,620
Δ^4-androstenedione	20,100
Testosterone	12,470
Estrogens	None

administration of androgens to primates for prolonged periods of time has been shown to cause such a lesion.[85] This is shown diagrammatically in Fig. 10.

There are other important experiments which have been performed with these persistent-estrus rats. If the estrogenic "positive feedback" mechanism is interrupted by removing one ovary and resecting most of the second (or inactivating its hormones by transplanting it to the portal circulation), the character of pituitary function changes and luteinization of the remaining ovary occurs.[86, 87] Still another experiment affirms this line of thought: Electrical stimulation of the hypothalamus of persistent-estrus rats fails to produce ovulation unless the animals are pretreated with large doses of progesterone (causing a storage of pituitary gonadotropin).[74]

These experiments may well suggest an answer to the perpetual question, why wedge resection restores cyclic function in some women with polycystic ovaries. By analogy with these rodent experiments, consider the endocrine situation which may develop in certain instances of human polycystic ovarian disease. Characteristically, menarche occurs at about the normal age and there is some menstrual function for a time. The histories often suggest that this phase of early ovarian function is anovulatory. If, for some reason, the human hypothalamic "cycling center" does not straighten matters out, the ovary remains exposed to a more or less tonic gonadotropic stimulus, without the beneficial gonadotropin-accumulating influence of progesterone. Thus the vicious circle of noncyclic GRF secretion, noncyclic and possibly qualitatively abnormal gonadotropin secretion, and finally uninterrupted estrogenic positive-feedback develops. Ultimately certain ovarian steroid-biosynthetic enzymes become exhausted. (In this connection it is worth noting that the testis, under its *non*cyclic gonadotropic control biosynthesizes androgens physiologically. When the ovary is exposed to noncyclic gonadotropic influences, its production of female hormones breaks down and it

reverts to a testicular pattern of steroidogenesis.) Chronic exposure of the ovary to these gonadotropic stimuli produces cystic changes, and the constant presence of high local concentrations of androgens may account for the ovarian fibrosis. Now, the surgeon inflicts a major trauma on the ovary, especially if he follows the customary recommendations to resect a *generous* portion of tissue and to extend his incision *well down into the hilus,* where the major blood supply is found. This procedure so affects the ovary that its steroid production is greatly reduced or perhaps even interrupted for a time. Positive feedback and the vicious circle are broken; secretion of gonadotropin is slowed or halted and accumulation can occur; the exhausted ovarian steroidogenic enzymes may be able to recover. If the cycling center is intact, it may be able to take over and initiate a rhythm which the rested pituitary and ovary may be able to follow. The fact that infertility is corrected less often than the menstrual rhythm may be due to mechanical problems, irreversible pathology of the ovary, or to as yet unknown factors. Leclercq[88] examined a successfully resected polycystic ovary some years later and described its appearance as "entirely normal." Others, including ourselves, have observed recurrence of the symptoms and again a typical gross pathology at the time of the second wedging. Whether these changes persisted or recurred is impossible to say.

There are, of course, many additional and perhaps very important factors which this tentative working hypothesis has not included. Puzzling instances of unilateral polycystic disease have been reported.[89–91] There is the question of the intrinsic responsiveness of the ovarian tissue to gonadotropin. Many animal experiments have shown that the administration of gonadotropin will produce a type of polycystic ovary in the hypothyroid animal, whereas that same treatment will be ineffective in euthyroid controls.[92, 93] The excessive enlargement of human polycystic ovaries in response to gonadotropin[94, 95] or clomiphene is an observation which requires further clarification. Taymor and Barnard[96] have recently supported the observations of Ingersoll and McArthur[97] of an intermittent elevation of urinary LH in some patients with polycystic ovaries and found these changes in patients with both normal-sized and enlarged ovaries. Many technical difficulties as well as troublesome day-to-day variation in gonadotropin output beset these investigations; improved methods and additional studies may explain the "normal" results and hopefully provide correlations with the neurohypophyseal studies in rodents.

At all events, these advances in ovarian biochemistry and neurohypophyseal relationships have suggested new approaches for research on the polycystic ovary problem and a working diagram which can be tested and will no doubt be modified, perhaps greatly, by future observations. The clinical

implications of the findings we have summarized are equally important. A more flexible attitude and a critical re-examination of our diagnostic criteria is evidently needed. Which cases of "secondary amenorrhea" or "functional uterine bleeding" might be associated with polycystic ovaries? What functional or in vitro studies will improve our ability to select those cases most likely to benefit from wedge resection, and what factors are present in those cases where surgery fails? Would the action of progesterone in the persistent-estrus rat be duplicated in the human—i.e., would there be a beneficial result from giving large doses of progesterone postoperatively to promote storage of gonadotropin in the pituitary? These and many other vital questions await further research in polycystic ovarian disease.

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REFERENCES

1. MARTIN, A. Ergebnisse der Ovarien und Tubenresektion. *Verhandl. deutsch. ges. Gynäk. 4:*242, 1891.
2. MATTHAEI, F. Uber die Ovarialresektion. *Zentralbl. Gynäk. 34:*828, 1894.
3. WALDO, R. Cyst of the distal end of the left fallopian tube and cystic degeneration of the right ovary removed one year after the removal of a portion of the left fallopian tube and small cysts from the right ovary. *Am. J. Obst. & Gynec. 32:*444, 1895.
4. BULIUS, G., and KRETSCHMAR, C. *Angiodystrophia Ovarii.* Enke, Stuttgart, 1897.
5. GOLDSPOHN, A. Resection of the ovaries. *Am. J. Obst. & Gynec. 70:*934, 1914.
6. MCGLINN, J. A. The end results of resection of the ovaries for microcystic disease. *Am. J. Obst. & Gynec. 73:*435, 1916.
7. KOHLER, R. Beeinflussung der Menstruation durch Reduktion der Ovarien. *Zentralbl. Gynäk. 44:*1695, 1923.
8. AMENOBAR, V. Resektion und Einschrankung des Eierstockblutkreislaufes zur Behandlung der sklerocystischen Oophoritis. *Ber. ges. Gynäk. Geburtsh. 2:*435, 1924.
9. STEIN, I. F., and LEVENTHAL, M. L. Amenorrhea associated with bilateral polycystic ovaries. *Am. J. Obst. & Gynec. 29:*181, 1935.
10. EVANS, T. N., and RILEY, G. M. Polycystic ovarian disease. *Am. J. Obst. & Gynec. 80:*873, 1960.
11. FITZGERALD, J. A., and CROSSLEY, J. L. Clinical vagaries of the Stein-Leventhal ovary. *New York J. Med. 55:*1179, 1955.
12. NETTER, A., LAMBERT, A., and HASKELES, M. Syndrome de Stein-Leventhal. A propos de 4 cas. *Compt. Rend. Soc. Franc. Gyn. 28:*127, 1958.
13. ROBERTS, D. W., and HAINES, M. Is there a Stein-Leventhal syndrome? *Brit. M. J. 1:*1709, 1960.
14. PERLOFF, W. H., CHANNICK, B. J., HADD, H. E., and NODINE, J. The Stein-Leventhal ovary: A manifestation of hyperadrenocorticism. *Fertil. & Steril. 9:*247, 1958.
15. GOLDZIEHER, J. W., and GREEN, J. S. The polycystic ovary. I. Clinical and histological features. *J. Clin. Endocrinol. 22:*325, 1962.
16. IANNACONE, A., GABRILOVE, J., SOHVAL, A., and SOFFER, L. The ovaries in Cushing's syndrome. *New England J. Med. 261:*775, 1959.
17. VARA, P., and NIEMINEVA, K. Small cystic degeneration of ovaries as incidental finding in gynecological laparotomies. *Acta obst. gynec. scandinav. 31:*94, 1951.

18. SOUTHAM, A. Personal communication.
19. VAGUE, J., PICARD, D., VITRY, G., GALINIER, L., FAVIER, G., and MILLER, G. Cytologic study of the sex chromatin in various endocrinopathies. *Ann. endocrinol. 17*:51, 1956.
20. WAIS, S. Bilateral polycystic ovaries with negative chromatin pattern. *Acta endocrinol. suppl. 51*:615, 1960.
21. NETTER, A., BLOCH-MICHEL, H., SALOMON, Y., THERVET, F., DEGROUCHY, J., and LAMY, M. Study of karyotypes in Stein-Leventhal syndrome. *Ann. endocrinol. 22:* 841, 1961.
22. CERVINO, J. M., RAVERA, J., GROSSO, O. F., POU DE SANTIAGO, A., MORATO MANARO, J., and MUSSIO FOURNIER, J. C. Syndrome of Stein-Leventhal in 2 sisters. *Ann. endocrinol. 17*:355, 1956.
23. TRACE, R. J., KEATY, E. J., and MCCALL, M. L. An investigation of ovarian tissue and urinary 17-ketosteroids in patients with bilateral polycystic ovaries. *Am. J. Obst. & Gynec. 79*:310, 1960.
24. GEMZELL, C. A., TILLINGER, K. G., and WESTMAN, A. Hirsutism: a clinical study of ovarian pathology and the urinary excretion of 17-ketosteroids. *Acta endocrinol. 30*:387, 1959.
25. PLATE, W. P. Pathologic anatomy of the Stein-Leventhal syndrome. *Bull. Soc. Roy. belge gynéc. et obst. 27*:671, 1957.
26. GOLDZIEHER, J. W., and AXELROD, L. R. The polycystic ovary. II. Urinary steroid excretion. *J. Clin. Endocrinol. 22*:425, 1962.
27. SZENAS, P., and PATTEE, C. J. Studies of adrenocortical function in obesity. *J. Clin. Endocrinol. 19*:344, 1959.
28. GOLDZIEHER, J. W., and AXELROD, L. R. A study of methods for the determination of total, grouped, and individual urinary 17-ketosteroids. *J. Clin. Endocrinol. 22:* 1234, 1962.
29. AXELROD, L. R., and GOLDZIEHER, J. W. The polycystic ovary. III. Steroid biosynthesis in normal and polycystic ovarian tissue. *J. Clin. Endocrinol. 22*:431, 1962.
30. MAHESH, V. B., and GREENBLATT, R. B. Isolation of dehydroepiandrosterone and 17-hydroxy Δ^5-pregnenolone from the polycystic ovaries of the Stein-Leventhal syndrome. *J. Clin. Endocrinol. 22*:441, 1962.
31. MACDONALD, P. C., VANDE WIELE, R. L., and LIEBERMAN, S. Precursors of the urinary 11-desoxy-17-ketosteroids of ovarian origin. *Am. J. Obst. & Gynec. 86*:1, 1963.
32. GOLDZIEHER, J. W., and LAITIN, H. Evaluation of the pituitary-adrenal axis in simple hirsutism. *J. Clin. Endocrinol. 20*:967, 1960.
33. NETTER, A., JAYLE, M. F., MUSSET, R., LAMBERT, A., and MAUVAIS-JARVIS, P. A functional test for the Stein-Leventhal syndrome. *Ann. endocrinol. 21*:590, 1960.
34. MAUVAIS-JARVIS, P. Studies on the endocrine anomalies encountered in the course of the Stein-Leventhal syndrome. *Bull. et mém. Soc. méd. hop. Paris. 77*:611, 1962.
35. BAULIEU, E. E., MAUVAIS-JARVIS, P., and CORPECHOT, C. Steroid studies in a case of Stein-Leventhal syndrome. *J. Clin. Endocrinol. 23*:374, 1963.
36. SHEARMAN, R. P. The role of the adrenal cortex in the Stein-Leventhal syndrome. Australian New Zealand *J. Obst. & Gynaec. 1*:24, 1961.
37. GIUSTI, G., BORGHI, A., TOCCAFONDI, R., and BIGOZZI, U. The frequency of androgenic adrenal hyperfunction associated with Stein-Leventhal type of changes in the ovary. *Acta endocrinol. suppl. 51*:405, 1960.
38. PELLEGRINI, J., PIOTTI, L., and FERRARI, E. Persistence of abnormalities of steroid metabolism after bilateral wedge resection in 3 cases of Stein-Leventhal syndrome. *Acta endocrinol. suppl. 51*:623, 1960.
39. LEVENTHAL, M. L. Functional and morphologic studies of the ovaries and suprarenal glands in the Stein-Leventhal syndrome. *Am. J. Obst. & Gynec. 84*:154, 1962.

40. SOHVAL, A. R., and SOFFER, L. The influence of cortisone and adrenocorticotropin on urinary gonadotropin excretion. *J. Clin. Endocrinol. 11*:677, 1951.
41. VAN TIENHOVEN, A. The effect of massive doses of corticotrophin and of corticosterone on ovulation of the chicken. *Acta endocrinol. 38*:407, 1961.
42. LAYNE, D. S., MEYER, C. J., VAISHWANAR, P. S., and PINCUS, G. The secretion and metabolism of cortisol and aldosterone in normal and in steroid-treated women. *J. Clin. Endocrinol. 22*:107, 1962.
43. GEMZELL, C. A., DICZFALUSY, E., and TILLINGER, K. *Ciba Found. Colloq. on Endocrinol. 13*:191, 1960.
44. BARRACLOUGH, C. A. Production of anovulatory sterile rats by single injections of testosterone propionate. *Endocrinology 68*:62, 1961.
45. PARKES, A. S. Androgenic activity of the ovary. *Recent Progr. in Hormone Res. 5*:101, 1950.
46. GOLDZIEHER, J. W., and AXELROD, L. R. Adrenal and ovarian steroidogenesis in the sclerocystic ovary syndrome. *Acta endocrinol. suppl. 51*:617, 1960.
47. SMITH, O. W., and RYAN, K. J. Biogenesis of estrogens by the human ovary: Formation of neutral steroid intermediates from progesterone-4-C-14, androstenedione-4-C-14 and cholesterol-4-C-14. *Endocrinology 69*:970, 1961.
48. WARREN, J. C., and SALHANICK, H. A. Steroid biosynthesis in the human ovary. *J. Clin. Endocrinol. 21*:1218, 1961.
49. STARKA, L., MATYS, Z., and JANATA, J. Identification of dehydroepiandrosterone in human sclerocystic ovaries. *Clin. chim. acta 7*:776, 1962.
50. KASE, N., KOWAL, J., and SOFFER, L. Androgen biosynthesis from progesterone-7-H-3 and 17-hydroxyprogesterone-4-C-14 by normal and abnormal human ovaries. Proc. 44th Meeting Endocrinol. Soc. 1962.
51. NOALL, M. W., ALEXANDER, F., and ALLEN, W. M. Dehydroisoandrosterone synthesis by the human ovary. *Biochim. et biophys. acta 59*:520, 1962.
52. SEEMAN, A., and SARACINO, R. T. Contribution to the study of the formation of androgens by the ovary. *Acta endocrinol. 37*:31, 1961.
53. MAHESH, V. B., GREENBLATT, R. B., AYDAR, C. K., and ROY, S. Secretion of androgens by the polycystic ovary and its significance. *Fertil. & Steril. 13*:513, 1962.
54. DIGNAM, W., PION, R., LAMB, E., and SIMMER, H. Plasma androgens in women. II. Patients with polycystic ovaries and hirsutism. *Acta endocrinol.* (in press).
55. DORFMAN, R. I., SORCINI, G., BRUST, N., NIGHTINGALE, M., and FORCHIELLI, E. Testosterone in human blood. Proc. 44th Meeting Endocrinol. Soc. 1962.
56. AXELROD, L. R., and GOLDZIEHER, J. W. Mechanism of biochemical aromatization of steroids. *J. Clin. Endocrinol. 22*:537, 1962.
57. FALCK, B. Site of production of oestrogen in rat ovary as studied in microtransplants. *Acta physiol. scandinav. suppl. 47*:163, 1959.
58. HILLIARD, J., ARCHIBALD, D., and SAWYER, C. H. Gonadotropic activation of preovulatory synthesis and release of progestin in the rabbit. *Endocrinology 72*:59, 1963.
59. SALHANICK, H. A. Discussion. *Am. J. Obst. & Gynec. 84*:162, 1962.
60. ZANDER, J. Steroids in the human ovary. *J. Biol. Chem. 232*:117, 1958.
61. ZANDER, J., FORBES, T. R., VON MUNSTERMANN, A. M., and NEHER, R. Δ^4-3-keto-pregnene-20α-ol and Δ^4-3-keto-pregnene-20β-ol, two naturally occurring metabolites of progesterone; isolation, identification, biologic activity and concentration in human tissues. *J. Clin. Endocrinol. 18*:337, 1958.
62. ZANDER, J., BRENDLE, E., VON MUNSTERMANN, A. M., DICZFALUSY, E., MARTINSEN, B., and TILLINGER, K. G. Identification and estimation of estradiol-17β and oestrone in human ovaries. *Acta obst. gynec. scandinav. 38*:724, 1959.
63. SIMMER, H., and VOSS, H. E. Androgens in the human ovary. *Klin. Wchnschr. 38*: 819, 1960.

64. SCHILD, W. Qualitative and quantitative identification of free and conjugated estrone, estradiol-17β and estriol in biological material. II. Estrogen determinations in various tissues and body fluids. *Ztschr. ges. exper. Med. 135:*13, 1961.
65. KECSKES, L., MUTSCHLER, F., THAN, E., and FARKAS, I. Paper chromatographic isolation of estrone, estradiol-17β and estriol from human ovaries. *Acta endocrinol. 39:*483, 1962.
66. WILSON, H., LIPSETT, M. B., and RYAN, D. W. Urinary excretion of Δ^5-pregnenetriol and other 3β-hydroxy Δ^5 steroids by subjects with and without endocrine disease. *J. Clin. Endocrinol. 21:*1304, 1961.
67. PICKETT, M. T., and KELLIE, A. E. Urinary excretion of pregnanetriol in the menstrual cycle. *Acta endocrinol. 41:*129, 1962.
68. BURGER, H. G., and SOMMERVILLE, I. F. Further evidence for an ovarian source of urinary pregnanetriol. *Acta endocrinol. 43:*95, 1963.
69. GURPIDE, E., MACDONALD, P. C., VANDEWIELE, R. L., and LIEBERMAN, S. Measurement of the rates of secretion and of peripheral metabolism of two interconvertible compounds: Dehydroisoandrosterone-dehydroisoandrosterone sulfate. *J. Clin. Endocrinol. 23:*346, 1963.
70. GURPIDE, E., ANGERS, M., VANDE WIELE, R., and LIEBERMAN, S. Determination of secretory rates of estrogens in pregnant and nonpregnant women from the specific activities of urinary metabolites. *J. Clin. Endocrinol. 22:*935, 1962.
71. DOMINGUEZ, O. V., FRANCOIS, G. D., WATANABE, M., and SOLOMON, S. Progesterone secretion in man. Proc. 44th Meeting Endocrinol. Soc. 1962.
72. SHORT, R. V., and LONDON, D. R. Defective synthesis of ovarian steroids in the Stein-Leventhal syndrome. *Brit. M. J. 1:*1724, 1961.
73. MCCANN, S. M. Recent studies on the regulation of hypophysial luteinizing hormone secretion. *Am. J. Med. 34:*379, 1963.
74. BARRACLOUGH, C. A., and GORSKI, R. A. Evidence that the hypothalamus is responsible for androgen-induced sterility in the female rat. *Endocrinology 68:*68, 1961.
75. NIKITOVITCH-WINER, M. B. Induction of ovulation in rats by direct intrapituitary infusion of median eminence extracts. *Endocrinology 70:*350, 1962.
76. KOTZ, H. L., and HERRMANN, W. A review of the endocrine induction of human ovulation. III. Estrogen. *Fertil. & Steril. 12:*196, 1961.
77. GORSKI, R. A., and BARRACLOUGH, C. A. Adenohypophyseal LH content in normal, androgen-sterilized and progesterone-primed sterile female rats. *Acta endocrinol. 39:*13, 1962.
78. ROTHSCHILD, I. Corpus luteum-pituitary relationships: The effect of progesterone on the folliculotropic potency of the pituitary in the rat. *Endocrinology 70:*303, 1962.
79. TAKEWAKI, K. Some aspects of hormonal mechanism involved in persistent estrus in the rat. *Experientia 18:*1, 1962.
80. HUANG, W. Y., and PEARLMAN, W. H. The corpus luteum and steroid hormone formation. *J. Biol. Chem. 237:*1060, 1962.
81. VINSON, G. P., NORYMBERSKI, J. K., and JONES, I. C. The conversion of progesterone into oestrogens by the mouse ovary. *J. Endocrinol. 25:*557, 1963.
82. CLARKE, E., and SELYE, H. The action of steroid compounds on the vaginal epithelium of the rat. *Am. J. M. Sc. 204:*401, 1942.
83. DODDS, E. C. In *Biochemical Contributions to Endocrinology.* Stanford Univ. Press, Palo Alto, 1957.
84. TAKASUGI, N., BERN, H. A., and DEOME, K. B. Persistent vaginal cornification in mice. *Science 138:*438, 1962.

85. SCOTT, R. B., and WHARTON, L. H. The effect of testosterone on experimental endometriosis in Rhesus monkeys. *Am. J. Obst. & Gynec. 78:*1020, 1959.
86. FLERKO, B., and BARDOS, V. Luteinization induced in constant estrus rats by lowering estrogen production. *Acta endocrinol. 37:*418, 1961.
87. DESCLIN, L., FLAMENT-DURAND, J., and GEPTS, W. Transplantation of the ovary to the spleen in rats with persistent estrus from hypothalamic lesions. *Endocrinology 70:*429, 1962.
88. LECLERCQ, E. Le syndrome de Stein et Leventhal. *Bull. Soc. roy. belge gynéc. et obst. 28:*493, 1958.
89. SIEGLER, A. A unilateral ovary with polycystic disease. *Am. J. Obst. & Gynec. 64:* 431, 1952.
90. REMOUCHAMPS, L., and GHISBRECHT, P. F. Considerations on the syndrome of virilization of ovarian origin. *Ann. endocrinol. 15:*229, 1954.
91. ARGUELLES, A. E., SALABER, J. A., RICCA, A., and CHEKHERDEMIAN, M. Adrenocortical function in cases of polycystic ovaries. *Obst. y ginec. latino-am. 17:*1, 1959.
92. MEITES, J., and CHANDRASHEKAR, B. The effects of induced hyper- and hypothyroidism on the response to a constant dose of pregnant mare's serum in immature rats and mice. *Endocrinology 44:*368, 1949.
93. THORSOE, H. Development of polycystic ovaries following thyroidectomy. *Acta endocrinol. 40:*161, 1962.
94. STAEMMLER, H. J. Influence of gonadotropic hormone on ovarian dysgenesis. *Arch. Gynäk. 187:*711, 1956.
95. KEETTEL, W. C. Discussion. *Am. J. Obst. & Gynec. 77:*803, 1959.
96. TAYMOR, M. L., and BARNARD, R. Luteinizing hormone excretion in the polycystic ovary syndrome. *Fertil. & Steril. 12:*501, 1962.
97. INGERSOLL, F. M., and MCARTHUR, J. W. Longitudinal studies of gonadotropin excretion in the Stein-Leventhal syndrome. *Am. J. Obst. & Gynec. 77:*795, 1959.