THE EFFECT OF ANDROGENS AND ESTROGENS ON HUMAN SEBACEOUS GLANDS*

JOHN S. STRAUSS, M.D., ALBERT M. KLIGMAN, M.D., Ph.D. AND PETER E. POCHI, M.D.

This paper deals with the mechanism of action of androgens and estrogens on the human sebaceous gland. Our experimental objectives were the following: 1) to quantitate the effects of these hormones on sebaceous gland function: 2) to determine their mode of action, whether directly on the target organ itself or through systemic pathways; and 3) to analyze the "antagonism" between androgens and estrogens on the sebaceous gland.

MATERIALS AND METHODS

Most of the observations have been made on the glands of the face, because the fully developed glands of this region are very large and sebum secretion is high (1–5).[1] We have centered our attention particularly on the large sebaceous follicles which are almost peculiar to this region (6). Before puberty, the sebaceous glands are tiny and insignificant, sometimes consisting of undifferentiated anlage with occasional nests of lipid-laden cells. In contrast, the postpubertal sebaceous follicles are unique in that the usual size relationships are inverted, the glands having acquired an extraordinary size and the hair having been reduced to a mere vestige. Furthermore, the sebaceous follicles contribute most of the surface lipid on the face. These are the follicles which become involved in acne, a disease which we have under continuing study and which has been a major stimulus to our interest in sebaceous gland physiology.

The sebaceous gland is a holocrine gland which delivers its product, sebum, by the casting off of lipid-filled cells. The output of sebum, with certain qualifications, as will be noted, is directly proportional to gland size (6–8). The productivity of the gland can be measured either directly by gravimetric assay of collected sebum or indirectly by histologic assessment of gland size. The former is far more precise.

Gravimetric assay. The procedure has been described elsewhere (8). The sebum produced on the forehead from an area usually 6.45 square centimeters in size is collected for a three hour period. The area is delineated with adhesive tape to prevent exchange of sebum with the surrounding skin. The sebum is trapped on thin absorbent cigarette papers held in place by gauze and a rubber bandage. Subsequently, the lipid is extracted from the papers with ether and weighed. This method of trapping the sebum as it is produced overcomes one of the major difficulties which in the past has plagued quantitative studies of sebum output, namely the extraordinary tendency for sebum to flow away and be lost (6). With the above technic, we have found that sebum production is relatively constant for any one subject on repeated testing. Nevertheless, individual values do vary, sometimes considerably so. Thus, it is the trend of multiple readings that is of significance.

Histology. Deep punch biopsy specimens, four to seven millimeters in diameter, have been taken from symmetrical non-bearded areas of the cheek before and after hormone administration. Serial sectioning is required for a reliable estimate of gland size, because the glands vary in size and shape from follicle to follicle. This method is useful only for comparatively gross differences.

QUANTITATIVE EFFECTS OF ANDROGENS ON THE SEBACEOUS GLANDS

1) Systemic Administration of Androgens

There is substantial evidence from animal experimentation that the sebaceous glands are under androgenic control (9–16). Testosterone increases the size of the glands of immature rats;

[1] Volunteer subjects were drawn from the following sources: 1) Massachusetts Correctional Institution, Walpole, Massachusetts, John E. Gavin, Superintendent; 2) Walter E. Fernald State School, Commonwealth of Massachusetts, Malcolm J. Farrell, M.D., Superintendent; 3) Woodbine state Colony, Woodbine, New Jersey, H. VonBulow, Superintendent; 4) Vineland State School, Vineland, New Jersey, Miles E. Drake, M.D., Superintendent; and 5) Philadelphia County Prison, Holmesburg, Pennsylvania.

the glands of the adult animal undergo atrophy after orchiectomy. While it is generally felt that the human sebaceous gland is also androgen-sensitive, the evidence is scanty. After administering testosterone to castrate and eunuchoid males, Hamilton observed increased oiliness of the skin (17). However, no quantitative measurements were made. In six prepuberal boys Rony and Zakon observed histologic enlargement of the pubic sebaceous glands when 25 milligrams of testosterone propionate were given three times a week for two weeks (18). Furthermore, indirect evidence that the glands are under androgenic control is afforded by the emergence of acne in association with androgen secreting tumors or androgen therapy.

Experimental

A. *Prepuberal subjects.* Fourteen prepuberal children, eight boys and six girls, seven to eleven years of age, were given methyl testosterone[2] orally daily for periods of 7 to 12 weeks. Five boys and three girls received 50 milligrams daily; three girls received 75 milligrams daily; and three boys received 100 milligrams daily. The glands were studied in cheek biopsy specimens before and after treatment. The final biopsy was usually done at the end of treatment (seven to twelve weeks). In one subject, a specimen was removed at three weeks.

In every case, except for two boys who received 100 milligrams daily, there was substantial enlargement of the glands (Figure 1), although the degree of enlargement varied from subject to subject. The enlargement was already evident in the three week specimen. By histologic criteria, the boys and girls seemed to respond similarly, although this cannot be considered final in view of the crudity of the method. The individual differences in responsiveness are probably genetically determined. One year later biopsy specimens of two boys, not yet embarked into puberty, showed regression of the glands. In most of these children, slight oiliness of the face was noted while the androgen was administered and the follicular openings appeared enlarged. In no case did acneform lesions appear.

Sebum output was gravimetrically determined in fifteen prepuberal boys, seven to 13 years of age, who received 100 milligrams of methyl testosterone daily. In ten, there was a definite increase, strikingly so in five (Figure 2). In three, the increase was slight. Two were unresponsive.* Sebum output fell to the control level rapidly when the methyl testosterone was discontinued.

Next, the speed of response was determined. One hundred milligrams of methyl testosterone orally daily caused histologic enlargement in two prepuberal girls after seven and thirteen days respectively. The gravimetric data also indicated definitely increased sebaceous activity in two to three weeks (Figure 2). Because of the danger of epiphyseal closure, the drug was not continued for more than two months, and thus it was not possible to determine whether maximal glandular stimulation had been reached. However, in many of the subjects, the output even after this short period was definitely in the adult range. We tentatively feel that androgen stimulation can rapidly transform the skin into the postpubertal type, anatomically and functionally. Within limits (children less than five years of age were not studied) this androgen-induced metamorphosis is not dependent on age or whether the individual is about to enter puberty.

The minimally effective androgenic stimulus may also be considered an index of gland sensitivity. Two prepuberal boys received the small dose of 5 milligrams of methyl testosterone orally daily. Six weeks later, the glands had greatly enlarged. In three of six prepuberal boys, 5 milligrams of methyl testosterone orally daily caused a modest increase in sebaceous secretion. This dose appears to be close to the threshold. As will be pointed out, 5 milligrams of methyl testosterone orally daily is less than the estimated adult endogenous secretion of androgens, thus highlighting the great sensitivity of the gland to this hormone. We did not quantitatively determine the dose-response relationship, but did show that 100 milligrams of methyl testosterone orally daily brought about a much greater increase in sebaceous secretion than 5 milligrams orally daily.

B. *Post-puberal males.* Six adult males, between 20 and 30 years of age, were given 100 milligrams of methyl testosterone orally daily. Post-treatment biopsy specimens at six weeks were compared to controls. No changes were seen histologically or clinically. Similarly, three adult males received up to 300 milligrams of methyl testosterone daily for eight weeks. Sebum production did not increase in any case.

C. *Post-puberal females.* Eight post-puberal females in the second and third decade received 100 milligrams of methyl testosterone daily orally for six weeks. Histologically, four showed slight enlargement of the sebaceous glands. Two other females received 200 milligrams of methyl testos-

* We cannot account for the complete androgen insensitivity of occasional prepuberal subjects. Attention is called to the fact that the subjects in these particular studies were mental defectives with a variety of associated physical abnormalities and stunted, retarded growth. Lack of absorption may be one factor.
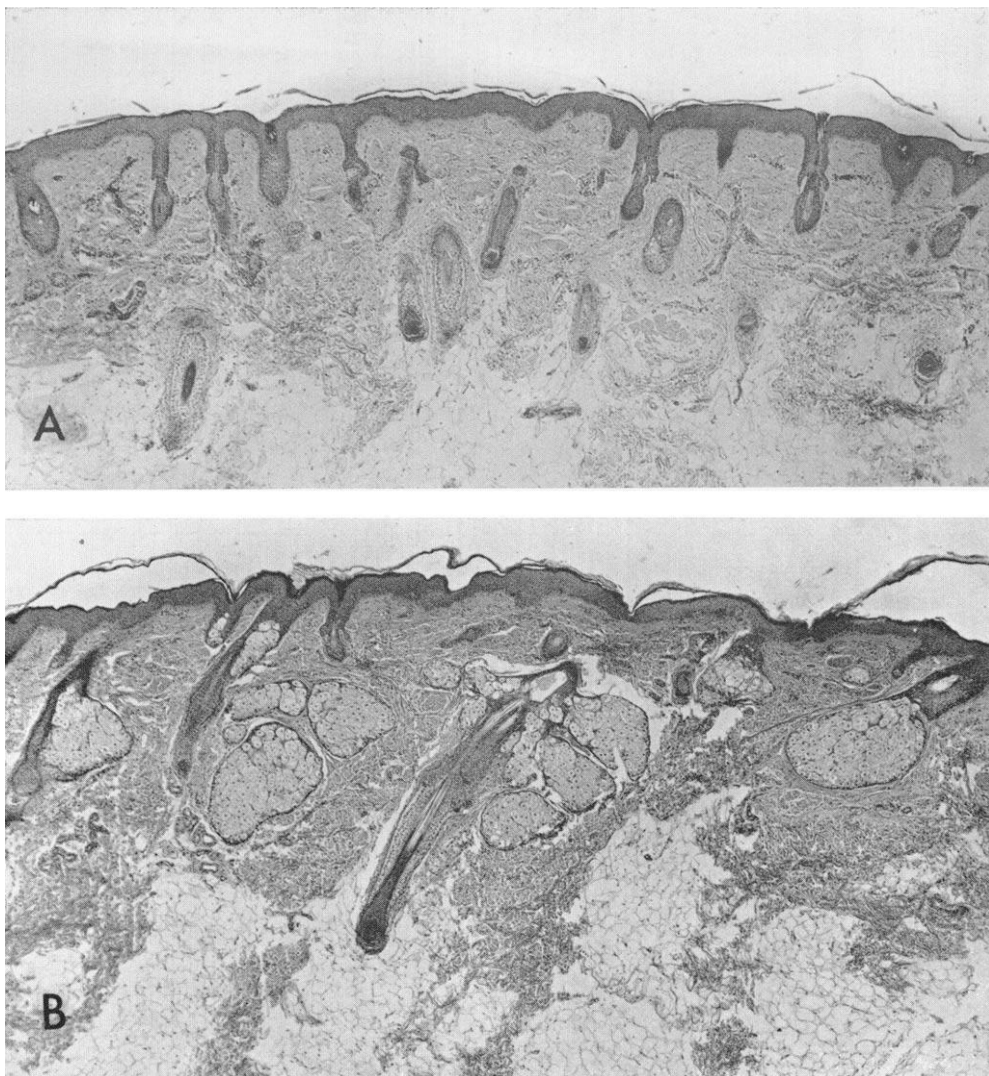
FIG. 1. Enlargement of the prepuberal sebaceous glands of the cheek of an eleven year old boy given 100 milligrams of methyl testosterone orally daily for twelve weeks. A) Pretreatment specimen. Sebaceous glands are small lateral outpocketings of undifferentiated cells. B) Post-treatment specimen. The glands and the follicles have greatly enlarged. Note that the corium has thickened too. This magnification is the same as A (hematoxylin and eosin, × 39).

terone daily for six weeks. One developed glandular hyperplasia; the other did not respond. Unfortunately, the more sensitive gravimetric assay method was not used.

D. *Aged females.* The sebaceous glands of the female are thought to undergo involution partially in old age, probably reflecting a diminished hormonal stimulation (19, 20). Three elderly females, over 65 years of age, received 100 milligrams of methyl testosterone daily orally for six to eight weeks. In each case, sebum production increased slightly, but not as rapidly as in the prepuberal subjects (Figure 3).

Comment

Exogenous androgen brings about prompt enlargement and increased secretion of the sebaceous glands of subjects in the prepuberal age group. The degree of enlargement with an adequate stimulus varies from subject to subject and probably depends more on the genetic responsiveness of the target organ than on the dose. The gland is highly androgen-sensitive. As little as 5 milligrams of methyl testosterone
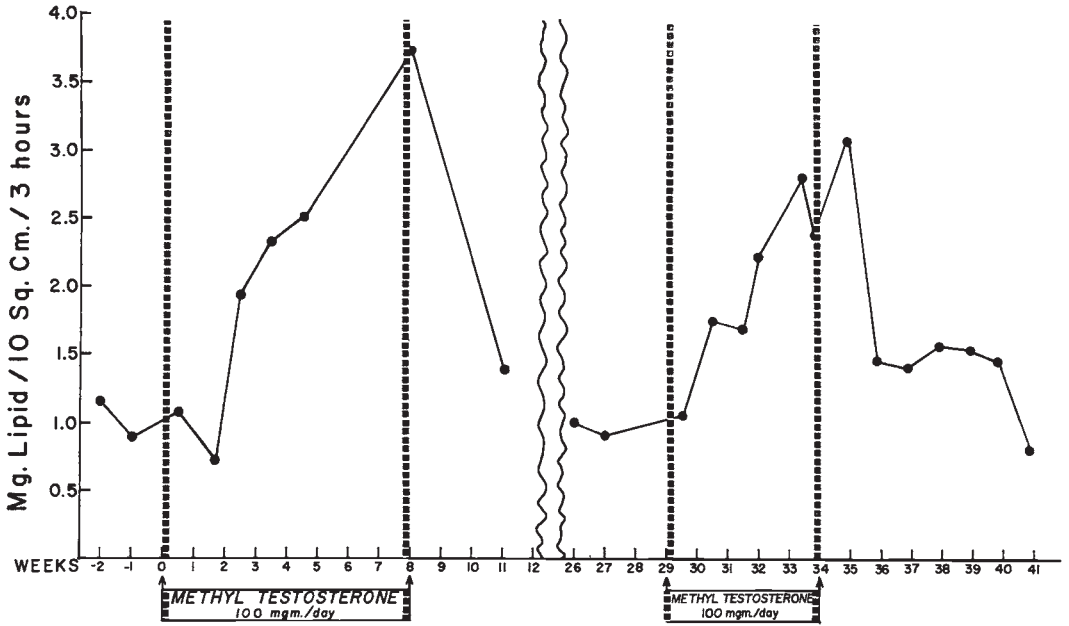
FIG. 2. Sebum output of an eleven year old prepuberal male given two courses of methyl testosterone orally (100 milligrams per day). Sebaceous secretion promptly rose with androgenic stimulation and quickly declined to the original level when the drug was stopped.
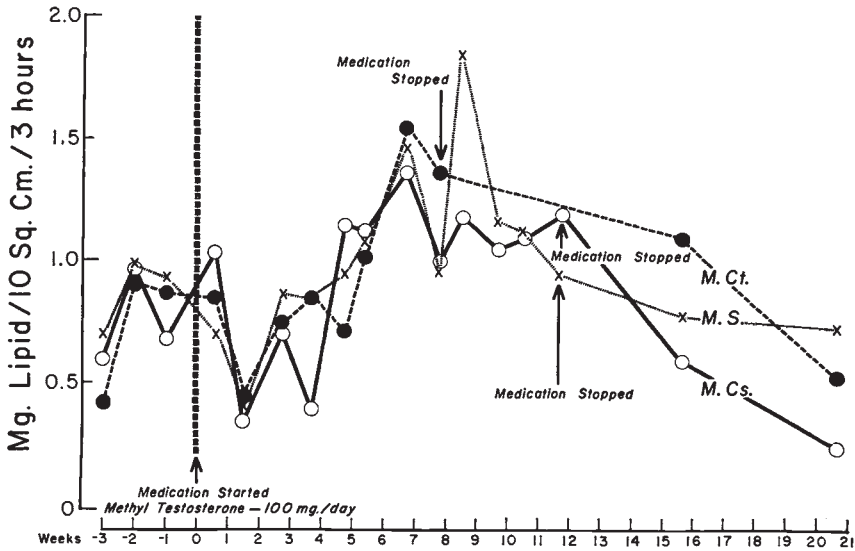
FIG. 3. Sebum production of three elderly post-menopausal females given 100 milligrams of methyl testosterone orally daily. Sebaceous secretion slightly but consistently increased while the drug was taken; it decreased when the androgen was stopped.

daily causes enlargement which becomes evident in two to three weeks.

Male sex hormone is not a single compound but a family of substances derived primarily from the adrenal glands and the testes (21). Individual androgens vary greatly in potency. Even the determination of total androgen secretion poses great technical problems. However, Fukushima, et al., basing their estimates on studies with radioactive testosterone, suggest that the daily

endogenous production of androgen in the human adult male, measured as testosterone, is about 17 milligrams per day and certainly no more than 36 milligrams per day (22). Since oral methyl testosterone has one-third the potency of circulating testosterone (23), our routine 100 milligram dose falls in the upper portion of the normal range, while the minimally effective dose of 5 milligrams is well below the average level. Presumably, the endogenous secretion of androgen in the adult male is probably in excess of that required to maintain maximal sebaceous gland function.

The extraordinary androgen sensitivity of the glands has been demonstrated in still another way. 17-alpha-ethynyl-19-nor-testosterone (Norlutin®) is a synthetic progestin with no significant androgenic activity in animal assays utilizing weight increases of the seminal vesicles and ventral prostate gland of immature castrated rats (24). Yet many female infants, born of mothers receiving this compound during pregnancy, have varying degrees of pseudohermaphrodism (25, 26). We have found that 20 milligrams of this compound causes enlargement of the prepuberal sebaceous glands (27, 28). Accordingly, we have suggested that the human prepuberal sebaceous gland provides a delicate biological test system for detecting androgenicity (28).

Though we have focused on the sebaceous gland, androgens have far reaching effects on prepuberal skin. The effect is one of general stimulation; the corium thickens greatly, the vasculature increases, the follicles lengthen, etc. (Figure 1).

At first we thought it surprising that the glands of adult males did not enlarge after strong androgenic stimulation. One need only realize, however, that in the post-adolescent male the glands of any given subject are already responding to endogenous androgens which are ordinarily in excess of that required for maximal effect.

The situation is somewhat different in the post-puberal female. Despite some controversy, females on the average secrete less sebum than males (3, 29–31). We have confirmed this in our own gravimetric studies (unpublished data). Acne is less severe and less frequent in females (32). Also, the endogenous secretion of androgen by the female is about two-thirds that of the males. The fact that exogenous testosterone

causes slight enlargement in some post-adolescent females probably indicates that the endogenous androgen output is somewhat less than that which would enable the glands to achieve their full responsiveness.

2) *Local Application of Testosterone*

The question with which we are concerned here is whether testosterone exerts a direct action on the target organ. Topical application, by presumably concentrating the drug locally in the skin, permits one to decide whether this is the case or not. One has to be aware, however, that the changes produced by such a potent, readily absorbed hormone might be part of a generalized response following absorption of the drug through the skin. To prove that testosterone acts directly on the target organ, one must show that glandular enlargement occurs earlier and to a greater degree at the local site. Therefore, a series of observations have to be made, all of which are controlled by determining any changes of the contralateral untreated side.

An alternate method would consist of superficially injecting the testosterone in a repository form. We abandoned this approach when we found that injection of depot testosterone caused dermal inflammation and partial involution of the glands. Dermal inflammation has been found to cause atrophy of the glands invariably (33).

Experimental

This study utilized prepuberal subjects exclusively because of the high androgenic sensitivity of their glands. The scalp is another area where the glands reach great size in adults. Therefore, after determining that the glands of the face and scalp respond in a parallel and proportionate fashion to androgen, we utilized the scalp in some cases.

In four prepuberal boys, 9 to 11 years old, 10 per cent testosterone propionate[3] in Hydrophylic Ointment, U.S.P., was applied once daily to one side of the scalp. A control biopsy specimen was removed beforehand; biopsy specimens were obtained from the treated and contralateral control sites after the application had been continued for three weeks. Although there was modest enlargement in the control areas indicating systemic effects through absorption, the glands of the

[3] Testosterone propionate supplied by G. Kenneth Hawkins, M.D., Schering Corporation, Bloomfield, New Jersey, and by Porter F. Crawford, M.D., Hubert C. Peltier, M.D., and Harold L. Upjohn, M.D., The Upjohn Company, Kalamazoo, Michigan.

treated area underwent a striking increase in size in each case (Figure 4).

In two other prepuberal males, 10 per cent testosterone ointment was applied to one cheek once daily for two months. There was great and

FIG. 4. Sebaceous gland enlargement produced by daily inunction of approximately 0.1 gram of 10 per cent testosterone propionate ointment for three weeks on the scalp of a prepuberal boy. A) Control specimen with small glands. B) Glands have enlarged at the site of application. C) Slight or questionable enlargement of glands of untreated control site. Biopsy specimen taken at same time as B. (Hematoxylin and eosin, A, B, & C × 55).

equal glandular enlargement on both sides showing that percutaneous absorption is sufficient to achieve maximal effects throughout the skin when inunctions of androgen in a high concentration are carried out for many weeks.

Comment

The evidence is compelling that testosterone has a direct local action on the sebaceous gland. Enlargement occurs to a far greater degree at the treated site (three-week experiment) and is followed by a generalized uniform response due to systemic absorption (two-month experiment). The inunction consisted of approximately 0.1 to 0.2 grams of ointment daily, which would therefore contain 10 to 20 milligrams of testosterone propionate. Even if only 10 per cent (1 to 2 milligrams) of testosterone propionate were absorbed, androgenic effects could be expected according to the data previously given. Perhaps by progressively reducing the concentration of hormone, one could find a threshold amount which would limit the enlargement to the site of application.

The present findings correlate well with other studies which show a direct effect of androgens on the pilosebaceous apparatus of man (34–36). When applied locally, testosterone causes increased hair growth on the abdomen (34), in the axilla (35, 36), and in the pubic area (36). To extend these observations, 10 per cent testosterone propionate ointment was applied to one axilla of four male prepuberal subjects, five, seven, ten, and eleven years of age. In the two oldest boys, the first hair growth occurred at the site of application. After three months, terminal hairs had developed both in the untreated axilla and in the pubes, indicating systemic absorption. The other two boys did not respond to the topical androgen.

Enlargement of the cock's comb is commonly used as a test of androgenicity (37). Morato-Manaro and Albrieux applied an androgen to one half of a comb which had been completely divided surgically (38). Greater enlargement occurred in the treated half of the comb, thus further authenticating the direct action of testosterone.

QUANTITATIVE EFFECT OF ESTROGEN ON THE SEBACEOUS GLANDS

1) *Systemic Administration of Estrogens*

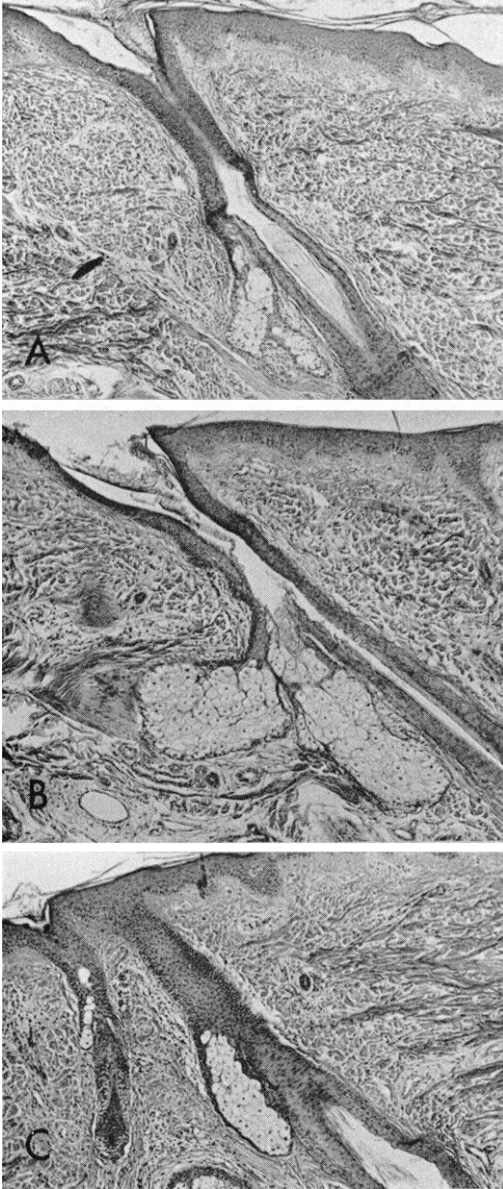It is commonly accepted that estrogens have a suppressive effect on human sebaceous secretion.

The evidence usually cited is the reported therapeutic value of estrogens in acne vulgaris. Nevertheless, with the exception of the few cases studied by Jarrett (39), one searches in vain for objective, controlled clinical studies which unequivocally demonstrate inhibition of sebaceous secretion. As a matter of fact, it will become all too clear that the improvement of acne usually claimed for estrogens, whether topical or oral, has been achieved with amounts too small to inhibit sebaceous output. Indeed acne is far too variable and fluctuating a disease to be used casually as a biological measurement of sebaceous gland physiology. The time is overripe for direct quantitative assessment, leaving aside the thorny problem of acne.

In animals, on the other hand, the issue has been settled beyond doubt. Ebling obtained what he describes as "limited reduction" of the sebaceous glands in rats weighing 40 to 60 grams with the subcutaneous injection of 1.0 gamma of estradiol benzoate daily for thirty days. However, when 100 gamma of estradiol benzoate were given under the same conditions, the decrease in the sebaceous glands was marked (11). Others have confirmed the depression of sebaceous glands in animals with large amounts of estrogen (15, 40–43). On a comparative weight basis, even the smallest dose that Ebling used, which would be equivalent to a daily subcutaneous dose of 1.5 milligrams of estradiol benzoate for a 70 kilogram human, is more than twice as large as the maximum therapeutic replacement dose for humans (44, 45); Ebling's higher dosage is obviously excessive.

Experimental

Of necessity all of the subjects were postpuberal males and females with good glandular development. The highly potent synthetic estrogen, ethynyl estradiol,[4] was used in most of the studies. In the experiments to be described, when the dose was to exceed 1.0 milligram of ethynyl estradiol, it was approached slowly over a period of seven to ten days to avoid nausea and vomiting.

Fifteen milligrams of ethynyl estradiol orally per day, the largest dose used, were given to three adult males for six weeks. Histologically, the glands became extremely atrophic and usually were reduced to small nests of undifferentiated cells. This shrinkage and involuting effect seemed to be more or less restricted to the glands, exempting the follicle and corium. Under the impact

---

[4] Ethynyl estradiol (Estinyl[R]) supplied by G. Kenneth Hawkins, M.D., Schering Corporation, Bloomfield, New Jersey.

of estrogen, the entire skin does not revert to its prepuberal condition.

Four more males, between sixteen and twenty years of age received 10 milligrams of ethynyl estradiol daily for eight weeks with effects identical to that of the higher dose.

Thereafter, twenty young adult subjects (sixteen males, four females) received 5 milligrams of ethynyl estradiol daily for periods up to two months. Again, the glands in every case became almost completely atrophic, reverting to the prepuberal state in those with the greatest change (Figure 5). There were individual differences in the final degree of involution, but these could not be correlated with original gland size. In all of the subjects, the skin was subjectively and objectively less oily.

Five milligrams of ethynyl estradiol greatly suppressed sebaceous secretion in three subjects (Figure 6). The decrease was detected in two to three weeks, although the maximum effect occurred later. This was a reversible change; sebum output returned to normal eight to twelve weeks after the drug was stopped. Similarly by histologic examination of biopsy specimens from the cheek of five adult males who received 5 milligrams of ethynyl estradiol daily, there was beginning involution at three weeks and by four weeks the decrease was unmistakable. Five weeks after the drug was stopped, restoration was well under way and by nine weeks the glands had regenerated fully.

Seven young adult subjects (four males, three females) received 2.5 milligrams of ethynyl estradiol daily for six weeks. Here the histologic decrease in gland size was less. Moreover individual responses were more variable. One female did not respond at all.

Sebum output was gravimetrically estimated in three males and two females who received 1.0 milligram of ethynyl estradiol daily. In each case, sebum production was reduced significantly (Figure 7). It should be emphasized that 1.0 milligram is still many times the physiological replacement dose.

At this point, the greater sensitivity of gravimetric assay became apparent. By histologic examination, doses of 1.0 and 0.5 milligrams failed to cause atrophy of the glands in two groups of five males each. By contrast, in six adult males 0.5 milligram of ethynyl estradiol definitely suppressed sebaceous secretion (Figure 8). The lowest dose evaluated was 0.25 milligram which gave a questionable slight reduction in sebum production. This is probably the threshold dose as measured by sebum production. The disparity between histologic and gravimetric suppression by estrogens is an exception to the well-founded principle that sebaceous secretion is proportionate to gland size. We tentatively hold that threshold doses of estrogen may have the special effect of inhibiting the proliferation of sebaceous cells without a corresponding shrinkage of glandular size.

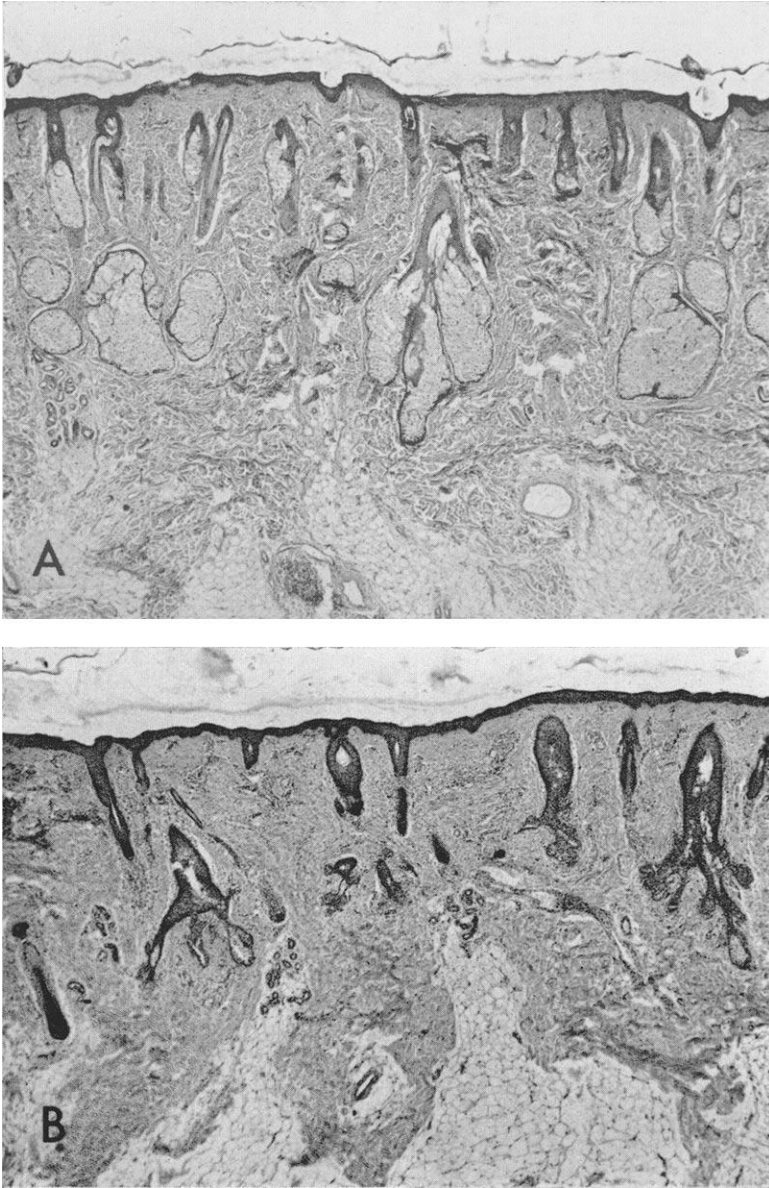Three young adult males received 50 milligrams

FIG. 5. Depression of sebaceous glands of adult male by 5 milligrams of ethynyl estradiol orally daily for six weeks. A) Pre-treatment specimen with lipid-laden, large sebaceous glands. B) After treatment, the glands, which have been almost completely wiped out, are reduced to nubbins of undifferentiated cells. (Hematoxylin and eosin, both A and B. × 32).

of diethylstilbestrol[5] orally daily for six to eight weeks. In each case there was moderate histologic reduction in gland size.

Finally, two young adult males received 20 milligrams, and two males received 40 milligrams of conjugated estrogenic substances (Premarin®)[6] orally daily for six weeks. The 40 milligram dose caused a slight decrease in gland size while the lower dose had little or no effect.

[5] Diethylstilbestrol supplied by Hubert C. Peltier, M.D., Harold L. Upjohn, M.D., and Porter F. Crawford, M.D., The Upjohn Company, Kalamazoo, Michigan.

[6] Conjugated estrogenic substances (Premarin[R]) supplied by Ayerst Laboratories, New York, New York.
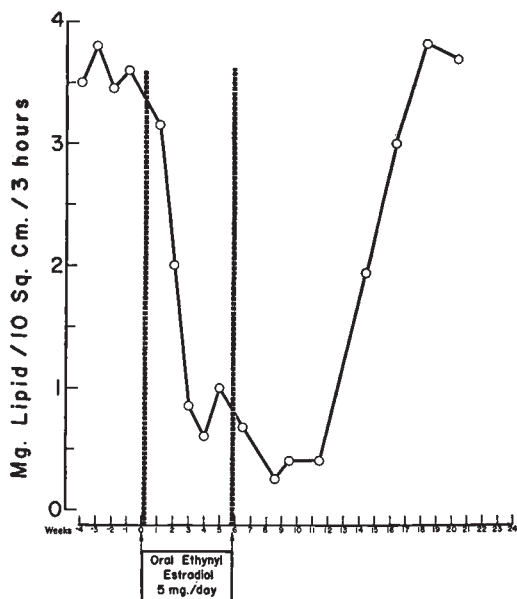
FIG. 6. Suppressive effect of 5 milligrams of ethynyl estradiol administered daily on the sebaceous glands of an adult male. Sebum output has declined greatly after three weeks, reaching a maximum in four to six weeks. It takes 9 to 12 weeks for the secretory activity to recover fully after stopping the drug. The changes in two other adult males were similar.
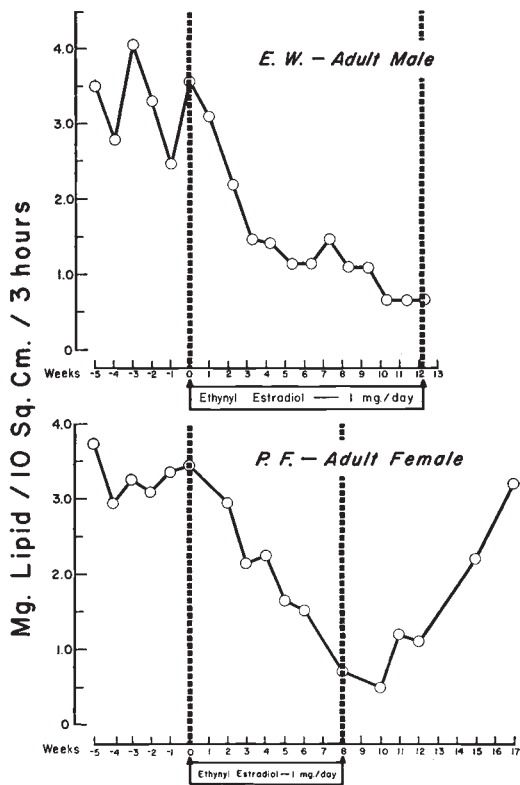
FIG. 7. Suppression of sebum output by 1.0 milligram of ethynyl estradiol administered daily to an adult male and female. Similar changes occurred in two other males and one other female.

Comment

It is quite evident that extremely high unphysiologic amounts of estrogen are necessary to cause the glands to revert to the prepuberal rudimentary state. For purposes of comparative analysis, the replacement dose of ethynyl estradiol, which is a rough estimate of the amount secreted endogenously, is usually less than 0.05 milligram per day (44, 45). Five milligrams of ethynyl estradiol invariably produced almost complete obliteration of the glands. Histologically, 2.5 milligrams daily (approximately fifty times the physiologic amount) was close to the threshold while 1.0 milligram daily had no effect. With the more sensitive gravimetric assay, 0.25 milligram of ethynyl estradiol appears to approach the threshold. Even this dose is at least five times the physiologic amount.

It is a fair estimate that ethynyl estradiol is twenty times more potent than diethylstilbestrol and thirty times more active than conjugated estrogenic substances (44, 45); hence, 50 milligrams of diethylstilbestrol, approximately equivalent to 2.5 milligrams of ethynyl estradiol,

could be expected to suppress the glands moderately by histologic criteria. The 20 milligram dose of Premarin®, corresponding to less then 1.0 milligram of ethynyl estradiol, was without effect histologically in agreement with expectations. Forty milligrams of this substance constituted a histologic threshold dose. It should be noted that these agents were not evaluated by the more sensitive gravimetric method of assay. At the doses used these drugs undoubtedly would have caused some decrease of sebaceous secretion. In any case, the amount required to produce almost complete histologic involution is so huge as to represent a pharmacologic, not a physiologic, effect.

A further qualification is necessary. All of these studies were of relatively short duration (less than two months). What effect small doses of estrogen administered over much longer periods of time would have is not known.

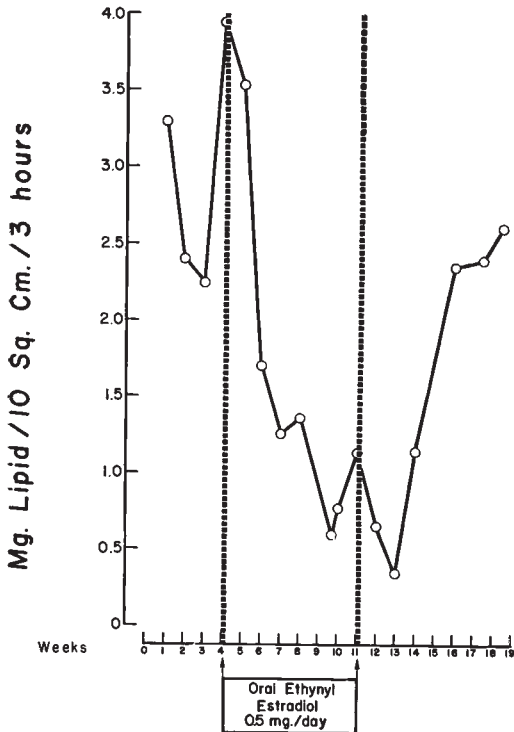With these high doses, it is not surprising that

FIG. 8. Suppression of sebum output by 0.5 milligram of ethynyl estradiol administered daily to an adult male. The drop is similar to that occurring with higher doses of ethynyl estradiol.

the characteristic systemic effects of estrogen were displayed in every subject. In the male, these consisted of softening of the testicles, loss of libido, breast enlargement and increased areolar pigmentation. It is noteworthy that the larger dosages of 5 to 15 milligrams had feminizing effects which were no greater than with 1.0 milligram. Every female developed areolar pigmentation and amenorrhea followed by withdrawal bleeding when the drug was stopped. These effects were reversible since the drug was generally not given for more than two months.

2) Local Application of Estrogen

As with testosterone we sought to discover whether ethynyl estradiol acted directly on the effector organ or via a central mechanism. Here, too, if one is to prove a direct inhibitory effect at the target organ, it will be necessary to show a suppressive effect at the application site which is clearly greater than that occurring in the control, untreated site. Obviously adults must be used as subjects.

Experimental

We first determined the threshold dose which would just cause glandular suppression at the treated site. Approximately 0.1 to 0.2 gram of 1.0 per cent ethynyl estradiol in Hydrophylic Ointment, U.S.P., was applied to the forehead of four adult males once daily for seven to ten weeks. Gravimetrically, sebum output definitely decreased in three subjects. This decrease was already evident in two to three weeks. In the fourth subject, there was no significant suppression of sebum output.

A 0.5 per cent ethynyl estradiol ointment was applied daily to the forehead of five more adult males. In only one of these did sebaceous secretion decrease. It would appear, then, that the minimal effective topical concentration of ethynyl estradiol lies between 0.5 and 1.0 per cent. It should be noted, however, that all subjects, including those whose sebum output was unaffected, experienced the usual galaxy of estrogenic side effects.

To determine whether estrogen acts directly on the target organ, the sebaceous gland, the experimental design of the topical androgen study was followed. The estrogen ointment was applied locally to one cheek, and the changes in the sebaceous glands of both cheeks were followed histologically. A control biopsy specimen was removed beforehand.

Six post-puberal males, between the ages of 15 and 20 served as subjects. Four received 10 per cent ethynyl estradiol ointment once daily; two received 10 per cent beta-estradiol ointment once daily. The biopsies taken at six weeks revealed a marked and equal decrease in systemic action. Feminization was evident in all six subjects, obviously reflecting percutaneous absorption.

Since histologic examination is not suitable to serial follow-up, the more sensitive gravimetric technique was used in the remaining studies. The estrogen ointment was applied to one side of the forehead and sebum output was determined on both sides simultaneously at various intervals.

Ten per cent ethynyl estradiol in Hydrophylic Ointment, U.S.P., was applied to one side of the forehead of two adult males once daily. Three more received 5 per cent ethynyl estradiol ointment. These concentrations greatly exceed the threshold dose. In all of these subjects, there was an equivalent decrease in sebaceous secretion on both sides (Figure 9).

A threshold concentration of 1.0 per cent ethynyl estradiol ointment was applied to one side of the forehead of three adult males daily. In two subjects, sebum production decreased on the treated side. However, an equally great suppression of sebum output occurred on the untreated control side (Figure 10).

Comment

With neither the high (5 to 10 per cent) nor the threshold (1 per cent) concentration of
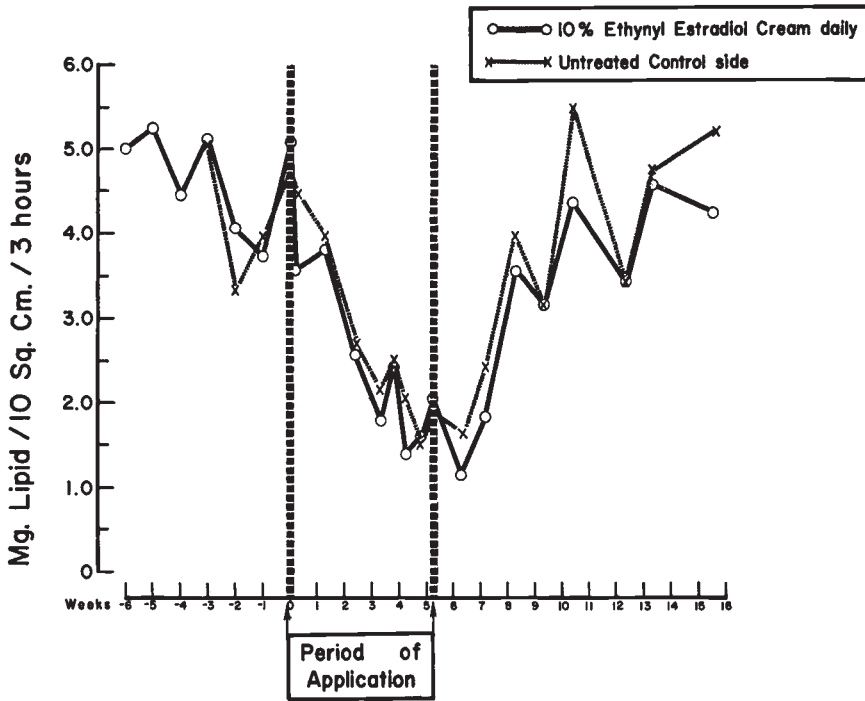
FIG. 9. Decreased sebum output after daily inunction of approximately 0.1 gram of 10 per cent ethynyl estradiol ointment to one side of the forehead of an adult male. Sebum production was assayed simultaneously on both treated and untreated sides. Sebaceous secretion decreased sharply but equally on both sides.

ethynyl estradiol ointment was there any evidence of earlier or greater suppression of sebaceous secretion at the treated site. This result indicates that the effects obtained required systemic absorption prior to producing the observed results on sebum secretion.

ANTAGONISM BETWEEN ANDROGENS AND ESTROGENS

It is more of a social than a biological truth that maleness and femaleness are opposites. Endocrinologically, human beings may be considered ambosexual since both adrogens and estrogens are present in all normal males and females, although there are marked differences in the proportions of the various sex steroids. The gonads are not the only source of sex hormones. For example, in the male, orchiectomy only reduces urinary androgens by one-third; adrenalectomy essentially eliminates the remaining source of androgens (46). Ketosteroid excretion in the female is two-thirds of that of a male (47). Since the adrenals are the predominant source of androgens in both males and females,

it is not surprising that urinary 17-ketosteroid excretion is not that much greater in the male.

It is customary in clinical medicine to think of androgens and estrogens as counteracting or antagonizing each other. For instance, high dosages of testosterone are masculinizing and suppress menstruation in females (48). Conversely, high doses of estrogen feminize the male and ameliorate prostatic cancer, an androgen dependent malignancy (49, 50). It should be realized however that these are biologically complex situations. Such antithetic responses probably involve indirect interactions of several systems. There is no clear understanding of how such effects are brought about.

The issue before us now is to define experimentally wherein lies the so-called "antagonism" between estrogens and androgens. The literature often implies that circulating estrogens and androgens are struggling or competing with each other for a target organ such as the uterus or the sebaceous gland. If the "antagonism" is at the target organ, there are several theoretical ways in which this might occur. For example,
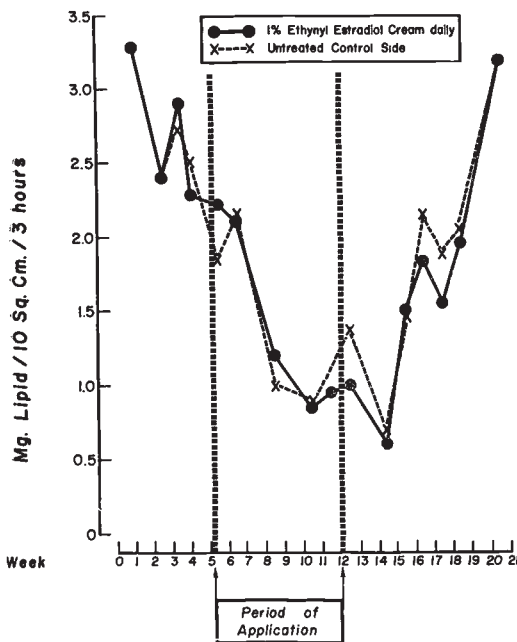
FIG. 10. Decreased sebum output after daily inunction of approximately 0.1 gram of 1.0 per cent ethynyl estradiol ointment to one side of the forehead of an adult male. Sebum production was assayed simultaneously on both treated and untreated sides. Sebaceous secretion decreased sharply but equally on both sides.

estrogens could block androgens in the same way that atropine pharmacologically inhibits acetylcholine at the end-organ; or the struggle might be compared to the pharmacologically opposing actions of methyl nicotinate and nor-epinephrine on the cutaneous capillaries. Nicotinate-induced vasodilation is quickly transformed into vasoconstriction by the injection of norepinephrine (51).

The following experiments test whether estrogen has a direct "neutralizing" or "blocking" effect on testosterone at the target organ. First, however, it will be helpful to summarize the facts established to this point: 1) testosterone has a direct local effect on the sebaceous glands; quite small amounts are effective; 2) estrogens do not appear to act locally; very large unphysiological amounts are necessary for suppression.

Experimental

Our reasoning in designing these experiments was that if estrogens counteract androgens at the target organ, for every dose of androgen, there should be an approximately corresponding dose of estrogen which would cancel its effect. Higher amounts of androgen would require proportionate increases in estrogen.

Three different experiments were done. First, 10 milligrams of methyl testosterone and 2 milligrams of ethynyl estradiol were simultaneously administered orally daily to a prepuberal boy for nine weeks. There was a prompt marked rise in sebaceous secretion (Figure 11). If one compares the rise in sebum output when this same subject was given androgens alone (Figure 2), it will be seen that the response was identical. The estrogen did not prevent the androgen from exerting its growth stimulating action on the gland.

Next, three normal adult males received concomitantly 1.0 milligram of ethynyl estradiol and 5 milligrams of methyl testosterone. There was no suppression in sebaceous secretion (Figure 12). This small amount of methyl testosterone, which is less than the endogenous secretion of androgen maintained sebaceous secretion in the face of a suppressive dose of estrogen.

In the final experiment, sebaceous secretion was first maximally suppressed by large amounts of estrogen (1 to 20 milligrams of ethynyl estradiol). At that point, a small amount of methyl testosterone (5 to 10 milligrams) was added so that the prepared subjects received both agents concomitantly daily. The result was clear cut. Regardless of the amount of estrogen given, the androgen promptly increased sebum output in all five subjects. The most dramatic illustration of the ability of a small amount of androgen to overcome the suppressive effect of excessive estrogen is given in Figure 13. While this man was receiving the massive dose of 20 milligrams of ethynyl estradiol daily, sebum production rose significantly during two courses of 10 milligrams of methyl testosterone orally.

Comment

These studies rule out the possibility that estrogens antagonize androgens at the level of the target organ. In review, the three separate findings are: 1) the sebaceous glands of the prepuberal child will respond to exogenous androgen even in the presence of excess estrogen; 2) if a small quantity of androgen is given to a normal adult, even large amounts of estrogen have no inhibiting effect; and 3) after the glands have been suppressed by estrogens, the introduction of small amounts of androgen exogenously will cause prompt increase in sebum output despite continuing excessive estrogen.

These findings possibly seem to indicate that the effect of large, quite unphysiological dosages of estrogen is to block the supply of androgen available to the sebaceous gland. When
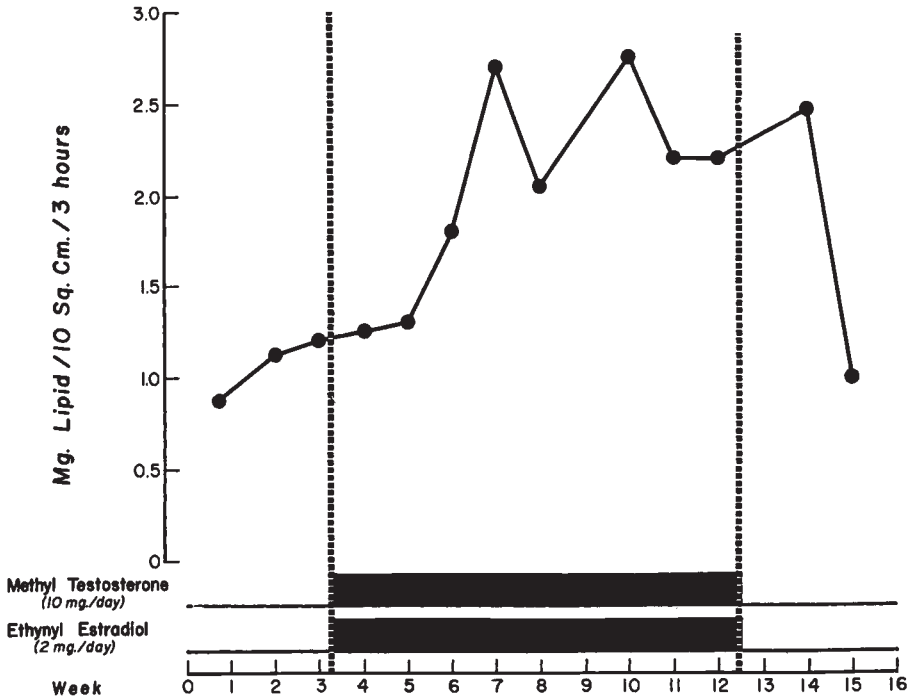
FIG. 11. Sebum output in an eleven year old prepuberal boy receiving 10 milligrams of methyl testosterone and 2 milligrams of ethynyl estradiol daily orally simultaneously. The estrogen did not block the expected rise in sebum output from the androgen. This is the same subject as illustrated in Figure 2.
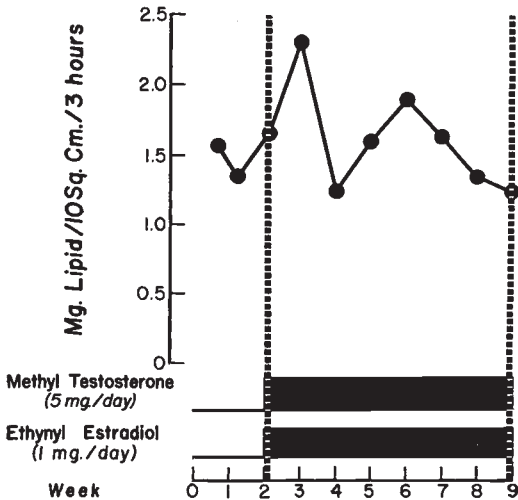

FIG. 12. Sebum output in an adult male receiving 1.0 milligram of ethynyl estradiol and 5 milligrams of methyl testosterone orally daily simultaneously. Normally, this amount of estrogen would have suppressed sebum output. However, the androgen maintained sebum output at a normal level.

threshold amounts of androgen are available, the presence of estrogens even in extraordinary quantities is without effect. This suggests possibly that there is no *physiologic* antagonism between androgens and estrogens in the case of the sebaceous gland.

In many organ systems, casual analysis suggests an androgen-estrogen antagonism at the target organ. However, many studies indicate that hormones with opposing actions on a specific target organ do not exhibit competitive inhibition at the target organ site. Moore and Price studied the effect of the simultaneous administration of androgens and estrogens on the sex structures of rats (52, 53). Testosterone causes normal development of the seminal vesicles and prostate in the castrate male rat. When estrogen is administered simultaneously, the development of these accessory sex structures is not inhibited. In spayed female rats, estrogens induce vaginal cornification. When testosterone is administered simultaneously, cornification proceeds normally. One may note that this example is the reverse of the situation that we have studied; that is to say, androgens do not in this case block a physiological effect of estrogen. All these studies demonstrate that, providing the specific regulatory sex hormone is present, the presence of its "antagonist" is immaterial.
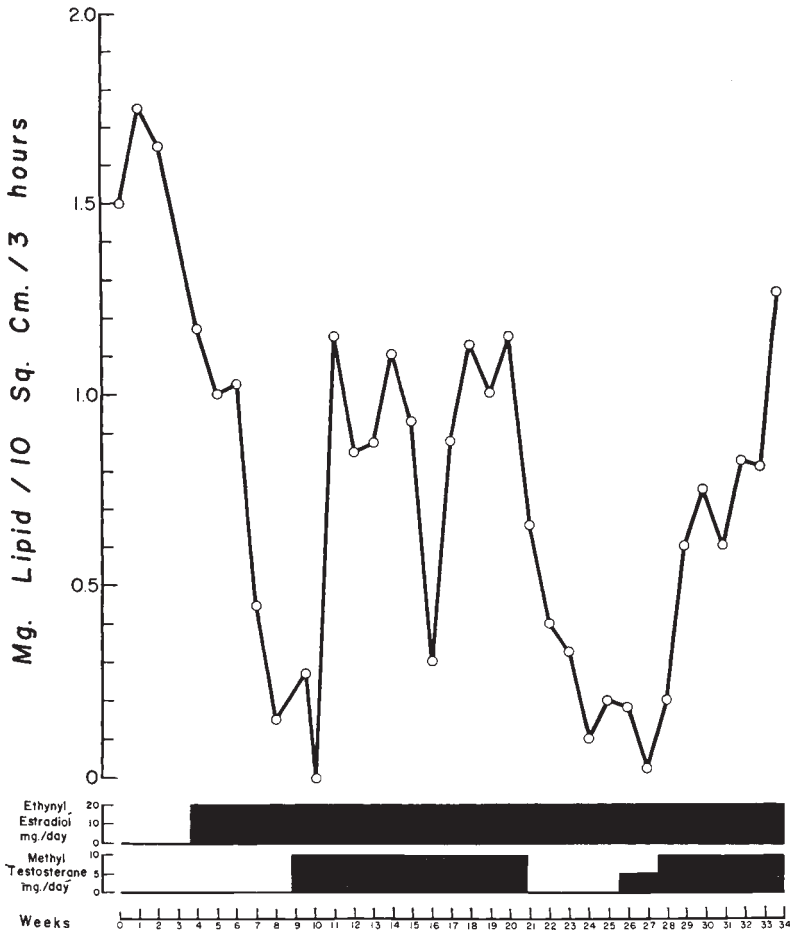
FIG. 13. This figure again illustrates the nature of the "antagonism" between androgens and estrogens. First, ethynyl estradiol was given to an adult male until sebaceous secretion was suppressed; then methyl testosterone was added to the regime while continuing the same dose of estrogen. Sebum output promptly rose. As long as some exogenous testosterone is available, the inhibitory effect of estrogen is cancelled. Note that as little as 10 milligrams of methyl testosterone overrides the effect of 20 milligrams of ethynyl estradiol.

Another tissue in which the opposing effects of androgens and estrogens can be studied is the cock's comb. The comb is extremely androgen sensitive (37). Investigators have found that in the intact cockerel large amounts of estrogens can inhibit normal comb development (54, 55). However, even in the face of excessive estrogen, comb development proceeds normally if small amounts of exogenous androgen are supplied (54, 56). Comb growth also is normal when chorionic gonadotropin is given simultaneously with estrogens (54). Furthermore, when estrogens and androgens are administered simultaneously to capons, male characteristics (growth of the comb) and female characteristics (development of

plumage) occur simultaneously (58). Each target organ responds to its appropriate hormone.

It is only fair to mention that controversy does exist in respect to estrogen-androgen interactions on the cock's comb. The cock's comb, unlike the sebaceous gland, is structurally a complex organ (59, 60), and experimental results may be interpreted in different ways. Some investigators, using the castrated cock instead of the cockerel as in the above studies, find that estrogens, locally or systemically administered, inhibit the growth stimulating effect of androgens on the comb (61, 62). Furthermore, when estrogens are applied topically to one half of the surgically divided capon's comb after androgens have been

applied to both halves, much greater atrophy occurs on the estrogen treated portion of the comb (38, 63, 64). This seems to be unassailable evidence for a direct local effect of estrogen. Closer scrutiny raises some doubts. One of the two reports involved the use of only two birds (38). The other contains a gross inconsistency. The authors applied varying amounts of estrone to one half of the divided comb of capons (63, 64). The combs had been previously stimulated by the topical application of testosterone to both halves of the comb. While they noted significant suppression of comb growth when 100 and 200 gamma of estrone were applied, there was no depression with 160 gamma. If 100 and 200 gamma are effective, how can 160 gamma be ineffective?

Assuming as we have, that estrogens exert their effect systemically, how is this brought about? There is considerable data supporting the concept that estrogens inhibit the pituitary production of gonadotropins; this in turn decreases the production of androgens by the testicle. Payne has demonstrated marked histologic changes in the pituitary glands of fowl treated with estrogens (65). This is indirect anatomical evidence. Moore and Price (52, 53) found that estrogens caused atrophy of the accessory male sex organs, an effect which was overcome by administering gonadotropins or transplanting isogenetic pituitary tissue. In addition, Boas and Ludwig, in a well controlled study, found that the administration of chorionic gonadotropin prevented the estrogen induced atrophy of the cock's comb (54).

In human beings, too, the administration of estrogens to males decreases androgen production (57, 66–68). This probably reflects decreased pituitary output of gonadotropins (69), as in the animal experiments. Indeed, the reduction of the high gonadotropin levels in the post-menopausal female by exogenous estrogens is great enough to have been used as an assay for estrogens (70).

If the gonads were the chief source of androgens, the explanations given to this point would be sufficient. However, even in the male, it is the adrenal which produces the major amount of androgen. It is necessary to postulate that estrogens, in some way, suppress the synthesis of androgen by the adrenal. This could be a direct effect, or an indirect one operating through the pituitary. Unfortunately, experimental medicine provides no clear cut answer one way or the other. There is, however, recent evidence that estrogens cause a further depression of androgen secretion in castrated human males (68, 71).

SUMMARY AND CONCLUSIONS

The quantitative effect of estrogens and androgens upon human sebaceous glands has been investigated using histologic and gravimetric methods.

Androgens, even in less than physiologic doses, cause great enlargement of the sebaceous glands of the prepuberal male and female in two to three weeks.

Androgens, even in high doses, cause no further enlargement of adult male's sebaceous glands. By contrast, the glands of some adult females increase moderately as do the partially involuted glands of aged females.

Topical application of testosterone propionate has a greater local growth stimulating effect on the prepuberal gland at the treated site as compared to the untreated side. Testosterone acts directly on the sebaceous glands.

Estrogens suppress sebaceous secretion only with unusually high, pharmacologic rather than physiologic, doses. Maximal histologic suppression is attained by about 2.5 milligrams of ethynyl estradiol, approximately fifty times the replacement dose. The threshold suppressive dose measured by gravimetric technics is about four times the physiological dose.

A 1.0 per cent ethynyl estradiol ointment, applied once daily, approaches the topical threshold level. Lesser concentrations are ineffective in suppression of sebum production.

The topical application of a 1.0 to 10 per cent ethynyl estradiol ointment is equally suppressive on the treated and control sides.

The inhibiting effects of large dosages of estrogen are not manifested when a small amount of androgen is given concomitantly. Estrogen does not antagonize or block the action of androgen peripherally at the target organ. The regulation of the sebaceous gland is solely by means of androgens. The suppressive effect of extraordinarily high doses of estrogen is probably due to the reduction in the endogenous synthesis of androgen.

REFERENCES

1. MONTAGNA, W.: The Structure and Function of Skin, Chap. 6, The Sebaceous Glands, pp. 255–293. New York, Academic Press Inc., 1956.

2. ROTHMAN, S.: Physiology and Biochemistry of the Skin, Chap. 12, Sebaceous Gland Excretion, pp. 284–308. Chicago, The Univ. Chicago Press, 1954.

3. EMANUEL, S.: Quantitative determinations of the sebaceous glands' function, with particular mention of the method employed. Acta Dermat. vener., **17:** 444–456, 1936.

4. PROSE, P. H., BAER, R. L. AND HERRMANN, F.: Studies of the ether-soluble substances on the human skin. II. Quantitative studies of the ether-soluble substances on the skin surface of patients with acne vulgaris. J. Invest. Derm., **19:** 227–235, 1952.

5. HODGSON-JONES, I. S., MACKENNA, R. M. B. AND WHEATLEY, V. R.: The study of human sebaceous activity. Acta Dermat. vener., **32:** Suppl. 29: 155–161, 1952.

6. KLIGMAN, A. M. AND SHELLEY, W. B.: An investigation of the biology of the human sebaceous gland. J. Invest. Derm., **30:** 99–125, 1958.

7. MIESCHER, G. AND SCHÖNBERG, A.: Untersuchungen über die Funktion der Talgdrüsen. Bull. Schweiz. Akad. Med. Wiss., **1:** 101–114, 1944.

8. STRAUSS, J. S. AND POCHI, P. E.: The quantitative gravimetric determination of sebum production. J. Invest. Derm., **36:** 293–298, 1961.

9. DEGRAAF, H. J.: Endocrine influences on sebaceous glands. Acta Brev. Neerl., **12:** 67–68, 1942.

10. DEGRAAF, H. J.: De invloed van Geslachtshormonen op de smeerklieren. Ned. T. Geneesk., **87:** 1450, 1943.

11. EBLING, F. J.: Sebaceous glands. I. The effect of sex hormones on the sebaceous glands of the female albino rat. J. Endocr., **5:** 297–302, 1948.

12. MONTAGNA, W. AND KENYON, P.: Growth potentials and mitotic division in the sebaceous glands of the rabbit. Anat. Rec., **103:** 365–380, 1949.

13. HAMILTON, J. B. AND MONTAGNA, W.: The sebaceous glands of the hamster. I. Morphological effects of androgens on integumentary structures. Amer. J. Anat., **86:** 191–234, 1950.

14. HASKIN, D., LASHER, N. AND ROTHMAN, S.: Some effects of ACTH, cortisone, progesterone and testosterone on sebaceous glands in the white rat. J. Invest. Derm., **20:** 207–212, 1953.

15. EBLING, F. J.: The action of testosterone and oestradiol on the sebaceous glands and epidermis of the rat. J. Embryol. Exp. Morph., **5:** 74–82, 1957.

16. EBLING, F. J.: The action of testosterone on the sebaceous glands and epidermis in castrated and hypophysectomized male rats. J. Endocr., **15:** 297–306, 1957.

17. HAMILTON, J. B.: Male hormone substance: A prime factor in acne. J. Clin. Endocr., **1:** 570–592, 1941.

18. RONY, H. R. AND ZAKON, S. J.: Effect of androgen on the sebaceous glands of human skin. A.M.A. Arch. Derm., **48:** 601–604, 1943.

19. KIRK, E.: Quantitative determinations of the skin lipid secretion in middle-aged and old individuals. J. Geront., **3:** 251–266, 1948.

20. SMITH, J. G., JR.: The aged human sebaceous gland. A. M. A. Arch. Derm., **80:** 663–671, 1959.

21. DORFMAN, R. I. AND SHIPLEY, R. A.: Androgens. Biochemistry, Physiology, and Clinical Significance. New York, John Wiley & Sons, Inc., 1956.

22. FUKUSHIMA, D. K., BRADLOW, H. L., DOBRINER, K. AND GALLAGHER, T. F.: The fate of testosterone infused intravenously in man. J. Biol. Chem., **206:** 863–874, 1954.

23. DORFMAN, R. I. AND SHIPLEY, R. A.: Androgens. Biochemistry, Physiology, and Clinical Significance, Chap. 19, Androgen Preparations and Methods of Administration, pp. 376–388. New York, John Wiley & Sons, Inc., 1956.

24. McGINTY, G. A. AND DJERASSI, C.: Some chemical and biological properties of 19-Nor-17-Alpha-Ethynyltestosterone. Ann. N. Y. Acad. Sci., **71:** 500–515, 1958.

25. GRUMBACH, M. M., DUCHARME, J. R. AND MOLOSHOK, R. E.: On the fetal masculinizing action of certain oral progestins. J. Clin. Endocr., **19:** 1369–1380, 1959.

26. WILKINS, L.: Masculinization of female fetus due to use of orally given progestins. J. A. M. A., **172:** 1028–1032, 1960.

27. STRAUSS, J. S. AND KLIGMAN, A. M.: The effect of progesterone and progesterone-like compounds on the human sebaceous gland. J. Invest. Derm., **36:** 309–319, 1961.

28. STRAUSS, J. S. AND KLIGMAN, A. M.: Androgenic effects of a progestational compound, 17 - Alpha - Ethynyl - 19 - Nortestosterone (Norlutin), on the human sebaceous gland. J. Clin. Endocr., **21:** 215–219, 1961.

29. SUZUKI, S.: Zur Physiologie und Pathologie der Talgsekration, besonders bei Lues. Jap. J. Derm. & Urol., **20:** 203–213, 1936.

30. KVORNING, S. A.: Investigations into the pharmacology of the skin fats and of ointments: II. On the occurrence and replenishment of fat on the skin in normal individuals. Acta Pharmacol., **5:** 262–269, 1949.

31. BRUN, R., ENDERLIN, K. AND deWECK, A.: Variations de la couche sébacée de l'avant suivant l'age et le sexe. Acta Dermat. vener., **35:** 311–317, 1955.

32. BLOCH, B.: Metabolism, endocrine glands and skin diseases, with special reference to acne vulgaris and xanthoma. Brit. J. Derm., **43:** 61–87, 1931.

33. STRAUSS, J. S. AND KLIGMAN, A. M.: Pathologic patterns of the sebaceous gland. J. Invest. Derm., **30:** 51–61, 1958.

34. WHITAKER, W. L.: The stimulation of human hair production by the topical application of testosterone. Univ. Bull., **8:** 46–47, 1942.

35. HURXTHAL, L. M.: Sublingual use of testosterone in seven cases of hypogonadism: Report of three congenital eunuchoids occuring in one family. J. Clin. Endocr., **3:** 551–556, 1943.

36. ALBRIEUX, A. S. AND MUSSIO FOURNIER, J. C.: The local action of testosterone propionate on the development of axillary hair in man. J. Clin. Endocr., **9:** 1434–1436, 1949.

37. DORFMAN, R. I. AND SHIPLEY, R. A.: Androgens. Biochemistry, Physiology, and Clinical significance, Appendix C. Androgen Bioassay Methods, pp. 543–555. New York, John Wiley & Sons, Inc., 1956.

38. MORATO-MANARO, J. AND ALBRIEUX, A.: The effect of sex hormones on the combs of castrated and normal cocks. Endocrinology, **24:** 518–522, 1939.

39. JARRETT, A.: The effects of stilboestrol on the surface sebum and upon acne vulgaris. Brit. J. Derm., **67:** 165–179, 1955.

40. HOOKER, C. W. AND PFEIFFER, C. A.: Effects of sex hormones upon body growth, skin, hair and sebaceous glands in the rat. Endocrinology, **32:** 69–76, 1943.

41. EBLING, F. J.: Sebaceous glands. II. Changes in the sebaceous glands following the implantation of oestradiol benzoate in the female albino rat. J. Endocr., **7:** 288–298, 1951.

42. EBLING, F. J.: Changes in the sebaceous glands and epidermis during the oestrous cycle of the albino rat. J. Endocr., **10:** 147–154, 1954.

43. BULLOUGH, W. S. AND LAURENCE, E. B.: Experimental sebaceous gland suppression in the adult male mouse. J. Invest. Derm., **35:** 37–42, 1960.

44. BENEDICT, P. H.: Commercial Preparations of Endocrine Products Used in Gynecology; in Progress in Gynecology, Vol. II, pp. 793–801. edited by J. V. Meigs and S. H. Sturgis, New York, Grune and Stratton, 1950.

45. GOODMAN, L. S. AND GILMAN, A.: The Pharmacological Basis of Therapeutics, Second Edition, Chap. 68, Estrogens and Progestogens, pp. 1589–1608. New York, The Macmillan Company, 1956.

46. HARRISON, J. H., LEMAN, C., MUNSON, P. L. AND LAIDLAW, J. C.: Hormone excretion before and after castration and adrenalectomy. New Engl. J. Med., **252:** 425–428, 1955.

47. DORFMAN, R. I. AND SHIPLEY, R. A.: Androgens. Biochemistry, Physiology, and Clinical Significance, Chap. 20, The Excretion of Androgens and 17-Ketosteroids in Various Clinical Conditions, pp. 389–450. New York, John Wiley & Sons, Inc., 1956.

48. HERTZ, R.: Physiologic effects of androgens and estrogens in man. Amer. J. Med., **21:** 671–678, 1956.

49. HUGGINS, C. AND HODGES, C. V.: Studies on prostatic cancer. I. The effect of castration, of estrogen and of androgen injection on serum phosphatases in metastic carcinoma of the prostate. Cancer Res., **1:** 293–297, 1941.

50. WHITMORE, W. F.: Hormone therapy in prostatic cancer. Amer. J. Med., **21:** 697–713, 1956.

51. STOUGHTON, R. B., DEOREO, G. AND CLENDENNING, W.: Effects of intradermal injection of vasopressors in normal and diseased human skin. A. M. A. Arch. Derm., **82:** 400–407, 1960.

52. MOORE, C. R. AND PRICE, D.: The question of sex hormone antagonism. Proc. Soc. Exp. Biol. Med., **28:** 38–40, 1930.

53. MOORE, C. R. AND PRICE, D.: Gonad hormone functions, and the reciprocal influence between gonads and hypophysis with its bearing on the problem of sex hormone antagonism. Amer. J. Anat., **50:** 13–71, 1932.

54. BOAS, N. F. AND LUDWIG, A. W.: The mechanism of estrogen inhibition of comb growth in the cockerel with histologic observations. Endocrinology, **46:** 299–306, 1950.

55. PARKES, A. S. AND EMMENS, C. W.: Effect of androgens and estrogens on birds. Vitamins Hormones, **2:** 361–408, 1944.

56. KOSIN, I. L. AND MUNRO, S. S.: Effect of sex hormones, separately and combined, on the proliferation and hydration of combs and cloacae of male chicks. Endocrinology, **30:** 102–106, 1942.

57. BIRKE, G., FRANKSSON, C. AND PLANTIN, L. O.: Carcinoma of the prostate: A clinical and steroid metabolic study. Acta Chir. Scand., **109:** 129–149, 1955.

58. JUHN, M., D'AMOUR, F. AND WOMACK, E. B.: The effects of simultaneous injections of the female and male hormones in capons. Amer. J. Physiol., **95:** 641–649, 1930.

59. HARDESTY, M.: The structural basis for the response of the comb of the brown leghorn fowl to the sex hormones. Amer. J. Anat., **47:** 277–324, 1931.

60. LUDWIG, A. W. AND BOAS, N. F.: The effects of testosterone on the connective tissue of the comb of the cockerel. Endocrinology, **46:** 291–298, 1950.

61. MUHLBOCK, O.: A difference of effect between the oestrogenic hormones and diethylstilbestrol. Nature, **143:** 160–161, 1939.

62. HOSKINS, W. H. AND KOCH, F. C.: The inhibition of comb growth in cockerels and capons by estrone. Endocrinology, **25:** 266–274, 1939.

63. MARTIN, J. E., GRAVES, J. H. AND DOHAN, F. C.: Studies of steroid antagonism. I. Local inhibition by estrone of the comb stimulating action of testosterone propionate. Amer. J. Med. Sci., **223:** 695–696, 1952.

64. MARTIN, J. E., GRAVES, J. H. AND DOHAN, F. C.: Local inhibition by estrone of testosterone-induced growth of the capon's comb. Amer. J. Vet. Res., **14:** 141–146, 1955.

65. PAYNE, F.: The cytology of the anterior pituitary of the fowl. Biol. Bull., **82:** 79–111, 1942.

66. HAMBURGER, C. AND SPRECHLER, M.: The influence of steroid hormones on the hormonal activity of the adenohypophysis in man. Acta Endocr., **7:** 167–195, 1951.

67. BIRKE, G., FRANKSSON, C. AND PLANTIN, L. O.: On the excretion of androgens in carcinoma of the prostate. Acta Endocr., **15:** suppl. 17, 1–33, 1954.

68. O'CONOR, V. J., JR., DESAUTELS, R. E., PRYOR, J. W., MUNSON, P. L. AND HARRISON, J. H.: Studies of hormonal changes in relation to cancer of the prostate: A progress report. J. Urol., **81:** 468–473, 1959.

69. MUNSON, P. L. AND PARLOW, A. F.: Quoted by O'Conor, V. J., Jr., Desautels, R. E., Pryor, J. W., Munson, P. L. and Harrison, J. H.: Studies of hormonal changes in relation to cancer of the prostate: A progress report, J. Urol., **81:** 468–473, 1959.

70. TOKUYAMA, I., LEACH, R. B., SHEINFELD, S. AND MADDOCK, W. O.: Depression of gonadotropin excretion as a method for assay of estrogens in human subjects. J. Clin. Endocr., **14:** 509–521, 1954.

71. BURT, F. B., FINNEY, R. P. AND SCOTT, W. W.: Steroid response to therapy in prostatic cancer. J. Urol., **77:** 485–491, 1957.