The general effects of castration, in addition to the loss of spermatogenesis, have been known since our earliest historical records. It is not the purpose of this paper to review the earlier studies which emphasize the effects of castration of the male, of transplantation of gonads, and of cryptorchidism on the various physiological, psychical, morphological, and histological changes in the body as a whole and in secondary sex characters and organs. Excellent reviews on the older general and more purely morphological aspects have been written by Lipschütz (1924), Pezard (1928), Sand (1926, 1932, 1933), Moore (1932) and Steinach (1936). From these and numerous other studies some have concluded that the hypothetical internal secretion of the testicle must have a profound effect on skeletal growth, muscular tone, metabolic rate, hemoglobin and oxidase content of blood and tissues as well as on the control of the secondary sex characters and accessory reproductive organs of the male. This review shows that only very few of these physiological reactions are sufficiently rapid and definite to be used as assay methods in the purification and isolation of the male sex hormones.

The action of testis-tissue extracts and concentrates on the rate of metabolism. Brown-Séquard and others have associated the fatigue and "lowered vitality" of advanced age with loss of the internal-secreting function of the testis; hence the early claims of rejuvenation by a hypothetical testicular hormone. The experiment carried out by Brown-Séquard (1889) upon himself is not only the earliest of its type in the field of the testicular hormone but in endocrinology in general. At the age of 72 he prepared very dilute and crude aqueous extracts from dog or guinea pig testes and injected them in 1 cc. amounts subcutaneously into himself, irregularly over a period of two weeks. He claimed a remarkable return of physical and mental endurance and of normal
intestinal function which lasted for four weeks. He admitted the possibility of suggestion having been the main factor in his case. Equally unconvincing were the ergographic investigations conducted by Fritz Prüfer (1896) and Zoth (1898) upon themselves with glycerol and “Brown-Séquard extracts” of testis tissue. These claims have never been confirmed by others even with more concentrated and more carefully prepared extracts. Nevertheless these observations received remarkable publicity and even today the general claims of action on metabolic rate and “well-being” are still assumed by some. Weil (1920) concluded that the injection of an acid-aqueous extract equivalent to 30 grams fresh testis tissue per kilogram body-weight produced increased CO₂-production in guinea pigs, especially when he used immature or castrated males. He found ovarian extracts to produce the opposite effect. Aude’s (1927) studies on capons and cockerels indicated a fall in basal metabolic rate following castration and a rise following the intraperitoneal injection of crude, unconcentrated testis-tissue extracts. He could not observe an effect on the rate of regression or a stimulation of growth of the capon comb in parallel with these effects on metabolism. Hence he concludes that the oxidation stimulant and the hormone acting on the secondary sex characters must be two different substances. Kuznetsov (1928) likewise reports no effect on the capon comb but a tonic effect upon the cardiac muscle, blood vessels, and on the metabolic processes with Kravkov’s perfusate prepared from bull testes. Ptaszek (1928) concludes from experiments on two dogs that castration is followed by a lowered metabolic rate, then by an adjustment to a higher level due to overactivity of other endocrines and lastly by a continuous lower rate. In the last condition he reports a rise in metabolism after six injections of “testis-tissue extract.” Korenechevsky and associates (1921, 1925, 1928) report that crude emulsions from testis or prostate tissue increased nitrogen excretion in two castrated dogs, but that the gaseous exchange data were contradictory. On the basis of scanty and variable results, they nevertheless conclude that the prostate extract affects protein metabolism, but that the testis acts as a synergist. These studies were repeated on rabbits, normal, castrated, and thyroidectomized, with several types of extracts. With saline “emulsions” of rabbit testes or kidney, they claim, on the basis of very irregular results, a lowered nitrogen metabolism but with prostate “emulsion” alone or combined with the testis preparation, an increased nitrogen excretion. These effects from kidney or testis extracts alone were found more marked in the thyroidectomized than in the normal or castrated rabbit,
but the prostate extract alone did not raise the nitrogen excretion in the thyroidectomized animals. In the later studies, Korenchevsky (1928) at one time limits the protein-metabolism-stimulating action to crude lipin fractions from bull testis and prostate and in the next paper in the same issue, Korenchevsky and Young (1928) find a metabolism-stimulating activity in an aqueous filtrate from testis tissue which is free from the synergistic action on prostate extracts. Korenchevsky (1926) also reports that castration combined with thyroidectomy in two rabbits results in a lethal fall in body temperature when the animals are cooled and hence he suggests that the gonads are involved in the regulation of body temperature. His reports (1930) that cryptorchidism in rats leads to a less marked response, but nevertheless a response in the same direction in the atrophy of accessory sex organs, in thyroid atrophy and in obesity development are suggestive of two hormonal factors, one from the seminiferous elements and the other from the Leydig cells, or of a gradual degeneration of the entire interstitial tissue. He (1930) found indications that cryptorchid pigs, however, gain weight faster and deposit more retroperitoneal fat than the castrated controls. He (1932) further observes that during the early stages of cryptorchidism in rats, there exists a higher metabolic rate as measured by gaseous exchanges; from this he concludes that the stimulation probably arises from the rapid resorption of the seminiferous cells. Recent studies by Holt, Keeton, and Vennesland (1936) do not confirm Korenchevsky's observations on obesity development in castrated male rats. In fact, Gertrude von Wagenen (1928) actually observed lowered body weights in castrated male rats. She confirmed Moore's (1922) earlier observations that castration usually leads to animals having shorter than normal bodies.

From in vitro studies on tumor tissues, Nakamura and Zuzuki (1930) conclude that crude sex-hormone preparations increase the respiration. Wu (1936), however, finds that the respiratory activity of cerebral sections is not altered by castration.

The studies above are at best only suggestive of a testis-tissue humoral control in basal metabolic rate and growth. The biological effects are not uniform and definite enough to encourage one to undertake these criteria for the necessary systematic quantitative fractionation of the very crude extracts used thus far. Furthermore, the slight and doubtful effects observed from extracts may be due to other extractives which may be present in other tissues and therefore are not specific. More recent studies on more definitely purified concentrates of the lipin frac-
tion which have been standardized in terms of capon units on the basis of the comb-growth response likewise lead to inclusive results on metabolism. Bühler (1933), on the basis of very scanty experimental evidence, arrives at the most startling conclusion that 50 to 150 capon units of "Proviron" (Schering-Kahlbaum) act specifically on the creatine metabolism in the male by decreasing creatinuria. Kochakian and Murlin (1935) found injections of a male-hormone concentrate from urine to produce a slight increase in fat metabolism, a decrease in protein metabolism (as measured by urea excretion) and no change in the carbohydrate metabolism in a thin castrated dog. In the fat castrated dog, carbohydrate metabolism is decreased. Unpublished data in the author's laboratories, obtained by E. W. Wallace, on castrated rats with highly purified concentrates from human urine and testis tissue respectively in daily doses of 5 to 10 capon units, reveal no effect on the basal metabolic rate.

Detection and assay of androgens by the comb-growth response in the capon. The first and most conclusive experimental evidence that the testicle controls the size of cockerel combs through a humoral mechanism was given by Berthold (1849). In fact, it is the first clear-cut evidence for any internal secretion by transplantation of the organ involved. Berthold even showed that amputated combs and wattles from a capon are regenerated by the transplantation of testes. The first investigator to produce comb growth in normal young hens by the repeated injection of a saline extract from cockerel testes over a period of months was Walker (1908). Pezard (1911) likewise first produced comb growth in black Orpington capons by injecting twice weekly over a period of five months the "extract from one-tenth of a cryptorchid hog testis." In spite of the very small amount of material used, it is worth noting that the long period of injection led to crowing and to sexual behaviour. These studies, although very clear cut, were only of qualitative value and limited in application on account of the time factor involved. It remained for McGee (1927, 1928) to fractionate and concentrate the material so that the substance could be detected in a five-day assay and to show that the activity is found in the lipin fraction from bull-testis tissue. The qualitative and specific distribution of this activity in bull testis and epididymis was shown by Gallagher (1928). The hormone has also been extracted from calf, swine, and stallion testes, but only one positive result has been reported on ram testes (Womack and Koch, 1930). The routine processing of a 500-pound lot of ram testes by Gallagher and Koch led to negative results.
The first quantitative method as devised by Gallagher and Koch (1929, 1930) was based on the minimum dose necessary to produce a detectable comb growth in five days. They succeeded in purifying the active lipin fraction to such a degree that 0.01 mgm. per day for five days produced a detectable comb growth in brown Leghorn capons. Gallagher and Koch (1930) from their quantitative studies concluded that daily injections for five days are more satisfactory than shorter or longer periods for assay purposes, that an increase in length plus the increase in height of the comb of 3 to 7 mm. is a desirable range. They defined the capon unit as the amount which, injected per day for five days, yields an average of 5 mm. increase in length and height of the combs on at least five brown Leghorn capons. They furthermore determined the "characteristic curve" establishing the relation of dosage to comb-growth response. Later experiences have confirmed these findings but have also shown (Womack and Koch, 1930; Womach, Koch, Juhn and Domm, 1931; Koch and Gallagher, 1934) that the comb-growth response value is affected by the intensity of light to which the capons may be exposed and by the initial length of the comb, but that the age of the capon and its repeated use does not influence the response seriously. To correct for the light factor, all assays must, therefore, be conducted in parallel with an assay of a standard preparation. Proper corrections must also be made for the initial comb-length. Unpublished data from the author's laboratory also show that the same "characteristic curve" in comb growth response holds for the variations in dose of crystalline androsterone, trans-dehydro-androsterone, androstenedione, and androstanediol.

Greenwood, Blyth and Callow (1935), by using essentially the same method of assay, also established the "characteristic curve" for crystalline androsterone. They report that the amount and character of the solvent used influences the response, but that initial comb-length was not a serious factor in their studies.

Freud, de Fremery, and Laqueur (1930, 1931, 1932) measure the comb-growth response by photographing the shadows cast by the comb before and after injection and determining the change in area of the shadows by a planimeter. They define a unit as the minimum daily dose which, introduced in two injections per day for four days, causes on the fifth day, an increase of 15 per cent in the area in over 50 per cent of the capons. To complete an accurate assay by this method may require four to five weeks because the minimum dose must be determined directly and is not calculated from a "characteristic curve." Although
these authors have confirmed the influence of illumination on the comb-growth response, they do not consider it necessary to correct this by assaying a standard in parallel with the unknowns. They also do not correct for the initial comb-size unless the capon weighs less than 1.5 kgm.

Gradstein (1934, 1935) has devised a method based on measuring the comb size by means of the photo-electric cell.

Ogata and Ito (1933) and Ogata, Hirano, and Tonaka (1934) also use a modified planimeter method for measuring the change in area of the photographic shadows of combs. They measure only the trapezium area exclusive of the barbles and thus eliminate serious errors in the planimetric measurements. They attempt to correct for the light factor by keeping the capons in a basement room during the assay period. The effect of the initial comb-size on the growth response is ignored although the data clearly show that this is a factor. The relation of per-cent increase to increase in dose is expressed approximately by a straight line for a range covering 10 to 30 per cent increase in area, but beyond that the curve becomes ellipsoidal in shape.

With pure hormone solutions, it is now feasible to apply the hormone solution to the surface of the comb or to inject a sterile solution directly into the comb. By these procedures, the response is much greater per unit weight of hormone (Ruzicka and Tschopp, 1934, and Greenwood and Blyth, 1935).

At the meeting of the League of Nations Committee held in July 1935, the international unit of male hormone was established as 100$\gamma$ of crystalline androsterone and it was recognized that it is essential to assay all preparations in parallel with a standard androsterone preparation. Henceforth it is very desirable to have all studies expressed in terms of this international unit.

Detection and assay of androgens by the response in the accessory sex organs in mammals. The biological bases of these assays were largely developed as a result of the pioneer castration, transplantation, and cryptorchidism investigations by Steinach (1894) and Moore (1926–1928). The earliest approach to the production of an active extract from testis was reported by Ancel and Bouin (1906) when they injected dilute glycerol extracts of cryptorchid testes for nine months into three castrated guinea pigs and observed the length and weight of the penis, and of seminal vesicles as compared with the length and weights of these organs in normal and untreated castrated animals. Stern and Battelli (1923) likewise made a few observations on the change in the guinea-pig
semenal vesicle as a result of the injection of a testis-tissue perfusate. They themselves say that the experiments were too few “permettre des conclusions définitives.”

*Spermatozoön motility test.* In connection with this test it is interesting to note that Stigler (1918) considered the epididymis to contain a substance which is favorable to the vitality, motility, and “ripening” of the spermatozoa. In 1925 Benoit observed that when an epididymis was ligated in the guinea pig, the spermatozoa retained their motility longer when testes remained than when the animals were castrated. Moore (1922, 1926, 1927, 1928) independently announced the same results in the guinea pig and suggested this phenomenon as a qualitative indicator for the testis hormone. In 1928 Moore and McGee employed

**TABLE 1**

<table>
<thead>
<tr>
<th>LABORATORY</th>
<th>NUM-BER OF INJECTIONS PER DAY</th>
<th>DAYS INJECTED</th>
<th>NUM-BER OF CAPONS USED</th>
<th>RESPONSE FOR A UNIT</th>
<th>EQUIVALENT IN* INTERNATIONAL UNITS (DAILY DOSES)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butenandt et al.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>20% area increase</td>
<td>1.5–2.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gallagher and Koch</td>
<td>1</td>
<td>5</td>
<td>5</td>
<td>Increase  ( \sim ) in ( L + H ) of 5 mm.</td>
<td>1.0</td>
</tr>
<tr>
<td>Laqueur et al.</td>
<td>2</td>
<td>4</td>
<td>4–12</td>
<td>15% area increase</td>
<td>approx. 1.0</td>
</tr>
<tr>
<td>Ogata, Hirano, Tanaka</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>20% area increase</td>
<td>&gt;2.0</td>
</tr>
<tr>
<td>Tschopp</td>
<td>1</td>
<td>6</td>
<td>5</td>
<td>20% area increase</td>
<td>0.7</td>
</tr>
</tbody>
</table>

* The international unit adopted by the League of Nations’ Committee in 1935 is 100γ androsterone.

this test on treated and untreated guinea pigs whose testes had been removed, but with the epididymides left intact in the scrotum. The hormone preparations employed represented crude lipin fractions obtained by McGee from bull testes and from bull brain tissue. Only the former prolonged the motility of spermatozoa in the isolated epididymides. Subsequently Moore and Gallagher (1930) determined the minimum dose, expressed as capon units, given daily to retain spermatozoön motility in the properly prepared guinea pig for the 35 days. The results were quite variable. Negative results were reported on 0.009, 0.018, and 0.09 capon unit and positive on 0.04, 0.07, 0.08, 0.12, and 0.17 capon unit per day for 35 days. Expressed in terms of international units, this would call for 0.1 to 0.2 I. U. per day for 35 days.
Obviously this is a very sensitive qualitative test, but it is of no value for quantitative purposes because the time factor is too long and the quantitative differences in individual guinea pigs are extremely variable.

_Prostate reactions._ This test was first developed on a histological basis by Moore, Gallagher, Price, and Koch (1929, 1930). The complete replacement experiments demonstrated that a perfectly normal rat prostate can be regenerated in 20 days although the animal may have been castrated for 85 to 90 days previously and even before sexual maturity. By the cytological reaction, Moore and Gallagher found this test to detect as little as 0.1 capon unit (as testis-tissue concentrate) if injected daily for 20 days, but for normal cytology it required 2 to 3 capon units per day for 20 days. Hansen (1933) attempted to establish the quantitative value of the cytological test and shortened the period of injection to 10 days. By this modification he could detect as low as 1.5 capon units distributed over the 10 days. The method is, however, not adaptable to quantitative studies because of the remarkable animal variations and the delay due to the histological technique.

On the basis of the linear measurements or the weight of the prostate, the method is much more rapid than the histological procedure, but by no means as reliable as the capon method from a quantitative point of view. The weight of the prostate is a better quantitative measure than the linear measurement. The animal variations are, however, still so great that the quantitative interpretations may be very misleading. Furthermore, the quantitative response is influenced by the amount of estrogenic material and probably by the amount of another synergist which may be present in the unknown. Nevertheless, the value of the information obtained by this method in parallel with the capon method cannot be overestimated. This will be brought out most strikingly in differentiating between various androgens.

Korenchevsky and his associates (1932, 1933, 1935) have published extensively on the rat prostate and seminal-vesicle weights in castrated rats when injected with androgenic substances. They recommend that the rats for comparative studies be from the same litter and state that two for the unknown and two for the control “are sufficient to obtain accurate results.” The rats should be castrated not less than 30 days before use and preferably before they are 30 days old. Injections are made twice daily for 7 days. The seminal vesicles and prostate glands are weighed together after 2 to 3 days in Allen’s modification of Bouin’s fixative. The prostate gland is then weighed separately. For doses of 200, 450, 600, and 900μ androsterone, the per cent increases
THE MALE SEX HORMONES

in prostate weight over the untreated castrates when calculated per 200γ androsterone were 48, 48, 47, and 51 respectively. The rat unit is recommended as the amount necessary to cause a 40 per cent increase in the prostate weight as compared with the uninjected castrated control rats.

Laqueur, Freud, Dingemanse, etc. (1931, 1934, 1935), have also employed the weight of the prostate gland in castrated rats as a measure of the androgenic action of various preparations. They have not published their method in detail.

The weight method on the prostate response has been investigated in the author's laboratories in collaboration with David Duncan (unpublished) for some time on castrated rats of various ages and on infantile rats. The results are extremely variable thus far.

Seminal-vesicle reaction. As a cytological test this was developed independently by Moore, Gallagher, Hughes, and Koch (1929, 1930) by Loewe and Voss (1929, 1930) and by Martins (1930). Either rat or mouse may be employed. In the castrated rat it is not as sensitive as the cytological prostate reaction. The minimum daily dose for 20 days which can be detected is 0.2 to 0.4 capon unit (as testis-tissue concentrate) and the dose necessary for normal regeneration of the seminal vesicle was found to be 6 to 7 capon units (Moore and Gallagher). Hansen (1933) also studied this reaction more quantitatively and concluded that a fair quantitative response holds over a range of 0.4 to 7.0 capon units per day for 10 days. Martins (1930) applied the test qualitatively on immature male and on castrated adult mice. Loewe and Voss (1929, 1930, 1931) describe their histological grading system in the castrated mouse in detail and claim that the test is very sensitive and very reliable for quantitative assay. The limitations of this method are the same as for the cytological prostate test.

On the linear measurement or weight basis, the seminal vesicle test appears somewhat more satisfactory than the prostate method. The reaction in weight increase is, however, also influenced by the presence of estrogenic material or other synergists. Korenchevsky and his associates (1932, 1933, 1935) have carried out extensive studies on the development of the seminal weight-method. Their technique is the same as on the prostate and the per cent increase in seminal vesicle plus prostate per capon unit of androsterone given daily for 7 days is also 40 per cent over the non-treated castrate. They actually prefer to take the per cent increase in the combined weight of seminal vesicle and prostate than the prostate or seminal vesicle alone because they consider
the increase in prostate weight alone to correspond to the capon test whereas the combined seminal vesicle plus prostate reaction represents the "whole male sexual activity." Needless to say such statements are very confusing. This is particularly true in view of the synergistic action of estrogenic and other substances. Laqueur and his associates (1931, 1934, 1935) have used an unpublished method based on the seminal-vesicle weights in distinguishing between different natural forms of androgenic substances. Ogata and Hirano (1934) call a rat unit the quantity of material which, in three daily injections, causes a 50 per cent increase in weight of the seminal vesicle over that of the untreated castrated rat. Tschopp (1935) castrates rats at 70 to 80 grams body weight. Four weeks later they are injected daily for 20 days. The average weights of the seminal vesicles from 3 to 10 rats are taken for each dosage of the male hormone. By his method the increase in weight of the seminal vesicles does not increase proportionally at the same rate for different artificially prepared male hormones. Tschopp in fact shows that crystalline theelin in daily doses of 50 to 100\(\gamma\) daily for 3 weeks stimulates seminal vesicle growth much more than the same or even higher weights of androsterone. Wang and Wu (1933) also find the seminal vesicle test non-specific. Butenandt and his associates (1935) and the writer with David Duncan (unpublished) have employed the seminal vesicle weight reaction in normal immature rats as a test for the male hormone preparations. This is a fairly satisfactory method providing one avoids the use of the test when gonad-stimulating factors are present. In fact Zaharesco-Karaman, Proumbaru, and Fotin (1935) use the same principle in testing urine for pregnancy, hydatiform mole, or chorioepithelioma.

The influence of estrogenic substances on the seminal vesicle and prostate weight and structure. Freud (1933) was the first to report that theelin acts as a "pacemaker" for the male hormone in the seminal vesicle reaction. He pointed out how important it is to consider estrogenic impurities in applying this male hormone assay-method on concentrates and called attention to the specific action of the estrogenic substance in stimulating growth of the smooth musculature of the seminal vesicle. These observations have been confirmed by de Jongh (1934, 1935), Korenechevsky and Dennison (1934, 1936), and by Overholser and Nelson (1935). This estrogenic action is not eliminated by hypophysectomy (de Jongh) nor is it counteracted by progesterone (David, Freud, and de Jongh, 1934). David, Freud, and de Jongh (1934) also observed that in castrated mice the theelin produces stratified epithelium
at the orifice of the ducts from the prostate and seminal vesicles. The stratified epithelium is not produced by the pure male hormone but instead a spongy glandular structure. Heringa and de Jongh (1934) also observed that in young castrated mice, the theclin changes the connective tissue layer in the ampulla of the vas deferens from the fine collagen bundles and the reticulin network to a thick layer with increased intrafibrillar material which appears to be mucoid in character. These observations led Laqueur and de Jongh (1935) to consider prostatic hypertrophy as due to an improper balance of male and female hormones. Hence male hormone should counteract these actions. This de Jongh has demonstrated experimentally in part by increasing the dose of male hormone or decreasing the theelin dosage.

*Other mammalian accessory sex-organ reactions to male hormone.* The normal histology of the epithelium of the vas deferens and of Cowper’s gland has also been regenerated in the castrated rat with male hormone concentrates by Vatna (1930) and Heller (1930, 1932) respectively. These reactions require 0.4 to 1.0 capon unit per day for 20 days as a minimum qualitative test and 6 to 7 units per day for complete restoration to the normal.

That the seminal vesicle and prostate of the castrate guinea pig can be maintained at a secretion level by male hormone concentrates has been shown by Moore and Gallagher (1930) and Kabak (1931) by the application of the Battelli electric ejaculation test (1922). The minimum daily dose necessary to produce a positive ejaculate is, however, of the order of 1.7 to 3 capon units.

The weight of the penis in the castrated rat has also been followed by the Korenchevsky group in parallel with their studies on the seminal vesicle and prostate. The rate of increase in weight with increasing doses follows a curve of the same character as those for seminal vesicle and prostate. The different androgenic substances act very differently on the different end organs, however. This will be covered in more detail later in this review.

The preputial glands of the castrated mouse have been regenerated by male hormone preparations by Voss (1931). In castrated rats, Dingemanse, Freud, and Laqueur (1935) have also shown an increase in the weights of the preputial and periurethral glands with male-hormone concentrates. Korenchevsky (1935) calls attention to the appearance of similar structures on the vagina at the base and in front of the bladder in ovariectomized rats on daily doses of 175 to 700 γ androstane-diol-3,17. He reminds us of Virchow’s statement that the periure-
thral glands found in the female are similar in structure to the male prostate.

**Other biological reactions.** Keck (1932, 1933, 1934) and Witschi (1936) have demonstrated that the bill pigmentation of the English sparrow is controlled by the male hormone. They have shown that the normal pigmentation can be restored in the bill of the castrated adult English sparrow in 10 to 20 days by the daily injection of 0.6 to 1.5 capon units. They suggest the method for quantitative purposes.

Glaser and Haempel (1931, 1932) suggest the production of the mating pigmentation in the castrated carp, *Cyprinus amarus*, or castrated *Rhodeus amarus* as the criterion for the male hormone. A fish unit is the minimum amount of substance necessary to produce the mating pigmentation in three out of four fish of the same weight in 6 to 7 hours after one intramuscular injection. André Beaume (1935) suggests using the non-castrated male fish, but it must not be in the spawning season and must be kept in water at 14 to 15°C. The fish are, however, rather susceptible to toxic substances found in urine extracts.

**The quantitative extraction of the male hormone.** The quantitative extraction of the male hormone from tissues, blood, and urine has grown in importance with every advance in our knowledge of the sex hormones. This is particularly true for urine. The historical development of this subject clearly illustrates how incompletely the first extractions of urine were carried out. Thus Loewe and Voss (1929) reported extracting sufficient hormone activity from 3.6 liters of men's urine to give a positive seminal vesicle regeneration test in the castrated mouse. Funk, Harrow, and Lejwa (1929, 1930) by chloroform extraction increased the yield of hormone per liter appreciably. Womack and Koch (1930) and Gallagher and Koch (1930) obtained still higher values in adult human male and female urines but no detectable amount in the urines from boys under 10 years of age or from bull calves. A systematic investigation on the completeness of extraction of urine in various types of continuous extractors and with various solvents has, however, shown that the content of human male urine is distinctly higher than the earlier studies indicate. Gallagher, Koch, and Dorfman (1935) recommended boiling the urine for two hours with the addition of one-tenth the volume of commercial hydrochloric acid and then extracting in a special extractor with at least ten volumes of benzene per volume boiled urine.

Although they found no difference in the yield of male hormone on extracting acidified unboiled urine and acidified urine boiled for two
hours respectively, recent unpublished observations by Gallagher, Peterson, and Koch (1936) show that a liberation and then a loss or destruction of androgenic material takes place within the two-hour period. The best yield has been obtained thus far by extracting the urine after boiling with hydrochloric acid for fifteen minutes.

The apparatus is based on the counter-flow principle and is so constructed that the benzene is very finely divided by being forced through a 40- to 80-mesh sintered glass filter before it comes in contact with the urine. By this procedure they obtain 18 to over 100 international units male hormone and 80 to 110 international estrogenic units per liter men’s urine. Re-extraction of the urine yielded no further hormones. Gallagher, Koch, and Dorfman (1935) have also obtained practically the same yield of male hormone by adsorption on “dicalite,” a commercial diatomaceous earth, and subsequent elusion by hot alcohol. The estrogenic material is not removed by dicalite.

Human urine appears to be unique with respect to male-hormone content, as is shown by the tabulation below:

<table>
<thead>
<tr>
<th>Age and Species</th>
<th>International units androgen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human urine male (per 24 hours)</td>
<td>40-100</td>
</tr>
<tr>
<td>Human urine female (per 24 hours)</td>
<td>30-100</td>
</tr>
<tr>
<td>Stallion urine (per liter)</td>
<td>8</td>
</tr>
<tr>
<td>Bull urine (per liter)</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Ram urine (per liter)</td>
<td>4</td>
</tr>
<tr>
<td>Rat urine (per liter)</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

The yield of hormone from bull testis-tissue still is exceedingly low. It varies from 50 to 150 grams fresh testis-tissue per international unit. All attempts to increase the yield by various methods of hydrolysis and by re-extraction have failed to improve the yield (Womack and Koch, 1930).

The extraction of the hormone from blood has been even less satisfactory. By applying the routine alcohol extraction to whole bull’s blood, Gallagher (1928) could not detect it although he injected the equivalent of 200 cc. blood per day. Womack and Koch (1930) obtained comb growth on the equivalent of 300 to 600 cc. bull’s blood. In view of these low values it is difficult to explain the results of other workers. Thus, Busquet (1927, 1928) reported that he produced crowing, treading, and comb growth in young and old capons by giving daily by mouth for 7 to 10 days, 5 cc. blood from bull, ram, or stallion. Blood from castrates gave negative results. Muto (1928) also reported that
he could retard the rate in weight decrease of the seminal vesicles in castrated mice by injecting the blood from the spermatic vein or the testicular lymph from a normal male mouse. Muto's evidence is of doubtful value because he has not allowed for animal variation and because the rate of regression of a seminal vesicle is an exceedingly poor criterion for hormone action. McCullagh, McCullagh, and Hicken (1933) report positive results on two capons by injecting the ether extract from 50 cc. oxalated human blood which had been dehydrated by 50 to 75 grams anhydrous sodium sulphate. They record similar results on human spinal fluid. Obviously careful quantitative studies are necessary in order to establish complete extraction methods on body fluids and tissues before we can hope to have our quantitative studies be of any value in diagnosis and in investigations on the distribution in normal and pathological conditions.

EXTRACTION AND PURIFICATION OF THE MALE SEX HORMONES, ANDROSTERONE AND DEHYDROISONANDROSTERONE, FROM URINE. The first natural source to yield a pure crystalline male hormone was human urine. Butenandt (1931, 1932) used as his starting material the raw oil obtained by chloroform extraction of acidified male human urine. An ether solution of this oil loses about 90 to 95 per cent by weight of inert material by shaking eight to ten times with 10 per cent sodium hydroxide. The neutral fraction left on evaporating the water-washed ether solution after repeated hydrolysis and subsequent partition between 50 to 70 per cent alcohol and petroleum ether leaves an alcohol-soluble oil which contains one Butenandt capon-unit per 300γ. By treating the latter with hydroxylamine, Butenandt obtained a crystalline product which no doubt was a mixture of oximes. The carefully purified oxime of melting point 215° contained approximately 1 Butenandt capon-unit per 100γ, but the comb-growth response was delayed and unusually prolonged. This indicates that the oxime itself is not active but that it is gradually hydrolyzed to the free active form in the organism. In-vitro hydrolysis did not yield the hormone in pure form directly. However, after careful sublimation at 80 to 85°C., at a pressure of 0.0001 mm. mercury, a substance was obtained of M.P. 178°, which Butenandt reported to contain 1 capon unit per 1 to 2γ. To this substance he then assigned the formula C_{18}H_{26}O_{2}; he considered it to be a saturated hydroxyketone. Later (1932) analysis of a substance of M.P. 160° led to the formula C_{19}H_{30}O_{2} or C_{18}H_{28}O_{2}. On the basis of the empirical formula, C_{19}H_{30}O_{2}, and the chemical characteristics of the substance, he then suggested the structure now adopted for androsterone.
These conclusions were confirmed and supported by additional data by Butenandt and Tscherning (1934). The general procedure adopted by them for the preparation of androsterone is given below. The final product has, however, only an activity of 1 Butenandt capon-unit per 150 to 200γ whereas for his first crystals, Butenandt (1931) reported an activity of 1 to 2γ per capon unit. No one has been able to duplicate the production of so potent a substance nor have subsequent assays by Butenandt confirmed these earlier claims. It is very probable that the substances separated by Butenandt in 1931 and in 1934 are identical but that in the earlier assays on the 1931 product an unknown error was introduced.

A second male-hormone fraction was separated as a by-product from fraction 10αγ (p. 168) in 1934 by Butenandt and Dannenbaum. This substance (C₁₉H₂₄ΟCl) was found to be an ester-chloride derivative of dehydroisoandrosterone. It is very probable that the chloride is an artefact, obtained from the parent substance, dehydroisoandrosterone, which usually accompanies androsterone in men’s urine. The chloride ester very likely is formed during the extraction of the acidified urine by boiling with chloroform. The relation of the dehydroisoandrosterone and chloride ester thereof to androsterone was proved by Butenandt and his associates (1934, 1935). In the later (1935) studies, they concluded that men’s urine contains practically equal amounts of androsterone and dehydroisoandrosterone. This statement is based on the use of digitonine, which precipitates the dehydroisoandrosterone, but not the androsterone. The yield of the two substances in pure form is approximately 20 mgm. of each per 100 liters of urine. The actual amount present probably is more nearly 20 to 40 times that quantity.

Butenandt and Tscherning method for the preparation of androsterone from men’s urine

Men's urine (1 capon unit* = 200 cc.)
\[\downarrow\]
Concentrated, acidified, and extracted with CHCl₃

Chloroform extract (1 capon unit = 150 to 100 mgm.)

Extracted with KOH
\[\rightarrow\] Alkali soluble (90 to 95%)

Neutral, undissolved part (1 capon unit = 9 to 10 mgm.)

Distillation with steam
\[\rightarrow\] Volatile fraction (30 to 35%)

Non-volatile fraction (1 capon unit = 6 mgm.)

Alkaline hydrolysis
\[\rightarrow\] Saponifiable fraction (45%)
Non-saponifiable fraction I (1 capon unit = 3 to 4 mgm.)
Acid hydrolysis → Hydrolyzed material (15 to 20%)

Non-saponifiable fraction II (1 capon unit = 2.8 to 3 mgm.)
Partition between benzene and petroleum ether → Benzene soluble 10a, α (12%)

Petroleum-ether soluble
Extraction by 60% alcohol → Petroleum-ether soluble fraction 10a, γ (51%)
(This used for the chlorketone)

60% Alcohol-soluble 10a β (1 capon unit = 1 to 1.4 mgm.)
Oxime formation
Oxime of M.P. 215 to 221°
Hydrolysis by dilute acid
Androsterone, C_{18}H_{24}O_{2}, M.P. 178° (1 capon unit = 150 to 200γ)

*Butenandt capon unit = 150 to 200γ androsterone or 1.5 to 2.0 I. U.

Gallagher and Koch (1934) also prepared concentrates from men's urine, but did not obtain the pure substances in crystalline form. However, it is interesting to note that the potency, 100γ per international unit, of the final concentrate (see flow sheet, below) was of the same order as that of androsterone.

Gallagher and Koch fractionation procedure of the male-hormone activity from men's urine

Men's urine
Acidified and boiled with 10% (by volume) commercial HCl.
Extracted with benzene in a continuous extractor.

Benzene extract (1 S. C. U.* = 10 mgm.)
Evaporated, dissolved in ether, and extracted with 10% NaOH → NaOH

Ether extract (1 S. C. U. = 1 mgm.)
Distilled at low pressure at 150°C. → Distillate

Residue (1 S. C. U. = 0.5 mgm.)
Solution in 70% ethanol → "Tar"

70% Ethanol solution (1 S. C. U. = 0.4 mgm.)
Evaporated, dissolved in 50% methanol → "Tar"
50% Methanol solution (1 S. C. U. = 0.3 mgm.)
Shaken with petroleum ether → Petroleum ether extract

\[ \text{Partition between 50\% CH}_2\text{OH and CCl}_4 \] → 50% Methanol (1 S. C. U. = 1 mgm.)

CCL₄ solution (1 S. C. U. = 0.17 mgm.)
Distilled at low pressure up to 200°C. → Residue

Distillate (1 S. C. U. = 0.1 mgm.)

* S. C. U. = standard capon unit = 150 to 160\(\gamma\) androsterone or 1.5 to 1.6 international units.

**EXTRACTION AND PURIFICATION OF THE MALE HORMONE, TESTOSTERONE, FROM TESTIS TISSUE.** The McGee (1927), Gallagher and Koch (1929, 1934) process carried the activity of the concentrate from bull testis-tissue to approximately ten times that of the urine concentrates. This clearly indicated that the male-hormone activity obtained from these two sources is due to different substances. The method employed is to extract the finely ground tissue at room temperature with four volumes by weight of 95 per cent alcohol for three to five days. After concentrating the alcoholic extract under diminished pressure to an aqueous sludge, it is thoroughly extracted with benzene. The benzene extract is evaporated to dryness and the residue dissolved in acetone. The acetone solution is chilled at \(-10^\circ\text{C.}\) for 24 hours and filtered. After the acetone is removed by evaporation, the solids are dissolved in hexane and thoroughly shaken with 70 per cent alcohol. The alcohol is removed by evaporation, the solids dissolved in ether and this solution shaken with 10 per cent aqueous NaOH. Further purification by the use of immiscible solvents similar to those employed in the fractionation of the urine extract yielded products approximately ten times as potent as androsterone by the comb-growth assay method.

Dingemanse, Freud, Kober, Laqueur, and Münch (1930, 1931) also fractionated the bull testis-tissue extracts by means of solvents to a stage where 0.05 to 0.10 mgm. contained the potency of one of their capon units. This material, when submitted to high vacuum distillation at 80 to 112°C. yielded a particularly active fraction at 90°C. and 0.001 mm. mercury pressure. The product was not in pure crystalline form. In 1935, David, Dingemanse, Freud, and Laqueur announced the separation of crystals of exceptional potency. David (1935) describes
the process as follows. After fractionation of the partially purified activity between petroleum ether and 70 per cent alcohol, the substance was recovered in benzene and shaken with 75 per cent sulphuric acid. The acid solution after dilution with water was extracted by ether and the residue from the ether solution submitted to high vacuum distillation at 110° to 130°. The distillate on recrystallization yielded the pure crystalline product. The acetate and oxime derivatives were prepared. These observations and the fact that no phenol character was detected led him to conclude that one alcohol and one carbonyl group must be present. He could not find evidence for an unsaturated bond. The substance melted at 154° to 154.5°, possessed a specific rotation of +109° and analyzed C₁₉H₂₈O₂. The activity was reported as 7 to 10 times that of androsterone by the capon test. It is the only crystalline hormone of high potency which has been separated from testis tissue.

Frattini and Maino (1930, 1932, 1935) reported the separation of the testis hormone in crystalline form a number of times. They have never given conclusive evidence that they have actually separated a pure substance. Furthermore, the procedures, yields, properties, and potency of their product do not inspire confidence in their claims. In their first publication they consider 3000 grams of fresh tissue to be equivalent to 3 mgm. of their product and claim that 3 mgm. is a capon unit, that it possesses estrogenic action, that it is water soluble, and exists as a sodium salt. In 1932 and 1935, they state that 500γ of their product in ten days produces 40 to 50 per cent increase in the comb length. They do not give any analytical data on composition or on the formation of derivatives of their substance.

Ogata and Hirano (1933, 1934) also claim to have separated the male hormone in pure form from hog testicles. They report that the non-saponifiable fraction remaining after the saponification of a 70 per cent alcoholic extract with Ba(OH)₂ when subsequently purified by resolution in absolute alcohol and then treated with hot hexane in ether solution yielded a deposit on cooling which in doses of 480γ constituted a capon unit. In their second report they also saponify boar testes with 5 per cent aqueous KOH for several hours and extract with ether. To remove cholesterol, the usual treatment with acetone and hexane was applied. After separating inert materials from 70 per cent alcohol and acetic-ether solutions, the soluble fraction in absolute alcohol was precipitated by hexane, then redissolved in 50 per cent ethyl alcohol and allowed "to evaporate for a few days in a cool place." The material
which separated was recrystallized from 50 per cent ethyl alcohol. It had a melting point of 128 to 130° and an activity of 1 capon unit per 850γ given daily for two days. The yield was 5 mgm. from 42 kgm. of tissue. No data on composition or derivatives were given.

The proof of the structure of androsterone, dehydroisoandrosterone, and testosterone and the preparation of various related compounds by degradation studies on sterols and bile acids. The chemical investigations on androsterone, dehydroisoandrosterone, and testosterone as prepared from urine and testis tissue respectively gave the empirical formulae, C_{19}H_{30}O_2, C_{19}H_{28}O_2, and C_{19}H_{26}O_2 respectively and suggested these structural formulae:

\[ \text{ANDROSTERONE} \quad \text{DEHYDROANDROSTERONE} \quad \text{TESTOSTERONE} \]

The relations of androsterone to dehydroisoandrosterone and to the chlorketone were clearly shown by Butenandt and Dannenbaum (1934) and Butenandt, Dannenbaum, Hanisch, and Kudszus (1935). Furthermore, the fact that pure theelin (oestrone), C_{18}H_{22}O_2, was converted into an octa-hydrogen derivative by intensive hydrogenation and that this new compound possesses comb-growth stimulating properties as shown by Schoeller, Schwenk, and Hildebrand (1933), and Dirscherl and Vos (1934) also supported the conclusion that the male hormones no doubt contain the hydrogenated phenanthrene ring. The final proof that the structural formulae are correct awaited the synthetic evidence. This evidence is now quite complete as a result of degradation studies on sterols and bile acids. Incidentally the investigations on sex hormones and vitamin Ds have stimulated the production, characterization, and degradation of sterols so intensively that new and remarkable structural relations among the sterols, bile acids, certain vitamins, hormones, and cardiac glucosides have been discovered at a breathtaking pace and for the first time the long suspected relations between certain of these groups are now well established. Up to the present, twenty-seven related substances of male-hormone character as well as androsterone, dehydroisoandrosterone, and testosterone have been
prepared by the synthetic degradation of sterols. The structural relations of these to each other are shown on pp. 180–189 and a more detailed chemical discussion of each is given below.

\((\text{cis})\)-\textit{Androsterone} (3-\textit{epi}-hydroxy-aetio-allocholanone-17) was first prepared from cholesterol by Ruzicka, Goldberg, Meyer, Brünger, and Eichenberger (1934, 1935). Theoretically it would appear a relatively simple matter to protect the alcohol group in position 3 by acetylation, to hydrogenate the 5,6 double bond, and then to destroy the side chain in position 17 by oxidation. However, the control of the oxidation, the further complication of stereo-isomerism on carbon 5 as well as the presence of six other asymmetric carbon atoms in the cholane ring presented numerous difficulties. It was already known that rings C and D in various known sterols and bile acids are identical in configuration, and that the junction between C and D is \textit{trans}. It also appeared probable that the linkage between B and C also is \textit{trans}. It therefore had to be assumed that cholesterol and androsterone are identical with respect to the positions discussed, but it remained to be determined whether after the saturation of position 5,6, we require the dihydrocholesterol or coprosterol ring. Finally it was a surprise to learn that even the asymmetry of carbon atom 3 was involved and that androsterone is a derivative of the \textit{epi} form of dihydrocholesterol and not of coprosterol. These relations were pointed out and solved by Ruzicka et al. (1934, 1935). The structural relations and the terminology are reproduced below:
The steps involved in the preparation of androsterone by Ruzicka et al. (1934) are a, the hydrogenation of cholesterol to dihydrocholesterol; b, the oxidation of the dihydrocholesterol to cholestanone by chromic acid; c, reduction of the cholestanone to a mixture of the two forms of dihydrocholesterol; d, separation of the epidihydrocholesterol from the reduction mixture; e, conversion into the acetate to protect the alcohol group; f, oxidation of the side chain; g, separation of androsterone acetate as the semicarbazone; h, hydrolysis of the semicarbazone and crystallization of the androsterone.

Butenandt, Dannenbaum, Hfanisch, and Kudszus (1935) proved the preparation of androsterone from cholesterol with dehydroandrosterone as an intermediate. The steps involved were a, cholesterol acetate is brominated and then oxidized by chromic acid to form the dibromketone; b, removal of bromine by zinc dust to form dehydroandrosterone; c, separation of the semicarbazone of dehydroandrosterone; d, hydrolysis; e, conversion of the dehydroandrosterone into the 3-chloroketone by treatment with thionyl chloride or with alcoholic HCl. This chloroketone was identical with the chloroketone Butenandt and Dannenbaum (1934) previously separated from urine and which they converted into androsterone acetate by hydrogenation and subsequent vigorous treatment with potassium acetate in glacial acetic acid. This treatment not only removes the chlorine in position 3 but also forms the epimer form of androsterone acetate. In other words, the original "dehydroandrosterone" is really the 5,6 unsaturated iso-androsterone.

Dalmer, Werder, Honigmann, and Heyns (1935) proved the relation of androsterone to the natural bile-acid group, which is, that the OH group in position 3 is the epi form in both cases, but that in position 5 the natural bile acids and coprosterol are identical, whereas cholesterol, sitosterol, stigmasterol, and androsterone yield or are of the opposite configuration or allo form. They accomplished this a, by preparing 3-hydroxy-allocholanic acid from hyodesoxycholic acid obtained from hog bile; b, by repeated alternate treatments thereof with Grignard reagent, acetylation, and oxidation until the C₁₇ side chain was removed step by step and replaced by the ketone oxygen as in androsterone. These investigators also prepared the same 3-hydroxy-allocholanic acid from the two hydrogenated sterols obtained from sitosterol (C₂₉H₄₉O) and stigmasterol (C₂₉H₄₉O).

Dirscherl (1935) used einchol (C₂₉H₄₉O), a sterol obtained from cinchona bark as the starting material. After hydrogenation he carried the dihydroeinchol through essentially the same steps first used so successfully by Ruzicka et al. (1934) and obtained androsterone.
Marker (1935) and Marker, Whitmore, Kamm, Oakwood, and Blatterman (1936) carried out the preparation of androsterone through the following steps: a, treatment of cholesterol with PCl₅ to form cholesteryl chloride; b, conversion into 3-chlor-5,6-dibromcholestanol; c, oxidation by chrolic acid and debromination to form 3-chlor-Δ⁵-androstenone (3-chlor-Δ⁵-dehydroisoandrosterone); d, hydrogenation of the latter to form 3-chloroisoandrosterone; e, the hydrolysis of this yielded androsterone. These investigators and Ruzicka (1934) avoided the bromination step by a, hydrogenating the cholesteryl chloride to form cholesteryl chloride, or b, by forming dihydrocholesterol before introducing the chloride. The cholesteryl chlorides formed by the two latter methods did not agree in melting points nor did the chlorandrosterones which were obtained by chrolic-acid oxidation agree in melting points. However, the chlorandrosterone of Marker, et al., on hydrolysis with potassium acetate yielded androsterone. It appears then that in the vigorous treatment with potassium acetate we have a Walden rearrangement from the isoandrosterone to the form of androsterone which occurs in urine.

Isoandrosterone (3-hydroxy-ñetioallocholanone 17) has also been prepared from cholesterol by Ruzicka et al. (1934) and from stigmasterol by Butenandt and Cobler (1935). The former group followed essentially the same methods as for androsterone from dihydrocholesterol only it was not necessary to shift the position of the OH and H on carbon atom 3. Butenandt and Cobler (1935) formed as an intermediate oxidation product allopregnanolone which is of interest because it is obtained from progesterone by hydrogenation and reduction of the 3-keto group. By the use of Grignard reagent and chrolic-acid oxidation, the allopregnanolone was converted into isoandrosterone. The allopregnanolone has also been separated from corpus-luteum extracts. Butenandt, Tscherning, and Hanisch (1935), and independently Wallis and Fernholz (1935) also prepared the isoandrosterone by the catalytic hydrogenation of dehydroisoandrosterone obtained from cholesterol.

The other two isomers theoretically possible from cholesterol are the 3'-hydroxy-ñettocholanone-17 and 3'-hydroxy-ñettocholanone-17. These are more closely related to coprosterol and were also prepared by Ruzicka et al. (1934). They were not found active in the capon in doses of 1000γ.

Androstanol-17-one-3 (17-hydroxy-ñettocholanone-3) is obviously another isomer of androsterone with an exchange in the OH and O positions. Butenandt, Tscherning, and Hanisch (1935) prepared this by oxidizing isoandrostenediol-monoacetate-17 with chrolic acid and
also by the hydrogenation of testosterone. The high activity, 25γ per capon unit, is of interest because of the close relationship to testosterone.

Dehydroisoandrosterone (Δ⁴-3-hydroxy-aetio-chenone-17 or Δ⁴-isandrostenol-3-one-17), as previously stated was first separated from urine by Butenandt, Dannenbaum, Hanisch, and Kudszus (1934, 1935). They also prepared it from the 3-chlorketone-17 derivative separated as an artefact in the fractionation of urine extracts and finally they prepared it from cholesterol and stigmasterol by the following steps: a, formation of dibromide of cholesterol acetate; b, oxidation by chromic acid to form the acetate of isandrosteronedibromide; c, debromination by zinc dust in acetic acid; d, separation of the dehydroisoandrosterone semicarbazone; e, hydrolysis of the semicarbazone derivative to free dehydroisoandrosterone. Oppenauer (1935) also prepared the substance by essentially the same methods from γ-sitosterol. Ruzicka and Wettstein (1935) and Wallis and Fernholz (1935) carried out practically the same procedure as that of Butenandt in the preparation of the substance from cholesterol.

It is now generally agreed that the dehydroandrosterone obtained from urine is identical with the synthetic degradation product described above. This leads to the conclusion that dehydroisoandrosterone is the better name and that carbon 3 has the same configuration in cholesterol, stigmasterol, isandrosterone, and dehydroisoandrosterone. This is further confirmed by the fact that dehydroisoandrosterone is precipitated by digitonin whereas the 3-epi compounds, such as bile acids and androsterone are not precipitated (Schoeller, Serini and Gehrke, 1935). In fact the Butenandt group (1935) makes use of this reaction in the separation of the dehydro form from urine.

Ruzicka, Fischer, and Meyer (1935) have also been able to shift the double bond in Δ⁴-trans-dehydroandrosterone to the 4-5 position by the usual treatment with hydrochloric acid dissolved in absolute alcohol thus producing Δ⁴-trans-dehydroandrosterone.

Androstanediol-3-cis-17-trans (dihydroandrosterone or 3-epi-hydroxy-allo-aetiocholanol-17-trans). After Schwenk and Hildebrandt (1933) and Girard, Sandulesco, and Fridenson (1933) had prepared oestradiol (dihydro-oestrone or dihydrotheelin) from theelin by reduction of the ketone group and found the product to possess greater oestrogenic potency it is not surprising that similar reduction studies were carried out on androsterone. Butenandt and Tscherning (1935) reduced androsterone to the diol by sodium in propyl alcohol. Ruzicka, Goldberg, and Meyer (1935) carried out the reduction by hydrogen in presence of
platinum oxide in acid and alkaline solutions. From the table, pp. 180–189 it is obvious that this diol also is more active than the ketone from which it is obtained.

However, by the reduction of the ketone-17 group to a secondary alcohol we have introduced another asymmetric carbon atom. As a result we now have possible four different androstanediols-3-17. The form discussed above has been assumed to be the 3-cis-17-trans form, that is position 3 is as in androsterone and in position 17, the OH group is considered to be trans to the CH$_3$ group on carbon 13. Obviously the 17-trans reduction product of isoandrosterone, that is, androstanediol-3-trans-17-trans is also possible. It has in fact been prepared by reduction of isoandrosterone by Butenandt, Tscherning and Hanisch (1935) and by Ruzicka, Goldberg, and Rosenberg (1935). Still another isomer, the 3-trans-17-cis diol, has also been prepared by Ruzicka and Goldberg (1936) from isoandrosterone and from androstenediol-3-trans-17-cis. The 3-trans-17-cis diol does not appear to have been identified as yet although it may easily be present in some of the products of reduction of androsterone. The comparative biological assays on these different androstanediols indicate that in position 3 the cis form raises the potency whereas in the 17 position it appears that there is very little difference between the potencies of the cis and trans forms (Ruzicka and Rosenberg, 1935, 1936).

17-Methylandrostanediol (17-methyl-3-epihydroxy-allo-aetio-cholanol-17). This substance was obtained in good yield by treatment of androsterone with methyl magnesium iodide by Ruzicka, Goldberg and Meyer (1935). It is of about the same order of activity as androstanediol in the capon, that is, about three times as potent as androsterone. The configuration of carbon 17 has not been determined.

17-Methylandrostanol-17-one-3 (17-methyl-3-keto-allo-aetio-cholanol-17). By first preparing the 17-methylandrostanediol from androsterone from the action of the methyl magnesium-iodide reagent and then oxidizing cautiously with chromic acid, the alcohol in position 3 was converted to a ketone group (Ruzicka, Goldberg, and Rosenberg, 1935). This substance is approximately six times as active as androsterone in the capon test.

3,17-Dimethylandrostanediol-3,17 (3,17-dimethyl-allo-aetio-cholanediol-3,17). This substance was obtained by the interaction of methyl magnesium iodide with the diketone, androstanedione-3,17. The very low potency indicates that methylation at position 3 lowers the activity of the saturated compound (Ruzicka, Goldberg, and Meyer, 1935).
17-Ethyl-cis-androstane-3,17 (17-ethyl-3-epihydroxy-allo-aetio-cholan-17). This was prepared in the same way by ethyl magnesium iodide with cisandrosterohe. This product is approximately five times as active as androsterone in the capon test (Ruzicka, Goldberg and Meyer, 1935).

17-Ethyl-trans-androstane-3,17 (17-ethyl-allo-aetio-cholan-3,17). By the same reaction applied to the transandrosterone, Ruzicka, Goldberg, and Meyer (1935) prepared this trans-isomer of exceedingly low activity. This again emphasizes the importance of the configuration of carbon atom 3.

17-Ethyl-androstan-17-one-3 (17-ethyl-3-keto-allo-aetio-cholan-17). The same investigators prepared this substance by the cautious oxidation of 17-ethyl-trans-androstane-3,17 with chromic acid. Its activity has not been reported, but it probably is of high order of activity.

Androstanedion-3,17 (allo-aetio-cholane-3,17). This product was prepared by Butenandt and Tscherning (1934) and Ruzicka, Goldberg, and Meyer (1935) from androsterone by mild oxidation with chromic acid. It has been prepared similarly from androstanolone (Butenandt, Tscherning, and Hanisch, 1935), isoandrosterone (Butenandt and Cobler, 1935), and androstanedion (Ruzicka and Rosenberg, 1936). It is approximately one-half as active as androsterone in the capon.

Testosterone (Δ4-androstenol-17-one-3 or Δ4-3-keto-aetio-chol-17). Testosterone was first separated in crystalline form from bull testis-tissue by Laqueur et al. (1935). The chemical characteristics are very different from those of androsterone. This was not surprising because numerous studies on concentrates from testis tissue and urine respectively convinced many of us that the hormones from the two sources are not identical. It was first suggested chemically by the destruction of the testicular product by basic hydrolysis (McGee, Juhn and Domnn, 1928; Gallagher and Koch, 1933, 1934). Biological differences were first clearly proved by Laqueur de Fremery, Freud, de Jongh, Köber, Luchs and Müneh (1931) who found 1400 per cent variations in ratios of capon assays to rat seminal-vesicle and prostate reactions on different urinary and testis-tissue concentrations and different fractions of each. This has been repeatedly confirmed by Freud and Laqueur (1934), Freud (1934), Dingemanse, Freud, and Laqueur (1935), David and Freud (1935), and by Callow and Deanesly (1935). Matsuzaki (1933) and Ogata and Hirano (1933, 1934) also reported that treatment of an acetone solution of the testis-tissue
concentrate with potassium permanganate causes destruction of the activity on rat seminal vesicles but not on the capon. They could not confirm the lability of the comb-growth stimulating factor to alkaline hydrolysis, but reported loss of seminal-vesicle stimulation as a result of such treatment. Many of these observations on urine and testis-tissue concentrates have been clarified as a result of the excellent biological results obtained from the various synthetic degradation products already discussed, but the artificial preparation of testosterone and various derivatives thereof by synthetic degradation methods, very soon after its first separation from testis-tissue concentrates, gave the best answer.

It may be recalled that trans-dehydroandrosterone was found to possess very low potency; that when it was saturated and oxidized to androstanedione, the activity was slightly improved, but that when the 17 position was reduced to form androstanol-17-one-3, a very active product was formed. Furthermore, reduction of androsterone to the androstanediol also increased the potency. The instability of the testis-tissue concentrates in alkaline solutions and the similar behaviour of progesterone also suggested that possibly the real male hormone as found in the tissues is a derivative of Δ^4-aetio-cholenone-3. Stimulated by these findings, the studies on the artificial preparation of derivatives of the unsaturated (cholene or androstene) series were already under way at the time the Laqueur group first announced the isolation of testosterone from testis tissue. At practically the same time, Butenandt and Hanisch (1935) and Ruzicka and Wettstein (1935) announced the artificial preparation of testosterone, identical in physical, chemical, and biological properties with natural form. The process involved the following steps: a, reduction of Δ⁵-dehydroandrosterone to Δ⁵-androstanediol; b, preparation of the 3,17-diacetate of the diol; c, partial hydrolysis to form the 17-mono-acetate-androstenediol; d, formation of the dibromide in order to protect the double bond; e, oxidation by chromic acid in the cold and debromination by zinc dust or sodium iodide to form Δ⁴-androstenol-17-one-3-17-acetate; f, mild saponification to form the free testosterone. Ruzicka, Wettstein, and Kägi (1935) later carried out the same steps beginning with Δ⁴-androstenediol-3-acetate-17-benzoate. The maximum absorption spectrum of the substance at 238 μμ is correlated with the location of the unsaturated bond in the 4–5 position. The relation to cholestenone and progesterone is thus confirmed. The further relation of testosterone to an isomer of androsterone was shown by hydrogenating testosterone to form androstanol-17-one-3 (Butenandt, Tscherning, and Hanisch, 1935).
THE MALE SEX HORMONES

$\Delta^5$-Androstenediol-3-trans-17-cis ($\Delta^5$-aetio-cholenediol-3,17). Ruzicka and Wettstein (1935) and Butenandt and Hanisch (1935) prepared this substance from $\Delta^5$-dehydroandrosterone by reduction with sodium in propyl alcohol or nickel catalyst in alcoholic solution.

$\Delta^4$-Androstenedione-3,17 ($\Delta^4$-aetio-cholenedione-3,17). Ruzicka and Wettstein (1935) and Butenandt and Kudszus (1935) prepared this by $a$, brominating dehydroandrosterone; $b$, oxidizing by chromic acid in acetic acid to form the diketone-dibromide; $c$, debrominating in acetic acid by zinc. Mild oxidation of testosterone also produced the androstendionc thus confirming the structural relations. In this connection the very recent observations by Butenandt and Dannenberg (1936) on $\Delta^1$-androstenedione and Butenandt and Riegel (1936) on 6-oxytestosterone are of interest because they find both of the products to be practically free from androgenic action but to possess some estrogenic properties. When we recall that $\Delta^4$-androstenedione is a very potent androgen, this result on the $\Delta^1$-androstenedione is very remarkable.

17-Methyl-testosterone ($\Delta^4$-17-methyl-androstenol-17-one-3). This was prepared by Ruzicka, Goldberg, and Rosenberg (1935) by $a$, treatment of dehydroandrosterone with methyl magnesium iodide; $b$, bromination; $c$, mild oxidation by chromic acid for 20 hours; $d$, separation of the dibromide as such; $e$, debromination by zinc, and $f$, sublimation.

17-Ethyl-testosterone ($\Delta^4$-17-ethyl-androstenol-17-one-3). Ruzicka and Rosenberg (1936) and Butenandt, Cobler, and Schmidt (1936) proceeded as follows: $a$, prepared $\Delta^5$-17-ethyl-androstenediol; $b$, brominated to the dibromide; $c$, oxidized at room temperature by chromic acid, and $d$, debrominated by zinc.

$\Delta^2$-17-Methylandrostenediol-3,17 ($\Delta^2$-17-methylaetiocholenediol-3,17 and $\Delta^2$-17-ethylandrostenediol-3,17 ($\Delta^2$-17-ethylaetiocholenediol-3,17). Ruzicka, Goldberg and Rosenberg (1935) prepared these substances by treatment of dehydroandrostrone in ether solution by the Grignard reaction. The ethyl derivative was also prepared by Butenandt, Cobler, and Schmidt (1936).

Androstanol-3-amine-17 (17-amino-3-epihydroxy-allo-aetiocholane) and $\Delta^2$-androstolenol-3-amine-17 ($\Delta^2$-17-amino-3-hydroxy-aetiocholene) were prepared by Ruzicka and Goldberg (1936) by reducing the oximes of androsterone and transdehydroandrosterone respectively in alcoholic solution with metallic sodium.

THE RELATIVE ACTIVITIES OF THE VARIOUS CONCENTRATES AND OF PURE PREPARATIONS. Table 2 on pages 180 to 189 lists in alphabetical order the names, structural formulae, and physical constants of 32
In the second column the initials of authorities quoted on activity are given above the horizontal division and below are given the sources from which the substances have been prepared. In the third column, the horizontal division separates the melting point and specific rotation values.

<table>
<thead>
<tr>
<th>NAME FORMULA</th>
<th>AUTHORITY*</th>
<th>M.P.</th>
<th>PHYSIOLOGICAL ACTIVITY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>[α]</td>
<td>γ Equiv.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>to 100γ</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Androsterone</td>
</tr>
<tr>
<td>Androstanediol-3-cis-17</td>
<td>B. &amp; T.</td>
<td>221–223°</td>
<td>25</td>
</tr>
<tr>
<td>or Dihydroandrosterone</td>
<td>T. &amp; R.</td>
<td></td>
<td>23</td>
</tr>
<tr>
<td>or 3-epi-hydroxy-aetio-allocholanol-17</td>
<td>D. &amp; P.</td>
<td></td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>Androsterone</td>
<td></td>
<td>+12.6° at 23°</td>
</tr>
<tr>
<td>Androstanediol-3-trans-17-trans</td>
<td>B. &amp; T.</td>
<td>164–168°</td>
<td>600</td>
</tr>
<tr>
<td>or Isodihydroandrosterone</td>
<td>T. &amp; R.</td>
<td></td>
<td>520</td>
</tr>
<tr>
<td>Substance Description</td>
<td>Molecule Structure</td>
<td>熔点/沸点</td>
<td>B. &amp; T.</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Androstanediol-3-trans-17-cis or 3-hydroxy-aetioallocholanol-17-cis</td>
<td><img src="image" alt="Molecule" /></td>
<td>178-179°</td>
<td></td>
</tr>
<tr>
<td>Androstanediol diacetate or Dihydroandrosterone diacetate</td>
<td><img src="image" alt="Molecule" /></td>
<td>159-160° 162-163° +12.5° at 23°</td>
<td>B. &amp; T.</td>
</tr>
<tr>
<td>Androstanedione-3,17 or aetio-allocholanedione-3,17</td>
<td><img src="image" alt="Molecule" /></td>
<td>125</td>
<td>B. &amp; T.</td>
</tr>
<tr>
<td>Androstane-3,17 or aetio-allocholanedione-3,17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Androstanol-3-amine-17 or 17-amino-3-epihydroxyaetio-allocholane</td>
<td><img src="image" alt="Molecule" /></td>
<td>187-188° &gt;1000</td>
<td>T. &amp; R.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NAMES FORMULAE</th>
<th>AUTHORITY*</th>
<th>M.P.</th>
<th>Capon test</th>
<th>Order of potency on rat</th>
<th>On minimum weight basis</th>
<th>On capon unit basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androstanol-17-one-3 or 3-keto-aetio-oZZocholanol-17</td>
<td>B. &amp; T.</td>
<td>178-180°</td>
<td>25-30</td>
<td>2</td>
<td>25-30</td>
<td>2</td>
</tr>
<tr>
<td>Androstane monooacetate, or dehydroandrosterone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Androstenediol-17-one-3 or 3-keto-aetio-oZZocholanol-17</td>
<td>B. &amp; T.</td>
<td>178-180°</td>
<td>25-30</td>
<td>2</td>
<td>25-30</td>
<td>2</td>
</tr>
<tr>
<td>Androstane monooacetate, or dehydroandrosterone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ⁵-Androstenediol-3-trans-17-cis or Δ⁵-aetiocholenediol-3-trans 17-cis</td>
<td>B. &amp; T.</td>
<td>178°</td>
<td>650</td>
<td>7</td>
<td>25-30</td>
<td>2</td>
</tr>
<tr>
<td>Transdehydroandrosterone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ⁵-Androstenediol-3-trans-17-cis or Δ⁵-aetiocholenediol-3-trans 17-cis</td>
<td>T. &amp; R.</td>
<td>175-178°</td>
<td>625</td>
<td>5</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Transdehydroandrosterone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ⁵-Androstenediol-3-trans-17-cis or Δ⁵-aetiocholenediol-3-trans 17-cis</td>
<td>D. &amp; P.</td>
<td>182-183°</td>
<td>235</td>
<td>6</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Transdehydroandrosterone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ⁵-Androstenediol-3,17-benzoate-17 or Δ⁵-aetiocholenediol-3,17-benzoate-17</td>
<td>T. &amp; R.</td>
<td>220.5-222°</td>
<td>6</td>
<td>10</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Compound</td>
<td>Source</td>
<td>mp 18°</td>
<td>mp 28°</td>
<td>mp 38°</td>
<td>mp 48°</td>
<td>mp 58°</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>Δ⁴-Androstenediol-3-trans-17</td>
<td>B. &amp; T.</td>
<td>128.5-130°</td>
<td>&gt;150(?)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>or Δ⁴-aetiocholenediol-3-trans-17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ⁴-Transandrostenediol diacetate</td>
<td>T. &amp; R.</td>
<td>165-166°</td>
<td>159.5°</td>
<td>-56.5° at 18°</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ⁴-Transandrostenediol monoacetate</td>
<td></td>
<td>147-148°</td>
<td></td>
<td>-62.4° at 18°</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ⁴-Androstenedione-3,17</td>
<td>B. &amp; T.</td>
<td>160°</td>
<td>120</td>
<td>5</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>or Δ⁴-aetiocholenedione-3,17</td>
<td>T. &amp; R.</td>
<td></td>
<td>100</td>
<td>6</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>D. &amp; P.</td>
<td></td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Transdehydroandrosterone or testosterone</td>
<td>+185° at 18°</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ⁴-Androstenol-3-amine-17</td>
<td>T. &amp; R.</td>
<td>160-182°</td>
<td>&gt;1000</td>
<td>8</td>
<td>&gt;11</td>
<td>&gt;11</td>
</tr>
<tr>
<td>or Δ⁴-17-amino-3-hydroxy-aetiocholene</td>
<td></td>
<td></td>
<td>Δ⁴-transdehydroandrosterone oxime</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAMES FORMULA</td>
<td>AUTHORITY*</td>
<td>M.P.</td>
<td>PHYSIOLOGICAL ACTIVITY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td>------------</td>
<td>------</td>
<td>------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Capon test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Order of potency</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>On minimum weight basis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>On capon unit basis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(cisi)-Androsterone or 3-epi-hydroxy-aetio-allocholan-one-17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. &amp; T.</td>
<td>182-183°</td>
<td>100</td>
<td>3</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>T. &amp; R.</td>
<td></td>
<td>100</td>
<td>4</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>D. &amp; P.</td>
<td></td>
<td>100</td>
<td>4</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>4H-Human urine, sterols, bile acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3H at 18°</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(trans)-Androsterone or isoandrosterone or 3-hydroxy-aetio-allocholanone-17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D. &amp; T.</td>
<td>174-175°</td>
<td>700</td>
<td>8</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. &amp; R.</td>
<td></td>
<td>770</td>
<td>7</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D. &amp; P.</td>
<td></td>
<td>700</td>
<td>7</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sterols</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Androsterone acetate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H. &amp; T.</td>
<td>160-161°</td>
<td>100</td>
<td>(delayed)</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. &amp; R.</td>
<td></td>
<td>100</td>
<td>4</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compound</td>
<td>T. &amp; R.</td>
<td>178-178.5°</td>
<td>185-185.5°</td>
<td>137-138° and 148° (two forms)</td>
<td>300</td>
<td>5 to 6</td>
</tr>
<tr>
<td>----------</td>
<td>---------</td>
<td>------------</td>
<td>------------</td>
<td>--------------------------------</td>
<td>-----</td>
<td>--------</td>
</tr>
<tr>
<td>Androsterone benzoate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Androsterone succinate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dehydroisoandrosterone or Δ⁴-3-hydroxy-actio-cholenone-17 or Δ⁴-androstenol-3-trans-one-17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human urine, sterols</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+10.9° at 18°</td>
<td></td>
</tr>
<tr>
<td>3,17-Dimethylandrostanediol-3,17 or 3,17-dimethylactio-allocholane-diol-3,17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Androstenedione</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&gt;700</td>
</tr>
<tr>
<td>17-Ethyl(cis)androstanediol-3,17 or 17-ethyl-3-epiandroxy-actio-allocholanol-17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>143-144°</td>
</tr>
<tr>
<td>NAMES FORMULAE</td>
<td>AUTHORITY&lt;sup&gt;a&lt;/sup&gt;</td>
<td>M.P.</td>
<td>PHYSIOLOGICAL ACTIVITY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td>------------------------</td>
<td>------</td>
<td>------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Found in or prepared from</td>
<td>[α]</td>
<td>Capon test</td>
<td>Order of potency on rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17-Ethyl(trans)androstenediol-3,17 or 17-ethyl-3-hydroxyetio-allocholanol-17</td>
<td>T. &amp; R.</td>
<td>204–205°</td>
<td>600</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17-Ethylandrostanol-17-one-3 or 17-ethyl-3-keto-etio-allocholanol-17</td>
<td>T. &amp; R.</td>
<td>137–138°</td>
<td>600</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ&lt;sup&gt;2&lt;/sup&gt;-17-Ethylandrostenediol-3,17 or Δ&lt;sup&gt;2&lt;/sup&gt;-17-ethylactio-chenenediol-3-trans-17</td>
<td>T. &amp; R.</td>
<td>190–200°</td>
<td>600</td>
<td>6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Equivalent to 100 γ androsterone
<table>
<thead>
<tr>
<th>Compound</th>
<th>Formula</th>
<th>M.p. (°C)</th>
<th>T. &amp; R.</th>
<th>D. &amp; P.</th>
</tr>
</thead>
<tbody>
<tr>
<td>17-Ethyltestosterone</td>
<td>C_{29}H_{40}O_2</td>
<td>184-185</td>
<td>T. &amp; R.</td>
<td></td>
</tr>
<tr>
<td>or Δ^1Δ^5-17-Ethyl-17-one-3</td>
<td></td>
<td></td>
<td>D. &amp; P.</td>
<td></td>
</tr>
<tr>
<td>or Δ^1Δ^5-17-Ethyl-3-keto-aetiocholenol-17</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17-Methyl(cis)androstanediol-3,17</td>
<td>C_{29}H_{40}O_2</td>
<td>143°</td>
<td></td>
<td></td>
</tr>
<tr>
<td>or 17-methyl-3-epihydroxy-aetiocholanol-17</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17-Methyl(trans)androstanediol-3,17</td>
<td>C_{29}H_{40}O_2</td>
<td>211-212°</td>
<td></td>
<td></td>
</tr>
<tr>
<td>or 17-methyl-3-hydroxy-aetiocholanol-17</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

17-Ethyltestosterone or Δ^1Δ^5-17-Ethyl-17-one-3 or Δ^1Δ^5-17-Ethyl-3-keto-aetiocholenol-17
<table>
<thead>
<tr>
<th>NAMES FORMULA</th>
<th>AUTHORITY*</th>
<th>M.P.</th>
<th>PHYSIOLOGICAL ACTIVITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Found in or prepared from</td>
<td>[α]</td>
<td>Capon test</td>
<td>Order of potency</td>
</tr>
<tr>
<td>17-Methylandrostanol-17-one-3 or 17-methyl-3-keto-aetio-allocholanol-17 or 17-methylidihydrotestosterone</td>
<td>T. &amp; R.</td>
<td>192-193°</td>
<td>25</td>
</tr>
<tr>
<td>Androsterone</td>
<td>D. &amp; P.</td>
<td></td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ^1-17-Methyl(trans)androstenediol-3,17 or Δ^1-17-methylaetiocholenediol-3-trans-17</td>
<td>T. &amp; R.</td>
<td>204°</td>
<td>525</td>
</tr>
<tr>
<td>Transdehydroandrosterone</td>
<td>D. &amp; P.</td>
<td></td>
<td>155</td>
</tr>
</tbody>
</table>
| Compound | Origin | Melting Point | Ref. | Blend | Content | Test | Test
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>17-Methyltestosterone or A(^4)-17-methylandrostenol-17-one-3 or A(^4)-17-methyl-3-keto-aetiocholenol-17</td>
<td>T. &amp; R., D. &amp; P.</td>
<td>163-164°</td>
<td>70</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Testosterone or A(^4)-androstenol-17-one-3 or A(^4)-3-keto-aetiocholenol-17</td>
<td>B. &amp; T., T. &amp; R., D. &amp; P.</td>
<td>154.5-155°</td>
<td>13</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Testosterone acetate</td>
<td>T. &amp; R.</td>
<td>139-141°</td>
<td>13</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Testosterone benzoate</td>
<td></td>
<td>194-196°</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
substances of male-hormone character in parallel with the comparative biological activities on the capon and the castrated rat.

The order of activity on the capon has been studied by Butenandt and Tscherning (1935, 1936), Tschopp and Ruzicka (1935, 1936), and Deanesly and Parkes (1935, 1936). Deanesly and Parkes measured the increase in length and height of the comb for their method of assay. The other values are based on the planimetric method. The three groups agree remarkably well on the order of activity and on the dose equivalent to 100γ of androsterone. The only serious exceptions on the dosage basis are Δ^5- androstenediol-3-trans-17-eis and Δ^5-17-methyl-(trans)-androstenediol-3,17, where Deanesly and Parkes obtain about one third to one half the values of the other groups, and for 17-methyltestosterone, where the reverse is true. These discrepancies suggest a qualitative as well as a quantitative difference in the comb reaction, but it is also possible that differences in the time period and in the distribution of the total dose in the various assays as indicated in table 1, may account for the discrepancies. In addition to the substances listed we should consider several other isomers and related compounds. It may be recalled that the two 3-hydroxy-17-keto isomers of androsterone obtained from coprosterol and epi-coprosterol respectively were inactive in the capon in 1-mgm. doses. Tscherning (1936) also reports that Δ^5-3,17-hydroxy-aetio-cholenone-7 as acetate in 2-mgm. doses and Δ^4-17-hydroxy-aetio-cholenedione-3,6 as acetate in 1-mgm. doses are inactive in the capon.

The tabulation of the comparative activities on the basis of mammalian assays seems less justified because the methods are less reliable from a quantitative point of view. The relationship of dose to response in per cent increase in weight of seminal vesicles and prostate in the castrated rat is considered to be expressed by a straight line by Korenchevskey and Dennison (1935) over a dosage range of 200γ to 900γ for androsterone, but for a range of 25γ to 350γ of androstanediol, the curve is hyperbolic. Obviously it is difficult to compare the potencies of different substances when such complications are involved. More extensive data on the mammalian test on a great variety of pure substances as published by Callow, Deanesly, and Parkes (1935, 1936) and Tschopp (1936) reveal this same difficulty. Hence absolute weights of hormones are not indicated in table 2. The order of activities given in table 2 is based on the weights of seminal vesicles and prostates obtained at one to three dosage levels. Different levels had to be chosen because of the necessary widespread in the dosage. In view of these complica-
tions the order of activity in the rat based on the capon-unit basis is also of doubtful value.

POSSIBLE BIOLOGICAL ORIGIN OF SEX HORMONES FROM CHOLESTEROL. I

An inspection of table 2 shows that the orders of activity are not the same on capons and rats. In fact they are not even the same for the seminal vesicle and prostate reactions. The former findings are in harmony with the comparative assay findings which the Laqueur group
(1931, 1935) reported on various urine and testis-tissue concentrates. A difference in seminal-vesicle and prostate reaction was first observed by Moore, Price, Hughes, and Gallagher (1930) who found the prostate
reaction much more sensitive to testis-tissue concentrates than the seminal-vesicle test in the castrated rat. This was confirmed more quantitatively on the histological basis by Hansen (1933). Recently Callow and Deanesly (1935) compared androsterone, androstanediol, testis-tissue concentrates and urine concentrates. They show that androsterone affects the prostate mainly and the seminal vesicles relatively slightly, but that androstanediol or testis-tissue extract acts about equally well on both accessory sex organs. In their hands, the injection of theelin with androsterone did not correct the effect on the seminal vesicles nor did such combinations bring about, per capon unit of androsterone, the same quantitative effect on the seminal vesicles and prostate as urine concentrates. They, therefore, suggest that other factors are present in urinary concentrates. However, Dingemanse, Freud, and Laqueur (1935) observed that per capon unit the seminal-vesicle reaction from testis-tissue concentrates is greater than from urine preparations. Deanesly and Parkes (1936) report that the relative potency of testosterone over androsterone is five or six times on the capon, two and one-half to five times on the rat prostate, and ten times on the rat seminal vesicle. Tschopp (1936) also calls attention to the greater effect of androsterone on the prostate and states that the normal weight-ratio of seminal vesicle to prostate is in general maintained better by the unsaturated series of male sex-hormones than by the saturated group. Korenchevsky and Dennison (1935) state that in the normal adult rat the weights of seminal vesicles and prostate are approximately the same. In the 30-day castrate rat the average weights for seminal vesicles and prostate are 10 to 12 mgm. and 50 to 55 mgm. respectively. Obviously the rate of regeneration of the two organs when expressed in increase in absolute weight or in percentage increase over castrate weight must be higher in the seminal vesicle than in the prostate if both are to be brought back to the normal weights. Korenchevsky and Dennison (1935) attempted to show the difference in the value of androsterone and androstanediol by expressing the ratio of percentage increase in weight of prostate to that of seminal vesicle in castrate rat on various doses of the hormones. They report that on doses of 200γ to 900γ daily of androsterone, this ratio is 1.06 while for androstanediol on doses of 25γ to 350γ it is 0.64. Obviously they find androstanediol more effective on the seminal vesicles in relation to the effect on the prostate. In fact doses of 25γ androstanediol caused increases in weight of seminal vesicles and prostate of 80 per cent and 48 per cent respectively.
RELATION OF STRUCTURE TO ACTIVITY. Naturally the usual attempts have been made to correlate physiological potency with structure. The correlation is rather poor (Parkes, 1935; Deanesly and Parkes, 1936; and Ruzicka, Goldberg and Rosenberg, 1935).

On capons the negative results with the coprosterol and epicoprosterol derivatives show that in the saturated compounds a 5-cis configuration lowers the activity whereas the 5-trans form or unsaturation increases it. The 3-epi-(cis)-hydroxyl group or a 3-keto group in the saturated series increases the potency considerably over the 3-trans-hydroxyl. This is not the case in the unsaturated series. In general a secondary alcohol in position 17 leads to a more active compound than a tertiary alcohol, but both of these forms are more potent than the 17-keto form.

On rats the 3-epi-hydroxyl form is also more potent than the trans form; however, a 3-keto form is more potent than the 3-epi alcohol derivative. Another difference is that a tertiary alcohol in position 17 is more favorable than either a secondary alcohol or a 17-keto form. Unsaturation in the 4–5 position increases the activity over the corresponding saturated compounds studied thus far.

The potency of esters of various organic acids with various derivatives of the androsterone and testosterone series is of some interest. Butenandt et al. (1934, 1935) reported a delayed response in capons from androsterone acetate, androsterone oxime, and androstanediol acetate. Callow and Deanesly (1935) found androsterone benzoate practically inactive in capons and rats. Ruzicka, Goldberg, and Meyer (1935) and Callow (1936) found adrosterone benzoate when given in doses equivalent to 1000γ to 5000γ of androsterone to give irregular and delayed response in the capon. Callow calls attention to the differences in the quantitative but delayed response on such high doses. Deanesly and Parkes (1936) also report testosterone benzoate inactive in capons.1 Tschopp, however, finds androstanediol monoaetate and diaetate, androsterone acetate, androsterone monosuccinate, testosterone acetate, and androstenediol diaetate all about equal in activity to the respective free hormone forms. He finds one exception in androstanediol disuccinate, which in the capon is only about one-sixteenth as active as the free diol; in the rat the difference does not appear to be so great.

THE ACTION OF ESTROGENIC SUBSTANCES ON THE MALE SEX ORGANS AND ITS SIGNIFICANCE IN THE MAMMALIAN ASSAY AND IN NORMAL PHYSIOLOGICAL DEVELOPMENT. The injurious effects on the testicle of crude

1 This has been confirmed in the author’s laboratories.
lipoid-soluble female-hormone preparations from ovaries, placenta, corpus-luteum tissue, testis tissue, and pregnancy urine were observed repeatedly by Herrmann and Stein (1916, 1921), Fellner (1921), Steinach and Kun (1926), and Laqueur and de Jongh (1928). A careful analysis of the mechanism involved in these studies and independent studies with more carefully purified and standardized hormone preparations led Moore and Price (1930, 1932) to interpret the injurious effect on the testicle of the adult rat as due to the depressing action of theelin or of the male hormone on the gonad-stimulating activity of the anterior pituitary and not a direct sex antagonism action as interpreted by Steinach and Kun (1926). Moore and Price found that in the normal male the theelin treatment leads to degeneration of seminal vesicles and prostate approaching the castrate type with possibly a tendency toward a delay in this process in the castrated male. They conclude, however, that theelin neither stimulates nor directly depresses the accessory male sex organs. Moore and Price did not report a specific type of reaction on the seminal vesicles or prostate of either the normal or castrate rat; furthermore, the combined injection of theelin and male hormone in the castrate produced the same effect on these accessory sex organs as male hormone alone. Their injections were, however, limited to adult rats and to short periods of injection with relatively low concentrations of estrogenic material. More specific effects from estrogenic materials are, however, detectable by using young castrated mice or rats or by more prolonged and more intensive theelin treatment of normal or castrated mice or rats of various ages. The more recent studies clearly show this.

Freud (1933) showed that 40 mouse units of theelin or theelol often produced smooth muscle development in the seminal vesicles of young castrated rats especially if injected together with low doses of male-hormone preparations. Lacassagne (1933) injected adult castrated and adult and young male mice with six gamma of crystalline theelin (Girard) over long periods of time. After five months they observed retention of urine and atrophied seminal vesicles, but hypertrophy of the posterior prostate. Independently Burrows and Kennaway (1934) confirmed these observations on normal mice. They applied 0.01 to 0.1 per cent solutions of crystalline theelin (Girard) in benzene by means of a brush to the skin in the interscapular region twice weekly and observed scrotal hernia, atrophy of testicle and seminal vesicles, but enlargement of the posterior lobe of the prostate and obstruction of flow of urine. The stimulating effect of theelin on the seminal vesicles is also
obtained in the hypophysectomized castrated mouse (de Jongh, 1934). David, Freud, and de Jongh (1934) injected immature castrated rats for five to seven days twice daily with 0.2 to 10 gamma of theelin. They found the seminal vesicles, preputial glands, anterior prostate, and periurethral tissue to increase when the higher doses were used, but the histology showed stratified epithelium in place of the spongy columnar type normally found at the orifice of the ducts of the prostate and seminal vesicles. Heringa and de Jongh (1934) also produced a change in the connective tissue layer of the ampulla of the vas deferens by theelin. This consisted in changing the normal woven mass of collagen bundles and network of reticulin in the young castrated mouse to a thicker mucoid type. David, Freud, and de Jongh (1934) as a result of comparative studies with eleven pure estrogenic substances conclude that the estrogenic substances all cause thickening and cornification of the vaginal stratified epithelium in the female and likewise a change from the columnar epithelium to the stratified type in the seminal vesicles and prostate of the castrate. They furthermore find that the corpus-luteum hormone counteracts these effects in the female, but not in the male; however, male hormone counteracts these effects in the male, but not in the female. They consider estrogenic substances to possess three pharmacological actions on the secondary sex organs of both sexes, viz., a, stimulation of growth of smooth-muscle tissue and interstitium; b, stimulation of submucosa and stratified epithelium growth; c, stimulation of growth of submucosa and undifferentiated cylindrical epithelium in non-tubular organs. Korenchevsky and Denison (1935) studied the effect of 60 to 80 international units of theelin in normal and castrated rats with and without treatment with male-hormone concentrates. Although they found that theelin alone stimulates growth of the seminal vesicles and prostate and causes hypertrophy of the pituitary in the castrated rat, its effect in conjunction with the injection of male-hormone concentrates is primarily on the seminal vesicles. They, therefore, conclude that the rat-prostate method based on weight increase should be considered the equivalent to the comb-growth assay, but that the weight increase for seminal vesicles and prostate together constitutes “the whole male sexual activity” assay. These investigators and Overholser and Nelson (1935) conclude that theelin and the male sex-hormones act synergistically on the epithelial and smooth-muscle elements of the seminal vesicles. Callow and Deanesly (1935) and Tschopp (1936) do not consider the theelin action as a pacemaker or synergist. The data, however, indicate that
Theelin acting with testosterone or adrosterone in the young castrate rat produces greater increases in the weights of seminal vesicle, prostate, Cowper's and preputial glands than the sums of the independent actions of theelin and testosterone, thus in part supporting Korenchevsky's statement.

The fact that these estrogenic effects are obtained more easily in the young castrates on relatively low doses or in normal adult rats and mice after more prolonged treatment suggests that male sex-hormones may counteract these actions somewhat. Studies by Pfeiffer (1936) show that a slight development of smooth muscle takes place in the seminal vesicles of the male rat if castrated at birth, but that such animals are more susceptible to a smooth-muscle reaction in the seminal vesicle as a result of ovarian grafts. If the animals are considerably older or adult before castration, the ovarian transplants do not bring about the smooth-muscle reaction so readily. Pfeiffer suggests that the differentiation of the seminal vesicles in the more mature rats renders them more refractory to the theelin action. Wells (1936) also observes an inhibiting action of male hormone to theelin in the ground squirrel. During the season of low sexual activity, he finds theelin to produce marked hypertrophy in the accessory sex organs of normal as well as of castrated males. The character of the hypertrophy is of the nature previously described. During the breeding season these effects are less marked in the normal male but the accessory sex organs are injured to some extent. The interpretation is that during the season of sexual activity the male-hormone secretion depresses the theelin action. More direct evidence is given by Zuckermann and Parkes (1935, 1936) who found theelin to produce fibromuscular growth of the prostatic stroma and epithelial stratification and distention of the uterus masculinus in the immature monkey. When androstanediol (50 parts) was injected with theelin (1 part) the theelin effects on the prostate were suppressed. Progesterone (20 parts) did not suppress the effect from 1 part of theelin. van Wagenen (1935) also observed the smooth muscle hypertrophy of the seminal vesicles in immature monkeys as a result of 34 days' treatment with theelin. de Jongh (1935) found three capon units of testis-tissue concentrate daily to counteract the prostatic reaction from three gamma of theelin in the rat. Laqueur (1934) has attempted to apply these findings to prostatic hypertrophy in man on the assumption that in these patients there is an improper ratio or balance between male and female sex-hormones resulting in a relatively higher concentration of the estrogenic material than of androgenic activity. He reports
favorable results in about 60 per cent of the cases of prostatic hypertrophy treated by male-hormone therapy. Hamilton, Deming, and Allen (1936), however, found no estrogenic material in the urines of twelve patients before and after prostatectomy. Inasmuch as the urine of normal men always contains estrogenic material in appreciable quantities, these negative findings are very surprising.

It is obvious from the above considerations that we cannot be too cautious in the interpretation of biological assays made with crude extracts without the actual removal of the oestrogenic (phenolic) fraction from the androgens or non-phenolic fraction.

**OTHER PHYSIOLOGICAL STUDIES WITH PURE AND IMPURE MALE SEX-HORMONES.**

I. Action on other accessory sex organs.

a. Cowper's gland. Tschopp (1936) also found the increase in weight of Cowper's glands in the castrate rat to respond in about the same order as the increase in weight of the seminal vesicles after injection of various pure androgens. The absolute increase in weight is greater, but the percentage change less than for seminal vesicles. Testosterone was most potent and transdehydroandrosterone least.

b. Penis. The increase in weight of the penis in the castrate rat has been studied by Korenchevsky, Dennison, and Kohn-Speyer (1932, 1933, 1935), by Tschopp (1936), and by Ito and Kon (1935). Korenchevsky and Dennison find the average per-cent increase in weight of the rat penis per 100γ androsterone for doses of 200 to 900γ to be 11 per cent and per 100γ androstanediol for a range of 25 to 200γ to be 100 per cent. Tschopp reports similar results and essentially the same order of (activities for the various androgens as found on the increase in seminal-vesicle weight. Ito and Kon (1935) observed the effects from the high doses of a urinary concentrate in normal puppies and young rats after three to six weeks' and two months' treatment respectively. In the puppies, they observed marked increases in the weights of all the accessories, a fall in testis weight and marked erections. After six weeks, a fall in body weight was recorded. In young rats, they produced a protrusion of the enlarged penis, a partial cornification of the mucous membrane and the accumulation of coagulated semen therein. With loss in body weight and inhibition of thymus growth, they also observed hypertrophy of the liver.

c. Preputial tissue. The preputial tissue response has been studied by Voss (1931), Freud (1933), Korenchevsky et al. (1934, 1935), and Tschopp (1936). In castrated mice the testis-tissue concentrates caused regeneration of, and produced mitosis in the preputial glands.
In castrated rats, an increase in weight of the tissue was observed from 2 capon units daily and no effect from 30 mouse units of crystalline theelin (Freud). High doses of crystalline theelin (100\(\gamma\) or more) caused growth of the tissue and low doses enhanced the effect produced by 2 capon units of male-hormone concentrate. Korenchevsky and Dennison, however, report increases of 13 per cent and 30 per cent from 60 to 180 units of theelin in the castrated rat. With androsterone in doses of 200 to 900\(\gamma\) and androstanediol in doses of 25 to 200\(\gamma\), they observed average 18 per cent increases in weight of the tissue per 100\(\gamma\) of androsterone and 37 per cent per 100\(\gamma\) of androstanediol.

d. Periurethral tissue. Growth of periurethral tissue is also stimulated by androgens. Freud and Laqueur (1934) report this action in the immature male rat from urine concentrates on doses which had little or no effect on the seminal vesicles. Korenchevsky (1935, 1936) finds that 175 to 700\(\gamma\) of adrostenediol daily in the spayed rat caused development of otherwise inconspicuous periurethral tissue which is similar in structure to the prostate. Theelin did not produce this development.

e. Estrogenic action in the rat. 1. Theelin-like action. Butenandt and Kudszus (1935) and Butenandt (1936) suggest that the unsaturated series of androgens show more theelin-like action than the saturated series. They find 400 to 2000\(\gamma\) daily for 3 days, of androstenedione, dehydroandrosterone, or testosterone to produce vaginal introitus and estrus in the normal infantile rat. In doses of 70\(\gamma\) per day for 3 days, \(\Delta^5\)-androstanediol produced the same effect. Androsterone in 2000\(\gamma\) doses was found inactive. Tschopp (1936) and Deanesly and Parkes (1936) confirmed these conclusions on adrostenediol and testosterone. The latter authors injected once daily for 2 days and find the total doses of the following to give the theelin-like action: androstenediol (2 to 4 mgm.), dehydroandrosterone (2 mgm.), transandrostenedione (2 mgm.), methyl transandrostenediol (0.5 mgm.), methyl androstanediol (0.5 mgm.), 17-methylandrostanolone-17,3 (0.6 mgm.), and methyl testosterone (3 mgm.). They also report negative results with androsterone (2 mgm.). Obviously even the saturated androgens also possess a theelin-like action on the normal immature rat. It appears that androgens in high doses, as compared with theelin or theelol, do act upon the female sexual organs.

2. Theelol-like action on the spayed mouse or rat. Butenandt (1936) found four injections of 200\(\gamma\) each of \(\Delta^5\)-androstanediol in a period of 36 hours in spayed mice to produce estrus which lasted for 1 to 2 days. Deanesly and Parkes (1936) report similar high doses of androstanediol,
dehydroandrosterone, androstenediol, methyl transandrostenediol, and methyl androstanediol to produce estrus in the spayed immature rat. The following have given negative results thus far: androsterone, androstenedione, testosterone, and methyl testosterone. Warren (1935) independently confirmed the nonestrogenic activity of androsterone (1000\textmu g) in spayed mice. Tschopp (1936) independently also found transandrostenediol and its 17-methyl derivative to produce estrus in the spayed adult rat. Tschopp (1936) also observed growth of the uterus after such treatment. Korenchevsky, Dennison, and Simpson (1935) found 22 to 27 days' treatment of spayed adult rats with androsterone or androstanediol to cause increase in weight of the uterus and vagina. The effect from androsterone (1.8 to 3.6 mgm. daily) was slight on the uterus and negative on the vagina; from androstanediol (0.49 to 0.62 mgm. daily) very significant increases in weights were obtained, but the recovery was not complete. Korenchevsky (1936) likewise finds testosterone to stimulate growth "of all the atrophied sexual organs" in the ovariectomized rat.

f. Progesterone-like action. The close structural relations existing between progesterone and some of the unsaturated \Delta^4-androgens naturally suggests possible similarity in physiological action. These studies have been very limited, mainly because the doses even of progesterone are high. However, Klein and Parkes (1936) find the normal immature rabbit to give responses equivalent to 0.25 to 0.5 mgm. progesterone on 10 to 20 mgm. of methyl androstanediol, methyl-trans-androstenediol, methyl dihydrotestosterone, and methyl testosterone. Testosterone in 20 mgm. doses was less potent. It must be emphasized that the doses used are 40 to 80 times those of progesterone and that the rabbits were not ovariectomized. All other studies (Butenandt et al., 1934–36) indicate that progesterone is very specific if dosage is properly controlled.

g. Mammary glands. Selye, McEuen, and Collip (1936) report that 200\textmu g testosterone benzoate daily for 14 to 23 days caused mammary gland growth in normal and gonadectomized male and female rats.

II. Effects on other organs. a. Thymus. The inhibiting action of thymus implants or extracts on the estrous cycle in the normal mouse were demonstrated independently by Walter (1930) and Loewe and Voss (1931) and clearly indicate an antagonism between the ovaries and thymus. The studies of Evans and Simpson (1934) involving the chronic treatment of normal and castrated rats with gonadotropic hormone also show that the ovaries and testes are involved in inhibiting the growth of the thymus in the immature normal animal and in hasten-
THE MALE SEX HORMONES

ing the atrophic changes in the normal adult. Korenchevsky, Dennison, and Simpson (1935) showed that androsterone or androstanediol hasten the involution of the thymus in the castrated male and spayed female rats. Testosterone was found to show the same action. With impure testis-tissue extracts or theelin the results were more irregular. Ito and Kon (1935) observed the same effects on normal immature rats from a urinary male-hormone concentrate.

b. Suprarenals. Further evidence on interrelationships between the adrenals and gonads is accumulating. Cannon (1932) claims that adrenal cortex contains a substance which inhibits the gonads of mature albino rats, but Corey and Britton (1931) report that adrenal-cortex extracts induce precocious maturity in the same species. Eaton, Insko, Thompson, and Chidester (1929) find both activities in the suprarenal gland because by feeding dried suprarenal cortex to chicks they developed larger testes, but dried suprarenal-medulla-fed chicks developed smaller testes than normal. Oskar Klein (1931) also finds that adrenal-in-free cortical extracts stimulate development of the gonads in the young male but inhibit sexual development in the young female. Handovsky and Tammann (1928) also find a sex difference in the changes produced in carbohydrate metabolism in rabbits when adrenalectomy follows castration. Schiller (1935) reports that sex function was interrupted in six male and fifteen female rats after three days following complete adrenalectomy. Daily adrenalin injections are said to have brought about a return to the normal condition.

Korenchevsky, Dennison, and Simpson (1935) could maintain the normal suprarenal weights in gonadectomized male and female rats by 1.8 to 3.6 mgm. of adrosterone and 0.17 to 0.7 mgm. adrostanediol respectively. Theelin in daily doses of 180 international units was less effective in inhibiting the adrenal hypertrophy. In fact, in normal male rats, 43 days' treatment with daily doses of 60 to 180 international units of theelin caused hypertrophy of the suprarenals.

That the suprarenals may produce an androgenic substance is indicated by two lines of research. The first is that women with adrenal tumors present marked hirsutism and excrete abnormal amounts of androgenic material. The second is that three independent workers (Kendall, Wintersteiner, and Reichstein, 1936) have prepared an oxidation product from an adrenal-cortical principle which is found to be one-sixth to one-fourth as active as androsterone in the capon test.

c. Liver. In 1934 Korenchevsky and Dennison reported that in the castrated male rat, the liver decreases from the normal average weight
of 11 grams to 9.5 grams. A year later with Simpson they restored these weights almost to the normal weight by the same doses of adrosterone and adrostanediol used for the adrenal control referred to above. The same effects were produced in ovariectomized rats. No definite histological changes were reported.

d. Kidneys and heart. The same investigators report that gonadectomy leads to a slight fall in the weights of kidneys and heart. In both sexes, the normal average weights are almost regained by the prolonged use of androsterone or androstanedial as indicated under suprarensals above.

e. Sebright feather-pattern control. It is well known that the hen-feathered Sebright bantam possesses the same plumage in both sexes, but that castration leads to the capon type. Gallagher, Domm, and Koch (1933) injected highly purified male-hormone concentrates into Sebright capons and produced typical hen feathers. This was true even when the concentrates were freed from estrogenic material. Callow and Parkes (1936), however, could not produce hen-feathering in their Sebright capons by 0.5 to 1 mgm. of androsterone, whereas 1 to 2 gamma theelin brought about the change. They, therefore, conclude that the normal plumage of the Sebright cockerel is controlled by the secretion of estrogenic material. Gallagher, Domm, and Koch (1936), however, conclude that the estrogenic material is not necessary because they produced hen-feathering with androsterone, obtained by the synthetic degradation of cholesterol.

III. The X substance of the Laqueur school. This substance, found in testis-tissue extracts, when injected alone, shows no action on the seminal vesicles and prostate in the castrated rat, but when administered with a given dosage of testosterone it acts as an activator. This activation is not the same as the synergistic effect of theelin in its action with androsterone on the seminal vesicle because the X substance is inactive alone and because it does not activate androsterone. The substance also appears to activate dihydroandrosterone in the mammalian test. In hypophysectomized young castrated rats, the X substance also has no effect on the androsterone action, but the activating actions toward testosterone and dihydroandrosterone are the same as in the castrate. The X substance is non-estrogenic in character. The activity has been found in testes, liver, suprarensals, ovaries, blood (cow and gull), wheat seedlings, and urine. The lowest dosage found active thus far on the purest known form is 1 milligram. It is lipoid soluble. The main
observations on this factor were first announced by David, Dingemanse, Freud, and Laqueur (1935), Freud (1935), Freud, Dingemanse, and Polak (1935), and were later confirmed by Ruzicka, Wettstein, and Kägi (1935), Tschopp (1936), Callow and Deanesly (1935), and Deanesly and Parkes (1936). These observations probably also explain in part the discrepancies many of us have observed between capon and mammalian assays in various fractionation studies on urinary and testis-tissue concentrates.

IV. The testis-hormone control of the anterior pituitary. Innumerable clinical and experimental studies (for the earlier literature, see Biedl, 1913, and Lehmann, 1927) have established that gonadectomy in both sexes leads to hypertrophy of the hypophysis which is associated with characteristic histological changes in the anterior lobe and with alteration of the physiological activity thereof. The histological studies reveal an increase in the number and size of the basophiles which later undergo vacuolization with development of “signet-ring” or “castration” cells. The changes in physiological activity involve increased content or secretion of gonadotropic hormones as demonstrated a, by implantation of the hypophysis into normal immature animals; b, by the higher concentration of gonadotropic substances in the urine of certain species after castration, and c, by parabiosis studies on gonadectomized rats and mice united with normal animals of either sex and observing the changes in the gonads of the normal mate and in the accessory sex organs in both animals.

Is the anterior pituitary lobe under the control of the male sex-hormones which have been separated thus far or are other substances from the gonads more effective alone or together with the androgens now known? These questions cannot be answered satisfactorily at the present time. The discussion below very definitely indicates that several factors may be involved in the testis control of the anterior pituitary activity. The solution of these problems is complicated by the fact that the quantiative biological studies on the hypophysis yield exceedingly variable results on the basis of weight, histological and physiological changes in the pituitary and that these fluctuations are affected by age, sex, season, and species. An extensive survey of the literature on all phases of this problem cannot be undertaken here. Instead, the review is limited to a consideration of the effects of various extracts, concentrates, and pure hormones on the anterior pituitary. The effects will be considered on the basis of a, histological changes; b,
sex antagonism studies; c, changes in physiological activity of the anterior lobe as determined by implantation studies, and d, changes in physiological activity as determined by parabiosis studies.

a. Effects of extracts on the histology of the anterior pituitary lobe in the castrate. Fichera (1905) made the most remarkable claims as to the effect of extracts from cockerel testes on the pituitary in the capon 20 to 25 days after castration. He stated that his extracts produced marked restitution to the normal histology as early as 10 to 15 hours after the first or second injection. Fichera no doubt employed very crude and dilute extracts and very likely his histological methods were equally non-critical.

Lehmann (1927, 1928) from histological evidence on serial sections of the anterior pituitary in gonadectomized rats concluded that male castrates' pituitaries are corrected by implants or extracts of testis tissue, but not by transplants or extracts of ovaries. He claimed the same sex specificity for ovarian transplants and extracts in the spayed female rat. His extracts represented the crude non-saponifiable fraction, but nothing is stated as to the dosage employed. He concluded that the anterior pituitary possesses a marked sex specificity. In his second paper he found no sex differences in the treatment with placental extract injections or placental tissue by mouth and hence concluded that placental tissue contains male and female sex-hormones.

The qualitative sex specific reaction of the anterior pituitary body to sex hormones suggested by Lehmann (1927) have not been confirmed throughout. However, it is very evident that a sex difference of a quantitative character does exist in some species. This will be stressed from time to time in the later developments discussed below.

Hohlweg and Dohrn (1931, 1932) prevented the histological changes in the anterior pituitary lobe of gonadectomized rats of both sexes by the injection of Progynon. However, it required twelve times as much for the male as for the female and more for adult than for young castrates. The Progynon used may not have been free from male hormone and hence the positive action on the males might be due to the contamination. This would also explain why so high a dosage was necessary for males. More recent studies with pure preparations eliminate the validity of these suggestions.

McCullagh (1932) states that the doses of testis tissue or urinary hormone concentrates (lipin fraction) which correct the castration changes in the accessory sex organs of the castrated rat do not prevent the appearance of "castration cells" in the anterior pituitary, but that
water extracts from much less testis tissue correct the pituitary changes. He also emphasizes that with destruction of the germinal epithelium one observes the appearance of castration cells without the degenerative changes in the accessory sex organs. However, later McCullagh and Walsh (1935) in parabiosis studies find the lipin-soluble male-hormone concentrates to inhibit the gonad-stimulating activity of the castrated animal.

A series of studies by Nelson and collaborators has done much to clear up the quantitative aspects of some of the questions raised above. At one time Nelson (1934) was inclined to believe that only the estrogenic impurities in the lipin-soluble androgen preparations inhibited the anterior pituitary or prevented the appearance of castration cells in the castrated rat, but concluded (1935) that the male pituitary requires 5 to 6 times as much crystalline theelin for inhibition as the female. However, Nelson and Gallagher (1936) also find male-hormone concentrates, free from estrogenic material, to correct or prevent the castration changes in the pituitary, in both sexes. The differences in doses of the same preparation required to produce the same inhibition in the pituitaries of the two sexes are again observed. The relative efficiencies of estrogenic and androgenic substances are also very striking. They find that when the male-hormone concentrate contains traces of estrogenic activity (alkali soluble) the 30-day castrated female rat requires 2 capon units per day for pituitary control but that the male castrate requires 10 capon units under comparable conditions. This is the 1 to 5 ratio found for crystalline theelin. However, when the male-hormone concentrate is thoroughly freed from alkali-soluble estrogenic material, the dose of male-hormone concentrate required for the spayed female rat must be increased to 10 capon units per day to prevent histological changes in the anterior pituitary in 30 days. What dosage of the same estrogen-free material is necessary for the castrated male has not been determined.

Nelson and Gallagher (1936) also show that the addition of 0.78 rat unit of crystalline theelin to 4 capon units per day is as efficient in the spayed female as 10 capon units alone and better than either 7.5 capon units or 0.78 estrogenic rat unit alone. Furthermore, the estrogenic-free male-hormone concentrates from urine and testis tissue were equally effective per capon unit in the spayed female. This is especially remarkable when we recall the low order of potency of androsterone, as capon units, as compared with testosterone in restoring the accessory sex organs in the castrated male rat. The relative efficiency of theelin and male-hormone concentrates on the pituitary suggests a
biological rôle for the presence of estrogenic material in the male. In fact, Nelson and Gallagher (1935) consider it so effective that it is not necessary to hypothecate another male hormone for pituitary control. In other words, the combined action of the known androgenic and estrogenic substances is sufficient to account for the natural control of the anterior pituitary body.

b. Sex antagonism studies—Injury to the gonads. From time to time attempts have been made to demonstrate the feminizing and masculinizing actions of the sex hormones. Naturally the earlier studies involved the effects from gonad transplants. The leaders in these earlier studies (Steinach, 1910–20; Sand, 1922; Moore, 1922, and Lipschütz, 1925) arrived at very different conclusions and interpretations of their findings. Steinach and Lipschütz independently interpreted the results as due to direct sex-hormone antagonism. Sand did not subscribe to this interpretation but in fact claimed that a heterogonad transplant was successful only when implanted into the gonad tissue. Moore could not demonstrate true sex antagonism by transplantation studies because he succeeded in keeping transplants viable in various sites, immaterial whether the opposite gonad was left intact or removed. The loss of spermatozoa production in testis transplants, Moore could account for on the basis of temperature effects. He concluded that the internal secretion of the testis transplant was not inhibited by the intact ovary and that the intact ovary still continued to function normally.

The earlier studies with sex-hormone preparations also tended to support the sex-hormone antagonism hypothesis. Thus, Fellner (1921) showed that the injection of large doses of the crude lipin fraction from placenta, ovaries, or corpus luteum caused damage to the spermatogenic elements, but not to the interstitial tissue in the rabbit testicle after the previous removal of one testis. Fellner also observed that a similar extract from bull testis-tissue (equivalent to 4 to 11 bull testicles!) produced the same effect and stimulated development of the mammary glands. Stein and Herrmann (1921) reported similar effects from corpus luteum and placental extracts on the testes of rats, guinea pigs, and rabbits, but they also called attention to the atrophy or degeneration of the accessory sex organs and stimulation of the mammary glands. The effects were interpreted as evidence of sex-hormone antagonism brought about by the “female sex-hormone” in the various extracts studied. However, a present-day interpretation is that the action is due to the inhibiting action of either male or female sex-hormone on the pituitary as a result of which the normal gonadotropic activity of the
anterior pituitary is not manifested in the intact gonad and thus, secondarily, the accessory sex organs degenerate.

With the production of better standardized sex-hormone preparations of higher purity and potency and especially with the actual purification of the male-hormone concentrates, more quantitative studies on so-called sex antagonism and on pituitary inhibition became possible. The importance of purity cannot be overemphasized. There is considerable evidence that various extracts or lipoid fractions which are entirely free from estrogenic and androgenic actions nevertheless may show so-called sex antagonism action. No doubt this factor is involved in the studies cited above and in Lendle's (1931) attempt to standardize crude male-hormone preparations on the basis of the inhibition of estrus in the normal rat. Studies by Walter (1930), Loewe and Voss (1931), and by Duncan, Gallagher, and Koeh (unpublished) all show that estrus may be inhibited in the adult rat by various lipin preparations obtained from various sources, but free from or negligible in content of sex hormones. Moore and Price (1932) also call attention to the injurious effect of lipins from heart, liver, and brain tissue on the rat testis. Whether these sex-hormone-free lipins act directly on the intact gonads or indirectly through the pituitary has not been determined. Obviously injury to the testis morphology or activity is not limited to female sex hormones nor is the mechanism of the action necessarily the same for different substances. In any case the dosage and the time of treatment employed are very important considerations. Although it appears well established that estrogenic and androgenic substances inhibit the gonadotropic activity of the anterior pituitary body of both sexes and thus injure the gonads of both sexes indirectly, it does not necessarily follow that certain end organs may not be modified in type by the direct action of a sex hormone of the opposite sex. This is well illustrated by the experimental hermaphroditism produced by Voss and Loewe (1931) in the female guinea pig by crude male-hormone injection and by the simultaneous stimulation of comb growth and female plumage pigmentation in the Brown Leghorn fowl (Juhn, D'Amour and Womack, 1930) with mixtures of male and female sex hormones.

That the anterior pituitary is involved in the so-called sex antagonism observations is suggested by numerous preliminary studies, but the final correlation of these data and the application thereof to the problem of sex antagonism remained for Moore and Price (1930, 1932). That no direct antagonism between the male and female sex hormones exists was demonstrated by Moore and Price when they found a given dose of
male hormone to control the accessory sex organs of a castrate equally well with or without 12 rat units of estrogenic material. They call attention to similar evidence by Ihrke and D'Amour as to the negative effect of male hormone on the estrogenic action of a given dose of "estrin" in the spayed rat. In the intact male rat they found 12 rat units of "estrin" (from placental tissue) to inhibit the testis as shown by the degeneration of the seminal vesicles. This "estrin" effect was, however, easily counteracted by the injection of urinary gonad-stimulating material or by hypophyseal implants thus showing that the testis had been inhibited due to the lack of gonad-stimulating secretion from the pituitary gland. They also showed that 6 capon units of male hormone caused a similar injury of the immature rat testicle; this is also corrected by hypophyseal implants or by urinary gonadotropic preparations. The above and a great mass of similar evidence from their own experiments and from those of others were correlated and led them to announce the generalization that sex hormones inhibit the heterologous and homologous gonads only indirectly by first inhibiting the gonad-stimulating activity of the anterior pituitary lobe. In other words, so-called sex antagonism really does not exist because either sex hormone may inhibit the anterior pituitary of either sex and hence the gonad of either sex. Moore and Price, however, did not investigate the quantitative differences in the pituitary response of the different sexes to the sex hormones. Schoeller and Gehrke (1933) confirm the effect of crystalline theelin or of urinary male-hormone concentrates on the testicle of the young rat. In the adult rat, neither Moore and Price nor Schoeller and Gehrke observed the decrease in size of the testes after male-hormone treatment. In further support of the Moore and Price interpretation we have the observations by Freud (1932) that in the hypophysectomized rat, the sex hormones produce the normal reactions in the accessory sex organs but do not produce antagonistic action. Walsh, Cuyler, and McCullagh (1933) also found the urinary male-hormone concentrate to prevent the atrophy not only of the seminal vesicles and prostate but also of the testes in the hypophysectomized rat. These observations were based on only one sex-hormone treated animal, one normal control, and one hypophysectomized control.

The age and sex specificity characteristics of the anterior pituitary can be reintroduced to advantage at this point. The action of the sex hormones on the anterior pituitary lobe is not quite as simple as suggested by Moore and Price, if the findings of Sand (1918), Smeler
(1933), Hisaw et al. (1934), Wolfe (1935), Clark (1935) and Pfeiffer (1935, 1936) are significant. Sand (1918) and Smelser (1933) find that ovarian grafts in male guinea pigs never luteinize, but do so in the female. Hisaw et al. (1934) and Wolfe (1935) state that "estrin" injected into females in proper amounts causes the release of the luteinizing factor from the anterior pituitary and the former group concludes that "estrin" suppresses the follicle-stimulating factor only. These studies suggest that the adult male anterior pituitary of the rat contains the follicle-stimulating hormone and little or no luteinizing factor. Hellbaum (1935) demonstrated the same difference for young mare and stallion pituitaries. Clark (1935) has demonstrated the difference in gonad-stimulating power of hypophyseal implants in immature mice from rats of both sexes at different ages and also from the gonadectomized rats at different ages. She finds the anterior pituitaries from normal females to be more potent from birth to 20 days of age; the normal male anterior pituitary gradually gains in potency until the age of puberty when it becomes more active than the normal female ever appears to be. If rats are gonadectomized on the first day of life and their pituitaries then assayed sixteen to eighteen days later, the sex differences in gonad-stimulating activities of the pituitaries are negligible. However, the castrated males possess pituitaries which average an increased activity of 102 per cent over the normal males. In the females, the activity was increased on an average by 19 per cent as a result of ovariectomy. Pfeiffer (1935, 1936) has demonstrated that in the immature rat the anterior pituitary lobe has not differentiated, that potentially it may become either male or female in type and that the type it differentiates into is determined by the type of gonad secretion influencing it. Pfeiffer was able to convert the bipotential anterior pituitary of a prepuberally castrated male or female rat into the female or male type by implanting ovary and testis respectively. Lipschütz and Villagran (1935, 1936), however, report that normally the male hypophysis has 30 times the luteinizing power of that of the female, but that castration leads to a decrease of the luteinizing power in the male and an increase therein in the female. Lipschütz and Del-Pino (1936) find only slight differentiation of the anterior pituitary in man, except in cases of advanced pregnancy. These contradictory conclusions are no doubt due to different experimental procedures and probably also involve very different quantitative relations from the standpoint of the hormones involved. With the pure preparations of sex hormones now
available we can expect to control this variable better and it is to be hoped that the very suggestive conclusions of Pfeiffer may be confirmed more definitely.

**The reciprocal relation between certain end-organs and the testicles.**

There are many observations on the effects of light, temperature, diet, nutrition, etc., on the control of the size and activity of the testicles. Hibernation involves these factors and light is the most important in many species. In the ferret (*putorius vulgaris*), Bissonnette (1935) considers light to act through the pituitary on the germinal and interstitial cells of the testes, but he considers ferrets as well as other animals with definite seasonal sexual cycles to possess an "inherent sexual rhythm." He showed that the eyes are involved in the light control of the hair cycle in the female ferret. Johnson and Hanawalt (1930) conclude that hibernation in the thirteen-lined ground-squirrel is not altered by the injection of thyroxin or pituitrin or by feeding desiccated thyroid or thymus tissue in large doses. Zalesky (1935), from his studies, concludes that the distinct seasonal changes of the thyroid in this species are not correlated with the sexual cycle because castration or administration of sex hormones from pregnancy urine or other gonad-stimulating preparations had no observable effect on the thyroid. However, the administration of anterior pituitary substance during the season of low thyroid activity stimulated the thyroids to a higher level of activity than the highest in the normal rhythm. Wells (1935) and Wells and Moore (1936) also conclude that a periodic activity of the anterior pituitary is involved in the sexual rhythm in this species, but they found no evidence that light is a factor. However, they found that the testes and accessory sex organs can be stimulated to an active condition during the sexually inactive period. An incidental and interesting observation is that crystalline androsterone produced precocious spermatozoa formation in the immature ground-squirrel and also earlier than normal in the sexually inactive adult. Baker and Johnson (1936) confirmed the positive effects from urinary gonad-stimulating preparations in the sexually inactive ground-squirrel.

In birds and fowl, light or season plays a very important rôle in the size of the gonads and other endocrine organs. Riddle and Fisher (1925) called attention to the marked seasonal variation in the weights of the gonads and thyroids in pigeons and ring doves. No doubt light is an important factor in the gonad control in this species. In the common starling (*sturnus vulgaris*), Bissonnette and Wadlund (1932) found the control of gonad size spread over a wide range of wave lengths of
visible light and suggested that the anterior pituitary is involved. The most striking observations are reported by Buckner, Insko, and Martin, (1934) who observed that when white leghorn cockerels are raised without exposure to sunlight they develop exceptionally large and less erect combs, but relatively small testicles. However, when the cockerels are raised exposed to sunlight, their combs are smaller and more erect, but the testes are very large. Furthermore, removal of combs and wattles produced cockerels with small testes whereas in pullets no such effect was observed on ovarian weight. The studies by Womack and Koch (1931) and Koeh and Gallagher (1934) show that the comb is a more refractory end-organ to androgens when the capon is exposed to light than when kept in dim light. This suggests that the larger comb developed in the normal cockerel in dim light calls for less male sex-hormone and hence the testes are smaller. However, it does not necessarily follow that a smaller organ is secreting its hormone less rapidly. That the comb may act antagonistically on the testes is also shown by M. M. Zawadowsky (1935) who confirmed the observations of Buckner et al. that comb removal results in larger testes in cockerels. Zawadowsky (1935) also observed slight atrophy of the seminal vesicles and prostate in rats after feeding cocks' combs by mouth. Jores (1935) reports that the melanophore hormone-content of the pituitary body and eyes of white mice is 2 to 3 times greater in animals kept in the dark than it is in controls kept in light. He claims that the same relations hold for the content of pressor and oxytocic activities in guinea pigs' pituitary glands with and without light treatment. Hence, he concludes that the light control of the pituitary is through the eye. Further observations confirming the pituitary or light factor or both on the gonads were made on the sparrow by Kirschbaum and Ringoen (1936), and Witschi and Keck (1935), and by Warren and Scott (1936) on ovulation in the fowl.

Other interesting studies involving damage to the testes are presented herewith. Buchheim attempts to explain the loss of sexual desire in the firemen of the French navy as due to a harmful effect of fuel oil because he finds that he can produce the same changes in rats, guinea pigs, and rabbits by injecting 0.005 cc. or feeding 0.115 cc. "mazout." In rats, he observed atrophy of the seminal vesicles. The harmful effects of carbon monoxide on testis weight in rats observed by Williams and Smith (1935) are also suggestive in that connection. According to Myers, Vidgoff, and Hunter (1933) dried bull testis-tissue fed to rats in 0.2-gram doses five times weekly for 85 to 117 days causes marked
atrophy of the testes, prostate, and seminal vesicles, and finally aspermia. These effects cannot be due to the androgenic activity of the lipoid fraction from bull testis-tissue because the accessory sex-organs have also been injured. Compare these effects with those produced by Ito and Kon in puppies and young rats after 3 to 9 weeks treatment with a concentrate of urinary androgenic material. Here the atrophy was limited to the testes and thymus while the gonad accessories were hypertrophied. It is, nevertheless, possible that part of the injurious effect on the testes may be due to impurities in the urinary extract. Lastly, the atrophy of pigeons’ and doves’ testes as produced by Riddle et al. (1934) by prolactin also calls for an explanation.

c. The effects of sex hormones on the physiological activity of the anterior pituitary as determined by implantation studies. With the introduction of the hypophysis implantation method (Smith, 1927, 1929; Zondek and Aschheim, 1927; Smith and Engle, 1927) much fundamental information on the hypophyseal-gonad interrelationship was obtained. The observations which have a special bearing on the gonad control under discussion here in part confirm the more important conclusions arrived at by the other methods of approach.

From the studies of Meyer, Leonard, Hisaw and Martin (1930, 1931, 1932), Evans and Simpson (1929), Engle (1929), we know that the hypophyses of gonadectomized rats show greater gonadotropic activity than from non-castrates when implanted into immature rats and that the injection of estrogentic material into the gonadectomized or normal rat lowers the gonadotropic effect by the implantation method. So also the anterior lobe transplants from the cryptorchid rat produce greater gonad stimulation. From these studies we also conclude that the female hypophysis is more easily depressed by “estrin” than the male pituitary. Other sex differences are also found as elsewhere indicated in this review. Quantitative studies on the effects of androgens in lowering the gonadotropic activities as measured by the implantation method have not been conducted.

d. The testis control of the anterior pituitary as revealed by parabiosis studies. In 1921 Matsuyama and Yatsu independently showed that parabiosis of a normal female rat with a castrated male leads to marked ovarian and uterine hypertrophy with large ovarian follicles and considerable luteinization. Goto (1925), Fels (1929) and Kallas (1929) confirmed these observations. Goto attributed the stimulation of the female sex organs as due to a hypothetical “kastrohormone” received from the blood of the castrated male. Kallas had the advantage of the
discoveries of Zondek and Aschheim (1927, 1928) and Smith and Engle (1926, 1927). Hence he concluded that the gonad-stimulating substance must be of hypophyseal origin. Kallas also showed that parabiosis of a normal female rat with a spayed rat leads to estrus in the normal female and that if an anterior pituitary gonad-stimulating preparation is injected into the spayed animal it hastens the appearance of estrus in the normal mate. Since estrus never occurs in the spayed mate, the conclusion is that estrogenic material does not pass over in sufficient concentration to influence the accessory sex organs of the castrate. The present day interpretation is that the concentration of ovarian hormone in the castrated animal is not sufficient to inhibit or control the gonad-stimulating activity of the hypophysis but that the gonad-stimulating hormone from the castrate does pass into the normal mate.

This type of experimental animal has been employed in attempting to determine the nature of the substance or substances of gonad origin which normally control the gonad-stimulating activity of the hypophysis. Martins and Rocha (1929, 1930, 1931) report results therewith which suggest a certain degree of sex specificity in the hypophysis. They found that if the spayed female united with a normal female is injected every other day with testis pulp, there is no change in the estrus and corpora lutea formation in the normal animal. However, if a castrated male is parabiotically united with a normal female and the male is then injected with the testis-tissue pulp, the estrus is delayed. Martins showed that a rat with cryptorchid testes also produces estrus in the normal female mate. Since the anterior pituitary of the cryptorchid rats contains castration cells, but the seminal vesicles appear normal and the testes are free from spermatozoa, Martins concludes that the inhibiting agent normally cannot come from the interstitial cells but must come from the spermatogenic elements. The assumption that cryptorchidism does not influence the accessory sex organs is, however, not correct because Nelson (1934) has now shown that if such animals are observed long enough the seminal vesicles and prostate also show castration effects. Nelson points out that the hypophysis is the most sensitive to changes in male-hormone concentration, but that seminal vesicles and prostate can be maintained at lower levels, hence cryptorchidism leads to the hypophyseal changes first. Martins and Rocha also observed a sex difference in the type of gonad-stimulating effect produced in the normal female when joined parabiotically with a castrated male or spayed female. The castrated male mate led primarily to follicle formation but the spayed female union usually led to
more luteinization. It is of some interest to note that if the castrated male infantile rat is donor to an infantile normal female, we also observe the estrus phenomena. Obviously, the infantile male pituitary normally is inhibited by a substance which originates in the testis tissue, but which has not affected the accessory male sex organs. In other words, if testosterone or androsterone are responsible for the normal control of the hypophysis, the concentration must be so low that it does not act on the accessories. It will be recalled that the reverse is true for the adult rat, that is, it requires only about one-fourth capon unit daily to affect the seminal vesicles and prostate in the castrated rat, but over ten capon units daily to control the anterior pituitary. Martins and Rocha with "an intensive treatment of castrated males with testicular aqueous extracts . . . succeeded in keeping the hypophysis in normal condition and without influencing the accessory sex glands." These findings are exceedingly important because if fully confirmed it means that the substance involved is neither androsterone nor testosterone. Whether it is theelin, theelol, or dihydrotheelin, remains to be determined. It is, however, quite probable that it is not one of the estrogenic substances. McCullagh and Walsh (1935) have confirmed Martins and Rocha in part. They find that the anterior pituitary activity in the castrated mate of the parabiotic pair can be inhibited by the lipin-soluble male-hormone concentrate and also by "testis mush." However, with the lipin material it requires the equivalent of very much larger amounts of tissue than with the "testis mush." Furthermore, the former regenerates the seminal vesicle and prostate in the castrate while the "testis mush" has no such effect.

Witschi, Levine, and Hill (1932) show that when a male rat, which has been sterilized by x-ray one month previously, is joined parabiotically with a normal female it leads to continuous estrus. In spite of this hypophyseal overactivity as a result of loss of a testis hormone, the testes were 25 per cent heavier than normal and the accessory sex organs were distinctly enlarged. The testes were free from germ cells. This could be interpreted as a case where the greater gonad-stimulating activity of the anterior pituitary is manifested not only on the normal female but also on the sterilized male. Since the hypophysis had become more active as a result of the destruction of the germinal cells, the conclusion is that a testis control of hypophyseal activity resides in the germinative tissue. The radium sterilization of male rats which Moltrom and Cramer (1923) also found to produce "castration changes" in the hypophysis and which led to a great increase in the number of
interstitial cells in the testis may be considered an earlier confirmation of the x-ray effects.

**General conclusions on the testis control of the hypophysis.** That the known testicular and urinary male-hormone preparations can inhibit the hypophysis gonad-stimulating activity is generally granted. However, the dosage involved is tremendous and at least five times as high for the male as for the female rat. Estrogenic substances on the basis of weight are more potent, but the dose required for males is also approximately five times that necessary for females. Considerable evidence indicates that another substance is elaborated by the testis tissue. This hypothetical substance appears to be active in the immature animal, but does not act on the accessory sex organs. In the control of the anterior pituitary lobe it appears to be more effective or else higher concentrations thereof exist in the testis tissue than of the known androgens. The substance seems to disappear from the testis with destruction of the seminiferous elements.

**V. More involved and less definitely established effects.**

### a. Creatine and creatinine excretion.

The well known differences in creatine and creatinine excretion in relation to sex and puberty naturally suggested attempts at the control of creatine excretion by means of male-hormone therapy. Bühler (1933) concludes from the use of doses of 50 to 150 capon units of “Proviron” in creatinuria associated with lowered sexual activity in man that the male hormone acts specifically in the male in lowering creatinuria, that it inhibits creatine metabolism in women and is without action before puberty. The experimental evidence given is not as convincing as one might desire. Kun and Peczenik (1935) report that post-puberaly castrated rats develop a creatinuria if 20 cc. of milk (containing about 0.4 mgm. creatine) are given by mouth. Normal male rats do not develop creatinuria on this milk feeding. The creatinuria developed in the castrates varies in different rats and for the same rat from day to day. The creatine content in urine varies from 10 to 55 per cent of the total creatinine plus creatine. The injection of 25 capon units of Proviron (probably a urine concentrate) actually increased the creatine excretion, but 30 to 125 capon units reduced the creatine content to a range of 0 to 26 per cent. Normal female rats show the highest creatine excretion during the estrous period and in ovariectomized rats theelin injection (500 to 12,000 mouse units) increased the creatinuria. In castrate males, theelin did not reduce the creatinuria. Steinach, Kun, and Peczenik (1936) attempt to classify cases of male-hormone deficiency in man on the basis of the degree of
creatinuria observed as a result of a uniform intake of creatine from a
diet of hard cheese and dark meat. They conclude that true male-
hormone deficiency exists only when amounts of creatine greater than
10 per cent of the total creatinine plus creatine are found in the urine.
In the cases of "true male-hormone deficiency" this value varied from
10 to 27 per cent while in the "non-deficient" group the range was 0 to
9 per cent although the latter group were recognized as suffering with a
weak libido. They do not give any data on the changes in creatine
excretion as a result of androsterone benzoate therapy but state that the
"non-deficient" group was not benefited otherwise whereas the "true
male-hormone deficiency" cases were benefited as determined by other
criteria. Among these criteria are such subjective findings as increase
in muscular strength, less tendency to fatigue after physical or mental
exertion, improvement in initiative and mental concentration with
less sleeplessness and less pain in the extremities! The 5-mgm. doses of
androsterone benzoate used by these authors represent approximately
50 international capon units. In other words, they actually used smaller
amounts than the maximum doses used on rats. The reviewer feels
that the interpretation or evaluation of analytical data on creatine and
creatinine excretion are exceedingly uncertain on account of the difficul-
ties involved in the analytical methods. Slight differences in the
absolute excretion values are of very doubtful significance. Hence
Steinach et al.'s attempts to classify types of male-hormone deficiency
on such differences do not seem very well founded. The relation of
creatinine and creatinine metabolism to sex-hormone therapy should,
however, be followed more intensively.

Kochakian and Murlin (1935) find that the castrate dog does not
excrete creatine if kept on a creatine-free diet, thus indicating that
castration does not affect endogenous creatine metabolism, but that
the castrate may not be able to fix exogenous creatine as well as the
normal male.

b. Cell permeability studies. Duran-Reynals et al. (1929, 30, 32, 34)
accidentally observed that extract of testis tissue in Ringer's solution
diluted with an equal volume of glycerol, when injected, appears to
stimulate cell division locally. It also produces an increased permea-
bility of the tissues as shown by the extent of the area containing India
ink which was suspended in the extract. This activity is heat labile.
It is precipitated in vitro by antisera against homologous testis extracts
but no antagonistic action is produced in vivo. Although other organs
and tissues contain the same activity, the testis is the most potent source
found thus far. McClean et al. (1931, 33) find protamine salts and extracts of spermatozoa to produce the same effect on tissue permeability. McClean (1930) finds the extract to enhance the local lesions following intradermal injections of vaccine virus and diphtheria toxin. Pijoan (1931) and Terada (1934) also confirmed the original observation of Duran-Reynals in connection with the enhanced and spreading effects of the testis extract on the infectious activity of organisms introduced with the extract. The exact nature of the substance involved has not been determined, but it is obvious that it is not of lipoid character. It is heat labile in aqueous solution, but when precipitated by alcohol and ether, it can be heated in the dry form at 100°C. for 30 minutes without destroying the activity. Incubation with trypsin destroys it.

c. Blood coagulation. Differences in rate of coagulation of blood have frequently been associated with sex. Bablik (1935) briefly summarizes the evidence that castration in rabbits and women results in slower coagulation of blood, the treatment of male dogs with “folliculin” hastens the rate of coagulation of blood and that in amenorrhea and menopause, the blood coagulates more slowly. On the basis of very few experiments by himself on gonadectomized rabbits of both sexes, he concludes that the administration of either of two proprietary male-hormone concentrates lengthens the time of coagulation of the blood. “Menformon” had the opposite effect.

d. Composition of tissue proteins. Tadokoro (1927, 1930, 1931, 1933, 1934) claims to find many fundamental biochemical differences in the sexes. He emphasizes in particular the chemical and physico-chemical differences of the muscle and skin proteins in the sexes. He reports that the greatest differences in the composition of the proteins reside in their histidine, arginine, and cystine content. He must consider a male sex-hormone factor to control the quantitative composition of the proteins because he claims that the composition of the myosin and myogen fractions from the capon muscle-tissue two months after castration approach the composition of the corresponding muscle-proteins from the hen. He reports similar differences in the composition of hide proteins from cow and bull and the muscle proteins in male dog and the gonadectomized litter-mates. The data as presented by Tadokoro are by no means convincing.

e. Sex difference in resistance to drugs. Lee (1935) makes equally dubious claims on the basis of very poor experimental evidence. He claims that in mice and rabbits, there is a sex difference in the resistance to nicotine poisoning, the females being more resistant. His evidence
for this claim is very unsatisfactory but he concludes, nevertheless, on the basis of very limited studies, that luteoglandol (Roche) increases the resistance of spayed mice to nicotine.

Very good evidence of a male-hormone control of resistance to drugs has been obtained by Holek and Kanân (1936). They find the female albino rat more sensitive than the male to barbiturates of the type evipal, pernoston, and amytal, but not to barbital. This difference in sensitivity is measured by the duration of hypnosis and the rate of acute mortality. Ovariectomy does not produce an appreciable change but castration of the adult male leads to a marked prolongation of the hypnosis and increases the rate of acute mortality to evipal, pernoston, and amytal, but not to barbital. Prolonged treatment with 10 to 20 eapon units of male-hormone concentrates (also pure androsterone) per day decreases the recovery time in the spayed females to about one-third for a given dosage of the barbiturate; in the castrated male, the treatment prevented most but not all of the castration effects. In both cases the beneficial action is lost completely some weeks after the treatment has ceased.

Possible modes of biological formation of sex hormones. The occurrence of dehydroisonandrosterone and androsterone in human urine, the probable occurrence of testosterone in human testes, the assumption that the estrogenic substance in male urine is theelin, the presence of progesterone and allopregnanolone in the corpus luteum, the presence of pregnanediol in pregnancy urine, and the structural relations between these substances and cholesterol and bile acids suggests possible modes of their formation in the organism.

The assumption appears to be made quite generally that cholesterol necessarily is the precursor of all of these substances. However, inasmuch as we know practically nothing about the precursors or metabolism of cholesterol, it probably is just as likely that some of the sex hormones may be synthesized quite directly without passing through cholesterol. Whether carotinoids, vitamin A, squalenes, vitamin E, fatty acids, or carbohydrates are involved in these syntheses remains to be determined.

If we assume that cholesterol is the precursor, then the natural derivation of various sex hormones and related substances appears to be a relatively simple process on paper. These relations are shown on pages 191 and 192.

On page 192 the importance of cholestenone as a common hypothetical intermediate between cholesterol and the four substances cis- and trans-
dihydrocholesterol, progesterone, and $\Delta^1$-androstenedione, is obvious. The fact that progesterone is accompanied by allopregnanolone (Buten-andt and Mamali, 1935) in corpus-luteum extracts and that pregnanediol and allopregnanediol (Hartmann and Locher, 1935) occur in pregnancy urine suggests that dihydrocholesterol may be the immediate precursor of allopregnanolone or that progesterone yields both allopregnanolone and pregnanolone and that the latter is reduced to pregnanediol as a waste product.

The relations of cholesterol, cholestenone, and androstenedione to androgens and to estrogens are indicated on page 192. The presence of dehydroisoandrosterone in human urine suggests its direct formation from cholesterol by specific oxidation of the long side-chain in position 17. Testosterone might then be formed from it with androstenediol as an intermediate. However, testosterone could be formed quite as easily from cholestenone with androstenedione as an intermediate. In either case the reduction of the 17-keto group to a secondary alcohol makes probable the formation of two isomers if carried out in vitro; biologically these reactions are usually controlled to produce only one form. As a result of the very recent work of Ruzicka and Kägi (1936), we now conclude that the natural form of testosterone contains the OH group in the 17-trans position because these investigators were able to obtain two forms of testosterone which involved the configuration of carbon atom 17. They separated the two forms and found the trans form approximately thirty times as potent as the cis form by the capon test. In rats, the cis form produced no reactions in the seminal vesicle and prostate in doses forty times that of the trans form. From these and related studies they now conclude that an OH group in the cis position on carbon 17 of the cholane or choleene ring lowers the potency very strikingly.

Androsterone calls for very specific configurations on carbons 3 and 5. This could be formed a, from testosterone by reduction of the 3-keto group, hydrogenation of the 4,5 position and oxidation of the 17-hydroxy group; b, from androstenedione by simply reducing the 3-keto position and hydrogenating the 4,5 position; c, from a hypothetical specific intermediate androstanedione by simply reducing the 3-keto group. In all of these reactions, isandrosterone could also be formed in equal amounts but biological processes usually produce only one such isomer selectively. However, androsterone may also be derived from cholestenone, with epidihydrocholesterol as an intermediate. If we can assume that the species which secrete male sex-hormones all have
the same one, testosterone, just as adrenalin is found in all adrenals, then it would seem more likely that if cholesterol is the precursor we should have the degradation go by way of cholestenone and androstenedione or dehydroisoandrosterone. The formation of androsterone from testosterone appears more probable than from epidihydrocholesterol.

The assumption of the formation of the estrogens from androstenedione or from testosterone is more far fetched. In fact there is no direct evidence that such is the origin of theelin and the related estrogens. These processes all call for the specific removal of the methyl group in position 10 and for the production of various degrees of unsaturation of rings A and B. It appears much more likely that these unsaturated rings are built up more directly from precursors other than cholestenone.

REFERENCES


Battelli, F. C. R. Soc. de Physique et d'histoire naturelle de Geneve 39: 73, 1922.


Benoit, J. University of Strasbourg Thesis, 1925.


Biedl, A. Innere Sekretion 2: 107, 1912.


La masculinisation des chapons par le sérum de Taureau, considérée au point de vue de la loi des sénils différentiels et de la loi "tout ou rien." Compt. rend. Soc. Biol. 99: 1855, 1928.


Über die Umwandlung des Dehydro-androsterons in Δ4-Androsten-ol-(17)-on-(3) (Testosteron); ein Weg zur Darstellung des Testosterons aus Cholesterin. Berichte 68: 1850, 1935.


CLARK, H. M. A prepubertal reversal of the sex difference in the gonadotropic hormone content of the pituitary gland of the rat. Anat. Rec. 61: 175, 1935.


The pituitary gonadal relationship and the problem of precocious sexual maturity. Endocrinol. 15: 405, 1931.
EVANS, H. M. AND M. E. SIMPSON. Comparison of anterior hypophyseal implants from normal and gonadectomized animals with reference to their capacity to stimulate immature ovary. Am. J. Physiol. 89: 371, 1929.
FELLNER, O. O. Wirkung Placentar und Hodenlipoides auf die männlichen und weiblichen Sexualorgane. Pfliiger's Arch. 189: 199, 1921.
Über die männlichen Geschlechtsmerkmale der Leghornhühner, besonders über den Kamm, als Grundlage eines Testobjektes für die Eichung des männlichen Hormons. Pflieger's Arch. 228: 1, 1931.
THE MALE SEX HORMONES


GRADSTEIN, S. A photo-electric method of measuring the comb of capons in the


HANSEN, I. B. Rat seminal vesicles and prostate glands as quantitative indicators of testicular hormone. Endocrinol. 17: 163, 1933.


Cowper's gland and its reaction to castration and to different sex hormone conditions. Am. J. Anat. 50: 73, 1932.


HOLCK, H. AND M. KANAN. Manuscript in preparation, August, 1936.


JOHNSON, G. E. AND V. B. HANAWALT. Influence of thyroxine, pituitrin, and
desiccated thymus and thyroid on hibernation. Am. Naturalist 64: 272, 1930.


KLEIN, O. Der Einfluss der Nebennierenrinde auf die Entwicklung der männlichen Geschlechtsorgane. Endokrinol. 9: 401, 1931.


The biochemistry and physiological significance of the male sex hormones. J. Urol. 35: 382, 1936.


KORENCHENSKY, V. The influence of the removal of the thyroid, parathyroid, and sexual glands and of thyroid feeding upon the regulation of the body temperature of rabbits. J. Path. and Bact. 29: 461, 1926.


The influence of cryptorchidism and of castration on body weight, fat deposition, the sexual, and endocrine organs of male rats. J. Path. and Bact. 33: 607, 1930.
Histological changes in the organs of rats injected with oestrone alone or simultaneously with oestrone and testicular hormone. J. Path. and Bact. 41: 323, 1935.
The histology of the sex organs of ovarietomized rats treated with male or female sex hormone alone or with both simultaneously. J. Path. and Bact. 42: 91, 1936.
Simultaneous administration of testicular hormone with antuitrin and prolan or with desiccated thyroid. Biochem. J. 27: 1513, 1933.
Lacassagne, A. Métaplasie épidermoïde de la prostate provoquée chez la souris,


Lipschütz, A. The internal secretions of the sex glands. Williams & Wilkins, Baltimore, 1924.

On the influence of oestrin injections on the balance between the pre-hypophyseal gonadotropic hormones of the male rat. Quart. J. Exper. Physiol. 25: 100, 1933.


FRED C. KOCH


Das testikulaire Hormon; Extraktion und morphologische Wirkungen auf infantile und kastrierte männliche Mäuse. Endokrinol. 7: 180, 1930.


The regulation of the hypophysis by the testicle and some problems of sexual dynamics. Endocrinol. 15: 421, 1931.


Further observations on testicular extract and its effect upon tissue permeability. J. Path. and Bact. 34: 459, 1931.

MCCULLAGH, D. R., W. K. CUYLER AND J. T. FRAWLEY. The origin of the comb-


Seminal vesicle and prostate function as a testis-hormone indicator; the electric ejaculation test. Am. J. Anat. 45: 39, 1930.


Gonad hormone functions and reciprocal influence between gonads and hypophysis with its bearing on problem of sex-hormone antagonism. Am. J. Anat. 50: 13, 1932.


THE MALE SEX HORMONES


Effects of ovarian transplants upon the development and maintenance of the seminal vesicles and prostate gland of the albino rat. Anat. Rec. 1936 (in press).


Riddle, O. Reciprocal size changes of gonads and thyroids in relation to season and ovulation rate in pigeons. Am. J. Physiol. 73: 5, 1925.


Ruzicka, L. and H. R. Rosenberg. Bereitung des 17-Äthyl-testosterons und
THE MALE SEX HORMONES


Evidence of a correlation between the amount of gonad stimulating hormone present in the pituitary of the guinea pig and the stage of the reproductive cycle. Anat. Rec. 42: 38, 1929.


Stigler, R. Der Einfluss des Nebenhodens auf die Vitalität des Spermatozoen. Pfüger’s Arch. 172: 273, 1918.


ZALESKY, M. A study of the seasonal changes in the thyroid gland of the thir-


Neue Versuche (Hantelversuche) über die Wirkung orchitischen Extractes. Pflüger’s Arch. 69: 386, 1898.

ZUCKERMAN, S. The embryological interpretation of changes induced by oestrogens in the male reproductive tract. Lancet 135, January 18, 1936.


ADDENDUM. The modification of sex development in the chick embryo by male sex-hormones. Studies by Willier, Gallagher and Koch (1935, 1937) show the effects of injection of high concentrations of androgens into incubating hen’s eggs on the reproductive system of the 18- to 19-day-old embryo. Bull testis-tissue concentrates in doses of 15 to 300 international capon units per egg had no observable effect on the gonads or oviducts of either sex; the Wolffian ducts (potential male ducts) may have been slightly hypertrophied. Human-urine concentrates, freed from phenolic estrogens, in doses of 12 to 24 international capon units per egg, at times modified the left testis in the males into an ovotestis and produced an ovarian cortex in the right gonad of the females; the Wolffian ducts were consistently, tremendously swollen but the oviducts were rudimentary or absent in the female embryos. The results on the female with urinary concentrates are in part similar to those produced by pure theelin and theeol. The different effects produced by the two types of concentrates may possibly be correlated with the estrogenic-like action observed by some with androsterone and dehydro-androsterone. Undoubtedly the concentrates also contained toxic substances which caused death in many of the embryos.


