THE MALE SEX HORMONES[1]

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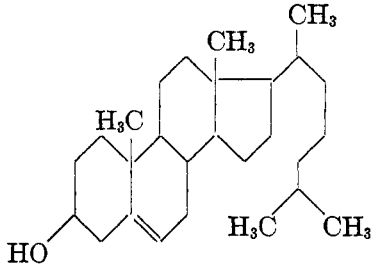
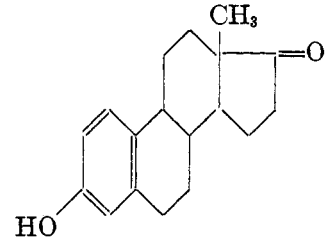
In the last few years investigations of the sex hormones have resulted in a remarkable increase in the knowledge of the relationship between chemical constitution and physiological activity. The astonishingly rapid development of the chemistry of the sex hormones was due to a fortunate accident: researches in this field were begun at the time that the study of the constitution of cholesterol was being brought to a close. Through the collective efforts of a number of investigators, especially Windaus, Wieland, Diels, and Rosenheim, it was possible in 1932 to propose with certainty formula I for cholesterol. In the meantime, the chemistry of the hormones of the sex gonads had reached a point which indicated the probable relationship to the sterols and thus permitted the use of the extensive knowledge in this latter field.



I
Cholesterol



II
Estrone

By the use of the Allen–Doisy test as a quantitative guide, Doisy in the United States and Butenandt in Germany succeeded in 1929 in isolating from pregnancy urine the first pure sex hormone, now generally known as estrone. This female hormone has the empirical formula $C_{18}H_{22}O_2$, and its chemical investigation in the laboratories of Butenandt, Doisy, and Marrian indicated the presence of a phenolic group, of a ketonic group,

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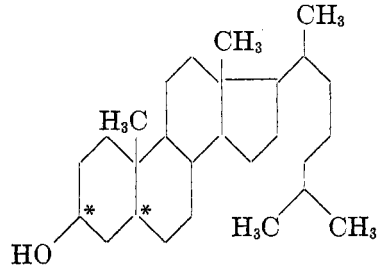
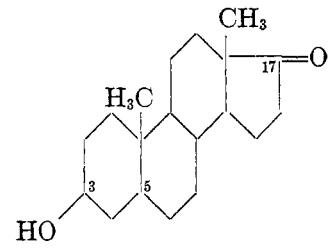
and of four rings. Since the only tetracyclic ring system previously observed in the animal kingdom had been found in the sterols and bile acids, it was assumed by Rosenheim and Bernal that the estrone molecule might be a derivative of the same skeleton. The simplest expression for estrone, on the basis of the known facts and on the assumption of a relationship to cholesterol, is given by formula II which was proposed by Butenandt. All the subsequent chemical work, of which the investigations of Cook in 1935 and of Marker in 1936 should be mentioned especially, proved to be in perfect agreement with this formula. Only the complete steric relationship of estrone to cholesterol remains to be determined.

Androsterone, the first male hormone to be isolated, was obtained from male urine by Butenandt and Tscherning in 1931. The cock's comb test, developed as a quantitative procedure by Koch, Moore, and Gallagher in 1929, was used as an assay method. If this hormone, $C_{19}H_{30}O_2$, which possessed both ketonic and alcoholic groups, was to be regarded as a simple derivative of a sterol, then it might be represented as a degradation product of a hydrogenated sterol (III) from which the side chain had been removed. Based on this assumption, formula IV for androsterone was proposed by Butenandt.



III
Cholestanol or a stereoisomer

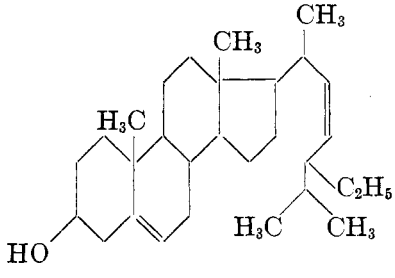


IV
Androsterone or a stereoisomer

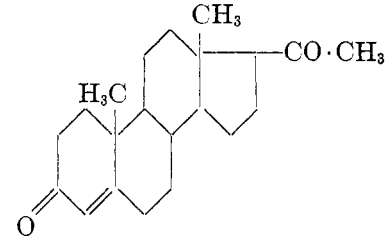
The amount of androsterone available for investigation was so extremely small that no support for this formula could be obtained by the usual degradation methods. In 1933, therefore, with the help of Goldberg, Meyer, and Brünger, investigations were undertaken in the laboratories of the Technische Hochschule in Zürich to determine the constitution of androsterone by an essentially synthetic procedure. The method which proved to be successful was the destructive oxidation with chromic acid of the acetates of the four available stereoisomeric dihydrosterols of formula III: cholestanol, epicholestanol, coprostanol, and epicoprostanol. These stereoisomers are distinguished by the configuration of carbon atoms 3 and 5 of the sterol nucleus. Fortunately, it was possible by the oxida-

tion of the saturated sterol acetates with chromic acid to obtain in small yields the acetates of the corresponding oxyketones (IV), which were then saponified. The four stereoisomeric oxyketones, $C_{19}H_{30}O_2$, were thus prepared, and that derived from epicholestanol proved to be identical with androsterone. In this manner the relationship between a sex hormone and the sterols was definitely established for the first time.

Very shortly thereafter both Butenandt and Fernholz, also by the use of synthetic methods, proved the constitution of the corpus luteum hormone, progesterone. Since this hormone was shown to be an unsaturated diketone, $C_{21}H_{30}O_2$, and on the assumption that it might be a sterol derivative, formula VI was proposed as a hypothetical expression for this substance. Subsequently, stigmasterol (V) was converted by stepwise degradation to a diketone identical with progesterone.



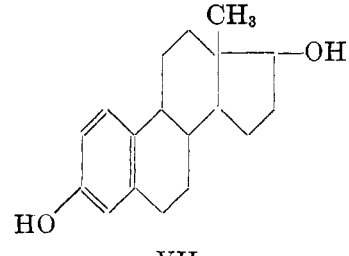
V
Stigmasterol



VI
Progesterone

The question now arose as to whether estrone, androsterone, and progesterone were the only naturally occurring compounds possessing sex hormonal action, and also as to whether synthetic compounds with similar physiological properties might not be prepared.

Schwenk and Hildebrandt discovered that the reduction product of estrone in which the ketonic group had been converted to a secondary alcohol (VII) has an activity several times greater than that of estrone itself. This substance, estradiol, was later isolated by Doisy from ovaries and proved to be the most active hormone of the estrane series.

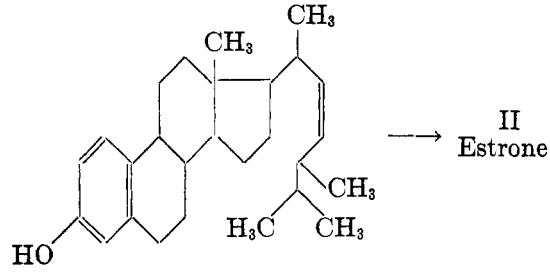


VII
Estradiol

In the corpus luteum no hormone other than progesterone has been discovered up to the present time.

Androsterone has been isolated only from urine and not from testes. Detailed investigations in many laboratories showed that, while qualitatively the agreement in the physiological activity of androsterone and testicular extract was good, marked quantitative differences appeared. Particularly striking was the difference observed between the action of androsterone and testicular extract on the combs of capons on the one hand, and on the accessory sex glands of castrated rats on the other hand. Measured in terms of cock's comb units, equivalent doses of androsterone and testicular extract when given to castrated rats led to the observation that the testicular extract has several times the activity of androsterone on the accessory sex glands. As a consequence androsterone cannot be regarded as the principal testicular hormone.

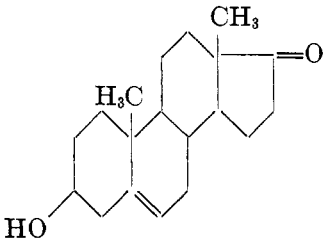
Butenandt somewhat later succeeded in isolating from urine a second male hormone, dehydroandrosterone (VIII), which differs from androsterone in being unsaturated and in the spatial arrangement of the hydroxyl group on carbon 3. By the application of the sterol degradation method described for the synthesis of androsterone, it was possible in collaboration with Wettstein to complete the synthesis of this hormone. Investigators in other laboratories were also successful in preparing dehydroandrosterone by the same procedure. This method of degradation of sterols obviously has general application, since Marker and coworkers have applied it very recently for the preparation of estrone from a transformation product (VIIa) of ergosterol.



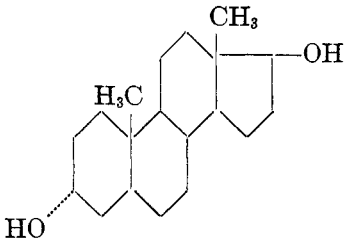
VII a

Dehydroandrosterone could not be responsible for the typical physiological action of the testicular extract since it has an activity less than androsterone. By hydrogenation, androsterone yields a diol (IX) the action of which on the cock's comb is about three times that of androsterone. Schematically, androstanediol can be compared to estradiol, but like androsterone and dehydroandrosterone it cannot be regarded as the

typical testicular hormone, since its action on the sex glands of rats is relatively feebler than that of androsterone.

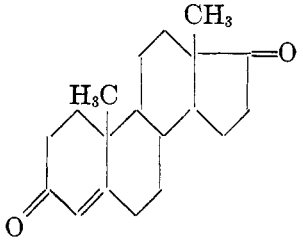


VIII
Dehydroandrosterone

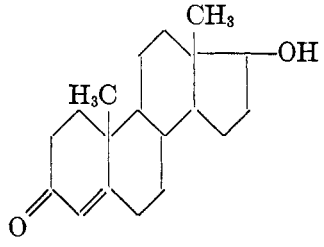


IX
Androstanediol

In addition to the difference in physiological activity there is also a noteworthy difference in chemical behavior between all of the previously mentioned androstane derivatives on the one hand, and the testicular extract on the other. The male hormone activity of testicular extract could be destroyed by boiling with alkali, while androsterone and the other known androstane derivatives were stable under these conditions. On the assumption that the testicular hormone is a cholesterol derivative, it appeared to us probable that it contained an α,β-unsaturated ketonic group, since such a linkage is relatively easily attacked by boiling alkali. The simplest compounds of this series analogous to androsterone are androstenedione (X) and androstenolone (XI).



X
Androstenedione



XI
Androstenolone

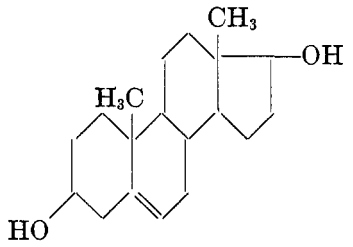
The problem as to whether either of these two compounds is the testicular hormone or not was investigated in collaboration with A. Wettstein and E. Tschopp. Androstenedione (X), prepared by the oxidation of dehydroandrosterone, had approximately the same physiological activity as androsterone as determined by the cock's comb test, but its action on the sexual glands of castrated rats was many times greater than that of androsterone. This gave the first experimental support to our hypothesis,

that the true testicular hormone might contain an α,β-unsaturated linkage. It seemed very likely, therefore, that the second compound, androstenolone (XI), which contains a hydroxyl group in the 17-position, might have an increased activity and might be the testicular hormone that had previously escaped isolation. Androstenolone was thus prepared synthetically by a stepwise transformation of dehydroandrosterone.

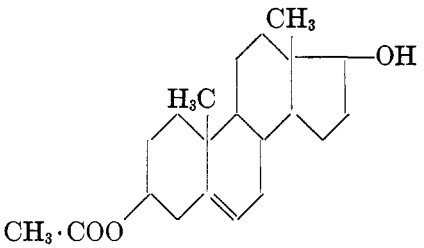
At this point, the synthetic approach to the problem coincided with the analytical procedure of Laqueur, David and their coworkers. These investigators succeeded in isolating a crystalline hormone from testes, which they named testosterone. This substance showed the same characteristic relationship between its action on the cock's comb and on the accessory sex glands as testicular extract. In view of the fact that androstenedione had shown the typical testicular hormonal action, David was led to attempt the oxidation of testosterone with chromic acid, and observed that the product obtained was identical with androstenedione. Simultaneously, it was possible to compare the synthetic androstenolone, prepared in our investigations, with testosterone. These proved to be identical and demonstrated the final proof of the structure of this important compound. During the same period Butenandt and Hanisch, while attempting to prepare new synthetic compounds with hormonal action, succeeded accidentally in obtaining testosterone by an exactly similar transformation.

As the yields of testosterone by the synthetic procedure were quite low, an improved method was sought and discovered in collaboration with Wettstein and Kägi. This permitted the production of testosterone on a large scale. Two essential improvements were developed. First, dehydroandrosterone was reduced to androstenediol (XII) with nickel and hydrogen without simultaneous reduction of the double bond. Second, two different ester groups were introduced in the 3- and 17-positions. Thus the monoacetate of dehydroandrosterone was reduced to the 3-monoacetate of androstenediol (XIII), and this substance was then benzoylated in position 17 (XIV). This made it possible to saponify the ester group in position 3 without affecting that in position 17. Partial saponification of the acetate-benzoate led to androstenediol 17-monobenzoate (XV) in good yield, and this compound gave testosterone by oxidation and alkaline saponification.

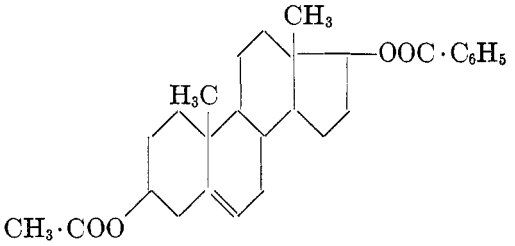
The close chemical relationship of the sex hormones introduces the question as to their origin in nature. Cholesterol might be considered as the single parent substance from which all of the sex hormones could be derived in a simple manner. The table on page 76 gives a hypothetical genesis of the sex hormones from cholesterol.



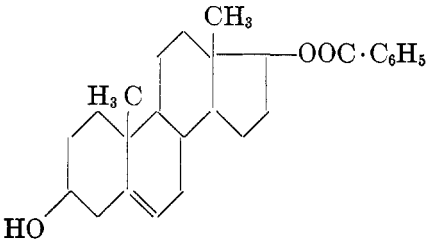
XII
Androstenediol



XIII
3-Acetate of androstenediol



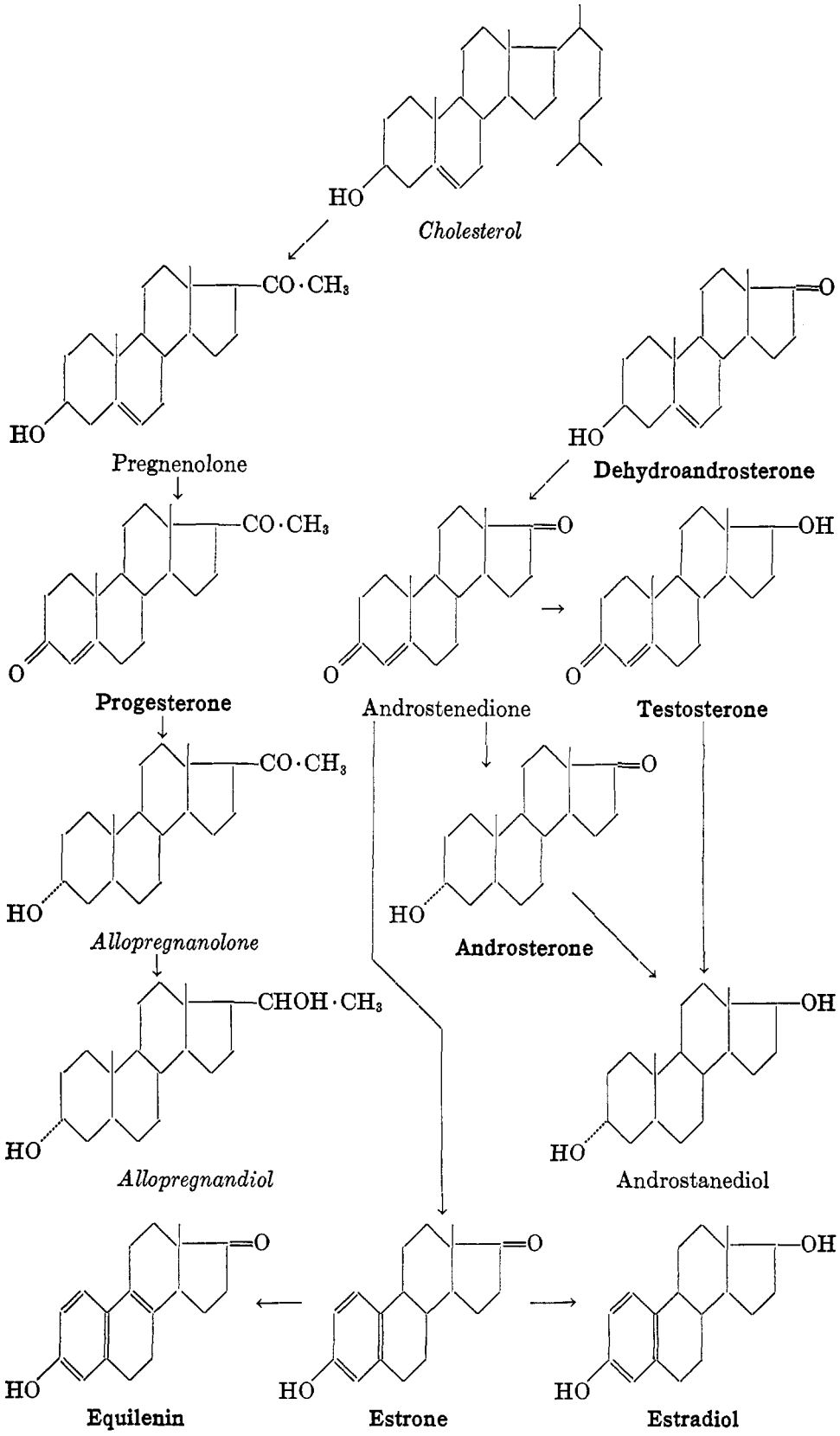
XIV
3-Acetate-17-benzoate
of androstenediol



XV
17-Benzoate of androstenediol

In the table, the hormones which occur in nature are printed in **boldface type,** related compounds found in the organism but without hormonal action in *italics,* and compounds prepared synthetically but not yet found in nature in ordinary type.

Closest to cholesterol chemically are compounds of the progesterone group. These have not yet been prepared in the laboratory by the direct oxidation of a sterol. The stages of the assumed transformation in the pregnane and the androstane series are closely analogous (horizontal rows


HO
Cholesterol
CO·CH₃
HO
Pregnenolone
O
Dehydroandrosterone
CO·CH₃
O
Progesterone
O
Androstenedione
OH
Testosterone
CO·CH₃
HO
Allopregnanolone
O
HO
Androsterone
CHOH·CH₃
HO
Allopregnandiol
OH
HO
Androstanediol
O
HO
Equilenin
O
HO
Estrone
OH
HO
Estradiol

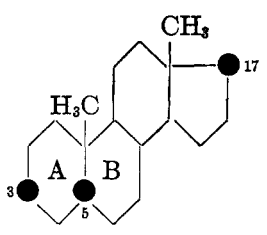
2 to 5 in the table). The first step may be the total or partial degradation of the side chain, resulting in the formation of the unsaturated ketones, pregnenolone and dehydroandrosterone. The second step may be the oxidation of the hydroxyl group in position 3 to a ketonic group. In the pregnane series the highest physiological activity is reached in the diketone, whereas in the androstane series the presence of the hydroxyl group in position 17 is essential for maximum activity.

It may be considered that the hormones of the estrane series are formed from an α,β-unsaturated ketone of the androstane series by the loss of methane. Thus, for example, the ovarian hormone, estradiol, may be produced from the testicular hormone, testosterone. Equilenin, the most completely dehydrogenated derivative of the sex hormone series which has yet been found, may be considered as a dehydrogenated estrone. Whether nature follows such transformations as these is completely unknown and remains as a problem of the future.

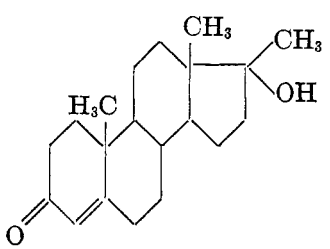
The relationship between physiological activity and chemical constitution in this general field is of unusual interest. Among the three groups of sex hormones, only in the androstane series is a relatively large number of synthetic and natural products which possess hormonal activity known. By slight chemical changes in progesterone, the activity is completely lost. In the case of estrone, the modifications of the molecule that are possible without disappearance of the activity are very limited.

Only the male sex hormones will be considered here, and in fact, it will be necessary to restrict the discussion to what appears now to be the most important of the physiological properties, namely the effect of the hormones in causing an increase in weight of the accessory sex glands of castrated rats. This property is of particular significance for the determination of the general and clinical value of the male hormones.

For the explanation of the results in the androstane series, the skeleton formula XVI is useful. The positions 3, 5, and 17 are specially designated, since changes in the molecule at these points are known to affect markedly the physiological properties. The differences between the various compounds thus far studied consist, first, in the spatial arrangement of the substituents at 3 and 5, second, in a modification of the functional groups at positions 3 and 17, and third, in the introduction of an olefinic linkage at carbon atom 5.



XVI



XVII
Methyltestosterone

The introduction of a double bond at carbon atom 5 causes only a slight increase in activity. The spatial arrangement of the substituents at each of the three centers of asymmetry is of much more significance. The *trans* position of rings A and B, which involves the steric configuration of carbon atom 5, occurs in the cholestane series and is of decided importance for hormonal activity. The analogous *cis* compounds, the coprostane series, show no activity. The "normal" spatial configuration of the hydroxyl group in position 3, as is found in the naturally occurring sterols, leads to a definitely lower hormonal activity than the epi configuration, but the difference is not as marked as that caused by changing the steric configuration of rings A and B. The steric configuration of the hydroxyl group on carbon atom 17 as it exists in testosterone leads to a considerably higher activity than the opposite configuration. If the hydroxyl group in position 3 or 17 is converted to the corresponding keto group, the physiological activity of the resulting molecule is not materially affected.

The introduction of a tertiary alcoholic group in position 17 leads to an unexpected result: methyltestosterone (XVII) was approximately ten to twenty per cent more active than testosterone itself.

In this connection Laqueur and his collaborators have made the more important observation that the action of testosterone on the male accessory glands is increased about five times by the presence of a substance which could be isolated from testes or from other animal organs. This X-substance, which is a mixture, acid in character, has in itself no hormonal activity. Miescher, Wettstein, and Tschopp could increase the activity of testosterone when they added a higher fatty acid or hydroxy acid to the hormone solution which was to be injected. Palmitic acid, for example, was found to be particularly effective, but an amount considerably larger than that of the X-substance was necessary to achieve the same effect as the latter.

The highest physiological activity toward accessory sex glands has been found in recent researches by my collaborators Miescher, Wettstein, and Tschopp. A series of esters of testosterone has been carefully investigated. It has been observed that the formic, acetic, and propionic esters show approximately five times the activity of testosterone, or about the same activity as the mixture of testosterone and X-substance. The butyric and valeric esters show still higher activities, about ten times that of testosterone. A further increase in the molecular weight of the ester group results in a rapidly decreasing activity. Thus, for example, the palmitic ester has an extremely low activity in contrast to a mixture of palmitic acid and testosterone. The quantitative action of testosterone esters is not influenced by the addition of a fatty acid.

The high activity of the testosterone esters upon accessory sex glands is

even more surprising when a comparison is made with the activity upon the capon's comb. The most active esters show only one-fifth to one-tenth the activity of testosterone on the capon's comb. Using capon units as a fundamental comparative basis, the activity of the esters of testosterone on the accessory sex glands is nearly fifty times that of testosterone.

The discussion of hormonal action has been limited to animal experiments. It would be of greater interest to discuss the action of these substances on the human organism, but the evaluation of the physiological action of these substances on humans will require years before the knowledge is as complete as that which now exists for castrated rats.

The significance of hormones in the realm of nature may be touched upon briefly. What is a hormone? Do the many synthetic compounds whose male hormonal activity equals or even surpasses that of naturally occurring hormones, really deserve the name "hormones" or are they merely "pseudo-hormones"? The futility of such questions becomes apparent when it is considered that a whole series of synthetic male hormones, having a definite although small estrogenic activity, has been described. Moreover, Parkes has observed that some of these same compounds have a definite corpus luteum hormonal action. The physiological properties of a single hormone and the chemical nature of a group of hormones with similar physiological activity cannot be sharply defined.

The viewpoint of earlier investigators in the hormone and vitamin field that these substances are highly specific, is no longer tenable. Other numerous examples might be cited. Reichstein has synthesized a whole series of compounds possessing typical vitamin C action. Windaus has recently shown that natural vitamin D contains one double bond and one methyl group less than calciferol obtained by irradiating ergosterol.

How did the vitamins and hormones achieve their important place in nature? The hormones appear to be derived either from proteins or from cholesterol and can be considered as metabolic substances to which the organism has so accustomed itself that they have become indispensable to the normal progress of life processes. The vitamins may be defined as plant substances whose presence in the animal organism has also become absolutely necessary. It is to be emphasized that no causal connection exists between the origin of the hormones or vitamins and their final physiological function in the organism. Why should substances that have become important for life merely by accident be specific and irreplaceable?

In conclusion, it appears that the animal body during its gradual evolution has adapted itself to the hormones and to the vitamins, until these substances have become essential. That other chemical compounds may be found to replace them was consequently to be expected and has, during the last few years, been experimentally established.