

The Artificial Preparation of the Male Sex Hormone.

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THE male sex hormone may be defined as a chemical compound produced in the testicle, and which in the male organism promotes the growth and function of the sex organs and glands, and also the development and maintenance of the secondary sex characteristics and sex instinct. The discovery of this hormone resulted from successful experiments on castrated male animals, in which the atrophy of the sex characteristics and organs was cured by implantation of the testicles of other adult animals. The first experiments in this direction date as far back as 1849, *i.e.*, long before there existed a science of hormones, when Berthold (Göttingen) successfully implanted fresh testicles into capons.

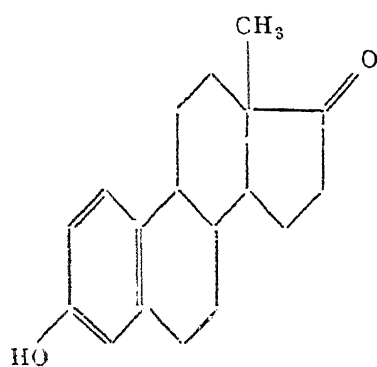
In 1929 Gallagher, Koch and Moore (Chicago) succeeded for the first time in preparing a really effective testicular extract which exhibited, in castrated animals, effects similar to those formerly obtained by grafting fresh testicles. These investigators also worked out the first practical biological test for the detection of the male sex hormone. It is the so-called capon test, which was subsequently improved by Funk, Laqueur and others, and which is based on the principle that the stunted comb of a capon increases in size by the injection of the male sex hormone, such increase being roughly proportional to the quantity of hormone injected. We call a capon unit the quantity of hormone which, with a definite technique, produces an increase of about 20% in the surface area of the comb.

With the help of this method, Butenandt (Göttingen) isolated in 1931 a male sex hormone in crystalline form from the urine of men; the injection into a capon of 0.3 to 0.4 milligrammes of the said hormone, in fractional doses, in the course of a few days produces a 20% increase in the surface area of the comb. The isolation of this hormone, called androsteron, is extremely laborious and up to the beginning of 1933 only 25 mg. of it had been isolated, for which quantity 50,000 litres of urine were required. Butenandt was able to establish that androsteron is a saturated oxyketone having the formula $C_{19}H_{30}O_2$ or $C_{18}H_{28}O_2$, and possessing four rings, although an exact chemical investigation was not possible at that time owing to the difficulty of obtaining sufficient quantities of the hormone. It was, however, possible to form a hypothetical picture of the probable structural formula of androsteron on the basis of the knowledge of the follicular hormone (theelin, oestrin) acquired in the meantime.

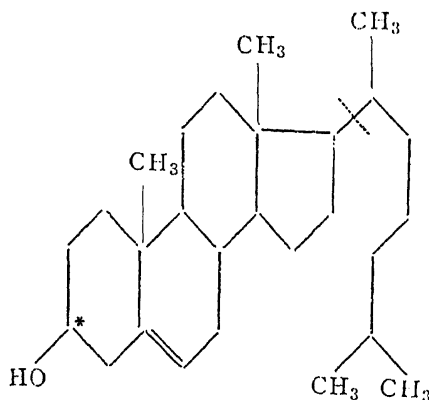
Following the discovery in 1923 of Allen and Doisy's test for the ovarian hormone, Butenandt and Doisy succeeded, independently and almost simultaneously in 1929, in isolating theelin in a crystalline form from the urine of pregnant women.

The chemical investigation of this substance by Doisy, Butenandt, Marrian and Cook, led to the assignment of formula I.

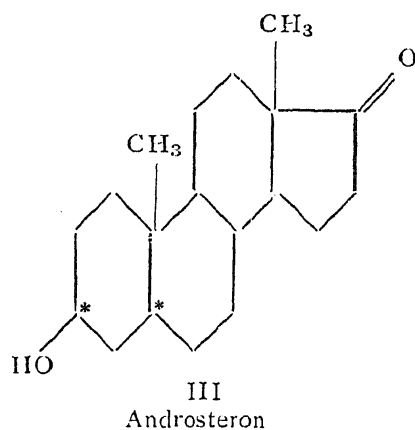
The simple manner in which this formula can be derived from cholesterol supports its correctness.



I
Theelin



II
Cholesterol

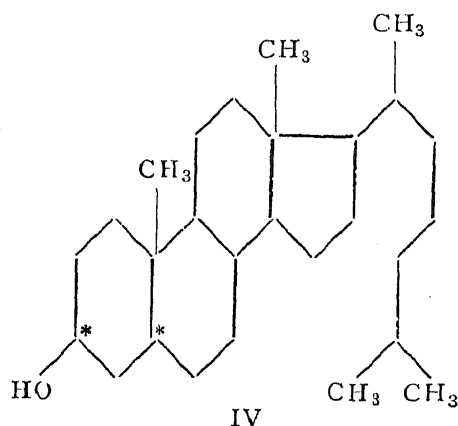


It is only necessary to consider that the terminal six ring of cholesterol is dehydrogenated with the formation of a phenol ring, and further, that the long side-chain is completely split off by oxidation with the formation of a ketone group.

Assuming that androsteron is also derived from cholesterol, the formula $C_{19}H_{30}O_2$ leads to the structural formula III which Butenandt proposed in 1933, as an intermediate product in the course of the hypothetical conversion of a hydrogenated sterol into theelin. The formation of a substance corresponding to formula III from hydrogenated sterol, requires only the splitting off of the long side-chain, in the same way as theelin is considered to be formed in the body by the oxidative degradation of a dehydrogenated sterol.

In view of the great difficulties which were to be expected in an attempt to determine the exact constitution of androsteron by entirely analytical methods, the author, together with his assistants, M. W. Goldberg, Jules Meyer, H. Brünnger and E. Eichenberger, decided to approach the question from another angle. An attempt was made to prepare the hormone artificially by following as closely as possible the method which nature probably uses for producing it in the body.

It was first of all necessary to investigate the question of the most suitable material to be used for the proposed work. We have discussed above the hypothesis of the derivation of theelin from cholesterol. However, up to now there are no facts at all showing that theelin is stereochemically identical with cholesterol. Further, in the artificial preparation of androsteron it was necessary to take into consideration the steric structure at the two positions marked with an asterisk in formula III. All hydrogenated sterols hitherto known differ one from another by a



Dihydrocholesterol and stereoisomeric sterols

different steric position at these two carbon atoms. Four different stereo-isomers of the formula IV are known: dihydrocholesterol, epi-dihydrocholesterol, koprosterol and epi-koprosterol. We have included all these stereo-isomers in our investigations. Before the splitting off of the side chain, the hydroxyl groups were protected by acetylation from the action of the chromic acid used as the oxidising agent. It must be emphasised, that according to the statements in the literature, it was very improbable that a ketone of the type of androsteron could result from the oxidation of an acetylated sterol corresponding to formula IV. This improbability has certainly deterred, up to now, other investigators from employing this exceedingly simple method for the solution of the problem of the male sex hormone. However, our optimism proved to be justified. By oxidation of the acetates of the 4 sterols named, we were able to isolate the corresponding 4 oxyketones. The oxyketone derived from epi-dihydrocholesterol proved to be identical in every respect, chemically, physically and physiologically, with the natural androsteron. On the other hand, the other three isomers are distinctly different from androsteron.

Although there was from the beginning a certain probability that androsteron might belong to the sterol group, no one could have expected that it was derived from epi-dihydrocholesterol. Girard, for instance, had considered the hypothesis of lithocholic acid being the mother-substance from which androsteron originates, while Butenandt thought koprosterol more feasible. No one had previously imagined the existence of a derivative of epi-dihydrocholesterol (or of epikoprosterol) in nature.

A comparison of the physiological action of the 4 stereo-isomeric oxyketones $C_{19}H_{30}O_2$ shows the importance of the steric

configuration for the hormone character. Whereas with androsteron (both natural and artificial) a capon unit amounts to 0.07 mg., one injection a day being made during six consecutive days, one unit of the oxyketone derived from di-hydrocholesterol is 0.5 mg. The two oxyketones derived from koprosterol and its epimer were ineffective in daily doses of 1 mg.

The synthetic preparation of androsteron permitted for the first time the complete elucidation of the constitution of a sex hormone. This is a rare case of the elucidation of the constitution of a natural product of intricate composition, by the artificial preparation of the substance before anything was known about the structure of the carbon skeleton.

In this case the method of elucidation was just the reverse of that usually employed: the first detailed publication of Butenandt on the chemical reactions of androsteron which appeared two months after our communication of the synthetic preparation contains no mention of a degradation product of androsteron which might have been identified with a compound of a known constitution.

The greater accessibility of synthetic androsteron permits the investigation of the question of whether there is only one male sex hormone, or if several compounds together are responsible for the effects observed. Butenandt has already discovered a second male hormone in the urine, dehydro-androsteron, which acts in the same manner as androsteron on the capon's comb, but is distinctly weaker. There are also several female sex hormones all of which, however, exhibit a weaker action than theelin. According to our present knowledge, theelin suffices for the production of the effects of the ovarian hormone. The results obtained up to now with androsteron do not contradict the assumption that it can exhibit all the effects which one expects from the testicular hormone. Let us now describe briefly the most important physiological investigations, which have been carried out by E. Tschopp in the "Ciba" Laboratories in Basle.

The capon test shows that an overdose

of androsteron causes an exceedingly pronounced increase in the size of the comb. For example, by painting the comb (according to Fussgänger's technique) daily for ten days with a 1⁰/₁₀₀ solution of androsteron we observed that the surface of the comb was increased sevenfold.

Furthermore, painting with a 0.5⁰/₁₀₀ solution of androsteron, the site where later the comb grows on newly hatched chickens.....causes, after a few weeks, the appearance of a comb of approximately the same size as that of cocks having attained their full development.

From a clinical point of view, it is interesting to note that with capons in which too small a portion of testicle has been preserved for the stunted comb to be able to grow, temporary injections of androsteron cause a prolonged growth of the comb. In completely castrated capons, on the contrary, the comb stops growing on cessation of androsteron treatment, whereupon a gradual atrophy of the comb to its initial size takes place. Such effects have already been observed following the administration of testicular extracts. In certain cases of testicular hypofunction, androsteron can act as a "hormone fillip" to stimulate the inactive generative glands into new activity. Investigations with mammals in that connection will be of great importance.

Furthermore, in castrated male rats, it was possible to obtain with androsteron a complete cytologic regeneration of the atrophied seminal vesicles (positive test according to Læwe-Voss). Finally the "wedding dress" picture of the male small fish called *Rhodeus amarus*, which is obtainable with testicular extracts, could also be produced with androsteron. All the experiments which have been carried out in the past with the various extracts exhibiting the action of the male sex hormone, and especially with testicular extracts, will be repeated with synthetic androsteron, which will subsequently also be tested clinically. These experiments will show whether androsteron, or any of its derivatives possessing stronger physiological properties, can completely play the rôle of the male sex hormone.