ENDOCRINES IN THEORY AND PRACTICE

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THE CHEMISTRY AND ASSAY OF MALE HORMONES

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The more obvious effects of castration, some of which have been known since early times, comprise atrophy or non-development—according to whether the operation is pre-pubertal or post-pubertal—of the accessory organs and secondary sexual characters. The former include the prostate, seminal vesicles, Cowper's gland, and penis; the latter, the comb of the fowl, the horns of the stag, and the body configuration, the deep voice, and the special distribution of hair in many species, including man. Grafting experiments showed that the control of the testes over the accessory organs and secondary sexual characters was probably endocrine in nature. In 1927 McGee prepared extracts of testis capable of restoring the atrophic comb of the capon, and an endocrine function of the testis was definitely established. Since then androgenic* substances have been found elsewhere in the body, but the effects of castration indicate that the testis is the essential and primary source. Various procedures such as x-irradiation, or ligation of the efferent ducts, which cause atrophy of the testis tubules, suggest the interstitial tissue as the site of origin of the testis hormone.

Detection and Assay of Androgenic Activity

Reversal of any of the changes which follow castration or the production of a state of hyperorchidism might be used to detect male-hormone activity. The Chicago workers, Moore, Koch, and Gallagher, used first the capon comb as a test object, but they subsequently described a number of tests on castrated rats and guinea-pigs, based on the cytological condition of the epithelium of the seminal vesicles, prostate, and Cowper's glands, the survival of motility of epididymal spermatozoa, and the power to ejaculate. [See Koch (1932) and Moore (1935) for reviews of this work.] Voss and Loewe (1930) attempted to assess gross size changes in the seminal vesicles of castrated mice, but finally used epithelial changes. Martins and Rocha (1930) measured the size of the seminal vesicles of the castrated mouse before and after treatment. Korenchevsky and his co-workers (1932-3) developed a method based on the weight of the seminal vesicles and prostate of castrated rats. In addition, such tests have been described as the increase of spontaneous activity of castrated mice, the growth of the crest of the male salamander, the production of the nuptial coloration of the male bitterling, growth of the ovipositor of the female bitterling, and blackening of the beak of the anoestrous or capon sparrow.

The only generally employed quantitative tests, however, are those based on production of growth in the comb of the caponized domestic cock or in the accessory

glands of the castrated rat. Of these the former is the easier and less equivocal test. The Second Conference on the Standardization of Sex Hormones (*Quart. Bull. Hlth. Org. L.o.N.*, 1935, 4, 618) recommended that "on the information before the conference the test based on the induction of growth in the comb of the capon is, at present, the only one sufficiently specific for the quantitative determination of activity in comparison with the standard preparation." The conference also agreed that the international standard preparation for male-hormone activity should consist of crystalline androsterone and that "the unit of activity shall be defined as the activity of 0.1 mg. of this standard as tested by a specific biological reaction; this weight is approximately the daily dose required to give an easily measurable response in the comb after five days." An increase in comb size of about 5 mm. in length plus height or 15 per cent. increase in area may be expected from one international unit (I.U.) daily for five days. Detailed techniques have been described by Dodds *et al.* (1930), Schoeller and Gehrke (1931), Freud *et al.* (1932), and Greenwood *et al.* (1935), as well as by the Chicago workers referred to above.

However, it is clearly desirable that quantitative work should be carried out on mammals. Increase in weight of the prostate and seminal vesicles of castrated rats is the simplest mammalian criterion of androgenic activity, and has been used extensively by Korenchevsky (1932 *et seq.*), Freud (1933 *et seq.*), Callow and Deanesly (1935), and Tschopp (1936). With the amounts of androgenic substance now available a readily assessable degree of enlargement can easily be obtained. The total weight of the seminal vesicles and prostate of the castrated rat is between 20 mg. and 50 mg. according to the technique of dissection, and increase to an average of 660 mg. has been produced by only ten days' treatment with male hormone (Parkes, 1936). The chief difficulty lies in the fact that the several accessory glands respond in different degrees to the different androgenic substances, so that the comparative activities of the different substances depend largely upon which organ is employed as a basis for comparison. Androsterone, for instance, is comparatively ineffective on the seminal vesicles, and produces quite an abnormal ratio of growth between these and the prostate. A second difficulty is that the effectiveness of a preparation is greatly influenced by the conditions of absorption (Deanesly and Parkes, 1936a).

Natural and Artificial Sources

The demonstrable male-hormone activity of testis tissue is comparatively small, as might be expected from the small proportion of interstitial tissue present. Thus extract equivalent to 0.5 kg. of tissue may be necessary to induce good comb growth in a five-day test. Favourable clinical results have been reported from time to time with dried tissue or testis extracts of low concentration, but in the absence of objective laboratory tests it is likely that these results are due to psychological rather than to direct physiological action. Testis extracts of sufficient potency, as measured by animal tests, for clinical use could only be prepared at enormous cost. The isolation of crystalline testosterone from bull's testes by David in 1935 was of great importance, but the process is not commercially economical.

* The terms "androgenic," in the sense of causing the development of male organs and characters, and "androgen," a substance with this property, have been used by American writers. They supply very necessary parallels to the terms "oestrogenic" and "oestrogen" applied to female sex hormones.

Loewe and Voss (1929) first reported that extracts capable of reversing castration changes could be prepared from the urine of normal men. This discovery led to the preparation of large quantities of androgenic concentrates for physiological and clinical investigation, but it is now known that the substances in the urine, androsterone and dehydroandrosterone, are different from the active substance, testosterone, isolated from the testis. Crystalline androsterone was first isolated in very small yield from urine by Butenandt in 1931, but it was later found that male hormone equivalent to about 3 mg. androsterone per litre is present. From androsterone the reduction product, androstenediol, has also been made in crude or in pure form. In 1934 Ruzicka and his collaborators prepared androsterone from cholesterol by way of *epicholesterol*. On the theoretical side this brilliant piece of work confirmed and amplified the details of the constitution for androsterone suggested by Butenandt (1933): practically, it has led to the artificial production on a manufacturing scale of both androsterone and testosterone, and to the ready accessibility of many related compounds for investigation of their physiological activity. The so-called "synthetic" pure male hormones are now far more readily available than the same substances from natural sources.

Although the compounds mentioned above account for most of the androgenic activity of the testis and of urine, it cannot be assumed that other active substances are not present. For instance, the isolation of a crystalline compound from boar's testes, tentatively identified with androstenedione, has been reported. The nature of the androgen detected in bull's blood, amounting to 1 to 2 units per litre, is not yet known. Finally, the substances are as yet unidentified to which the urine of normal women owes its androgenic activity (Womack and Koch, 1932; Koch, 1936), which is about the same as that of male urine, and the significance, if any, of the presence of male hormone in the female is unknown. The phenomena of virilism are outside the scope of this article, although its association with adrenal hyperplasia, and with the excretion of large amounts of androgenic material in the urine, coupled with the isolation from adrenals of a series of steroids which includes a weak androgen, adrenosterone (Reichstein, 1936) indicate that glands other than the gonads must be considered in the story of androgen metabolism.

Constitution and Nomenclature

Work now published and in progress on a multiplicity of compounds with androgenic activity makes it necessary to give some attention to the systematic nomenclature.

The natural or artificial androgens so far known are all simple derivatives of the tetracyclic hydrocarbon androstane (Formula I). This is the dimethyl derivative of fully hydrogenated *cyclopentenophenanthrene*, the skeleton of which (Formula II) is regarded as the schematic parent of all the steroids—that is, the sterols, bile acids, male and female hormones, cardiotonic aglucones, saponins, and toad poisons.

The systematic nomenclature of the male hormones is based on the root "androst—" with the addition of appropriate suffixes and of numbers in the usual way to indicate the nature and position of substituent groups. The numeration is somewhat illogical, for historical reasons, and it is fortunate that only five positions need be considered—namely, 3, 4, 5, 6, and 17 (Formula III).

The known derivatives of interest are either dihydroxy-derivatives (-diols), diketo-derivatives (-diones), or mixed hydroxy-ketones (-olones), with the groups in positions 3 and 17, and these may either be saturated (-ane-) or have an unsaturated linkage at 4:5 or 5:6 (Δ^4 - or Δ^5 -ene-). A series of 17-alkyl-derivatives (for example, 17-methyl-17-ol) has also been prepared. In certain cases, which need not be dealt with

here, it is necessary to consider the configuration (*cis*- or *trans*-) of the groups round carbon atoms 3, 5, and 17. Nearly all the possible permutations and combinations of these substituent groups and double linkages are represented among the artificially prepared compounds, which provide a happy hunting ground for the investigator of relations between chemical constitutions and physiological activity.

Formula I.—Androstane.

Formula II.—*cyclopentenophenanthrene*.

Formula III.—Numeration of androstane derivatives.

Formula IV.—Androsterone.

Formula V.—Testosterone.

The two compounds of greatest importance possess common names: androsterone, which is androstan-3-ol-17-one (Formula IV), and testosterone, which is Δ^4 -androst-17-ol-3-one (Formula V), and some names (of unfortunate ambiguity) have been derived from these—for example, dehydroandrosterone (Δ^5 -androst-3-ol-17-one) and dihydrotestosterone (androstan-17-ol-3-one), and are used in preference to the less euphonious systematic names.

Physical and Chemical Properties

The male-hormone compounds are colourless crystalline substances, readily soluble in most organic solvents, moderately soluble in oil, but very sparingly soluble in water or aqueous media. It is therefore necessary to administer them therapeutically in oil solutions. Such

evidence as is available indicates that they are relatively ineffective when given by mouth, and intramuscular injection is almost invariably employed. The stability to heat, light, and oxygen, so far as is at present known, indicates that there is little danger of deterioration under ordinary conditions of storage. Both testosterone and androsterone are secondary alcohols and therefore readily form esters, which have modified biological activity. The ketonic group is also able to combine with suitable reagents, but the compounds have so far been used only for chemical separation or characterization, and their physiological properties have not been systematically investigated.

Relative Activity of Principal Androgens

The chief compounds with which the clinician is likely to be concerned are testosterone, androsterone, the main active constituent of urine extracts, and androstenediol, which is a reduction product of androsterone not known to occur naturally. Tested on capons the same amount of comb growth is produced by 0.18 mg. testosterone, 0.30 mg. androstenediol, or 1 mg. androsterone. The relative activity on castrated rats cannot be stated simply, for in the first place it involves the different activities on the various accessory glands, and, secondly, conditions of administration and absorption exert a controlling influence on comparative activity. Roughly, androstenediol is about twice as active on the prostate as androsterone and about four times as active on the seminal vesicles. Given under conditions permitting of rapid absorption testosterone is only slightly more active than androstenediol, but where rapid absorption is prevented testosterone is much more effective.

Modifying the Effectiveness of Androgens— "X-Substances"

In the course of their work on testis extracts the Amsterdam group discovered that acidic fractions of extracts from testis, or even from other organs, inactive by themselves, increased the efficiency of certain male hormones when injected at the same time. Any idea of a strictly specific co-hormone and of synergy at the site of action of the hormone was dispelled when Miescher, Wettstein, and Tschopp (1936a) showed that the same adjuvant effect was exerted by a number of pure fatty acids, but only when the hormone was mixed with the fatty acid at the site of injection. The Amsterdam group maintains that the material from testis is of peculiarly high activity, but the balance of evidence is in favour of the view that there is a non-specific effect at the site of injection which may be simulated and replaced for clinical purposes by the choice of a suitable ester and a suitable solvent. The effect of esterification of the male hormones is very marked, as it is with the female hormones, and it seems likely that this is a result of influences on the availability, which depends in turn on absorption and ease of hydrolysis in the body. Androsterone is excreted in normal urine in a combined form, probably an ester with glucuronic acid, which is readily soluble in water and is inactive, perhaps because of rapid elimination. Water-soluble derivatives, the alkali salts of the hydrogen succinates of androsterone and androstenediol, have also been prepared in the laboratory, and, according to Korenchevsky *et al.* (1935), their activities are only one-ninth and one-third respectively of those of the free hormones. The important esters from the therapeutic point of view are those with simple fatty acids. Ruzicka and Wettstein (1936) have prepared the complete series of testosterone esters from formate to valerate and certain others, and Miescher, Wettstein, and

Tschopp (1936b) have tested these biologically, reaching the conclusion that optimal activity is possessed by the propionate. This ester, unlike free testosterone, retains effectiveness even with 10-day intervals between injections (Parkes, 1936).

Anomalous Activities of Male Hormone Compounds

The fact that extracts of testis and male urine might show oestrogenic properties has been known for a number of years, but this was usually held to indicate the presence of oestrogenic substances, probably of the oestrone group, in addition to male hormone. It is now known, however, that certain members of the androstane series are oestrogenic. Androsterone was originally reported not to show oestrogenic properties, but it now appears that heavy doses may do so (Wolff and Ginglinger, 1936). Butenandt and Kudzus (1935), however, showed that dehydroandrosterone, androstenedione, and testosterone caused opening of the vagina in the immature rat, and Butenandt (1936) later drew attention to the oestrogenic activity of *trans*-androstenediol, which will cause cornification of vaginal epithelium (Deanesly and Parkes, 1936b). These compounds will feminize the plumage of capon fowl. Androstenediol and testosterone cause enlargement of the uterus of the ovariectomized rat (Korenchevsky and Dennison, 1936). The dual activity of these compounds is important in view of the increasing evidence that prostatic enlargement in man is due to oestrogenic stimulation.

The 17-methyl and 17-ethyl derivatives of testosterone and certain of the other compounds possess the progesterone-like power to evoke progestational proliferation in the rabbit uterus (Klein and Parkes, 1936). Further, testosterone and androstenediol have the progesterone-like power to modify the action of oestrone on the rat vagina, resulting in extensive mucification, and, further, they have the progesterone-unlike power to cause mucification directly when given alone to the ovariectomized rat (Korenchevsky and Dennison, 1936). Testosterone also has some effect on the mammary glands (Selye, McEuen, and Collip, 1936). The biological significance, if any, of these anomalous activities is not yet obvious. Androstenediol (Zuckerman and Parkes, 1936) and also testosterone (Zuckerman, 1936) have the power to protect the male organism from the effects of oestrone.

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COAL-TAR NAPHTHA DISTILLATES FOR DESTRUCTION OF BED-BUGS*

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A number of experiments both in the laboratory and in the field have been carried out which show that the vapour of certain coal-tar naphtha distillates is lethal to the bed-bug (*Cimex lectularius* Linn.). Toxicity experiments have also been carried out by Dr. G. R. Cameron at University College Hospital Medical School, London, and these tests have shown that animals kept for prolonged periods in an atmosphere saturated with the vapour have not been injuriously affected in any way and afford some considerable assurance that no danger to man from this cause is to be apprehended.

A method for applying these naphtha distillates to the disinfection of houses has been developed, and consists essentially in spraying the walls and ceilings of rooms which have previously been made gas-tight by any of the usual methods of sealing. Special attention is given to likely harbourages such as skirting-boards, architraves, etc., a high-pressure hand syringe being used for spraying such harbourages. Gas masks with a suitable filter are worn during the process. To ensure satisfactory results it is important that sufficient vapour concentration be maintained, and for this purpose fumigation should not be begun at a temperature of less than 60° F. It is an advantage to work at as much above this temperature as is practicable.

The quantity of naphtha used in the experiments is on the basis of 1 gallon for each 750 cubic feet of room space. The rooms are kept sealed for eighteen to twenty-four hours, and at the end of this time are rapidly ventilated by opening doors, windows, fireplaces, etc. The vapour clears very quickly, and it is possible to enter a room after a few minutes' ventilation without experiencing any discomfort. It has, moreover, been shown that there is no tendency for a concentration of vapour to "build up" if after ventilation the doors and windows are again closed for several hours.

The desirability of a high initial temperature is emphasized, and only in very hot weather with air temperatures of 70° to 80° F. should pre-heating of houses be dispensed with. In the experiments a very effective paraffin stove, working on the primus principle, has been used, but in any case sufficient time must be given to warm walls, ceilings, etc., thoroughly. Owing to the inflammable nature of the naphtha, fires or other sources of heat must be extinguished, and, if portable, removed from the rooms before fumigation is started, and smoking and the use of naked lights must be prohibited while the naphtha

is being handled in any way. The experiments indicate that after a house has been warmed it is advisable in most instances to spray the roof space before the rooms are treated.

Results

Successful results have been obtained in the disinfection of upwards of two hundred houses and flats of varied constructional detail. Failure has been reported in a few cases, but this, we think, has been incidental to the development of a suitable technique for applying the naphtha. After fumigation for twenty-four hours dead bugs will sometimes be seen on the floors, but usually they will be found dead in their customary hiding-places—for example, behind skirting-boards, picture rails, etc.

The naphtha used is derived by the distillation of coal tar, and consists of a complex mixture of aromatic hydrocarbons and associated bodies, and the constituent or mixture of constituents which is toxic to the bed-bug is not known. The following specification has been drafted to govern the supply of coal-tar naphtha as used in these experiments.

SPECIFICATION FOR HEAVY COAL-TAR NAPHTHA

1. *Colour*.—Shall not be darker than a freshly prepared solution of 1 ml. of N/10 iodine in 1,000 ml. of distilled water.
2. *Specific Gravity*.—Not less than 0.835 and not greater than 0.910.
3. *Water*.—Shall be free from water and other visible impurities at 15.5° C.
4. *Distillation Range*.—Method as defined in B.S.I. Specification No. 479 for Coal-tar Naphthas.
Up to 160° C. not more than 5 ml.
Up to 190° C. not less than 90 ml.
5. *Flash-point (Abel)*.—Not less than 105° F.
6. *Tar Acids and Tar Bases*.—Not more than 0.25 per cent. of tar acids and 0.25 per cent. of tar bases.

Further research into various aspects of the question of the use of heavy coal-tar naphthas for the destruction of bed-bugs is in progress, and new methods for more effective application are in course of development. As will be seen from these preliminary notes the use of coal-tar distillates for the destruction of the bed-bug is still in the experimental stage, but the results so far achieved are promising and indicate that further research on these lines may produce valuable results.

As was to be anticipated failures have occurred, not, however, owing to defects in heavy naphtha as a toxic agent for destruction of bed-bugs, but owing to defects in the methods of employing it for that purpose. If, therefore, those responsible for the management of house property adopt this method of treatment for eradication of bed-bugs it is important that they should realize that the technique of fumigation with heavy naphtha is still the subject of test and research, and that the cause of any failure is first to be sought in the method used. In the event of failure, the circumstances of the particular case should be carefully investigated, especially in regard to the temperature at the time of fumigation, to the sealing of the premises, and to the distribution of the vapour.

The eighth International Congress of the Haute Culture Médicale will be held at Algiers from March 25 to April 6. There will be sessions on tropical and subtropical diseases, syphilography and dermatology, cardiovascular affections, blood disorders, and social medicine. During the congress there will be a special commemoration of the pioneers in the campaign against malaria, and also excursions, receptions, and entertainments; in the first of these, which will be broadcast, Dr. P. H. Manson-Bahr will participate. At the end it is hoped to arrange visits to Morocco and Tunis. The travel arrangements are in the hands of Messrs. Cook, Wagons-lits, and further information can be obtained from the congress secretary, M. Tomarkin, Institut Alfred Fournier, 25, Boulevard Saint-Jacques, Paris, XIV.

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