7-Dehydrosterol in the Sexual Organs of the Rat

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In a study of the distribution of vitamin A in the rat we examined the male reproductive system under ultraviolet irradiation screened with Wood's glass (Moore & Ward, 1951). Faint-yellow fluorescence suggested the presence of the vitamin in the caput epididymis and preputial glands of normally nourished animals. Attempts to confirm this conclusion by spectrophotometric methods, however, were impeded by the presence of well-defined absorption bands at 293, 282, 272 and 262 mμ., characteristic of 7-dehydrocholesterol or ergosterol. The intensity of the absorption suggested that one of these substances was present in unusually high concentration. It seemed of interest, therefore, to investigate whether the amount of the sterol could be influenced by dietary changes, including deficiency of vitamin A, and whether it could also be detected in the sexual organs of other species.

A preliminary account of our main findings has already been given (Moore & Ward, 1952). The purpose of the present communication is to give details of our spectroscopic data, and to report tests on an irradiated extract of rats' preputial glands for antirachitic activity. Since we have not isolated the substance responsible for the spectrophotometric absorption in pure form, and in amounts sufficient for its identification, we refer to it as 7-dehydrosterol. We consider that it is almost certainly 7-dehydrocholesterol, as implied by previous workers who have observed the same characteristic absorption spectrum in extracts made from tissues of higher animals (Glover, Glover & Morton, 1952).

EXPERIMENTAL

Methods. The organs under investigation were mostly taken from piebald rats, and were carefully dissected free from adipose tissue. In our earlier experiments they were first ground with quartz powder and anhydrous Na_2SO_4, and extracted in the cold with several portions of ether. The combined extracts were then evaporated to dryness under reduced pressure, and the fat was redissolved in a measured volume of ether for a preliminary examination with a Unicam S.P. 500 spectrophotometer. After the solvent had again been removed the fat was saponified on a boiling-water bath with 0·44 ml. of a saturated aqueous solution of KOH and 2 ml. of ethanol/g. fat. The unsaponifiable residue, dissolved in light petroleum (b.p. 40–60°) was fractionated by chromatographic adsorption on a column (1 cm. diameter, 5 cm. length) of Al_2O_3 (Brockmann, Grade 2) followed by elution with 100 ml. portions of light petroleum containing increasing quantities of ether. In later experiments the preliminary extraction with ether was omitted, and the tissues were digested directly in hot 25% ethanolic KOH.

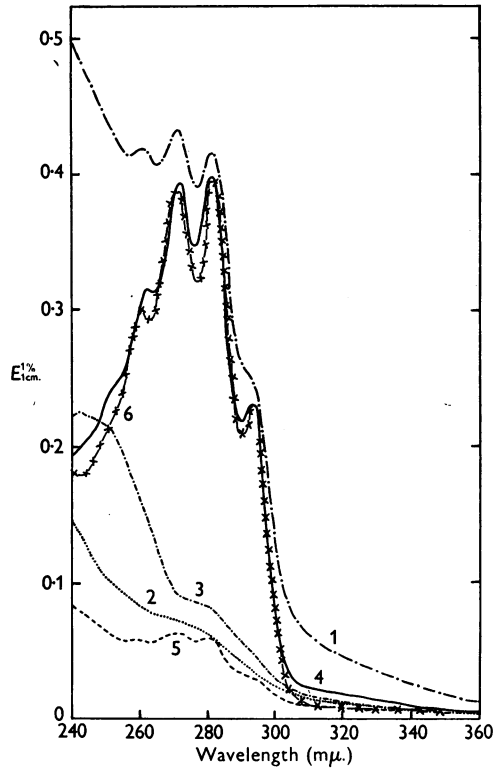

Fig. 1. Ultraviolet absorption spectra in ether of fractions obtained from the preputial glands of piebald rats by chromatography on alumina. 1, crude unsaponifiable matter ($\frac{1}{2}$ scale). 2–5, eluates obtained with light petroleum containing ether; 2, 5% ether; 3, 10% ether; 4, 20% ether; 5, 30% ether. 6, Pure 7-dehydrocholesterol reduced to same scale as 4.

With each procedure the extracts were usually examined spectrophotometrically at each stage. Extinction coefficients $(E)_{1\,cm.}$ have been calculated on the basis of an extract from 1 g. of the original tissue being dissolved in 100 ml. of solvent. It will be understood that E falls as the 7-dehydrosterol is lost during purification or divided between different fractions.

Fig. 1 shows how chromatography increased the clarity of the absorption spectrum of 7-dehydrosterol by the removal

of substances having non-selective absorption. The extract in this experiment was made from the preputial glands of male rats by direct digestion with KOH, and after adsorption of the unsaponifiable residue the 7-dehydrosterol was in this instance eluted by light petroleum containing 20 % of ether. The absorption maximum of the crude extract was at 272 mμ., but after chromatography the maximum was transferred to 282 mμ., with changes in the relative intensities of the other maxima to produce an absorption spectrum closely similar to those of the pure 7-dehydrosterols. This change was obviously due to the removal of substances having unselective absorption increasing in intensity at the shorter wavelengths.

The location of 7-dehydrosterol in the sexual organs of the rat. Fig. 2 indicates the regions in the sexual organs of the adult male rat in which 7-dehydrosterol was detected. The highest concentration, as judged by measurements on the crude fat,

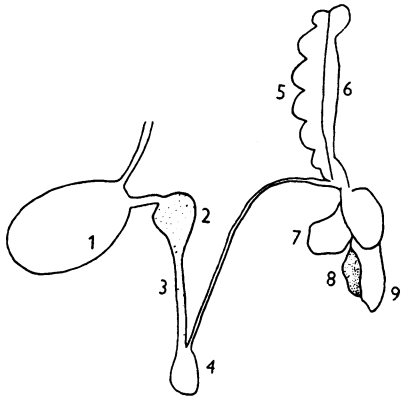

Fig. 2. Reproductive system of the male rat. The dotted areas indicate the positions at which the 7-dehydrosterol was detected. 1, testis. 2, caput epididymis. 3, corpus epididymis. 4, cauda epididymis. 5, seminal vesicle. 6, coagulating gland. 7, prostate. 8, preputial gland. 9, penis.

was found in the preputial glands; for piebald rats our richest extract had *E* (272 mμ.), 0·92. The next highest concentration was in the caput epididymis, with a maximum value for *E* (272 mμ.) of 0·22 for a similar crude preparation, and the corpus epididymis followed with *E* only 0·087. No absorption indicative of 7-dehydrosterol was observed in the testes, cauda epididymis, prostates, seminal vesicles or coagulating glands. In rats only 50 days old, which were still sexually immature, no selective absorption was found even in extracts of the caput epididymis.

In mature female rats the bulbi vestibuli, which appear to resemble the preputial glands of the male in form and function, contained about the same amount of 7-dehydrosterol, with *E*, 0·74. An extract was also made from the material which could be squeezed out from the bulbi vestibuli, and the spectrum of 7-dehydrosterol was again found with *E*, 1·31. The same methods of extraction failed to show any 7-dehydrosterol in the ovaries, oviducts and uteri. In an extract from one collection of vaginal plugs, the small lumps of cheese-like material which are left in the vagina after copulation, an absorption band was found at 268 mμ. and inflexions at 297, 278 and 258 mμ. This spectrum was consistent with that of 7-dehydrosterol displaced towards shorter wavelengths by superimposed general absorption. In an extract from another collection of plugs, bands or inflexions were again found not far removed from the positions characteristic of 7-dehydrosterol at 294, 280, 269 and 264 mμ. Additional bands of unknown significance, however, were also seen at 315 and 300 mμ., with inflexions at 348 and 320 mμ.

In order to find out whether similar intense selective absorption could be observed in a site recognized as being rich in cholesterol, we examined an extract made from rats' brain. When our experimental procedure was applied in the usual way no selective absorption was observed.

The effect of diet and the breed of the rat. When the caput epididymis of rats which were deficient in vitamin A was irradiated it did not show the faint-yellow fluorescence which was the starting point of these studies. Spectrophotometric comparisons between crude extracts made from the caput epididymis of rats deficient and adequate in vitamin A, however, indicated the presence of about equal amounts of 7-dehydrosterol. Thus for the deficient animals *E* (272 mμ.) was 0·19, as compared with 0·20 after supplements of 3 i.u. daily had been given and 0·22 after 320 i.u. daily for 15 days. In animals which had been kept on an adequate stock diet *E* was 0·19. For rats deficient in vitamin D, the one value recorded for *E* was 0·11, but since the rats were much younger than in the other groups the significance of this finding is questionable.

Dried brewer's yeast, which is rich in ergosterol, is a component of the basal diet used in this laboratory for inducing vitamin A deficiency, and it is also sometimes given to our stock animals. Although it is generally held that ergosterol is not absorbed into the tissues it was considered advisable to examine organs from wild brown rats, in whose diet yeast would be an unlikely constituent. Absorption characteristic of 7-dehydrosterol was again found, with *E* (272 mμ.), 0·18 for the caput epididymis and 1·54 for the preputial glands. These observations also indicated that the concentration of 7-dehydrosterols in these sites is not confined to one breed of rat.

The antirachitic activity of an irradiated extract of rats' preputial glands. The apparently high concentration of 7-dehydrosterol in the preputial glands suggested that extracts after a brief ultraviolet irradiation should be strongly antirachitic. In order to test this inference, the glands of forty normal rats, weighing 6·86 g., were treated with ethanolic KOH. The crude unsaponifiable residue weighed 0·35 g. and *E* (282 mμ.), calculated on the original tissue, was 0·61. On the basis of $E^{1\%}_{1\,cm.} = 310$ (Morton, 1942) for the pure substance, this was equivalent to a maximum of about 13·5 mg. of 7-dehydrosterol. By chromatography *E* was decreased to 0·20, equivalent to a total yield of only about 4·4 mg. of the sterol. The partly purified material was dissolved in ether and three portions, each containing about 1·3 mg. of 7-dehydrosterol, were irradiated for periods of 2, 3·5 and 5 min. at a distance of about 5 cm. from a large Hanovia ultraviolet lamp. Spectrophotometric examination of each solution indicated that this treatment caused loss of the bands at 262 and 294 mμ., but since no band appeared at 265 mμ. it was obvious that the efficiency of the formation of vitamin D was very low. Finally, the ether was evaporated, and the residue from each portion was dissolved in arachis oil for testing upon rachitic rats by the radiographic method (Bourdillon, Bruce, Fischmann & Webster, 1931).

During a curative period of 8 days groups of four animals each were given the irradiated products, in total doses corresponding to about 0·14 mg. of 7-dehydrosterol. Without allowing for losses during manipulation each treated rat received the product made from three of the original glands. In every animal advanced healing of the rickets was observed, corresponding to numbers 6–8 on the radiographic scale of healing (Bourdillon *et al.* 1931). In four rats which were dosed only with arachis oil the severity of the rickets remained unaffected, with a scale number of zero.

Organs from other animals. The extension of our observations to other animals was sometimes handicapped by difficulties in recognizing the parts of the genital system which corresponded to those already studied in the rat; in particular it was difficult to distinguish between the three regions of the epididymis.

In a human epididymis there was no evidence of 7-dehydrosterol, but bands at 278 and 284 mμ. were found in the part taken to be the cauda. The testis had no selective absorption in the ultraviolet, but contained carotene and lycopene. In the epididymis of the bull there was again no evidence of 7-dehydrosterol, but extracts from both the caput and the cauda had a band at 260 mμ. No selective absorption was found in the caput epididymis in the guinea pig, rabbit or ram, in glands in the rabbit resembling the rats' preputial glands, or in the preen gland of the duck.

DISCUSSION

Localized sites for the formation of 7-dehydrosterol. Glover *et al.* (1952) have studied the equilibrium between cholesterol and 7-dehydrocholesterol in the intestines of the guinea pig, and have shown that when cholesterol is administered the concentration of 7-dehydrocholesterol is greatly increased. It is clear that the desaturation is readily effected, presumably by the enzyme system which these workers postulate. Our own evidence on the distribution of 7-dehydrosterol in the sexual organs of the rat suggests further that its formation may be highly localized, and may take place in sites which are remote both from the route of absorption of dietary sterols and from the concentrations of cholesterol in the brain and nerves.

Thus the caput and corpus epididymis, which are rich in 7-dehydrosterol, are connected in one direction with the testes and in the other with the cauda epididymis, in neither of which could substantial amounts of 7-dehydrosterol be demonstrated. Similarly, isolated concentrations of the sterol occur in the preputial glands and bulbi vestibuli, but these glands differ from the epididymis in being associated with the genital system only in so far as their ducts are directed externally towards the openings of the penis and vagina, respectively. Another detached locus for the formation of 7-dehydrosterol has already been found by Morton & Rosen (1949) in the ovaries of the frog.

The extent to which 7-dehydrosterol was concentrated in the sex organs of our rats may be appreciated by comparing our values with those found by Glover *et al.* (1952) for the intestinal tissues of their guinea pigs, which were considered to be especially rich in 7-dehydrosterol. In the caput epididymis and preputial glands of the piebald rat our extinction coefficients observed on the crude extract, and roughly corrected for irrelevant absorption, indicated concentrations of 0·4 mg. and 2 mg. of 7-dehydrosterol/g. of wet tissue, respectively. In comparison, Glover *et al.* (1952) found the intestines of normally nourished guinea pigs to contain only 0·1–0·4 mg./g.; very much increased levels of 7-dehydrosterol were found in animals which had been given massive doses of cholesterol.

7-Dehydrosterol and the sex functions. Evidence suggesting a function for the high concentrations of 7-dehydrosterol in the genital system of the rat is scanty, but it is perhaps significant that we found indications of its presence in the plugs of clotted semen formed in the vagina after copulation. In rabbits which have been ovariectomized after fertilization, Feyel Cabanes (1949) has claimed that the administration of 7-dehydrocholesterol reduces the dose of progesterone which is necessary to maintain gestation.

Preputial exudate as a source of provitamin D. Hou (1930) reported that the material secreted by the anal preen glands of birds contains provitamin D. It was claimed that the bird distributes the secretion over its feathers with its beak, and is protected from rickets by ingesting some of the same material, after exposure to sunlight, during subsequent preenings. As yet we have no evidence that the rat imitates the bird by using the secretion of its preputial glands for grooming its fur, but it is clear that a rich source of provitamin would be available. Thus in each of two adult wild rats we found that the glands contained about 0·4 mg. of 7-dehydrosterol, which theoretically could yield 16 000 i.u. of vitamin D if efficiently converted. Although our experiments suggest that the preputial exudate could readily be activated by irradiation, the nocturnal habits of the rat raise doubts that a mechanism similar to that in the bird could be effective. Rosenberg (1953), moreover, has failed to confirm that the preen glands of geese, ducks and chickens contain provitamins, which have been detected in high concentrations in the feet.

Other animals. Our persistent failure to detect 7-dehydrosterol in the sex organs of animals other than the rat indicates that there must be at least minor differences in sterol metabolism between different species among the higher animals. Only single examinations were made on sex organs from man and the ox, which showed absorption bands not due to 7-dehydrosterol. Further research would be necessary to decide whether these bands are con-

sistently present, and if so whether they indicate important peculiarities in the sexual biochemistry of the animals concerned.

SUMMARY

1. A 7-dehydrosterol, presumably 7-dehydrocholesterol, was detected spectrophotometrically in high concentrations in the caput epididymis, corpus epididymis and preputial glands of male rats, and in the bulbi vestibuli of female rats. It could not be detected in other parts of the male or female genital systems.

2. The concentration of 7-dehydrosterol in its sites of deposition was not influenced by substituting a stock diet for an experimental diet, nor by varying the intake of vitamin A. About the same amounts of 7-dehydrosterol were found in wild brown rats as in tame piebald rats. No 7-dehydrosterol was detected in the rudimentary epididymides of sexually immature rats.

3. An irradiated extract from preputial glands had strong antirachitic activity.

4. No evidence of similar concentrations of 7-dehydrosterol was found in the male sex organs of the limited number of other animals investigated.

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The Activity of the Cytochrome System in Muscle and its Relation to Myoglobin

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Data secured during an investigation of factors affecting the percentage of myoglobin in muscle (Lawrie, 1950) had confirmed that a high concentration of this pigment is usually found in muscles of high physiological activity. This is of particular interest in view of the strong affinity of myoglobin for oxygen which enables it to act as an intramuscular oxygen store for the tissue's principal oxidizing catalysts, the cytochromes (Hill, 1936; Millikan, 1937). Keilin (1925) had early pointed out that all aerobic cells contain cytochromes, and that the concentration of cytochromes, as determined spectroscopically, is highest in the most actively respiring cells. Nevertheless, the concentration of cytochromes has been reported both as being higher (Fujita, Hata, Numata & Ajisaka, 1939) and lower (Hill, 1936) in myoglobin-rich or 'red' muscles than in the 'white' variety. It therefore appeared desirable to study the distribution of certain components of the cytochrome system in muscle and their relation to the content of myoglobin. A preliminary account of this work has already been published (Lawrie, 1952).

EXPERIMENTAL

An assessment of the cytochromes in muscle involves consideration of a series of components. Of these, only cytochrome *c* can be estimated quantitatively as protein. The determination of cytochrome *c* itself affords to a certain extent a measure of the cytochrome system as a whole, since its concentration in various tissues has been shown to follow closely the rate of O_2 uptake (Junowicz-Kocholaty & Hogness, 1939). However, it was the immediate relation of the cytochrome system to O_2 which was of interest in the present study. It was thus considered more appropriate to determine the system's power of catalysing the uptake of O_2 by assessing the activity of cytochrome oxidase (Keilin & Hartree, 1939; Slater, 1949*a*), which can be estimated by the rate of oxidation of ascorbic acid or *p*-phenylenediamine in the presence of excess cytochrome *c*. For the determination, the procedure of Slater (1949*b*) has been followed, as