CCCX. THE OCCURRENCE OF AN OESTROGENIC SUBSTANCE IN THE SEXUAL SKIN OF MONKEYS.

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OESTROGENIC substances can be recovered from the ovaries, placenta, urine, blood, faeces, bile, colostrum, testes, adrenals and pituitary [Doisy, 1932; Callow & Parkes, 1936]. Until recently there has been no evidence of their presence in tissues on which they specifically act. The present paper is an account of their occurrence in the sexual skin of monkeys, a tissue whose activity, it has been found, is controlled by oestrin.

The sexual skin in the female is generally restricted to the circumanal and circumgenital zones. These regions become highly coloured, and in certain species considerably swollen, during the first or follicular phase of the menstrual cycle (for details as to species variation, see Zuckerman [1930]). Male monkeys do not normally exhibit a sexual skin, but it has been found recently that the injection of oestrone induces considerable swelling of the anus, scrotum, and surrounding regions [Dohrn *et al.*, 1933; see Zuckerman & Parkes, 1936, for further references].

Both normal and experimental sexual skin and experimental scrotal swellings appear on section as a jelly-like avascular tissue. The tissue is either colourless or faintly blue; usually it is odourless, but occasionally it smells unpleasantly. The cut surfaces exude a pale straw-coloured grumous fluid, which in amount varies with the extent of surface and the degree of swelling.

The effect which sexual skin swelling has on the water balance is discussed elsewhere [Krohn & Zuckerman, 1936].

Material and technique.

Tissues from three female Hamadryas baboons (*Papio Hamadryas*), a female Chacma baboon (*P. porcarius*), a male Hanuman langur (*Presbytis entellus*), and sixteen male and female rhesus monkeys (*Macaca mulatta*), were used in this investigation.

Sexual skin swelling in baboons is considerable, and is confined to the skin immediately surrounding the vulva and anus. Corresponding changes do not occur in the Hanuman langur. In the rhesus monkey the changes vary with age. In the pubertal female the first phase of sexual skin activity mainly takes the form of a blister-like swelling of the tissues immediately ventral to the pudendal cleft, the swelling being somewhat pear-shaped. The body of the pear is in front, and is formed by the expansion of a transverse pubic flap of skin, usually referred to as the scrotal folds; the apex of the neck of the pear is formed by the praeputium clitoridis. The degree of blister swelling decreases in successive phases of sexual skin activity, and in adolescence the pubic swelling is not so much oedematous as rugose, and the area affected extends more and more away from the genital region. In some cases, lobulated swellings reach well above the iliac crests. In

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fully mature rhesus females swelling of the sexual skin is unusual, the only obvious change undergone by the skin being one of colour. The extent of the area that colours is variable, but the most intense coloration occurs in the sexual skin proper, the area immediately surrounding the pudendal cleft and anus.

The corresponding changes that can be experimentally induced in the young male rhesus show the same variations in character relative to the length of the time during which oestrin is administered. The first change noticed, towards the end of a week's injections of 100 γ of oestrone daily, is a thickening of the scrotal skin and an eversion of the anal margin. From that time onwards the changes are rapid. By the middle of the second week the anus usually forms a prominent tense and deep-red swelling, which begins to be resorbed about the end of the second week of injections. The scrotum and penile sheath become more and more swollen, the swelling, as in the pubertal female, being pale pink and somewhat translucent. As the period of injections extends, the scrotal swelling decreases, and the reactive area, as in the female, becomes more extensive.

The material examined comprised sexual skins, exudate of the sexual skin, and a number of other tissues, all of which are denoted in Table I.

Table I. *Tissues tested for oestrogenic action.*

Species	Number	Sexual skin or scrotum	Sexual skin exudate	Blood serum	Liver	Kidneys, spleen, pancreas	Stri- ated muscle	Extra- genital skin	Mam- mary gland
<i>Papio Hamadryas</i>	E.U. 2	x	—	—	—	—	—	—	—
"	E.U. 4	x	x	—	—	—	—	—	—
"	E.U. 5	x	—	x	—	—	—	—	—
<i>Papio porcarius</i>	O.M. 105	x	x	x	x	x	x	—	—
<i>Presbytis entellus</i>	O.M. 82	—	—	x	—	—	—	—	—
<i>Macaca mulatta</i>	O.M. 60	x	—	—	—	—	—	—	—
"	O.M. 63	x	—	x	—	—	—	—	—
"	O.M. 72	x	—	x	—	—	—	—	—
"	O.M. 73	x	—	—	—	—	—	—	—
"	O.M. 24	x	—	—	—	—	—	—	—
"	O.M. 36	x	—	—	—	—	—	—	—
"	O.M. 79	x	—	x	x	x	x	x	x
"	O.M. 19	x	—	—	—	—	—	—	—
"	O.M. 23	x	—	—	—	—	—	—	—
"	O.M. 25	x	—	—	—	—	—	—	—
"	O.M. 40	x	—	—	—	—	—	—	—
"	O.M. 41	x	—	—	—	—	—	—	—
"	O.M. 42	x	—	—	—	—	—	—	—
"	O.M. 135	x	—	—	—	—	—	—	—
"	O.M. 136	x	—	—	—	—	—	—	—
"	O.M. 137	x	—	—	—	—	—	—	—

Tissues from both normal and experimental animals were used, some of the normal animals being in an active, others in a quiescent sexual skin phase. The experimental animals had been injected either with oestrone alone, with both oestrone and progesterone, with oestrone and cholesterol, or oestrone and epicholestanol (the injections having been given primarily for the purpose of another investigation). Except where otherwise stated in Column 4 of Table II, all injections were given daily for 14 days.

Animals which had been injected with two preparations were included in the present investigation since the results of the injections showed plainly that the effects of oestrone dominated those of the other injected substance—at least in so far as external changes are concerned. The character of the scrotal and anal swellings in these animals was no different from that in animals injected with oestrone alone.

Care was taken in the treatment of the experimental animals to keep the point of injection as far from the scrotal region as possible, so as to obviate any

Table II. *Condition of animals studied.*

Designation of animal	Body wt. (kg.)	Oestrone (γ /day)	Progestosterone (γ /day)	Epicholesterol (mg./day)	Cholesterol (mg./day)	Days of treatment	Degree of sexual skin swelling at death	Oestrogenic activity of extracts of sexual skin
O.M. 24	3.4	50	—	—	—	14	Moderate	Negative
O.M. 36	2.5	50	—	—	—	14	Pronounced	"
O.M. 79	2.9	100	—	—	—	15	"	Positive
O.M. 19	3.1	200	500	—	—	14	"	Negative
O.M. 23	3.1	50	400	—	—	14	Slight	Positive
O.M. 25	4.3	50	300	—	—	14	Pronounced	Negative
O.M. 40	4.1	50	500	—	—	14	"	"
O.M. 42	4.0	50	1000	—	—	14	"	"
O.M. 135	2.1	100	—	—	5	14	Moderate	"
O.M. 136	2.4	100	—	5	—	14	Pronounced	"
O.M. 137	3.0	100	—	10	—	14	"	"
E.U. 4	10.0	Normal	—	—	—	Not treated	Full	Positive
E.U. 2	10.7		—	—	—		"	Negative
O.M. 105	14.6		—	—	—		Abnormally persistent	Positive: slight
E.U. 5	—		—	—	—		None	Negative
O.M. 60	4.9		—	—	—		"	"
O.M. 63	3.4	Normal	—	—	—	Not treated	"	"
O.M. 72	3.4		—	—	—		"	"
O.M. 73	5.4		—	—	—		"	"
O.M. 82	4.1		200	—	—		"	—
O.M. 41	3.6		50	750	—		"	Negative

possibility of the introduction of the hormone into tissue which was later to be extracted. Injections were given intramuscularly.

The cutaneous and subcutaneous tissues comprising the sexual skin or scrotum were completely dissected at autopsy from the underlying muscle. The swollen sexual skins in the three baboons, E.U. 2, E.U. 4 and O.M. 105, weighed 0.9 kg., 1.092 kg. and 3.574 kg. In the case of the male rhesus monkeys the anus and surrounding skin when swollen were occasionally included with the scrotum, the maximum weight of oedematous tissue extracted in the case of any male monkey being 165 g.

With the exception of two samples of exudate from the sexual skin, and certain of the blood sera, which were injected into test mice without previous extraction, all the tissues tested were first extracted for oestrin according to the method outlined by Parkes & Bellerby [1926].

EXPERIMENTAL RESULTS.

The preparations were tested for oestrogenic activity by the standard method on gonadectomized mice. Control tests were also run, with the same technique of injection and vaginal examination, using a suitably diluted solution of crystalline oestrone, kindly provided by the Department of Biological Standards, National Institute for Medical Research.

Clear positive results were obtained only with the sexual skin (or scrotum) of O.M. 23, E.U. 4 and O.M. 79, with the exudate of E.U. 4, and the livers of O.M. 79 and O.M. 105.

Sufficient material for complete biological assay was available only in the case of the first two, which contained the equivalents of 95 γ and 27 γ per kg. of tissue respectively. The amounts present in the other four were in the region of 6 γ per kg., 67 γ per litre of exudate, and 15 γ and 7 γ per kg. respectively.

Evidence of some oestrogenic activity (i.e. pro-oestrous smears) was also provided by several of the other tissues examined (e.g. O.M. 105, sexual skin,

O.M. 105, exudate of sexual skin). Completely negative results were obtained with the blood serum of O.M. 82, using amounts of extract equivalent to 11 ml. of serum, and with the blood serum of O.M. 79 (7 ml. of serum). A pro-oestrous smear was obtained with the equivalent of 50 ml. of the blood serum of O.M. 105. The equivalent of 60 g. of the striated muscle of O.M. 105 proved negative, as was also the equivalent of 30 g. of the extra-genital skin of O.M. 79. No indications of oestrogenic potency were given by extracts of the tissues of any of the normal animals which at autopsy were in a quiescent sexual skin phase.

Our failure to obtain more conclusive results was due not only to the small amounts of tissue available for extraction but also to the difficulty of securing emulsions for injection which were on the one hand sufficiently concentrated and on the other sufficiently fluid.

DISCUSSION.

Since our methods of extraction were relatively crude and did not include preliminary hydrolysis, the many negative results we obtained cannot be taken to indicate that the tissues concerned were free of oestrogenic substance. On the other hand, our positive results show that some free oestrin, the term being used to denote unspecified oestrogen, is present in the sexual skin, the fluid of the sexual skin and the liver. Since several swollen sexual skins gave negative results, no particular weight can be attached to the fact that negative results were always obtained with tissues from animals whose sexual skins were inactive. Moreover our data do not provide any explanation for the varying amounts of oestrogen found in the swollen sexual skins or oedematous scrota we examined. It is significant, however, that positive results were obtained with both the naturally and the experimentally active circumgenital skin, and that four out of fourteen swollen skins yielded active extracts, whereas none of the seven which were not swollen gave positive results.

The completely negative result obtained with the blood serum of O.M. 82 should be noticed in the evaluation of these facts. Frank *et al.* [1932] have shown that intravenously injected oestrin very rapidly ceases to be identifiable in the blood. Fee *et al.* [1929] found the same with the help of a heart-lung-kidney preparation. If no oestrogen could be extracted from the blood of an animal which had received as much oestrone as O.M. 82 had (200 γ daily for 39 days), it is very unlikely that the oestrogenic substance we did succeed in extracting from three sexual skins and two livers was in solution in the blood contained in these organs.

It is well known that large amounts of oestrin are present in the urine of pregnant women. Cohen *et al.* [1935] found that only 1 % of the oestrin present during the first 8 months of gestation is "free", the rest being "combined" (in the case of oestriol, in the form of an oestriolglucuronide; Cohen & Marrian [1936]). According to Zondek [1934], most of the oestrin injected into an experimental animal is converted into the "combined" form, and then stored in striated muscle. He believes that the process of combination takes place in the liver, and our finding of "free" oestrin in that organ may be related to his view. Frank *et al.* [1935] have recently been able to liberate, by some method of which the steps are not given, large amounts of oestrogen from striated muscle (up to 8000 M.U. per kg. dried muscle). A similar amount was extracted from a human uterus removed during the intermenstrual period; and, by postponing ether extraction to the later stages of purification, the yield from blood has also been increased fourfold [Frank & Goldberger, 1935]. In the circumstances it is reasonable to suppose that the yield from an active sexual skin would be

increased after preliminary hydrolysis; we propose to investigate this point as soon as further material and facilities become available.

The possibility that the presence of "free" oestrin in the sexual skin and in its interstitial fluid is indicative of selective concentration at a site of action requires investigation. It is not unlikely that oestrin is "absorbed" by the cellular elements of the swelling in the sense in which Zondek states it is by striated muscle. If it were, it would presumably be absorbed in the "combined" form. Accordingly, if the sexual skin proves to be a localized part of the body's subcutaneous tissues in which oestrin is stored, it may prove to be possible to relate the oedematous process which it undergoes to the fairly generalized oedema which is a not uncommon occurrence in human pregnancy, during which the major part of the oestrin elaborated becomes combined, presumably in the tissues.

Our finding of "free" oestrin in a tissue which is specifically sensitive to the hormone is also of interest in view of the opinion that the occurrence of oestrogenic substances in benign tumours is indicative of their aetiological responsibility for the condition [Geschickter *et al.*, 1934; Ingleby, 1935; Witherspoon, 1935]. The practical importance of this view clearly indicates the necessity for the further investigation of the probable normal selective concentration of oestrin at a site of action.

SUMMARY.

Various tissues of eight normal monkeys and thirteen monkeys which had been injected with oestrone were tested for oestrogenic potency. Positive results were obtained with the active sexual skin, the exudate of the sexual skin, and the liver.

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