612.62:612.018].612.461.269
612.62:612.018].577.81

THE SIGNIFICANCE OF THE OCCURRENCE OF ŒSTRIN IN MALE URINE.

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I. Introduction.

The work recorded in this paper was concerned with three related problems: (*a*) the reported presence of an œstrus-producing substance in male urine, and its identity with that obtained from female sources, (*b*) the significance of the occurrence in the male of what has been regarded as a female sex hormone, and (*c*) the nature of the process by which œstrin reaches the urine.

As regards the occurrence of an œstrus-producing substance in the male, it has been reported as present in the testis (1, 3), the blood (4, 6) and the urine (7, 8, 10). In most cases, however, vaginal cornification appears to have been the only criterion of œstrus used, and since this reaction is now known not to be entirely specific for œstrus (2) it seemed desirable to test extracts from male sources by at least one other criterion of œstrus. Investigation showed that the substance present in male urine was almost certainly œstrin, and it was then endeavoured to ascertain whether it had any essential connection with the testis, or whether its occurrence in the male was merely accidental.

Finally, the renal secretion of œstrin was studied by means of the isolated heart-lung-kidney preparation.

II. The presence of œstrin in male urine.

Samples of urine were collected from individual workers in the laboratory over a period of about 8 hours. Each sample was preserved by the addition of a little toluene during the collection. As soon as possible after collection the specimens were evaporated to a small bulk under reduced pressure and the concentrated residue dried with anhydrous

Na_2SO_4. This dried material was then thoroughly extracted with ether for several hours in a Soxhlet apparatus. The ether extract, after washing 4–6 times in a funnel with water, was evaporated to dryness, the last traces of solvent being removed *in vacuo*. The thorough washing of the ether extract with water has been found to be of considerable importance, since the extracts proved to be highly toxic to mice when this precaution was not taken.

The residue, after drying, was again treated with about 5–10 c.c. ether and filtered from small quantities of amorphous pigments that were not readily soluble in ether. The filtrate was then again evaporated to dryness, weighed and emulsified in 1–2 c.c. of 0·5 p.c. Na_2CO_3. The addition of a few drops of olive oil was found to result in smoother and more stable emulsions. This emulsion was then injected subcutaneously into one or two ovariectomized mice and the vaginal smears recorded every 12 hours. The results are shown in Table I.

TABLE I.

No. of extract	Source	Vol. urine c.c.	Weight total ether soluble material g.	Weight injected into each mouse g.	Mice used (O.M.)	Result
U.M. 5	A	1150	0·291	0·14	97	Cornified vaginal smear
					98	,, ,,
,, 6	B	800	—	—	113	Negative
					114	Cornified vaginal smear
,, 7	C	795	0·185	0·09	50	Negative
,, 9	C	630	0·199	0·09	53	Cornified vaginal smear
					54	,, ,,
,, 10	A	730	0·115	0·06	55	,, ,,
					57	,, ,,
,, 11	D	855	0·214	0·10	82	,, ,,
					84	,, ,,
,, 12	A	975	0·199	0·09	85	,, ,,
					86	,, ,,

These results confirm those of Loewe(10) and of Laqueur(8) and their co-workers who showed that substances producing cornification of the vagina of ovariectomized animals can be obtained from the urine of normal healthy human males. It was felt, however, that it would be unjustifiable to assume the identity with œstrin of the substance responsible unless criteria of œstrus other than cornification of the vagina were also satisfied. Examination of several of the test mice that gave positive vaginal reactions showed the uterus to be in the typical anœstrous condition. However, these animals were all killed towards the end of the induced cornification and any effect on the uterus may have passed

unobserved. In addition, from observations carried out on mice injected with placental œstrin, it seemed possible that amounts of the hormone sufficient to produce cornification of the vagina might be insufficient to produce growth of the uterus. Accordingly it was decided to work up a large amount of male urine and inject into a number of mice quantities of the extract which would undoubtedly cause growth of the uterus if the substance concerned was œstrin.

Forty litres of urine from nine different workers in the laboratory were collected under toluene and extracted four times with ether as soon as possible after collection. The ether was evaporated, and the aqueous residue heated for 10 minutes with 100 c.c. of 5 p.c. aqueous KOH. This partially saponified mixture was cooled, diluted with water and extracted with ether four times. The ether extract after being washed four times with water to remove excess of alkali and soap, was evaporated to dryness *in vacuo* and weighed. In this way 0·1456 grm. of a brown oily material was obtained. A portion of this was emulsified in 0·5 p.c. Na_2CO_3 with the addition of a few drops of olive oil and injected into two ovariectomized mice. Each mouse received about 24 mg. of the extract, which was given in six separate injections over 36 hours, in order to prolong the action. Vaginal smears were recorded every 4 or 5 hours. Both animals were killed 5 hours after the first signs of cornification in the vagina. In each case the uterus was in the typical swollen condition characteristic of œstrus.

There can be little reason to doubt that the substance occurring in male urine responsible for these phenomena is indeed identical in its physiological action with œstrin prepared from ovaries or placenta.

The problem now arose as to the source of this œstrin in the male. Two possibilities presented themselves. (*a*) That it was produced in the body. (*b*) That it was present in the food and was absorbed through the alimentary tract. The first hypothesis receives some support from the reports of the presence of œstrus-producing substances in testis (3). On the other hand, the fact that œstrus-producing substances can be obtained from various plant sources (5, 11) and that œstrin may have a slight activity when administered by the mouth (9) lends support to the latter alternative.

An attempt was made to investigate this problem by collecting and testing the urine of male rabbits before and after castration. Five extracts were made from quantities of urine from normal male rabbits, varying from 1500 to 3100 c.c., in the manner described in the last experiment. Each extract was injected into two ovariectomized mice.

It was found, however, that these extracts were frequently highly toxic and, in addition, only one of the five extracts made proved to have any œstrus-producing activity at all. Since it seemed to be impossible to demonstrate the presence of œstrin in the urine of the normal male rabbit with any regularity, this experiment was abandoned.

III. Renal excretion of œstrin.

McClendon, Burr and Conklin(12) state that œstrin may be detected in the urine some few hours after subcutaneous injection, but little information appears to be available with regard to the mechanism whereby it gets into the urine.

It was therefore decided to study the excretion of œstrin on the isolated kidney.

For this purpose the isolated heart-lung-kidney preparation was used. The operative technique was essentially the same as described by Starling and Verney(14). The kidneys were perfused at arterial blood-pressures between 90–120 mm. Hg and the renal blood flow varied between 80–120 c.c. after the urine secretion started. Blood temperature was kept between 36·0–37·5° C. Usually 500–1000 c.c. of blood were in circulation. When the urine flow had definitely started after the insertion of kidney into the heart-lung-circuit, from 1–2 c.c. of urine were rejected in order to avoid contamination with any urine previously secreted and remaining in the renal pelvis and tubules. A 10 c.c. sample of urine was then collected (usually during the next 15–30 minutes), after which either 100 rat units or 200 mouse units of œstrin were added to the circulating blood. A further one or two 10 c.c. samples of urine were collected during the next 20 minutes. Blood samples, each of 10 c.c., were also taken before and after the addition of the œstrin. These were extracted and tested for their œstrin content. The results are summarized in Table II.

Table II. Renal secretion of œstrin in heart-lung-kidney preparation.

No. of exp.	Blood in circulation c.c.	Amount of œstrin added	Amount of urine sample			Rate of secretion c.c. per 10 mins.			Urine test		
			1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
1	600	100 r.u.	7·5	9·8	—	3·7	7·5	—	Neg.	Active in 5 c.c.	—
2	550	120 r.u.	14·0	12·0	12·0	7·8	8·6	15·0	„	Active in 6 c.c.	Neg.
3	1000	200 r.u.	10·0	10·0	—	5·0	10·0	—	„	„	—
4	1640	200 m.u.	10·5	20·0	20·0	5·2	—	40·0	„	Neg.	Neg.
5	450	200 m.u.	20·0	20·0	—	8·0	5·0	—	„	„	—

The following conclusions may be drawn from these results:

(*a*) Small amounts of œstrin may be found in the urine shortly after a large dose has been added to the circulation. Only about 1 p.c. was, however, recovered from the urine. The isolated kidney, therefore, does not actively secrete or concentrate the hormone. Its occurrence in the urine is probably the result of diffusion through either the glomerular or tubular epithelium.

(*b*) The hormone disappears from the blood of the heart-lung preparation very rapidly. It was detected in none of the samples of blood that were taken.

To throw further light on the fate of œstrin in the preparation, two further sets of experiments were performed: (*a*) addition of œstrin to standing blood and subsequent extraction, (*b*) addition of œstrin to a heart-lung preparation and extraction of the component parts, heart, lung and blood. The results showed:

(*a*) That œstrin is not appreciably destroyed by incubation in standing blood for 3 hours either at room temperature or at 37° C.

(*b*) Only a small amount of œstrin (? 3 units and ? 2 units respectively) is retained by the heart and lung tissue, and a 25 c.c. blood sample gave negative results 25 minutes after the addition of 200 M.U. to the 425 c.c. of blood in circulation.

Œstrin added to a heart-lung-kidney preparation is thus not excreted, or adsorbed by the heart and lung, in appreciable quantities, and since it is not destroyed in standing blood, it is probable that the very rapid destruction observed is due to oxidation as the blood circulates through the lungs.

Since such a small percentage of the œstrin added to the blood of such a preparation is eliminated by the kidneys the presence of large amounts in the urine of pregnant women suggests that very large quantities must be produced at this time.

We wish to thank Prof. E. C. Dodds who kindly supplied us with much of the œstrin used in the excretion experiments.

The expenses of this research were defrayed by grants from the Medical Research Council and the Royal Society.

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