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URINARY OESTROGENS IN THE MATURE MALE MULE

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Summary. The urinary excretion of oestrogens in mature male mules was estimated by means of the method of Brown (1955).

It was found that oestrone was the major oestrogen present in the urine of normal mules. Oestradiol-17β was also present, but in considerably smaller amounts; oestriol could not be detected. None of these three oestrogens was detected in any of the samples of urine from castrated mules.

The oestrogen excretion in the mule was compared to that in horse and the significance of the difference found was discussed.

INTRODUCTION

In seeking an explanation of the sterility of mules, a consideration of endocrine factors may be of interest. Most of the papers published on this interspecific mammalian hybrid concern its gametogenic function and chromosomal set, in comparison with the horse or donkey (Benirschke, Brownhill & Beath, 1962; Trujillo, Stenius, Christian & Ohno, 1962). Some of the workers described also the structure of the mule testicular interstitial tissue (Makino, 1955; Becze, 1958; Nishikawa, 1959). The information on the hormonal activity of this animal is scanty. Also the hormonal picture of mule testis has not yet been studied and the pattern of steroid excretion in the mule is still lacking.

The purpose of this study was to find out the pattern of urinary oestrogen excretion in the mature male mule. The blood oestrogens in the mule are under investigation and the results will be published later.

MATERIAL AND METHODS

Five sexually mature mules and three castrated mules, all from Bulgaria, were used in the experiment. The animals were 4 to 10 years of age, and weighed 200 to 280 kg. Only single urine samples were collected, owing to the technical difficulties of collecting over 24 hr. The samples were stored in a refrigerator without preservatives until used.

For the chemical determination of oestrogens the method of Brown (1955) was used, as modified by Diczfalusy & Westman (1956) and Salokangas &

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Bulbrook (1961). The chromatographic separation of oestrogen 3-methyl ethers was performed on Samoore alumina, deactivated with 7·5% of distilled water; Kober chromogens were extracted with the 2% *p*-nitrophenol/tetrachloroethane reagent (w/v), according to Salokangas & Bulbrook (1961). All reagents were of a standard grade and manufacture as required for the method.

No attempt was made to differentiate between 'free' and 'bound' oestrogens except in one urine sample which had been examined immediately after collection, and where no detectable amounts of unconjugated oestrogens were found. Also, no attempt was made to determine the relative proportions of oestradiol-17β and oestradiol-17α, but paper chromatography of mild hydrolysed urine extracts showed the presence of a compound with an R_f value identical with that of cristalline oestradiol-17α. As the absorption spectra suggested the presence of oestradiol-17β in the urine, the results were expressed in terms of this compound. The results of the recovery experiments were in agreement with those obtained in previous study (Pigoń & Marchut, 1963). No correction for the methodological losses was made.

RESULTS

When the method of Brown was applied to the acid hydrolysed male urine samples interfering chromogens were found to be present in the final fractions. These chromogens were always present in the oestradiol fraction and occasionally also in the others, even when saponification as an additional step of purification was included. A helpful way of eliminating this non-specific

TABLE 1

OESTROGEN CONCENTRATIONS IN THE URINE OF FIVE NORMAL MALE MULES (MG/1000 ML)

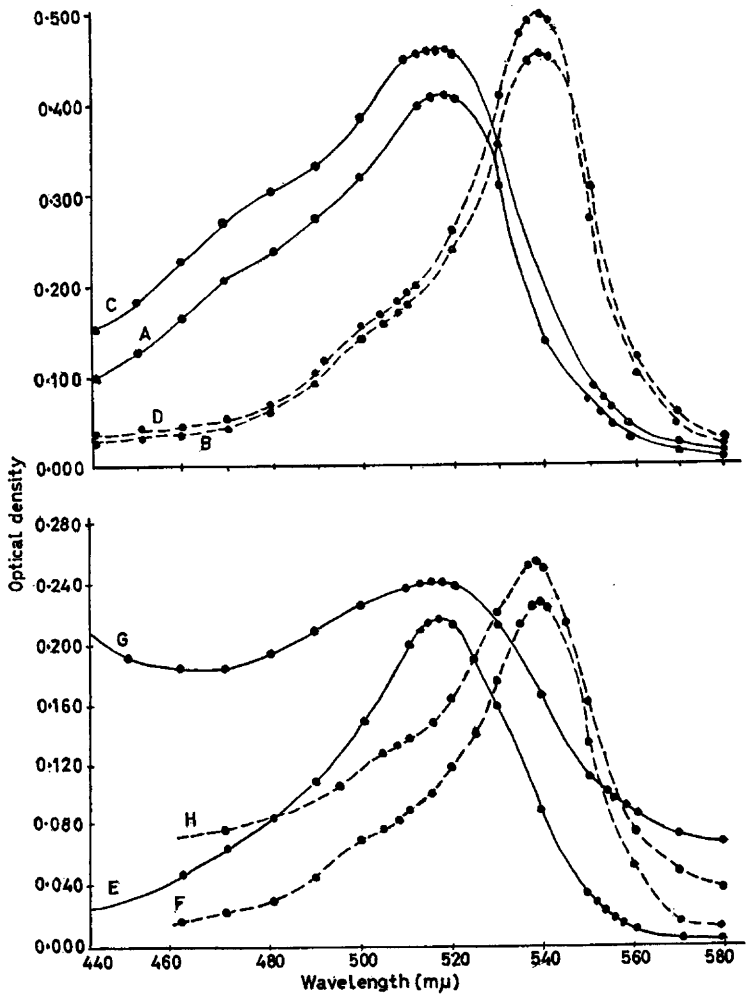
Animal No.	*Oestrone*	*Oestradiol**
1	2·91	0·303
2	0·521	0·108
3	1·38	0·276
4	0·492	0·088
5	1·06	0·230

* Includes oestradiol-17α and oestradiol-17β.

interference was the extraction of the Kober colour complex with a 2% solution of *p*-nitrophenol in tetrachloroethane. This procedure resulted in a considerable reduction of non-oestrogenic absorption due to urinary impurities and it increased considerably the sensitivity of the method; this was particularly important for the oestradiol-17β, since its titre in the urine was very low.

Text-fig. 1 shows the results of spectrophotometric examination of the mule urinary fraction of oestrone and oestradiol obtained by means of the original and modified Kober reaction. In the same figure are presented the spectral curves obtained by carrying out the reaction with the corresponding amounts of pure oestrone and oestradiol-17β methyl ethers. The absorption spectra of the urinary oestrone fractions can be seen to correspond well in both the original

and the modified Kober reactions with those of oestrogen standards. The spectrum of the oestradiol fraction, on the other hand, agrees reasonably well with the corresponding spectrum of standard only when the Kober reaction is combined with an extraction; in the original Kober reaction it shows a more flattened shape than the standard absorption curve, with higher spectrophotometric readings in the short wave range.



TEXT-FIG. 1. Absorption curves due to colour produced in Kober reaction; ———, without extraction; ------, after extraction with tetrachloroethane reagent; A, B, pure oestrone methyl ethers (10 μg); C, D, oestrone fractions from male mule urine (mule No. 2, 20 ml); E, F, pure oestradiol-17β methyl ethers (5 μg); G, H, oestradiol-17β fractions from male mule urine (mule No. 5, 12·5 ml); Beckman Model DU Spectrophotometer.

The results of the oestrogen assay carried out on the samples of urine collected from five normal male mules are shown in Table 1. They show that the major urinary oestrogen is oestrone, and that in addition, there are also present smaller amounts of oestradiol-17β. The oestriol fractions regularly showed a dark brownish colour when developed with the Kober reagent; no colour,

however, was obtained after Kober chromogen extraction, suggesting that little, if any, oestriol was present. None of these oestrogens was found in the urine of castrated mules.

DISCUSSION

From the evidence presented here it is justifiable to conclude that the male mule excretes at least two oestrogens. Oestrone is the principal urinary oestrogen. Oestradiol, excreted in amounts not exceeding 10 to 20% of oestrone, is a quantitatively less important metabolite. Oestriol was not detected in mule urine. However, to confirm the latter finding it would be necessary to use a more sensitive method for the detection and measurement of oestriol than that used in the present study.

The pattern of oestrogen excretion in the mule seems to be quite similar to that found in the horse (Pigoń, Lunaas & Velle, 1961), where the ratio of urinary excretion of oestrone to oestradiol was also about 10 : 1, and oestriol was absent from the urine.

Although the number of animals in the present study is too small to draw a valid comparison between horse and mule, the results obtained indicate that the secretion rates of oestrogens in the horse and its hybrid are different. It would appear that the mature mule excretes less oestrogen than the mature horse, and that the amounts of oestrogen excreted by a mature mule are of the same order as those present in the urine of a horse which had not yet reached the state of full sexual maturity. It may be that the low level of oestrogens excreted by the mule reflects weak steroid synthetic ability of its testes and can be related, at least to some extent, to the low degree of interstitial tissue development. On the other hand, there is also the possibility that the low oestrogen secretion is a characteristic which the mule 'inherited' from the jack-ass.

ADDENDUM

After the present paper was submitted for publication, the paper by King, Short, Mutton & Hamerton (1965) came to our notice. It is of relevance to the present work since it also concerns steroid production by the testes of equine hybrids.

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