

## A Rapid Method for the Quantitative Estimation of Urinary Oestrogens in the Pregnant Mare\*

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During an attempt to develop an easily practicable pregnancy test in the mare based on the presence of urinary oestrogens it was found that the procedure arrived at apparently allowed crude quantitative estimations of the oestrogens when specific photometric measurements were included. In this communication the applicability of the method for this purpose is evaluated.

The material consisted of urine samples collected from 60 mares during the months 5 and 6 after breeding. Of the urine 1 ml was pipetted into a thinwalled 100 ml flask and diluted with 10 ml water of room temperature. Care was taken to select uniform glassware. To the diluted urine 15 ml of conc.  $H_2SO_4$  were slowly added during swirling of the flask, and the reaction mixture (temperature ca. 110°C) was

\* A preliminary report on the application of the method as a pregnancy test has been presented at the 9. Nordiske Veterinærmede (Section E-5), Copenhagen 1962.

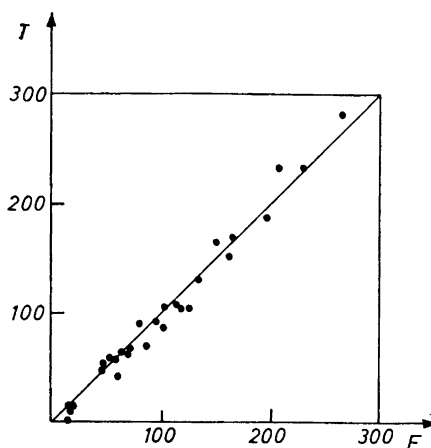


Fig. 1. Urinary oestrogen concentrations in mares calculated as  $\mu g$  oestrone per ml. Means of duplicate estimates obtained by colorimetry, T, and fluorimetry, F.

left warm for 5 min after which time it was diluted with 35 ml of water. Of this final dilution an aliquot of 15 ml was thoroughly cooled under the tap, and extracted with 5 ml of methylene chloride containing 2% (w/v) *p*-nitrophenol<sup>1</sup> which was then centrifuged and submitted to colorimetry. For fluorimetry an aliquot of 1 ml (corre-

Table 1. Precision of fluorimetric estimation of urinary oestrogens (calculated as oestrone) in the mare.

Range, $\mu g/ml$	Number, <i>n</i> , of duplicates	Standard deviation, <i>s</i> , of single estimates from their means, %	Fiducial range, <i>f</i> , of the mean, <i>M</i> , of two estimates ( $P = 0.05$ ), %
0-2	22	47.9	$M \pm 70.0$
2-6	11	43.3	$M \pm 67.5$
6-10	lacking	—	—
10-20	4	16.8	$M \pm 33.0$
20-40	lacking	—	—
40-100	11	8.6	$M \pm 13.4$
100 (standards)	18	11.9	$M \pm 17.6$
100-300	13	11.5	$M \pm 17.5$

$$s = \sqrt{\sum d^2 / 2n}, \quad d = \text{difference between duplicates as per cent of the mean.}$$

$$f = ts / \sqrt{2}$$

sponding to about 1/60 ml of urine) was similarly treated.

As standards 100  $\mu\text{g}$  of oestrone were taken through the procedure in the presence of 1 ml of urine from a non-pregnant mare. All urine samples and standards were developed in duplicate.

The optical densities were read at the wavelength of maximal absorption of oestrone, 533  $\text{m}\mu$ , and also 33  $\text{m}\mu$  to both sides. The corrected optical densities calculated as  $E_c = 2E_{533} - (E_{500} + E_{566})$  were assumed to be linearly related to the oestrogen concentration.

Fluorimetry of the methylene chloride extracts was performed with a Beckman model DU monochromator supplied with an attachment made in the laboratory (to be published) and a photomultiplier combination. As incident light an unfiltered beam of a wolfram continuum was used. The light emitted at a right angle of the incident beam was recorded at 550  $\text{m}\mu$  (maximal emission of oestrone) and at 520  $\text{m}\mu$ . The slit of the monochromator was kept at 0.1 mm corresponding to a half intensity band width of about 2.5  $\text{m}\mu$ . The difference between the light intensities at the two wavelengths was assumed to be due to oestrogens.

The results of colorimetric and fluorimetric analyses are plotted against each others in Fig. 1. It may be seen that the values of the oestrogen concentration obtained with both photometric methods were in good agreement. Estimates of the precision<sup>2</sup> of the fluorimetric method in different ranges of urinary oestrogen concentration are given in Table 1. It is evident that oestrogen concentrations below 10–20  $\mu\text{g}$  per ml could not be determined with any reasonable confidence. It is questionable to which degree positive values in this range represent oestrogens. At very low oestrogen concentrations the net fluorescence was always positive whereas the corrected optical densities were sometimes negative. Colorimetry at low concentrations tended to give erratic results apparently due to the relatively high unspecific (background) absorption and inadequacy of the correction formula applied.

During the months 5 and 6 after breeding the ratio between oestrone and equilin, which apparently are the major oestrogens excreted in the pregnant mare, seems to be high, but may decrease towards parturition<sup>3</sup>. The results of the method described for the estimation of total urinary oestrogens would be influenced by any variations in

this ratio since the chromogenic (and fluorogenic) value of equilin was much lower than that of oestrone.

The results reported indicate that the amounts of oestrogens being excreted in the pregnant mare are subject of large variations. Due to its practicability the crude method described might be of some value for further studies of these variations and of the possible variations of the time after breeding at which an appreciably increased excretion of oestrogens is initiated.

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## Isothiocyanates XLVI\*. Glucocappasalin, a New Naturally Occurring Thioglucoside

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*Capparis salicifolia* Griseb. (*Capparidaceae*) is a tree indigenous to the Chaco region of the Argentine. Paper chromatography of an extract of its seeds\*\* revealed the presence of two new thioglucosides with  $R_B$ -values<sup>1</sup> of 0.81 and 1.17 in butanol:ethanol:water (4:1:4). We here wish to report on the structure of the fastest migrating glucoside for which the name *glucocappasalin* is proposed.

After purification by ion exchange, counter-current separation of the two glucosides (380 transfers), and acetylation, *glucocappasalin tetraacetate* was obtained

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