Summary. With the method of Brown (1955), the urinary levels of oestrone were determined daily in three sows during the oestrous cycle and during the first 6 weeks after conception.

During the oestrous cycle, the patterns of the urinary oestrone levels were characterized by a rise of the levels associated with oestrus to a well-defined peak followed by a sudden decrease, usually to the lowest levels observed.

After conception, the levels of oestrone were not appreciably different from those during the oestrous cycle until at the end of the 3rd week at which time the excretion of oestrone increased. The week of maximal oestrone excretion in the three sows during early pregnancy lasted from Days 24, 25 and 27 after breeding.

Consistent fluctuations during the oestrous cycle or after conception of urinary oestradiol-fraction Kober chromogens were not recorded.

INTRODUCTION

In the urine of the pregnant sow, increased oestrogenic activity has been found in two periods of the gestation time, namely during the 4th and 5th weeks after breeding and during the last 6 weeks prior to parturition (Küst & Struck, 1934). Throughout both periods, the urinary levels of oestradiol-17β stay low, whereas the levels of oestrone are greatly increased (Velle, 1958a, b and 1959).

Oestrone, which appears to be the only oestrogen so far identified in the urine of the sow, has been isolated also from pig ovaries where it is found in addition to oestradiol-17β (Westerfeld, Thayer, MacCorquodale & Doisy, 1938). Whereas the amounts of oestradiol-17β in pig ovaries probably exceed those of oestrone, evidence has been obtained that the oestradiol-17β levels of the urine during the entire oestrous cycle stay very low and that, associated with oestrus, fluctuations may be recorded rather of urinary oestrone than of oestradiol-17β levels (Velle, 1958b). The data reported in this paper support this finding and indicate further, supporting previous findings (Velle, 1958b), that the levels of urinary oestrone after conception do not differ appreciably from those during the oestrous cycle until at the end of the 3rd week after
breeding, at which time the levels rise to attain a peak at the end of the 4th week.

MATERIAL AND METHODS

The material included three nulliparous sows of the Norwegian breed about 9 months of age. The oestrous cycle in one of the sows, No. 2, was prolonged, lasting almost 4 weeks compared to the normal duration of 3 weeks, as in Sows 1 and 3. All of the sows were successfully inseminated at the second oestrus exhibited during the observation period. Sow 3 was inseminated also at the preceding oestrus. Probably due to the conditions under which they were kept (see below), Sows 1 and 2 did not show very distinct heat symptoms and were inseminated at the time judged optimal from the appearance of the vulva (decreasing redness and incipient wrinkling of the skin). Judged from the response to hand pressure on the back, Sow 3 appeared to be in heat on the days of insemination. Sow 1 was allowed to live and delivered a litter of six at normal time, whereas Sows 2 and 3, which were slaughtered, had four and eleven foetuses, respectively.

Sows 1 and 2 were kept in narrow cages constructed for the purpose of 24-hr urine collection. The arrangements were such that some contamination of the urine with faeces could not be avoided. The urine samples of Sow 3 were collected at micturition. The urine samples were kept in deep-freeze until analyses could be undertaken.

For the quantitative determinations of oestrone and oestradiol-17β, the method of Brown (1955) with minor modifications was used. Of the urine samples, 100 or 25 ml were diluted to 200 ml with distilled water and refluxed with 30 ml of hydrochloric acid (11 n) for 1 hr. The oestriol fraction extracted with water from the light petroleum benzene solution of the ether-extract residuum was discarded. In the chromatographic procedure for the separation of the 3-methyl ethers of the oestrone-oestradiol fraction, the light petroleum was replaced by n-hexane (Diczfalusy & Lindquist, 1956). Colour development was performed according to Brown (1955) with Kober reagents prepared from sulphuric acid of the E. Merck AG manufacture.

The optical densities were read in a Beckman model DU spectrophotometer. Known amounts of oestrone and oestradiol-17β were added to urine prior to the hydrolysis procedure as standards and the net optical densities obtained used for the calculations of the results reported. The recoveries at the 10-μg level amounted to about 60%.

The fluorescence spectra of the Kober colour substances in tetrachlorethene (Ittrich, 1958) were recorded in a Beckman model DU monochromator equipped with a spectrofluorimetry attachment made in the laboratory (Lunaas, unpublished).

RESULTS

During the oestrous cycles in the three sows studied, most of the urinary levels of oestrone and oestradiol-17β were evidently low, but the corrected optical densities of both the oestrone and the oestradiol fractions developed in the
appropriate Kober reagents were always positive. To test the possibility that the urine might contain unspecific chromogens that could be eluted adjacent to the oestrone and oestriadiol 3-methyl ethers and give rise to positive corrected optical densities when developed with the Kober reagents, the method of Ittrich (1958) was used for the selective extraction from sulphuric acid of Kober colour substances due to oestrogens. In the random specimens examined, the sulphuric acid was diluted with water and extracted with tetrachlorethane containing $p$-nitrophenol. This procedure almost entirely eliminated the background absorption of both the oestrone and the oestriadiol fractions and gave an increment of the corrected optical densities of the oestrone fractions comparable to that found when the oestrone-3-methyl ether was similarly developed. The fluorescence spectra of the colour substances of the oestrone fraction in tetrachlorethane corresponded well to that of the colour substances of the oestrone-3-methyl ether in the same medium, having a peak at 550 to 552 m$\mu$ and a steep side towards lower wavelengths.

The corrected optical densities of the tetrachlorethane extracts of the oestriadiol fractions were, on the other hand, lower than in the Kober reagents indicating the presence in the oestriadiol fraction of unspecific chromogens which in the routine method would be recorded as oestriadiol. The fluorescence spectra of these extracts were broader than that of oestriadiol-17$\beta$ similarly developed, but had a definite peak corresponding to that of the reference substance.

As shown in Text-figs. 1, 2 and 3, the urinary levels of oestrone-fraction Kober chromogens fluctuated considerably during the oestrous cycle. During oestrus, indicated by swelling of the vulva, a well-defined peak of the levels was common to all the cycles studied. Generally, these high levels were suddenly followed by very low levels, usually the lowest observed during the cycle. In Sow 3, another peak of increased levels occurred during the luteal phase of the cycle. During the luteal phase of the cycle in the two other sows, the levels were also generally higher than immediately after the oestrous peak, but a common pattern was less evident. The oestriadiol fraction Kober chromogen during the oestrous cycle were maintained at low levels that were usually somewhat higher than the lowest levels observed of the oestrone fraction chromogens and the fluctuations were small and erratic with no clear pattern corresponding to the stages of the cycle.

Following conception, the urinary levels of oestrone- and oestriadiol-fraction chromogens were not much different from the levels in the preceding oestrous cycle until the end of the 3rd week after insemination, at which time the levels of oestrone increased rapidly to attain a peak at the end of the 4th week. During the period of relatively large oestrone excretion which lasted until the beginning of the 6th week, probably significantly increased levels of oestriadiol-fraction chromogens were occasionally encountered.

**DISCUSSION**

Oestriadiol-17$\alpha$, which is not separated from oestriadiol-17$\beta$ in the chromatographic method of Brown (1955), would probably be destroyed under the
TEXT-FIG. 1. Urinary secretion of oestrone (per 24 hr) in Sow 1 during the oestrous cycle and the first 6 weeks after conception. The numbers on the abscissa are of the days before and after successful insemination (arrow). The number in the litter was six.
TEXT-FIG. 2. Urinary secretion of oestrone (per 24 hr) in Sow 2 during the oestrous cycle and the first 6 weeks after conception. The numbers on the abscissa are of the days before and after successful insemination (arrow). There were four foetuses.
TEXT-FIG. 3. Concentration of urinary oestrone in Sow 3 during the oestrous cycle and the first 6 weeks after conception. The numbers on the abscissa are of the days before and after successful insemination (second arrow). There were eleven foetuses.
Urinary oestrogen levels in the sow

hydrolysis conditions selected in the present investigation (Velle, 1958b). For a recording of the fluctuations of the urinary levels of oestradiol-17β during the oestrous cycle in the sow, the methods used for chromatography and quantitative determination were evidently not adequate. The oestradiol-fraction material, when developed in the Kober reagent, gave rise to low corrected optical densities which could not solely be ascribed to oestradiols and the variation of which, during the oestrous cycle, was small and without any clear pattern.

By the extraction procedure of Ittrich (1958), which seems specific for the separation of the colour substances due to oestrogens from other colour substances formed from urinary material in the Kober reagent, indications were obtained that the oestrone fractions were relatively pure. It is, therefore, assumed that the fluctuations observed of the urinary levels of oestrogen fraction chromogens during the cycle largely represented variations of the excretion of oestrone.

The fluctuations of urinary oestrone levels during the oestrous cycle are presumably due to cyclic production of oestrogens in the ovaries. The oestrogens so far reported to be present in the ovaries of the sow are oestradiol-17β (MacCorquodale, Thayer & Doisy, 1935) and oestrone (Westerfeld et al., 1938). The failure of the method used in the present investigation to demonstrate appreciable fluctuations of oestradiol-17β levels in the urine during the oestrous cycle could indicate that this oestrogen, if released from the ovaries, is undergoing metabolic transformations prior to excretion. Evidence that oestradiol-17β is transformed peripherally in the sow has been obtained (Lunaas, unpublished) by parenteral administration of the steroid, which does not result in substantially increased urinary levels of this oestrogen but in urinary excretion of oestrone. The fluctuations of urinary oestrone during the oestrous cycle in the sow could thus be contributed to by ovarian release of oestradiol-17β.

It is reasonable to assume that the rather precipitous drop in the oestrone excretion observed during oestrus is timed with the rupture of the follicles, and the possibility exists that the determination of urinary oestrone in the sow may offer information on the time of ovulation. For this purpose, the pattern of oestrone excretion during oestrus could be more closely studied by analysis of the fractionated 24-hr urine sample. It may be seen that the sows conceived upon insemination at various stages of the oestrone excretion associated with oestrus, but that the litter sizes in two instances were small, which could indicate that the times of insemination were not optimal.

Knowledge of the maximal concentrations of oestrone that can be encountered in the urine of the non-pregnant sow has some practical implications since chemical methods for the quantitative determination (Velle, 1960) and detection (Lunaas, 1961) of urinary oestrone excreted during the 4th and 5th weeks of the gestation time may be applied as pregnancy tests in this species. The mean value of 6-0 µg (range 1.8 to 20.5) oestrone/litre urine reported in sows during oestrus (Velle, 1960) corresponds well to the value found in Sow 3 at the time of insemination. During the peak excretion in this sow prior to insemination, however, the urinary concentration of oestrone approached the tenfold value. This is also consistent with previous findings in a sow prior to standing heat (Velle, 1958b). In Sow 2, the maximal amount of oestrone
excreted during oestrus was about 150 µg/24 hr, which in small urinary volumes would give rise to considerable concentrations of oestrone. Urine samples collected from sows with normally cycling ovaries may, therefore, occasionally contain amounts of oestrone possibly important for the interpretation of pregnancy tests based on the occurrence of urinary oestrogens.

The duration of the highly increased oestrone excretion, the time of maximal excretion as well as the amounts of oestrone excreted in pregnant sows during the 4th and 5th weeks appear to be subject of variations (Lunaas, 1961). Determination of the oestrone concentration, as a method for pregnancy diagnosis, has given favourable results when the urine samples were collected between Days 25 and 30 after breeding (Velle, 1960). In two of the sows followed in the present investigation, maximal excretion was found during this period. In Sow 2, however, the maximal values occurred around Day 30 after insemination and the excretion as late as on Day 26 was less than 300 µg/24 hr or only twice the maximal amounts excreted prior to conception. The oestrous cycle studied in this sow was prolonged, lasting almost 4 weeks, and the number of embryos was small (four).

The results indicate that determination of urinary oestrone in the sow may be of value for the study of the reproductive functions in this species. It is interesting to note that the time of increasing oestrone excretion after conception coincides with the time during which the foetal membranes become expanded and vascularized, namely during the 3rd and 4th weeks after breeding (Corner, 1921). For the survival and development of the embryos, this period of the gestation time is presumably very critical and further studies on the significance of oestrone excretion seem justified.

ACKNOWLEDGMENTS

This investigation has been supported financially by the Agricultural Research Council of Norway. The author is indebted to Miss Aud Mortensen for technical assistance.

REFERENCES


