Investigation on the Excretion of Sex Hormones by Sheep

E. G. BASSETT, O. K. SEWELL and E. P. WHITE, Ruakura Animal Research Station, Hamilton.

SUMMARY.

This paper describes recent investigations on the rate of excretion of the different sex hormones in the various stages of the life cycle of the normal ewe.

In comparison with many other species the sheep excretes very small amounts of sex hormones. It has thus been necessary to develop suitable methods of chemical extraction and biological assay.

For the same reason, it has been found necessary to extract and assay a whole twenty-four hour elimination of urine. To this end an apparatus has been designed for continuous urine collection from grazing ewes (1).

The final urine concentrates were so toxic to the test mice that bioassay was impossible. Chemical investigation identified the toxic substance as p-cresol and the method of extraction was modified so that its removal was ensured (2). A large variety of different chemical methods have been tried on pregnant ewes' urine, and there appears to be little difference between them in the amount of oestrogen extracted. The most convenient method has been found to be that of Pincus (3).

The results so far have been from relatively few animals and it is not yet possible to draw conclusions as to the typical excretion pattern of normal ewes. However, many technical difficulties have been overcome and some interesting information obtained.

Oestrogenic activity has been detected in pregnant ewes' urine only during the last few weeks before lambing, while androgens have been found in one case from the beginning of the third month of gestation. There appeared to be considerable variation between ewes both in the amounts of hormones excreted and in the stage of pregnancy at which they can first be detected. The greatest amount of oestrogen excreted during pregnancy was equivalent to 6 micrograms of oestrone, while for androgens the highest level of excretion was equivalent to 156 micrograms of androsterone.

From observations on a number of ewes it appeared that the urinary excretion of oestrogens maintained a fairly constant level through the breeding season (4). The values obtained were between 1-2 micrograms equivalent of oestrone in twenty-four hours. Preliminary investigations using a more sensitive bioassay method have suggested that extremely small amounts of oestrogen may be shown to be excreted during the anoestrous period.

REFERENCES:

1. Bassett, E. G. (1951). New Zealand J. Sci .Tech. In press.
2. White, E. P., Sewell, O.K. and E. G. Basett, (1950). Nature **166**, 269.
3. Pincus, G. (1945). J. Clin. Endoc. **5**, 291.
4. Bassett, E. G. and O. K. Sewell. (1951). Nature **167**, 356.

Discussion

Mr. CLARKE: In the small number of animals that Mrs. Bassett has examined she has been able to show very large differences. It is important therefore that methods should be developed to allow the handling of larger number of experimental sheep.

Mr. SWAN: Oestrogens are detoxified in the liver. Would it be possible to use liver-damaging substances to increase the sensitivity of the assay?

Mrs. BASSETT: We have not considered it.

Professor CAMPBELL: Can the chemists offer any hope of chemical methods replacing the laborious bio-assay methods? Have checks been made by injecting oestrogens in massive amounts to detect changes in urine concentration?

Mrs. BASSETT: Relatively high levels in the urine were obtained from small urine samples from dry ewes with stilboestrol implants of 1 gm. About 10 times the highest amount of activity found in the urine of pregnant ewes.

Mr. WHITE: In human work chemical methods have been used but there is little hope for using them on sheep urine samples, chiefly because of the very small quantities of hormone and large amounts of coloured impurities present. There is a faint hope in the case of the androgens.

Dr. WALLACE: Could the use of composite samples be used to study the changes in a flock of ewes, with a smaller expenditure of labour?

Mrs. BASSETT: Composite samples would be of some use but we still require information on the normal variation between animals.

Mr. McFARLANE: Bulking of urine samples may be dangerous if the sex of the lamb has any influence. Techniques are available using pieces of uterine tissue either in vitro or transplanted into the anterior chamber of the eye, and these may be useful.