It must be assumed, therefore, that under the influence of insulin the fixation of adrenalin in the tissues is increased, and this has been clearly demonstrated by Hökfelt[3]. The increased secretion of adrenalin after insulin administration may thus be a reaction to compensate for the lowering of the plasma adrenalin-level, rather than an effect of hypoglycæmia. Euler and Luft[4] have recently shown that insulin causes an increased urinary excretion of adrenalin, but not of noradrenalin. This observation would be difficult to reconcile with the reduction of plasma adrenalin-levels, unless it is assumed that the adrenergic amines of urine exist largely in the conjugated form. The conjugated amines would not be estimated by the method used for the analysis of blood.

H. Weil-Malherbe

Runwell Hospital,
Wickford, Essex.
May 18.

[1] Weil-Malherbe, H., and Bone, A. D., *J. Ment. Sci.*, **98**, 565 (1952); *Biochem. J.*, **52**, xi (1952). IIe Congrès Intern. Biochim., Résumés des Communications. 67 (1952).
[2] Weil-Malherbe, H., and Bone, A. D., *Lancet*, **264**, 974 (1953).
[3] Hökfelt, B., *Acta Physiol. Scand.*, **25**, suppl. 92 (1952).
[4] Euler, U. S. von, and Luft, R., *Metabolism*, **1**, 528 (1952).

Extremely High Alkaline Phosphatase Activity in the Vaginal Mucus of the Cow

In the course of an investigation on the phosphatase content of the vaginal smear of some non-pregnant cows and heifers, extremely high alkaline phosphatase activity was observed in the mucus at regular intervals corresponding with the ovarian cycle of the animal. The highest values were found on the day that mucus secretion begins, two days before the visible signs of heat (bellowing, unrest, willingness to copulate). On that day a relatively small but highly active amount of mucus is secreted. A second period of relatively high activity of the mucus is often seen between the eighth and the twelfth day *post œstrum*. The activity is rather variable in different individuals and the daily variations may be great, especially in periods of irregular mucus secretion.

The diagram indicates the concentration of alkaline phosphatase in the mucus of a normal, healthy cow. Phosphatase activity is expressed in King and Armstrong units[1] per 100 gm. of mucus. In our first series of experiments, the mucus was homogenized and dissolved in water; later, we used a 0·06 per cent magnesium chloride solution in order to obtain maximal activation. Since the amount of mucus which was secreted daily could not be estimated, the diagram does not give the total amount of phosphatase produced. During œstrum, mucus secretion is abundant, but the clear mucus is only slightly active.

Alkaline Phosphatase Activity (King and Armstrong Units per 100 gm.) in the Vaginal Mucus of Heifers

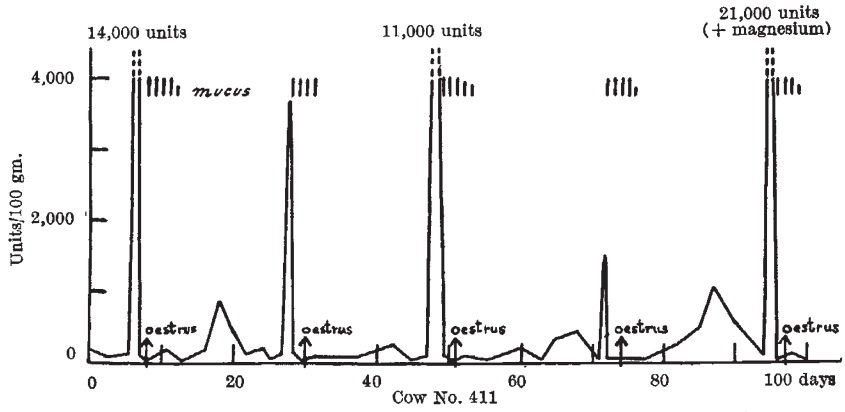
Date	21/4/52	23/4/52	28/4/52	1/5/52	4/5/52
No. 817	27,600	100*	100	86	—
,, 818	3,200	1,890	11,040	5,180	460
,, 819	1,040	220	200	104	290
,, 820	6,000	26,200	100*	610	3,000
,, 821	7,500	3,310	1,520	510	800
,, 822	9,500	1,240	320	760	700

* = œstrum.

Days of abundant secretion are indicated in the diagrams by vertical lines. The rapid fall in activity just before œstrum is an effect of dilution and cannot be ascribed to instability of the enzyme, since a sample of highly active mucus (14,000 King and Armstrong units per 100 gm.) retained its full activity while stored at room temperature for three days.

Large amounts of alkaline phosphatase are secreted by heifers, especially in the period of first œstrum. A characteristic picture of the very high and variable activities found in the mucus of six heifers is given in the accompanying table.

The alkaline phosphatase diagram probably gives a picture of the permeability of the vaginal epithelium and of the amount of enzyme stored in the mucosa. The influence of castration, hormones and diet (feeding of phosphates) is now under investigation. In a case of corpus luteum persistens, very low alkaline phosphatase values were found. Stable dry enzyme preparations, containing 50–100 per cent of the original activity, can be obtained from the mucus by precipitation with cold ethanol or acetone. Our most active preparations, 40–50 King and Armstrong units per mgm. nitrogen, showed a positive biuret, a strong Molisch and a negative phosphorus reaction.



Alkaline phosphatase activity (in King and Armstrong units per 100 gm.) in the vaginal mucus of a cow during five ovarian cycles. Days of abundant mucus secretion are indicated by short vertical lines; days on which full œstrus was observed are marked by an arrow

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G. A. van Klinkenberg

Rijksseruminrichting,
Prof. Poelslaan 35,
Rotterdam.
March 10.

[1] King, E. J., and Armstrong, A. R., *Canad. Med. Assoc. J.*, **31**, 376 (1934).