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α-Glucosidase Activity in the Reproductive Tract of the Ewe

T. O'Shea and B. E. Murdoch

Department of Physiology, University of New England, Armidale, N.S.W. 2351.

Abstract

α-Glucosidase activity has been estimated in the tissues and rinsings of the reproductive tract of the ewe. There were peaks of activity in the oviducal mucosa at pH 4·0 and 5·7. In the endometrium, caruncles and cervical mucosa the pH optimum occurred from pH 4·0 to pH 5·7. A sharp peak in the activity in the vaginal mucosa occurred at pH 5·7.

The only tissue in which changes in enzyme activity were consistently related to one endocrinological state of the ewe was the cervical mucosa. Cervical α-glucosidase activity was greater at oestrus than during the rest of the oestrous cycle, declined during early pregnancy, and increased in ovariectomized ewes following the injection of oestradiol-17β.

Introduction

The female reproductive tract contains glycogen which after degradation could augment the carbohydrate passing from the blood into the tract. In particular this glycogen may act as an indirect source of nutrition for spermatozoa and the developing conceptus before formation of the chorio–allantoic placenta (Zondek and Stein 1940; Reinius 1968; Finn and Porter 1975).

Although glycogen metabolism is controlled largely by the activity of glycogen (starch) synthase (UDP glucose : glycogen 4-α-glucosyltransferase, EC 2.4.1.11) (glycogen synthase) and glycogen phosphorylase (1,4-α-D-glucan : orthophosphate α-glucosyltransferase, EC 2.4.1.1), other enzymes are also involved. One such glycogen-degrading enzyme (Auricchio and Bruni 1967) is α-glucosidase (α-D-glucoside glucohydrolase, EC 3.2.1.20) which forms glucose from maltose and other linear oligosaccharides as well as from the outer chains of glycogen. It also catalyses the transfer of a glucosyl unit from maltose to glycogen (Hers and van Hoof 1966). α-Glucosidase activity has been observed in the rat uterus (Rao *et al.* 1971). As part of a study of enzyme activity in the genital tract of the ewe we have examined the activity of α-glucosidase in cyclic ewes, in pregnant ewes, and in ovariectomized ewes given exogenous hormone treatments.

In cyclic ewes periods of oestrogen and progesterone dominance alternate and therefore priming effects of each hormone on the actions of the other seem possible. Experiment A was an investigation of the effects of exogenous oestradiol-17β and progesterone on α-glucosidase activity in ovariectomized ewes which had been primed with oestradiol-17β. In experiment B the effects of priming with oestradiol-17β and/or progresterone on the effect of subsequent injections with oestradiol-17β were examined.

Materials and Methods

Experimental Animals

Adult Merino ewes were treated in one of three ways.

(1) *Naturally cyclic ewes.* Oestrus was detected by running ewes with raddled, vasectomized rams and examining them daily for raddle marks. Animals were slaughtered on the day of onset of oestrus (day 0), or at 1, 8 or 15 days after oestrus.

(2) *Mated ewes.* Ewes were mated with fertile rams and slaughtered either on the day of mating (day 0) or on days 8, 15, 22, 30 and 44 of pregnancy.

(3) *Ovariectomized Ewes.* Ewes were ovariectomized and, after a period of at least 30 days, were injected intramuscularly according to the following schedule.

Experiment A. Ewes in the following groups were injected with 30 μg oestradiol-17β on day 0 and killed on day 6.

Group 1: Controls; 1 ml peanut oil on days 3, 4 and 5.
Group 2: 30 μg oestradiol-17β on days 3, 4 and 5.
Group 3: 10 mg progesterone on days 3, 4 and 5.
Group 4: 30 μg oestradiol-17β and 10 mg of progesterone on days 3, 4 and 5.

Experiment B. Ewes in the following groups were killed on day 6.

Group 1: Controls; peanut oil on day 0, 30 μg oestradiol-17β on days 3, 4 and 5.
Group 2: 30 μg of oestradiol-17β on days 0, 3, 4 and 5.
Group 3: Peanut oil day 0, 10 mg progesterone day 1, 30 μg oestradiol-17β on days 3, 4 and 5.
Group 4: 30 μg oestradiol-17β on days 0, 3, 4 and 5 and 10 mg progesterone on day 1.

The steroids were administered in 1 ml of peanut oil.

Preparation of Tissues and Rinsings

Tissues of rinsings were prepared as described by Murdoch and O'Shea (1978).

Analytical Methods

α-Glucosidase activity was determined by an adaptation of the specific transglucosylation method described by Hers and van Hoof (1966). The modifications consisted of examining the enzyme activity over a pH range (Rao *et al.* 1971), and isolating the glycogen at the end of the reaction by the technique described by Thomas *et al.* (1968). The tissues were homogenized in 9 volumes of double-distilled water containing penicillin (1000 i.u./ml) and streptomycin sulphate (1 mg/ml). A stock substrate solution of 3·125 μCi of [U-^{14}C]maltose (7 μCi/μmol) and 60 mg glycogen per ml of double-distilled water was prepared. This was necessary as the background radioactivity of control samples was found to vary between pH values when the stock solution was stored at -20°C with citrate–phosphate buffer at different pH values.

For the assay, 25 μl of [^{14}C]maltose substrate, 25 μl of citrate–phosphate buffer (of the required pH), and 50 μl of tissue homogenate (or rinsing) were mixed and incubated for 4 h at 37°C. Control background radioactivity samples containing 50 μl of distilled water in place of the tissue homogenate were also run. The reaction was stopped by standing the test tubes in ice and 50 μl of the reaction mixture was spotted onto Whatman No. 3 filter paper and subjected to the washing and counting procedure described by Thomas *et al.* (1968) for the isolation of glycogen. α-Glucosidase activity is reported as [^{14}C]glucose incorporated from [^{14}C]maltose into glycogen per hour.

The protein concentration of the samples was determined by the biuret method (Wales *et al.* 1961).

Statistical Analyses

The data were subjected to analysis of variance. Where separation of means was performed by the multiple range test (Duncan 1955) the standard errors of the means (s.e.m.) were calculated from the analysis of variance.

Results

Effect of pH on the Assay

Using tissues from cyclic ewes, a preliminary experiment was carried out to examine the effect of pH during the assay. The pH at which maximum enzyme activity occurred varied between tissues (Fig. 1). The α-glucosidase activity in the oviducal mucosa showed optima at pH 4·0 and 5·7, these optima both being greater than the activity at pH 5 ($P < 0{\cdot}05$). A broad pH peak (pH 4·0–5·7) of maximum α-glucosidase activity was seen with the intercaruncular endometrium (endometrium), the caruncles and the cervical mucosa. A sharper peak (pH 5·5–6·3) occurred with the vaginal mucosa.

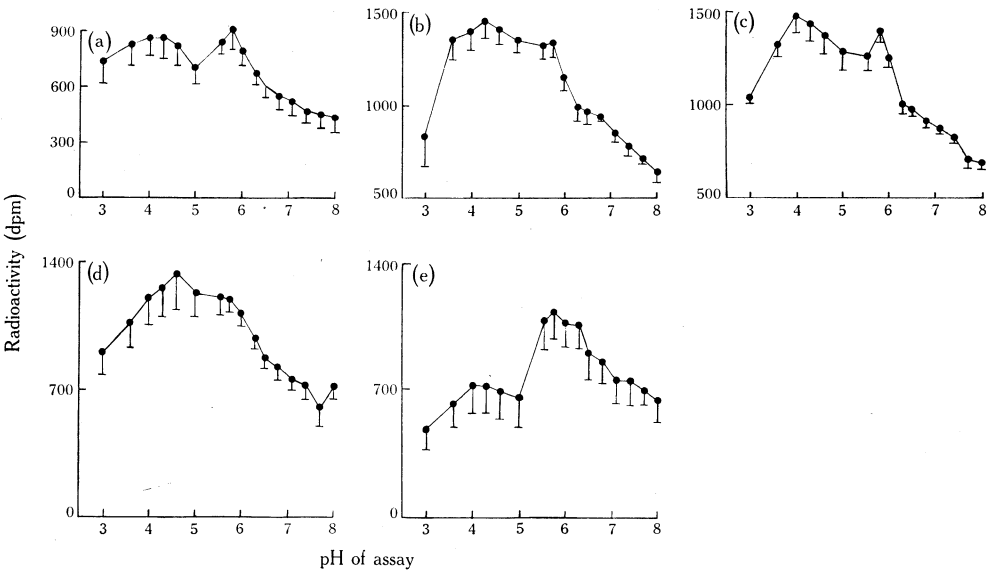

Fig. 1. Effect of pH on the assay of α-glucosidase. (*a*) Oviducal mucosa (s.e.m. = 45, d.f. = 71, $u = 6$). (*b*) Endometrium (s.e.m. = 72, d.f. = 70, $n = 6$). (*c*) Caruncular endometrium (s.e.m. = 48, d.f. = 71, $n = 6$). (*d*) Cervical mucosa (s.e.m. = 68, d.f. = 82, $n = 7$). (*e*) Vaginal mucosa (s.e.m. = 64, d.f. = 71, $n = 6$). Plotted points are the mean values for six or seven ewes.

Inspection of the data in this work led us to believe that there was an interaction between the effects of pH and stage of the oestrous cycle. In the subsequent experiments several pH values were used (pH 4·0, 5·0, 5·7, 6·0, 6·5, 7·1 and 8·0). These include the pH optima and other pH levels thought to be the ones at which there is interaction between pH and stage of the oestrous cycle. However, as in nearly all of the following results there was no interaction between pH and treatment, only the data related to the pH optima and neutrality (pH 7·1) are given.

Naturally Cyclic Ewes

Enzyme activity in the oviducal mucosa, endometrium, caruncles and vaginal mucosa did not change significantly during the cycle, whereas in the cervical mucosa (Fig. 2) it was greater at oestrus than at the other days ($P < 0{\cdot}01$).

In the oviducal mucosa α-glucosidase activity was greater at pH 4·0 and 5·7 than at pH 5·0 ($P < 0·05$). Rinsings from the uterus showed greater enzyme activity at day 15 than at day 1 ($P < 0·05$), but those from the cervix showed no significant effects (Fig. 4).

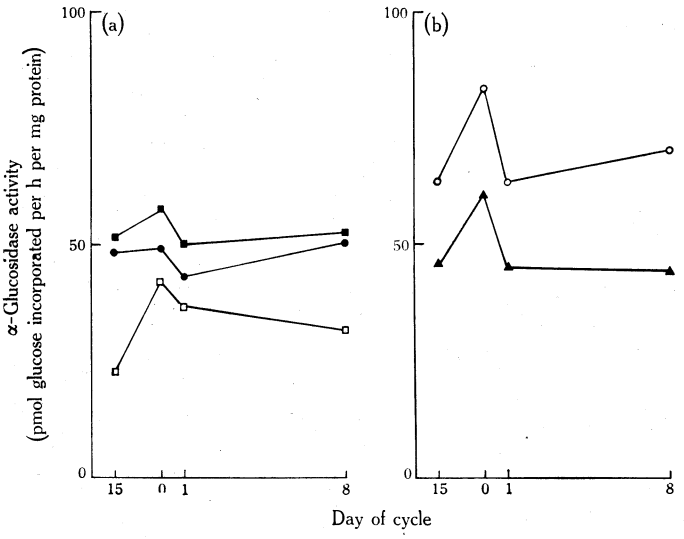

Fig. 2. α-Glucosidase activity during the oestrous cycle. (*a*) Oviducal mucosa (s.e.m. for pH values = 2·8, d.f. = 86). (*b*) Cervical mucosa (s.e.m. for days = 2·9, d.f. = 91). ● pH 4·0. □ pH 5·0. ■ pH 5·7. ○ Mean of pH 4·0, 5·0 and 5·7. ▲ pH 7·1. Plotted points are the mean values for five ewes.

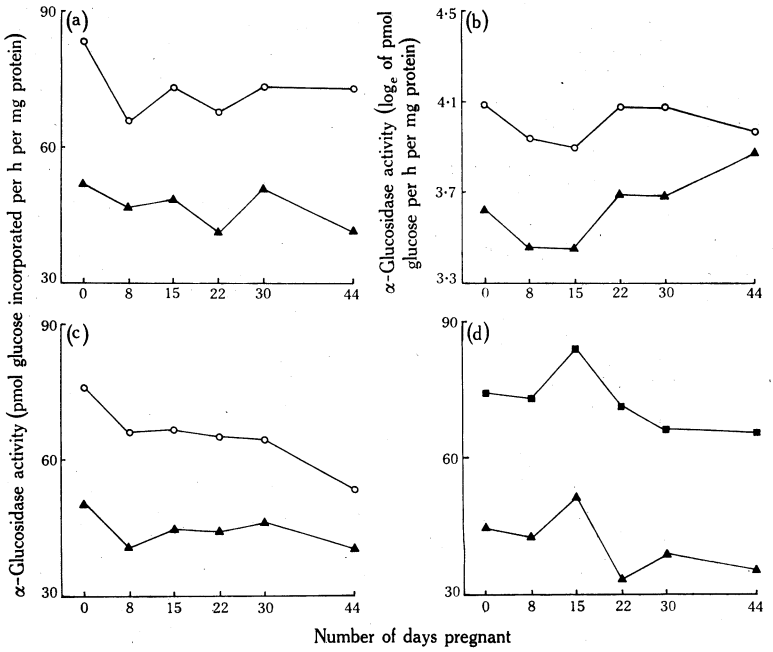

Fig. 3. α-Glucosidase activity during early pregnancy. (*a*) Endometrium (s.e.m. for days and pH = 2·2, d.f. = 137). (*b*) Caruncles (s.e.m. = 0·064, d.f. = 137). (*c*) Cervical mucosa (s.e.m. = 2·0, d.f. = 130). (*d*) Vaginal mucosa (s.e.m. = 2·2, d.f. = 120). ■ pH 5·7. ○ Mean of pH 4·0, 5·0, and 5·7. ▲ pH 7·1. Plotted points are the mean values for five ewes.

Pregnant Ewes

No significant changes were seen in enzyme activity in the oviducal mucosa. In the endometrium (Fig. 3) α-glucosidase activity was greater at oestrus than at day 15 ($P < 0{\cdot}05$) or at days 8, 22 and 44 ($P < 0{\cdot}01$).

Because there was greater variability in the data for the enzyme activity in the caruncles as pregnancy progressed, these data were transformed to logarithms for analysis. Caruncular α-glucosidase activity (Fig. 3) was greater ($P < 0{\cdot}05$) at days 30 and 22 than at day 15. The apparent increase at pH 7·1 from day 8 to day 44 approached significance ($P = 0{\cdot}05$). α-Glucosidase activity in the cervical mucosa (Fig. 3) was greater ($P < 0{\cdot}01$) at oestrus than at days 8, 15, 22, 30 and 44, and less at day 44 than at all other days ($P < 0{\cdot}01$).

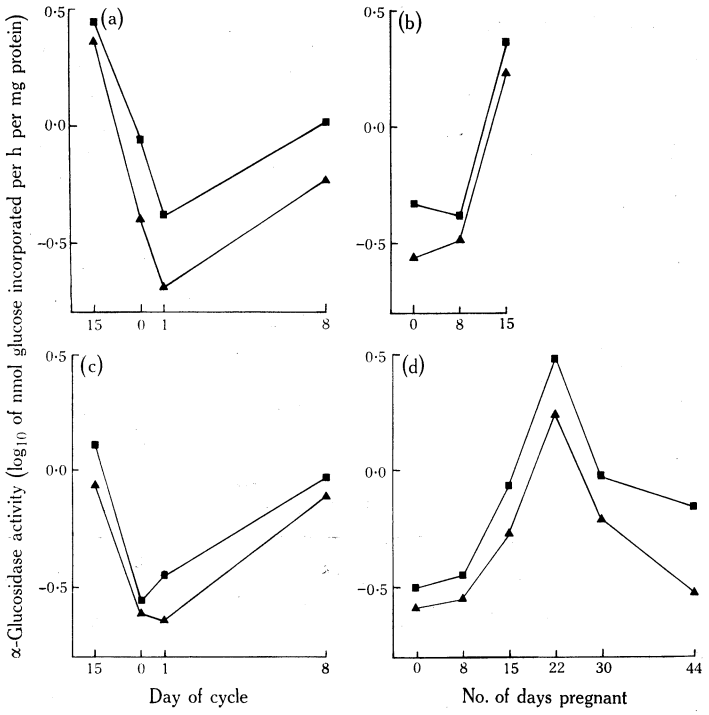

Fig. 4. α-Glucosidase activity in reproductive tract rinsings. (*a*) Uterine rinsings during the oestrous cycle (s.e.m. = 0·20, d.f. = 33). (*b*) Uterine rinsings in early pregnancy (s.e.m. = 0·15, d.f. = 27). (*c*) Cervical rinsings during the oestrous cycle (s.e.m. = 0·33, d.f. = 36). (*d*) Cervical rinsings in early pregnancy (s.e.m. = 0·18, d.f. = 52). ■ pH 5·7. ▲ pH 7·1. Plotted points are the mean values for five ewes.

In the vaginal mucosa enzyme activity was greater at oestrus than at day 44 ($P < 0{\cdot}01$), and greater at day 15 than at days 8, 22, 30 and 44 ($P < 0{\cdot}01$).

The uterine rinsings (Fig. 4) were not examined beyond day 15 because of the increase in size and attachment of the conceptus. There was greater α-glucosidase activity at day 15 than at days 0 and 8 ($P < 0{\cdot}01$). In the cervical rinsings the enzyme activity increased markedly from day 8 to day 22 and then declined ($P < 0{\cdot}001$) but remained higher at day 44 than at oestrus ($P < 0{\cdot}01$).

Ovariectomized Ewes

Only the data from those tissues where significant effects were seen are presented in Tables 1 (experiment A) and 2 (experiment B). Because the minute amount of protein in some rinsings could not be accurately measured the results for the rinsings are expressed in terms of the total rinsing.

Table 1. Effect of oestradiol-17β and progesterone on α-glucosidase activity in oestradiol-primed ovariectomized ewes

Values are the means for five ewes and are from the $\log_e$ transformed data expressed as picomoles of glucose incorporated from maltose into glycogen per hour per milligram tissue protein or per total organ rinsing

	Oviducal mucosa			Oviducal rinsing			Cervical mucosa			Cervical rinsing		
pH:	4·0	5·7	7·1	4·0	5·7	7·1	4·0	5·7	7·1	4·0	5·7	7·1
Control	3·39	3·51	3·01	3·87	3·72	3·52	3·54	3·83	3·43	5·39	5·14	4·85
Oestradiol	3·83	3·94	3·56	5·08	4·73	4·34	3·98	4·07	3·68	4·83	5·16	4·85
Progesterone	3·58	3·77	3·51	3·87	4·70	4·25	3·74	3·89	3·51	4·40	4·74	4·43
Oestradiol+ progesterone	3·43	3·63	3·29	3·56	4·25	3·95	3·72	3·99	3·54	4·33	5·58	5·30

Summary of the analyses of variance

Source of variation	d.f.	Variance ratios			
		Oviducal mucosa	Oviducal rinsing	Cervical mucosa	Cervical rinsing
Oestradiol (O)	1	3·05	3·34	5·34*	1·06
Progesterone (P)	1	0·00	0·41	0·10	1·82
O×P	1	13·89**	14·66**	3·27	4·21*
Error mean square	48	0·1122[A]	0·4847	0·0858	0·4714

[A] 45 degrees of freedom.
* $P < 0·05$. ** $P < 0·01$.

Table 2. Effect of priming with oestradiol-17β and progesterone on the α-glucosidase activity evoked by oestradiol-17β in ovariectomized ewes

Each value is the mean for five ewes and is from the $\log_e$ transformed data expressed as picomoles of glucose incorporated from maltose into glycogen per hour per milligram tissue protein

	Oviducal mucosa			Caruncles			Vaginal mucosa		
pH:	4·0	5·7	7·1	4·0	5·7	7·1	4·0	5·7	7·1
Control	3·95	4·18	3·75	4·08	4·01	3·79	3·37	3·87	3·45
Oestradiol	3·83	3·94	3·56	3·89	3·96	3·59	3·20	3·62	3·26
Progesterone	3·45	3·98	3·45	3·93	4·10	3·74	2·76	3·70	3·37
Oestradiol+ progesterone	3·61	3·45	3·48	3·90	3·77	3·54	3·15	3·99	3·53

Summary of the analyses of variance

Source of variation	d.f.	Variance ratios		
		Oviducal mucosa	Caruncles	Vaginal mucosa
Oestradiol (O)	1	1·21	8·41**	0·36
Progesterone (P)	1	9·95**	0·98	0·44
O×P	1	1·96	0·11	11·75**
Error mean square	48	0·0812[A]	0·0481	0·0743

[A] 45 degrees of freedom.
* $P < 0·05$. ** $P < 0·01$.

Injection of oestradiol-primed ewes with oestradiol increased the activity of the enzyme in both the oviducal mucosa and the oviducal rinsings but not when progesterone was given simultaneously (interaction: $P < 0{\cdot}01$). Priming with progesterone decreased α-glucosidase activity in the oviduct ($P < 0{\cdot}01$).

There were no significant effects of injection of the hormones on enzyme activity in the endometrium, but oestrogen priming resulted in a decline in α-glucosidase activity in the caruncles ($P < 0{\cdot}01$). Enzyme activity in the uterine rinsings was not significantly changed by the hormone injections.

Both priming with oestradiol and injection of oestradiol-primed ewes with oestradiol ($P < 0{\cdot}05$) increased enzyme activity in the cervical mucosa, but this effect was not significant with the priming treatment. Progesterone treatment decreased enzyme activity in the cervical rinsings but this effect was only just significant and was not seen when oestradiol was given simultaneously (interaction oestradiol-17β × progesterone: $P < 0{\cdot}05$).

Priming injections of either oestradiol or progesterone caused a decrease in enzyme activity in the vaginal mucosa but not when they were given together (interaction: $P < 0{\cdot}01$).

Discussion

In the present study, α-glucosidase activity in the cervical mucosa was increased by oestradiol administration to ovariectomized animals, a finding consistent with the higher activity of α-glucosidase in this organ at oestrus in mated and naturally cyclic ewes (Figs 2 and 3). The cervix of the ewe is known to function as a spermatozoal reservoir (Mattner 1966). The spermatozoa in this reservoir are closely associated with the mucosa, are mostly not removed by rinsing the cervix (Mattner 1968; Edey *et al.* 1975), and are separated spatially from the increased number of leucocytes induced in the genital tract by spermatozoa (Mattner 1968). The increased α-glucosidase activity in the cervical mucosa at oestrus may be related to this enzyme having a role in supplying metabolites from glycogen for the spermatozoal reservoir or in the formation of the cervical mucus. Although there has been no unequivocal evidence that any specific component of cervical mucus is of crucial importance in prolonging survival of spermatozoa in the cervix of the ewe, in the human it has been found that the viability of spermatozoa is markedly depressed in patients with low levels of glucose in the cervical mucus (Weed and Carrera 1970).

There was no change in α-glucosidase activity in the endometrium during the oestrous cycle or with hormone treatment of the ovariectomized ewes. The decrease seen in pregnant ewes after mating may possibly be due to an increase in the number of leucocytes shortly after mating, as was seen in the endometrium of mated oestrous goats and cattle by Mattner (1968), leucocytes being rich in lysosomes (Bainton and Farquhar 1968).

In the caruncles the increased enzyme activity during days 22 and 30 of pregnancy was only just significant but does occur at the time of implantation (Boshier 1969; Bryden *et al.* 1972) and may be related to this process (Boshier 1969). It is possible that neutral α-glucosidase activity became more important in the caruncles during pregnancy (Fig. 3). However, α-glucosidase occurs in the human placenta (Thanavala *et al.* 1974). This, coupled with the increasing variability of the caruncular data

during the same period, may suggest that the increased activity at pH 7·1 (not seen in any other tissue) was due to contamination with placental α-glucosidase. In opposition to this suggestion is the finding that human placental α-glucosidase has a pH optimum of pH 3·2–5·0 (Thanavala *et al.* 1974). The pattern of α-glucosidase activity seen in the uterine and cervical rinsings is consistent with its luminal appearance being increased by progesterone. However, progesterone treatment of ovariectomized ewes did not have any effect on enzyme activity in the uterine rinsings and decreased that in the cervical rinsings. The higher α-glucosidase activity in the uterine rinsings at day 15 of pregnancy may be just a byproduct of lysosomal actvities (Lejeune *et al.* 1963) at the start of implantation (Boshier 1969). Similarly, the increased activity seen in cervical rinsings at day 22 may be due to admixture of lumen contents from the uterus, and may again be associated with implantation.

The changes in α-glucosidase activity in the vaginal mucosa during pregnancy do not seem to be related to any physiological event or to the hormone experiments.

In the rat (Rao *et al.* 1971) α-glucosidase in the female reproductive organs has been reported to have several pH optima (pH 4·3, 5·6, 6·3 and 7·1) although the peak at pH 7·1 was absent in the uterus. The only tissue in the ewe with more than one pH optimum was the oviducal mucosa (pH 4·0 and 5·7). Again contrary to what is reported to occur in the rat (Rao *et al.* 1972), no evidence was found for an interaction between pH of the assay and treatment of ovariectomized ewes with oestradiol-17β or progesterone. These differences may be either true differences between species or be due to the different techniques used in the enzyme assays. In addition, in the present study the tests were carried out with mucosal tissues alone rather than with whole organs as used by Rao and co-workers (Rao *et al.* 1971, 1972).

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