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Activity of Enzymes of Glycogen Metabolism in the Reproductive Tract of the Ewe at Mating and during Early Pregnancy

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Abstract

The glycogen concentration and the activity of several enzymes of glycogen metabolism have been measured in the mucosal tissues of the oviduct, cervix and vagina, as well as in the endometrium and caruncles of ewes at days 0, 8, 15, 22, 30 and 44 of pregnancy. Enzyme activities were also determined in uterine and cervical rinsings.

Glycogen levels decreased after day 8 in the endometrium, caruncles, cervix and vagina. Independent glycogen (starch) synthase showed decreased activity in the cervical mucosa following mating and peaks of activity at days 0 and 15 in the oviduct. Following mating both independent and total glycogen (starch) synthase activities increased in caruncular tissue. α-Amylase activity declined from day 0 to day 44 in the oviducal mucosa, but showed a slight peak at day 15 in the endometrium. The levels of total and active glycogen phosphorylase did not change significantly in any tissue but did show a peak of activity in the uterine and cervical rinsings at days 8 and 15 respectively.

The results are interpreted as showing increased turnover of glycogen in the oviducal and cervical mucosa at mating, in the intercaruncular endometrium in the period before implantation, and in the caruncles from the start of implantation.

Introduction

The activities of certain enzymes in the reproductive tract of the ewe have been shown to fluctuate during early pregnancy (Hafez and White 1968; Boshier 1969; Murdoch 1970*a*, 1970*b*). Because these studies have been made in relation to implantation they have been confined to the uterine tissues. No systematic studies have been made of enzyme changes in the cervical, oviducal and vaginal mucosal tissues of the ewe during this period.

Glycogen may be secreted into the lumen of the oviduct (Reinius 1970), and the fertilized ovum remains in the oviduct of the sheep for 77–96 h (Clark 1934). If oviducal glycogen or glycogen degradative products are important for spermatozoa or the fertilized ovum, glycogen synthesizing or degrading enzymes in the oviduct may show higher activity at mating than later.

Glycogen is the principal polysaccharide in cervical secretions (Moghissi 1973) and the cervix in the ewe acts as a sperm reservoir for about 3 days (Dauzier and Wintenberger 1952; Mattner 1966). Thus if glycogen metabolism provides nutrients for sperm there may be greater activity of glycogen enzymes in the cervix at the time of mating.

Implantation is a late event in the ewe, commencing at 17–18 days after mating (Bryden *et al.* 1972). Consequently in early pregnancy the 'uterine milk' (histotrophe) which bathes the embryo contains the only possible source of external nutrients

(Amoroso 1952), and therefore greater metabolic activity might be expected in the glandular intercaruncular tissue before implantation is fully established. The non-glandular caruncular tissue only becomes important for embryonic metabolism during implantation, and, by analogy with rat decidua (Christie 1966), may show greater metabolic activity from this time. We have, therefore, examined some enzymes of glycogen metabolism in all of the mucosal tissues of the reproductive tract of the ewe during early pregnancy, both before and after implantation.

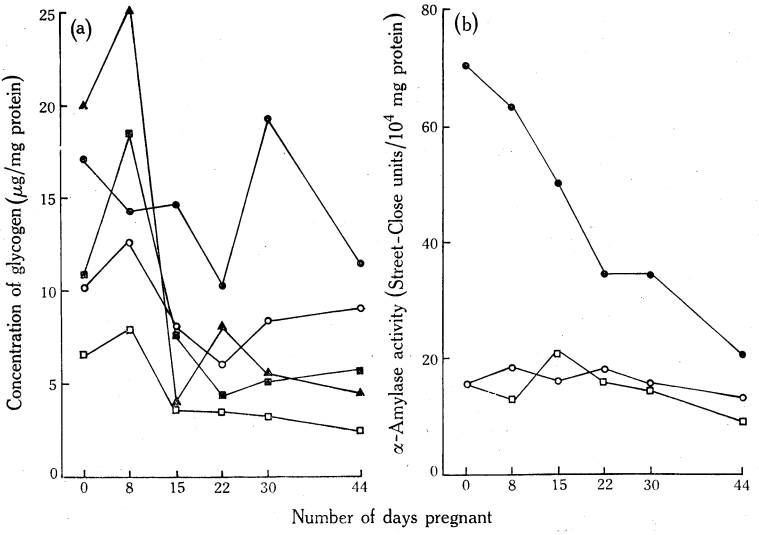

Fig. 1. (*a*) Glycogen in the reproductive tract of the pregnant ewe. ● Oviducal mucosa (s.e.m. = 2·40, d.f. = 14). □ Endometrium (s.e.m. = 0·88, d.f. = 18). ▲ Caruncles (s.e.m. = 2·54, d.f. = 18). ○ Cervical mucosa (s.e.m. = 1·57, d.f. = 18). ■ Vaginal mucosa (s.e.m. = 3·00, d.f. = 17). Plotted points are the mean values for four ewes.

(*b*) α-Amylase activity in the reproductive tract of the pregnant ewe. ● Oviducal mucosa (s.e.m. = 11·58, d.f. = 22). □ Endometrium (s.e.m. = 3·02, d.f. = 23). ○ Cervical mucosa (s.e.m. = 2·19, d.f. = 22). Plotted points are the mean values for five ewes.

Materials and Methods

Adult Merino 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.

The preparation of tissues and rinsings and the analytical methods were carried out as previously described (Murdoch and O'Shea 1978). The uterine rinsings from ewes killed on day 8 were examined for blastocysts and only tracts from which a blastocyst was recovered were used. Uteri from ewes at more advanced stages of pregnancy than day 15 were dissected and the embryonic fluids, embryos and their supporting membranes were carefully separated from the maternal tissues. The exposed endometrium and caruncles were blotted with filter paper and samples of the tissues taken with fine scissors and forceps. Uterine rinsings were taken only up to day 15 because the increase in size and attachment of the conceptus after this stage made rinsing and preparation of the tissues too slow.

Statistical Analyses

The significance of the data for tissues from the same region has been assessed by analysis of variance using the interaction error mean square to calculate the variance ratios which were then partitioned using orthogonal polynomials (Snedecor and Cochran 1969). In the figures only the

standard errors of the means (s.e.m.) derived from these analyses of variance are given, and the statistical significance of the results is given in the text. For comparison between tissues, an analysis of variance was carried out for each enzyme and separation of means was performed by the multiple range test (Duncan 1955). Where there was heterogeneity of variance in the raw data the data have been transformed to logarithms for the analysis.

Results

The means of the data for each tissue enzyme which showed a significant change are presented in Figs 1 and 2. To simplify the figures some of the results are given in the text.

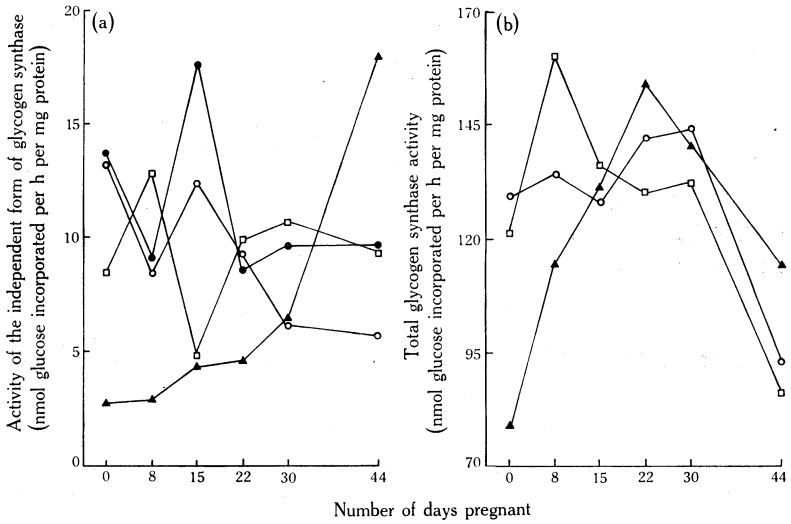

Fig. 2. (*a*) Activity of the independent form of glycogen synthase in the reproductive tract of the pregnant ewe. ● Oviducal mucosa (s.e.m. = 2·07, d.f. = 19). □ Endometrium (s.e.m. = 2·31, d.f. = 23). ▲ Caruncles (s.e.m. = 1·85, d.f. = 23). ○ Cervical mucosa (s.e.m. = 2·44, d.f. = 22). Plotted points are the mean values for five ewes.

(*b*) Activity of glycogen synthase (with added glucose-6-phosphate) in the reproductive tract of the pregnant ewe. □ Endometrium (s.e.m. = 17·0, d.f. = 23). ▲ Caruncles (s.e.m. = 14·9, d.f. = 24). ○ Cervical mucosa (s.e.m. = 16·1, d.f. = 22). Plotted points are the mean values for five ewes.

Glycogen (Fig. 1a)

The amount of glycogen in the oviducal mucosa was very variable but did not change significantly during early pregnancy ($P > 0{\cdot}05$). In the endometrium and vaginal mucosa glycogen decreased linearly between mating and day 44 ($P < 0{\cdot}01$). The endometrium had lower glycogen levels than all the other tissues ($P < 0{\cdot}01$). Caruncular glycogen decreased to a minimum value on day 15 after being maximal at day 8, and remained low until day 44 with a slight rise at day 22 ($P < 0{\cdot}01$). In the cervical mucosa glycogen concentration was highest at day 8 and lowest at day 22 when values were expressed as micrograms per gram wet weight ($P < 0{\cdot}05$). Although the same trend was apparent, there was no significant change when glycogen levels in the cervix were expressed as micrograms per milligram of protein.

α-Amylase (1,4-α-D-glucan glucanohydrolase, EC 3.2.1.1) (Fig. 1b)

The most striking change in the oviducal mucosa was the linear fall in α-amylase activity between mating and day 44 of pregnancy ($P < 0{\cdot}001$). Oviduct α-amylase activity was much greater than that in the other tissues ($P < 0{\cdot}01$) until day 44 by which time it was similar. The other tissues showed no significant changes except that on a units per gram wet weight basis there was a significant quadratic effect of time on endometrial α-amylase with a peak at day 15 ($P < 0{\cdot}025$). The level in the vaginal mucosa was $15{\cdot}47 \pm$ s.e. 0·98 Street-Close units/10^4 mg protein and that in the caruncles was $15{\cdot}62 \pm 1{\cdot}29$.

Glycogen phosphorylase (1,4-α-D-glucan : orthophosphate α-glucosyltransferase, EC 2.4.1.1)

There were no statistically significant changes in glycogen phosphorylase activity in any of the tissues. Mean values (micrograms of phosphate released per hour per milligram protein) $\pm$ s.e. for active and total glycogen phosphorylase were: oviducal mucosa $39{\cdot}0 \pm 4{\cdot}2$ and $75{\cdot}2 \pm 6{\cdot}5$; endometrium $18{\cdot}7 \pm 1{\cdot}6$ and $30{\cdot}5 \pm 2{\cdot}1$; caruncles $28{\cdot}4 \pm 2{\cdot}7$ and $32{\cdot}3 \pm 2{\cdot}8$; cervical mucosa $27{\cdot}5 \pm 2{\cdot}6$ and $47{\cdot}9 \pm 3{\cdot}0$; and vaginal mucosa $42{\cdot}8 \pm 4{\cdot}0$ and $63{\cdot}1 \pm 4{\cdot}7$.

Glycogen (starch) synthase (UDP glucose : glycogen 4-α-glucosyltransferase, EC 2.4.1.11) (glycogen synthase) (Fig. 2)

Oviducal mucosa independent glycogen synthase activity was greatest at mating and on day 15 ($P < 0{\cdot}01$). Total glycogen synthase activity in this tissue remained high ($143 \pm 4{\cdot}6$ nmol glucose incorporated per hour per milligram protein) at all times.

Because of the large variability the statistical analysis for the vaginal data was carried out on logarithm-transformed data. In both the endometrium and the vagina changes in glycogen synthase activity approached but did not reach significance ($P > 0{\cdot}05$). Mean values (nanomoles of glucose incorporated per hour per milligram protein) $\pm$ s.e. for independent and total glycogen synthase activities in the vaginal mucosa were $15{\cdot}9 \pm 3{\cdot}3$ and $154 \pm 11{\cdot}1$ respectively.

Independent glycogen synthase in the caruncles increased linearly between days 0 and 44 ($P < 0{\cdot}001$), the greatest rise occurring after day 30 ($P < 0{\cdot}01$). However, caruncular total glycogen synthase reached maximum activity on days 15 and 22 and then declined to day 44 ($P < 0{\cdot}01$).

Independent glycogen synthase in the cervical mucosa showed a linear decrease in activity between mating and day 44 ($P < 0{\cdot}05$).

Fig. 3 shows the mean values of the logarithms of the data for glycogen phosphorylase activities in the tract rinsings.

Uterine Rinsings

Glycogen phosphorylase activity increased during early pregnancy ($P < 0{\cdot}05$), that of the active form falling slightly at day 15 from its peak at day 8 ($P < 0{\cdot}05$).

Cervical Rinsings

As it was not possible to determine sufficient glycogen phosphorylase values after day 15, all those measured were placed in a single group (mean 30 days) and labelled 'later' in Fig. 3. Both forms of the enzyme showed a quadratic effect of time with peak values at day 15 ($P < 0{\cdot}05$).

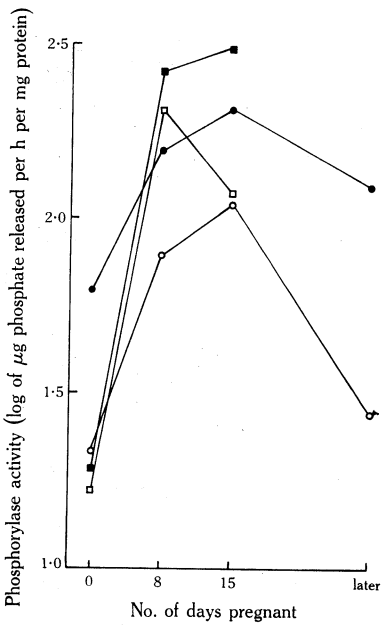

Fig. 3. Glycogen phosphorylase activity in rinsings from the reproductive tract of the pregnant ewe. □ Active form of the enzyme in the uterine rinsings (s.e.m. = 0·24, d.f. = 9). ■ Total enzyme in the uterine rinsings (s.e.m. = 0·23, d.f. = 9). ○ Active form of the enzyme in the cervical rinsings (s.e.m. = 0·26, d.f. = 14). ● Total enzyme in the cervical rinsings (s.e.m. = 0·23, d.f. = 14). Plotted points are the mean values for five ewes.

Discussion

α-Amylase has been found in the oviducts of women, sheep, rabbits and cows at levels greater than the corresponding serum levels (McGeachin *et al.* 1958). Its linear decline during early pregnancy, the peak activity of independent glycogen synthase at days 0 and 15, and the constant high level of glycogen suggest a more rapid turnover of glycogen in the oviducal mucosa at day 0 than at other days. The high α-amylase activity at this time is probably unrelated to capacitation (Bedford 1970), contrary to earlier reports (Kirton and Hafs 1965; Dukelow *et al.* 1966), but related to increased glycogen metabolism under the influence of the pro-oestrus oestrogen surge (Moore *et al.* 1969; Terqui *et al.* 1973).

Glycogen concentrations in the endometrium, caruncles, cervical mucosa and vaginal mucosa all tended to show a slight increase up to day 8 followed by a subsequent fall to lower values. This similar pattern in overall glycogen response masks different responses of individual enzymes in the various tissues to the same hormone environment.

The decline in endometrial glycogen during early pregnancy from the high level at and shortly after the pro-oestrus oestrogen surge confirms previous results (Murdoch 1970*b*) and is consistent with the claim that uterine glycogen levels are increased by oestrogen in this species (Bitman *et al.* 1967). The results failed to confirm the increase in endometrial total and active glycogen phosphorylase activities

at day 8 reported by Murdoch (1970*b*), although greatly increased activities were found in uterine washings on days 8 and 15. It seems likely that there is a small but statistically significant increase in endometrial α-amylase activity around days 15–22. Previous reports have also shown either significant (Murdoch 1970*b*) or non-significant (Hafez and White 1968) increases at this time. Endometrial glycogen synthase activity remained constant and thus there is a suggestion of increased glycogen degradation in the endometrium at days 8–22.

In the caruncular tissue glycogen decreased despite increasing glycogen synthase activity. The small glycogen peak at day 22 coincides with the maximal total glycogen synthase activity but the greatest increase in independent glycogen synthase occurs after this time. The only glycogen-degrading enzyme to show large changes in the caruncles is α-glucosidase which shows maximal values at days 22–30 (O'Shea and Murdoch 1978). Hafez and White (1968) have reported a peak in caruncular α-amylase activity at days 18–19. This peak was very transient and the negative findings in the present study do not therefore conflict with their results. Bryden *et al.* (1972) showed that, in the sheep, the first loose attachment of the chorion to the caruncular mucosa occurs on days 17–18. By day 31 villi, which appear on the surface of the chorionic sac, provide a closer attachment of the extra-embryonic membranes to the uterine caruncles. We interpret our results as showing that increased turnover of glycogen in the caruncles commences with the initiation of implantation.

Cervical glycogen levels did not change significantly although there was decreasing activity during early pregnancy of the independent form of glycogen synthase. In this tissue neither α-amylase nor glycogen phosphorylase altered with pregnancy but another glycogen-degrading enzyme, α-glucosidase, is more active at day 0 than at other days (O'Shea and Murdoch 1978). Thus again glycogen turnover may be more rapid at day 0, presumably in association with the supply of glycogen for cervical secretion (Moghissi 1973) and also perhaps increased supplies of metabolites for spermatozoan metabolism.

In the vaginal mucosa, glycogen levels decreased during pregnancy despite continuing high levels of glycogen synthase. The glycogen-degrading enzymes did not change, but α-glucosidase is higher during early than during later pregnancy (O'Shea and Murdoch 1978). Information, however, is not available on the activity of amylo-1,6-glucosidase (debranching enzyme) at this stage in this tissue. The results allow for no firm conclusion as to changes in glycogen utilization in the vagina during early pregnancy.

Acknowledgments

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