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Activity of Enzymes in the Mucosal Tissues and Rinsings of the Reproductive Tract of the Naturally Cyclic Ewe

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Abstract

The activity of several enzymes has been measured in the oviducal mucosa, endometrium, caruncles, cervical mucosa and vaginal mucosa as well as in uterine, cervical and oviducal rinsings of ewes at days 0 (oestrus), 1, 8 and 15 of the oestrous cycle.

Acid phosphatase activity was maximal during the luteal phase in the oviduct, endometrium and cervix, but not in the vagina where the highest level was on day 1. Alkaline phosphatase activity showed a significant rise at mid-cycle in the endometrium and caruncles as did glucose-6-phosphate dehydrogenase activity in the endometrium.

Glycogen (starch) synthase in the endometrium and cervical mucosa was greater at days 0 and 8 than at days 1 and 15. Vaginal and caruncular total glycogen (starch) synthase activity showed a significant rise at mid-cycle. Active and total glycogen phosphorylase activities were maximal on days 8 and 15 in the vagina. Glycogen concentration was greatest at days 8 and 15 in the cervix, while oviduct values were lower on day 1 than at oestrus or day 15.

In the oviduct rinsings alkaline phosphatase activity was greatest at day 1, whereas in cervical rinsings the activities of acid and alkaline phosphatases were less at oestrus than at days 8 and 15. The glycogen phosphorylase and β-glucuronidase activities in uterine rinsings were greater at days 8 and 15 than at oestrus.

Introduction

Ovarian steroids influence the metabolism of the uterus (Finn and Porter 1975). Murdoch and White (1968) demonstrated that the activities of certain enzymes in the endometrium and caruncles of the ewe fluctuate during the oestrous cycle. No systematic comparative studies, however, have been made in the ewe of changes in enzyme activity of the oviducal mucosa, cervical mucosa and vaginal mucosa. In view of this, the activities of several enzymes in the oviducal mucosa, endometrium, caruncles, cervical mucosa and vaginal mucosa as well as uterine, cervical and oviducal rinsings have been determined in normal cyclic ewes. Because of our interest in interactions of the female genital tract and sperm (see reviews by Thibault and Levasseur 1973; Austin 1975), emphasis was placed on times around oestrus, i.e. days 15, 0 and 1. Day 8 was included to contrast with the oestrous period.

The enzymes examined had already been reported in the literature to undergo changes in activity in some region of the female tract in one or more species (e.g. Rosa and Velardo 1958; Thiery and Willighagen 1963; Rubulis *et al.* 1965; Murdoch and White 1968). They were lactate dehydrogenase (L-lactate : NAD^+ oxidoreductase, EC 1.1.1.27) (LDH), glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate : $NADP^+$ 1-oxidoreductase, EC 1.1.1.49) (GDH), succinate dehydrogenase (succinate : (acceptor) oxidoreductase, EC 1.3.99.1) (SDH), active and total glycogen

phosphorylase (1,4-α-D-glucan : orthophosphate α-glucosyltransferase, EC 2.4.1.1), independent and total glycogen (starch) synthase (UDPglucose : glycogen 4-α-glucosyltransferase, EC 2.4.1.11) (glycogen synthase), aspartate aminotransferase (L-aspartate : 2-oxoglutarate aminotransferase, EC 2.6.1.1) (GOT), alkaline phosphatase (orthophosphoric-monoester phosphohydrolase (alkaline optimum), EC 3.1.3.1), acid phosphatase (orthophosphoric-monoester phosphohydrolase (acid optimum), EC 3.1.3.2), α-amylase (1,4-α-D-glucan glucanohydrolase, EC 3.2.1.1), and β-glucuronidase (β-D-glucuronide glucuronosohydrolase, EC 3.2.1.31).

Materials and Methods

Experimental Animals

Oestrus was detected by running adult Merino ewes with vasectomized rams fitted with Sire-Sine harnesses and examining the ewes daily for raddle marks. Animals were slaughtered by cutting the throat and dislocating the cervical vertebrae on the day of oestrus (day 0), or 1, 8 or 15 days after oestrus. (The ovaries of ewes on day 0 had prominent unruptured follicles while those of ewes on day 1 of the cycle had recently ruptured follicles or blood-spotted eruptions with newly formed corpora lutea.)

Preparation of Tissues and Rinsings

Following slaughter the reproductive tract was removed, placed in crushed ice, and divided into oviducts, uterus, cervix and vagina. Fatty and connective tissues of the broad ligament of the uterus were removed and all subsequent processing of the material was carried out at 4°C. Ice-cold calcium-free Krebs–Ringer solution (4 ml) buffered to pH 7·0 with tris (122 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 25 mM tris, 23 mM HCl) (Krebs–Ringer–tris) was introduced into each uterine horn with a syringe and a blunted 21 gauge needle. The rinsings were passed along each horn by gentle squeezing, and were collected into graduated centrifuge tubes at 4°C. The oviducts were flushed with 1 ml of the Krebs–Ringer–tris solution followed by injection of air, while the cervix was washed from the cranial end with 4 ml of Krebs–Ringer–tris followed by injection of 4 ml of air to ensure removal of the fluid. Cervical washings were shaken to break up any mucus present and all rinsings were centrifuged at 400 *g* for 10 min at 4°C to remove contaminating cells or debris (Wallace and White 1965). Any samples containing red blood cells in the precipitate were discarded. Collection of rinsings was completed within 20 min of slaughter.

After the flush had been performed the fallopian tubes were opened along one side with fine scissors, laid out on narrow perspex strips, and blotted with filter paper to remove any trace of the flushing fluid or secretions. The mucosal lining was then removed by gentle scraping with a scalpel blade and placed on a watch-glass at 4°C. Mucosal layers from the cervix and vagina were removed in a similar manner. The uterine horns were placed on a piece of filter paper and opened along the mesometrial side. The exposed endometrium and caruncles were blotted with filter paper and samples of tissue taken using fine scissors and forceps. As far as possible, an enzyme was estimated at the same time after slaughter in all ewes.

Analytical Methods

Tissues for LDH, GDH, SDH, GOT, alkaline and acid phosphatase, and α-amylase determinations were homogenized in 9 parts of calcium-free Krebs–Ringer–tris with a teflon Potter–Elvehjem homogenizer and then filtered through fine terylene. Samples for glycogen phosphorylase analysis were homogenized in 9 parts of a solution containing 0·1 M NaF and $2{\cdot}4\times10^{-3}$ EDTA adjusted to pH 6·1 (Leonard 1971) and filtered as above. Glycogen synthase preparations were made by homogenizing tissue in 4 volumes of 50 mM tris–HCl, 5 mM EDTA, and 250 mM sucrose (pH 7·8) and centrifuging at 16 000 *g* for 15 min at 4°C (Thomas *et al.* 1968).

The methods for measuring LDH, GDH, GOT, alkaline and acid phosphatase, α-amylase, and β-glucuronidase were based on those described by Bergmeyer (1963). LDH activity was measured by the decrease of optical density at 340 nm due to the oxidation of NADH in the presence of pyruvate at pH 7·5. A unit is the amount of LDH which changes the optical density of NADH at 340 nm by 0·001 in 1 min in a 3-ml assay mixture at 24–27°C (Wroblewski and La Due 1955).

GDH activity was determined at pH 7·5 by measuring the rate of formation of NADPH spectrophotometrically (Warburg *et al.* 1935). One unit of activity is the amount of enzyme in a 1-ml sample which at 25°C in a 3-ml assay mixture changes the optical density of NADPH at 340 nm by 0·001 in 1 min (La Due *et al.* 1954).

GOT activity was measured at pH 7·4 by determining the rate of formation of oxaloacetate colorimetrically as the 2,4-dinitrophenylhydrazone (Reitman and Frankel 1956). The optical density at 546 nm was converted to GOT units from the table in Bergmeyer (1963). A GOT unit is the amount of transferase in 1 ml of homogenate or rinsings which decreases the optical density of NADH at 340 nm by 0·001 in 1 min in a 3-ml assay mixture at 25°C (La Due *et al.* 1954).

Alkaline (pH 10·5) and acid (pH 4·8) phosphatase activities were determined by using p-nitrophenylphosphate as substrate (Bessey *et al.* 1946; Andersch and Szcypinski 1947). One phosphatase unit is defined as being the amount of enzyme, contained in a 1000-ml sample, which liberates 1 mmol (139 mg) p-nitrophenol at 37°C.

α-Amylase was estimated by the change in iodine colour of an amylase stock solution (Street and Close 1956) using 1/10 of the volume prescribed in Bergmeyer (1963) for the micromethod. Under these conditions one Street–Close unit would be contained in 10 ml of sample when 0·1 ml hydrolysed 0·2 mg amylose in 15 min at pH 7·0 and 37°C.

β-Glucuronidase activity was determined at pH 4·5 by the release of phenolphthalein from phenolphthalein glucuronide (Talalay *et al.* 1946; Levvy and Conchie 1966). Activity is reported as micrograms of phenolphthalein released per milligram of protein per hour at 37°C.

The reduction of neotetrazolium chloride to formazan with sodium succinate as substrate was used to determine SDH activity (Sobel and Eckstein 1959). The SDH activity of the homogenate was expressed as micrograms of formazan formed during 20 min at 37°C and pH 7·4.

Total and active glycogen phosphorylase activities were measured at pH 6·1 as described by Leonard (1957) and are reported as micrograms of phosphate liberated from glucose-1-phosphate per milligram of tissue protein per hour.

Independent and total glycogen synthase levels were determined by the 'rapid filter paper assay' described by Thomas *et al.* (1968) and are reported as nanomoles [^{14}C]glucose incorporated into glycogen per milligram of tissue protein per hour at 30°C and pH 7·8.

The glycogen concentration of the tissues was determined with the anthrone reagent of Seiffer *et al.* (1950) after the tissue was digested in 30% (w/v) KOH and the polysaccharide precipitated with ethanol.

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

Statistical Analyses

Analyses of variance were carried out on the data for each enzyme within each tissue and the separation of means was performed by the multiple range test (Duncan 1955). In the tables the standard errors of the means (s.e.m.) calculated from the analyses of variance are given with the associated degrees of freedom, and the statistical significance of the results is given in the text. Where there was heterogeneity of variance in the raw data, the data have been transformed to logarithms for the analysis. For simplification the tables do not include values for those enzymes which showed no significant change in any tissue.

Results

The activities of LDH, SDH and α-amylase did not change significantly during the oestrous cycle in any of the tissues.

Tables 1 and 2 give the mean results for the other enzymes measured in the reproductive tissues of the ewes at 15, 0, 1, and 8 days after the onset of oestrus.

Oviduct

Acid phosphatase activity was greater at mid-cycle than at oestrus ($P < 0·05$). GOT levels were lower on day 15 than at mid-cycle ($P < 0·05$), while total glycogen phosphorylase activity increased at day 8 above that of day 1 ($P < 0·05$). The glycogen concentration was lower on day 1 than at oestrus or day 15 ($P < 0·05$).

α-Amylase activity at oestrus (mean 33 units/10^4 mg protein) was not significantly greater ($P > 0{\cdot}05$) than that at the other days (means 14–19 units/10^4 mg protein). The activity of the other enzymes did not change significantly during the oestrous cycle.

Table 1. Enzyme activity of homogenates of the oviducal mucosa, endometrium, caruncles, cervical mucosa, and vaginal mucosa during the oestrous cycle

Values are the means of five ewes and are calculated as units per milligram protein (see text for definition of units). The standard errors of the means (s.e.m.) are from error mean squares with 16 degrees of freedom. Values with different superscripts differ significantly from each other

Tissue	Day of cycle	$10^4 \times$ Acid phosphatase activity	$10^4 \times$ Alkaline phosphatase activity	GOT activity	GDH activity
Oviducal mucosa	15	$5{\cdot}5^{ab}$	10·9	$14{\cdot}0^{a}$	33·6
	0	$5{\cdot}0^{a}$	15·6	$18{\cdot}3^{ab}$	32·8
	1	$5{\cdot}4^{ab}$	18·3	$15{\cdot}6^{ab}$	20·9
	8	$8{\cdot}1^{b}$	5·6	$24{\cdot}5^{b}$	28·5
	s.e.m.	0·94	4·34	2·90	7·99
Endo-metrium	15	$4{\cdot}4^{ab}$	$91{\cdot}2^{a}$	15·5	$6{\cdot}6^{a}$
	0	$4{\cdot}2^{a}$	$48{\cdot}8^{a}$	11·4	$6{\cdot}6^{a}$
	1	$8{\cdot}5^{ab}$	$95{\cdot}6^{a}$	13·3	$9{\cdot}4^{a}$
	8	$9{\cdot}6^{b}$	$187{\cdot}8^{b}$	15·3	$14{\cdot}4^{b}$
	s.e.m.	1·67	26·9	2·68	1·64
Caruncles					
	15	1·8	$27{\cdot}7^{a}$	7·5	3·0
	0	1·3	$8{\cdot}1^{a}$	8·1	4·0
	1	3·6	$21{\cdot}4^{a}$	8·2	6·2
	8	2·4	$62{\cdot}4^{b}$	9·8	7·9
	s.e.m.	0·74	7·81	2·44	1·54
Cervical mucosa	15	$11{\cdot}9^{ab}$	189·6	20·5	20·9
	0	$9{\cdot}8^{a}$	142·2	21·3	26·3
	1	$13{\cdot}6^{ab}$	102·8	19·1	26·5
	8	$19{\cdot}6^{b}$	230·2	24·3	25·7
	s.e.m.	2·46	44·2	3·69	4·76
Vaginal mucosa	15	$3{\cdot}4^{a}$	9·9	15·5	14·4
	0	$4{\cdot}8^{ab}$	9·3	17·3	26·5
	1	$8{\cdot}6^{b}$	17·3	16·1	24·9
	8	$5{\cdot}4^{ab}$	10·9	20·5	25·1
	s.e.m.	1·30	3·15	2·92	4·97

Endometrium

Alkaline phosphatase and GDH activities were maximal at mid-cycle ($P < 0{\cdot}05$) with the differences between days 8 and 0 significant at the 1 % level. Acid phosphatase values were greater at day 8 than at oestrus ($P < 0{\cdot}05$). Independent glycogen synthase activity was significantly greater at oestrus and mid-cycle than at days 1 or 15 ($P < 0{\cdot}01$), the peak at mid-cycle being greater than that at oestrus ($P < 0{\cdot}05$). Total glycogen synthase activity was lower on day 1 than at mid-cycle ($P < 0{\cdot}01$), oestrus or day 15 ($P < 0{\cdot}05$). Activity of the other enzymes and glycogen concentration did not change significantly during the cycle.

Caruncles

Caruncular alkaline phosphatase activity was significantly greater ($P < 0{\cdot}01$) at mid-cycle than at any other time. Total glycogen synthase increased in activity at mid-cycle ($P < 0{\cdot}05$) after having reached minimum values at day 1 and remained high at day 15. The independent form showed a similar, but not significant, change.

GDH, GOT and glycogen phosphorylase activities, and glycogen concentration did not differ significantly throughout the cycle.

Table 2. Activity of enzymes of glycogen metabolism in homogenates of the oviducal mucosa, endometrium, caruncles, cervical mucosa, and vaginal mucosa during the oestrous cycle

Values are the means of five ewes and are calculated as units per milligram protein (see text for definition of units). The standard errors of the means (s.e.m.) are from error mean squares with 16 degrees of freedom. Values with different superscripts differ significantly from each other

Tissue	Day of cycle	Glycogen (μg/g wet weight)	Glycogen phosphorylase[A]		Glycogen synthase	
			Active	Total	Independent	Total
Oviducal mucosa	15	1501[a]	26·2	57·7[ab]	10·0	120
	0	1287[a]	24·9	57·1[ab]	19·1	180
	1	677[b]	16·4	38·7[a]	23·4	117
	8	1072[ab]	37·8	73·0[b]	17·4	156
	s.e.m.	172	7·69	9·15	5·93[B]	19·9
Endo-metrium	15	764	17·6	36·8	3·3[a]	123[a]
	0	523	19·4	35·7	8·3[b]	125[a]
	1	627	18·1	30·3	2·3[a]	69[b]
	8	985	20·5	32·6	12·9[c]	163[a]
	s.e.m.	145	4·92	7·62	1·37	13·3
Caruncles	15	1129	30·7	41·2	3·1	119[a]
	0	1919	29·7	35·5	4·0	92[ab]
	1	1451	29·4	34·6	1·9	62[b]
	8	2125	43·3	50·8	4·6	116[a]
	s.e.m.	365	8·39	9·73	0·86	16·0
Cervical mucosa	15	1820[a]	33·4	60·9	3·1[a]	91[a]
	0	424[b]	38·0	52·4	14·2[b]	145[b]
	1	906[b]	26·6	43·3	4·7[a]	91[a]
	8	1508[a]	40·6	74·9	11·9[b]	144[b]
	s.e.m.	186[B]	8·73	11·9	1·68	10·1
Vaginal mucosa	15	2058	75·2[a]	100·5[a]	9·9	161[ab]
	0	1040	32·7[b]	49·8[b]	15·6	122[a]
	1	1669	30·0[b]	40·9[b]	9·5	126[a]
	8	1543	73·8[a]	95·6[a]	18·9	191[b]
	s.e.m.	370[B]	11·3	13·4	3·1	14·7[B]

[A] Means of four ewes; 12 degrees of freedom.
[B] 15 degrees of freedom.

Cervix

Acid phosphatase activity at mid-cycle increased above the level at oestrus ($P < 0{\cdot}05$). Both total and independent glycogen synthase levels were higher on days 0 and 8 with large falls in activity occurring by days 1 and 15 ($P < 0{\cdot}01$). Glycogen concentration was greater on days 8 and 15 than on days 0 and 1 ($P < 0{\cdot}01$). GOT,

GDH, α-amylase and glycogen phosphorylase activities remained unchanged throughout the cycle.

Vagina

The most striking changes in the vaginal mucosa were the increases in total and active glycogen phosphorylase and total glycogen synthase activities at days 8 and 15 and at mid-cycle respectively ($P < 0{\cdot}05$). Acid phosphatase activity was greater at day 1 than at day 15 ($P < 0{\cdot}05$).

Table 3 shows the mean logarithms of the data of the enzyme activities of rinsings from the reproductive tract of naturally cyclic ewes.

Table 3. Enzyme activity in rinsings of the oviduct, uterus and cervix during the oestrous cycle
Values are the means of the logarithms of the data from five ewes calculated per milligram of protein (see text for definition of units). The values in parentheses are the mean logarithms of the data calculated as total units per rinsing. The standard errors of the means (s.e.m.) were obtained from error mean squares with 16 degrees of freedom. Values with different superscripts differ significantly from each other

Tissue	Day of cycle	$10^4 \times$ Acid phosphatase activity	$10^4 \times$ Alkaline phosphatase activity	β-Glucuronidase activity	Glycogen phosphorylase	
					Active	Total
Oviduct	15	0·09	$1{\cdot}80^{a}$	1·08	—	—
	0	0·33	$1{\cdot}67^{a}$	0·74	—	—
	1	0·86	$2{\cdot}72^{b}$	0·81	—	—
	8	0·50	$1{\cdot}87^{a}$	0·95	—	—
	s.e.m.	0·29	$0{\cdot}18^{A}$	$0{\cdot}13^{A}$		
Uterus	15	—	—	$2{\cdot}29^{a}$	$2{\cdot}76^{a}$	$2{\cdot}94^{a}$
	0	—	—	$1{\cdot}43^{b}$	$1{\cdot}64^{b}$	$1{\cdot}84^{b}$
	1	—	—	$1{\cdot}01^{b}$	$1{\cdot}55^{b}$	$1{\cdot}70^{b}$
	8	—	—	$2{\cdot}44^{a}$	$2{\cdot}74^{a}$	$2{\cdot}82^{a}$
	s.e.m.			0·18	0·24	0·21
Cervix	15	$1{\cdot}24^{a}$	$3{\cdot}14^{a}$	$1{\cdot}59\ (1{\cdot}21)^{a}$	2·26	$2{\cdot}64\ (2{\cdot}20)^{a}$
	0	$0{\cdot}61^{b}$	$2{\cdot}37^{b}$	$0{\cdot}97\ (0{\cdot}64)^{b}$	1·40	$1{\cdot}89\ (1{\cdot}71)^{b}$
	1	$1{\cdot}00^{ab}$	$3{\cdot}07^{a}$	$0{\cdot}86\ (0{\cdot}87)^{ab}$	1·50	$1{\cdot}96\ (2{\cdot}12)^{ab}$
	8	$1{\cdot}31^{a}$	$3{\cdot}12^{a}$	$1{\cdot}32\ (1{\cdot}33)^{a}$	2·29	$2{\cdot}59\ (2{\cdot}23)^{a}$
	s.e.m.	0·15	0·12	0·27 (0·14)	$0{\cdot}36^{A}$	$0{\cdot}29^{A}$ (0·13)

[A] 15 degrees of freedom.

Oviducal Washings

The only significant cyclic change was the greater alkaline phosphatase activity at day 1 than at other days ($P < 0{\cdot}05$). Acid phosphatase, LDH (mean $417 \pm$ s.e. 69 units/mg protein), GDH (29 ± 9), and β-glucuronidase activities showed no significant changes.

Uterine Washings

Active and total phosphorylase and β-glucuronidase activities were greater at mid-cycle and day 15 than at days 0 and 1 ($P < 0{\cdot}01$).

Cervical Washings

LDH (mean $303 \pm$ s.e. 48 units/mg protein), GDH (34 ± 7), and GOT (35 ± 5) activities did not change significantly.

Acid phosphatase activity on days 8 and 15 exceeded that on day 0 ($P < 0{\cdot}05$) while alkaline phosphatase levels were significantly less on day 0 than on the other days ($P < 0{\cdot}05$). Although both glycogen phosphorylase and β-glucuronidase activities were less at oestrus than at days 8 and 15, the differences were significant ($P < 0{\cdot}05$) only when the data for total glycogen phosphorylase and β-glucuronidase were expressed as total activity per organ rinse.

Discussion

In the endometrium, caruncles, and uterine rinsings, for those enzymes showing significant changes, maximal enzyme activities occurred at day 8. These results confirm and extend the findings of Murdoch and White (1968) that in the ewe endometrial and caruncular acid and alkaline phosphatase activities are correlated with the growth and regression of the corpus luteum. This is not so in all species; for example in women and sows (Atkinson and Engle 1947; Hall 1950; Goode *et al.* 1965; Gautray *et al.* 1969) oestrogen appears to stimulate uterine alkaline phosphatase. Acid phosphatase activities in the oviducal mucosa and cervical mucosa are similar to that of the endometrium in that they are greater at day 8 than at day 1, whereas acid phosphatase activity in the vagina shows a different pattern in being greater at day 1 than at day 15. Thus besides species differences, there may be variations in hormonal influence on the activity of the same enzyme along the reproductive tract of the ewe.

The cyclic changes in SDH, GOT and α-amylase activities reported by Murdoch and White (1968) in the endometrium and caruncles were not confirmed. The changes in GOT reported by Murdoch and White (1968) were mainly due to a decrease in GOT activity in their synchronized animals at day 15 with no change in the naturally cyclic ewes. The other differences may be due to the different comparisons used by Murdoch and White in that they compared periods of the cycle (e.g. days 0–2 *v.* days 8–14) rather than specific days as in the present paper.

In the oviduct glycogen levels decreased during oestrus from a peak at day 15 to a minimum at day 1. This was despite a high level of glycogen synthase. The increase in oviducal α-amylase activity at oestrus did not reach significance, but studies on pregnant ewes have shown a steady decrease after oestrus in α-amylase activity in the oviduct (O'Shea and Murdoch 1978*a*). Thus the two catabolic enzymes measured had different patterns during the oestrous cycle with total glycogen phosphorylase showing a rise from day 1 to day 8, whereas α-amylase showed maximum activity at day 0. Similarly a difference in pattern between glycogen phosphorylase and α-amylase during the menstrual cycle has been reported in the cervical mucus of the Bonnet monkey (Sheth *et al.* 1975).

Cervical glycogen fell markedly at oestrus, despite the high level of activity of glycogen synthase. In this tissue α-amylase and glycogen phosphorylase remained constant, but the activity of acid α-1,4-glucosidase was at a maximum at day 0 in the cervical mucosa (O'Shea and Murdoch 1978*b*). Thus in the oviduct and cervix both synthesis and degradation of glycogen are increased at oestrus. In the vaginal

mucosa glycogen synthase activity was maximal at day 8 while that of glycogen phosphorylase was high at both 8 and 15 days. Thus synthetic and degradative activities are increased in this tissue during the period of progesterone dominance.

In the rat, rabbit, mouse and sheep the synthesis of uterine glycogen is dependent upon oestrogen (Hall 1965; Bitman *et al.* 1967; Gregoire *et al.* 1967; Cecil and Bitman 1968). Endometrial glycogen synthase activity was greatest at day 8 in the present experiments but the second peak in independent glycogen synthase activity at day 0 suggests both progesterone and oestrogen may be concerned with glycogen metabolism in the sheep endometrium. In a similar fashion total glycogen synthase activity in the rat uterus is increased following either progesterone or oestrogen treatment (Bo and Ashburn 1968). Caruncular total glycogen synthase activity appears to be associated with progesterone as its activity is greater at days 8 and 15.

The limited material available did not allow all enzyme activities examined in the tissues to be investigated in the rinsings. Murdoch and White (1968) have previously reported the levels of some enzymes in uterine flushings so these activities were not determined. In the present study all enzymes examined in each flushing were found to be present in measurable but variable quantities. As with uterine rinsings (Murdoch and White 1968), alkaline and acid phosphatases in the rinsings of the cervix showed changes in activity which correlated with the changes observed in enzyme activity in the tissues. However, acid phosphatase in the oviducal rinsings, and GDH in oviducal and cervical rinsings were unrelated to enzyme levels in the corresponding tissues. In such a comparison two points should be remembered. Firstly, the rinsings from the cervix would have included some uterine secretions (Mattner 1968), and secondly, the tissue samples analysed would have included other cells in addition to those concerned with formation of secretions in the lumen. In addition rinsings do not give values for the concentration of enzymes in tract secretions. However, where the same cyclical changes were observed when the data were expressed both per total enzyme recovered and per milligram of protein they indicate similar changes in the luminal fluid.

Where changes were observed, peak values were mostly at day 1 in the oviducal rinsings and at days 8 and 15 in the uterine and cervical rinsings. This again emphasizes that the period of greatest metabolic activity varies between the organs of the reproductive tract. It also may suggest that secretion of these particular enzymes by the uterus and cervix of the ewe may have little direct physiological importance for spermatozoa (Thibault and Levasseur 1973; Austin 1975).

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