Aust. J. Biol. Sci., 1980, **33**, 549–55

Acid and Alkaline Phosphatases in Histologically Defined Areas of the Sheep Uterus and Placenta: Histochemical and Microfluorometric Analyses

M. Javad Zamiri

Department of Physiology and Pharmacology, University of Queensland, St. Lucia, Queensland, 4067.

Abstract

Acid and alkaline phosphatases were studied in different structures of the sheep uterus and placenta by semiquantitative histochemistry of frozen sections (days 10–80 of pregnancy) and quantitative microfluorometry of freeze-dried sections (days 10–145 of pregnancy).

Histochemically, lowest activity for acid phosphatase was found in the uterine glands, but biochemically it was lowest in the maternal caruncles. Histochemical acid phosphatase activity in the uterine glands and maternal caruncles increased on day 80 of gestation; however, biochemically, the activity decreased in the glands but did not change in the maternal caruncles during pregnancy. Weak histochemical staining was found in the luminal epithelium on days 25 and 80 of gestation compared with other days, whereas biochemically the activity was high at these times.

Histochemically, alkaline phosphatase activity in the luminal epithelium, uterine glands and maternal caruncles was high and did not change during pregnancy. Fluorometrically, the activity decreased in the luminal epithelium but it increased in the uterine glands and maternal caruncles. Histochemical data showed higher alkaline phosphatase activity in the maternal caruncles and trophoblast than in the foetal cotyledons, but biochemically no activity was detected in the trophoblast and foetal cotyledons.

Introduction

The role of acid phosphatase [orthophosphoric-monoester phosphohydrolase (acid optimum), EC 3.1.3.2] and alkaline phosphatase [orthophosphoric-monoester phosphohydrolase (alkaline optimum), EC 3.1.3.1] in reproductive processes remains unclear. Acid phosphatase (ACP) release has been associated with lysosomal involvement in implantation and placentation in the sheep (Boshier 1969), man (Contractor *et al.* 1977) and mouse (Moulton *et al.* 1978). Alkaline phosphatase (ALP) activity in the luminal epithelium and trophoblast of the sheep has been associated with carbohydrate metabolism and the production of trophoblastic fibrinoid (Boshier 1969).

All spectrophotometric (Hafez and White 1968; Murdoch 1970*a*, 1970*b*) and histochemical (Boshier 1969; Murdoch 1972) studies of ACP and ALP in the uterus of pregnant sheep have been confined to the first few weeks of gestation. Histochemical findings have been reported mostly for the epithelial and caruncular tissues and other uterine tissues have not received much attention.

Data from histochemical and biochemical studies in some tissues have been contradictory (Chayen *et al.* 1969) but no comparative studies have yet been reported for the sheep uterus. The biochemical data which are obtained by spectrophotometric determination of enzyme activity of tissue homogenate give accurate information on enzyme levels but do not indicate the site of enzyme activity. The significance of

uterine enzymes, if any, in reproductive physiology could be more confidently assessed if they could be localized more precisely and their activity determined more accurately than by spectrophotometric methods. These objectives can be achieved by using the highly sensitive, quantitative, histochemical techniques of Lowry and Passonneau (1972) which allow determination of enzymic activity in minute amounts of tissue.

This paper describes the semiquantitative histochemical and quantitative microfluorometric analysis of ACP and ALP activity in histologically defined areas of the gravid sheep uterus and placenta.

Materials and Methods

Animals and Tissues

Merino ewes were used in these studies. On each of days 10, 15, 20, 25, 30, 80 and 145 after mating five ewes were killed and a segment of the uterine horns as well as placentomes from days 80 and 145 were immediately frozen in liquid nitrogen (Zamiri and Blackshaw 1979*a*). The tissues were stored at −80°C until processed. A small segment from the mid-part of the gravid (ipsilateral to the corpus luteum), and non-gravid (contralateral to the corpus luteum) uterine horns and placentomes from each of days 80 and 145 were dissected in a cold chamber (−25°C), placed on a specimen holder and then frozen in cold hexane kept on dry ice (−80°C). In this paper the term 'placentome', consisting of the maternal caruncle and foetal cotyledon, has been used for the functional units of the sheep placenta (Hafez and Jainudeen 1974).

Histochemical Techniques

Frozen sections (10 μm) from the mid-segment of the gravid and non-gravid uterine horns were cut in a cryostat at −25°C, mounted on microscope slides, air-dried and stained for ACP and ALP (Zamiri and Blackshaw 1979*b*). The incubation medium contained sodium-α-naphthyl phosphate (1 mg/ml), Fast Blue BB (1 mg/ml) and polyvinylpyrrolidone (75 mg/ml) in 100 mM acetate buffer, pH 5·0 (ACP) or in 100 mM Tris-HCl, pH 10·0 (ALP). Fresh incubation medium was pipetted on the sections which were incubated for 45 min at 37°C (ACP) or at room temperature (ALP).

To semiquantify enzymic activity, two sections from each uterine horn at different stages of pregnancy (days 10–80) were randomly selected and each section was scored (×500) on a scale of 0–5, from no activity (0), very weak (1) to very strong activity. The scores for the two sections were summed and used as the unit for the analysis of variance, but mean scores are given in the tables. Enzyme activity was determined in different uterine structures as previously described (Zamiri and Blackshaw 1979*b*).

Microfluorometric Techniques

Frozen sections (10 μm) were cut, freeze-dried at −35°C under vacuum and stored in vacuum tubes (−25°C) (Matschinsky *et al.* 1968; Lowry and Passonneau 1972). Before analysis vacuum tubes were warmed to room temperature (20°C), the vacuum broken and the sections were removed from the section holder.

A stereozoom dissecting microscope (Zeiss-DV4) with a wide range of magnification (×10–×40) was used for dissecting tissue sections. The section was held in place with a hair-loop or a hair point, and dissection was accomplished by a series of short vertical cuts through the section using a dissection knife which was made from a splinter of a razor blade mounted on a flexible bristle. Portions of the luminal epithelium, uterine glands, maternal caruncles, foetal cotyledons and intercotyledonary trophoblast (0·1–0·4 μg dry weight) were dissected, picked up and transferred to sample carriers with a hair point. Tissue portions were weighed on a quartz-fibre fishpole balance which was constructed by mounting a single quartz fibre horizontally at one end; it was enclosed in a small balance case made from a glass hypodermic syringe. Samples were placed on the free end of the fibre and the deflection of the fibre (proportional to the weight added) was measured on a 10-mm scale placed in the ocular of a horizontally mounted dissecting microscope (×10). These and subsequent procedures were carried out in an air-conditioned room at 20°C (see Lowry and Passonneau 1972 for details).

The methods for ACP and ALP assay were modifications of those described for quantitative histochemistry of these enzymes in the brain (Lowry *et al.* 1954), in which *p*-nitrophenyl phosphate

was replaced with α-naphthyl phosphate (Sigma, St. Louis, U.S.A.) using 100 mM acetate buffer (pH 5·0) for ACP or 100 mM Tris-HCl buffer (pH 10·0) for ALP. The concentrations of the constituents in the reagents, prepared fresh before use, were: 100 μM α-naphthyl phosphate, 5 mM MgCl, 0·05% (w/v) bovine serum albumin and 0·5% (w/v) Triton X100 (for ACP only). The detergent, Triton X100, was included in the medium for ACP to solubilize lysosomal membranes and release ACP. All solutions were prepared in deionized water.

Standards

A solution of 100 mM α-naphthol (Sigma, St. Louis, U.S.A.) was prepared in ethanol (A.R.) and stored at −25°C. This was then diluted with 0·5 M NaOH immediately before use to give final concentrations of standards in fluorometer tubes of 0·1–0·2 μM for ACP and 0·5–0·8 μM for ALP.

Assay Procedure

Fluorometer tubes were kept on ice and 0·5 ml reagent was added to each tube. Standards or tissue samples (ACP: 0·3–0·4 μg; ALP: 0·1–0·2 μg) were transferred to tubes and the tubes were incubated at 37°C for 60 min (ACP) or 30 min (ALP). At the end of incubation, 0·5 ml 1 M NaOH was added to each tube, mixed and cooled to room temperature before reading fluorescence in a Jasco (model FP-550) spectrofluorometer (Japan Spectroscopic Co. Ltd.). The cuvette carrier was modified to accommodate round-bottomed glass tubes (int. diam. = 7 mm; length = 60 mm), so that fluorescence could be directly measured in these tubes. One of the standards was set at ≃100 against one of the blanks (excitation wavelength, 330 nm; emission wavelength, 480 nm) and readings were made for other tubes. Calculations of enzymic activities were based on standards, using triplicates of samples, standards and blanks. The enzyme activity was expressed in moles of substrate converted per kilogram dry weight of tissue in 1 h.

Statistical Analysis

The data were analysed by analysis of variance on a PDP10 computer. Due to the sampling procedure, maternal caruncles sometimes could not be found in sections from one or both horns of some ewes and the caruncular enzymic activity was thus analysed by one-way analysis of variance. Student–Neuman–Keuhl (SNK) rank test was used to compare means, after tabular values were corrected for the appropriate degrees of freedom (Sokal and Rohlf 1969).

One ewe from each of days 10, 15, 30 and 80 ovulated from both ovaries; therefore, the results from these animals were not included in the analysis of variance.

Results

Acid Phosphatase

(*a*) *Histochemical*

Activity of ACP (mean score) in the luminal epithelium decreased on day 25 and lowest activity was recorded on day 80 (Table 1*a*). In uterine glands, the activity was generally low early in pregnancy. However, by mid-pregnancy, it increased to moderate levels. The activity in the caruncles decreased on day 25 but by day 80 it increased to very high levels. There was no ACP activity in the longitudinal myometrium and the blood vessels but the activity was very low (1·1±0·2) in the circular myometrium and stroma. There were no differences in ACP activity between uterine horns.

(*b*) *Microfluorometric analysis*

Activity of ACP in the luminal epithelium and glands was not different between the gravid and non-gravid uterine horns. In the luminal epithelium activity of ACP (Table 1*a*) was highest on days 15–25 and 80 of pregnancy and lowest activity was seen on day 145. In uterine glands the activity was high on day 10, but decreased throughout pregnancy so that on day 145 it was about 100-fold lower than on day 10. There were large variations in the enzymic activity of the uterine glands from the same animals. In maternal caruncles, ACP activity did not change during pregnancy.

In the foetal cotyledons, similar ACP activity ($0{\cdot}03 \pm 0{\cdot}01$) was found on days 80 and 145 of gestation; however, in the trophoblast the activity increased significantly ($P < 0{\cdot}05$) on day 145 ($0{\cdot}67 \pm 0{\cdot}10$) compared with day 80 ($0{\cdot}25 \pm 0{\cdot}10$) of pregnancy.

Table 1. Comparison of acid phosphatase (*a*) and alkaline phosphatase (*b*) activity in the pregnant sheep uterus by qualitative histochemical scoring (H) and quantitative microfluorometry (M)

Values given as μmoles substrate hydrolysed per milligram dry weight of tissue per hour. In each column values without any common superscripts are significantly different ($P < 0{\cdot}05$). * Activity higher in the gravid horn ($P < 0{\cdot}05$) except on day 15 from mating when it was higher in the luminal epithelium of the non-gravid horn

Days from mating	No of ewes	Luminal epithelium[A] H	Luminal epithelium[A] M	Uterine glands[A] H	Uterine glands[A] M	Maternal caruncles[B] H	Maternal caruncles[B] M
		(*a*) Acid phosphatase activity					
10	4	$4{\cdot}9^{a}$	$0{\cdot}20^{bc}$	1·7	$1{\cdot}46^{a}$	$3{\cdot}8^{ab}$ (6)	0·10 (4)
15	4	$4{\cdot}8^{a}$	$0{\cdot}30^{a}$	1·4	$0{\cdot}80^{b}$	$4{\cdot}2^{a}$ (6)	0·10 (4)
20	5	$4{\cdot}4^{ab}$	$0{\cdot}23^{abc}$	1·7	$0{\cdot}41^{bc}$	$3{\cdot}5^{ab}$ (4)	0·10 (5)
25	5	$3{\cdot}6^{b}$	$0{\cdot}25^{ab}$	1·6	$0{\cdot}34^{bc}$	$3{\cdot}0^{b}$ (7)	0·10 (5)
30	4	$4{\cdot}0^{ab}$	$0{\cdot}15^{c}$	1·2	$0{\cdot}16^{c}$	$3{\cdot}5^{ab}$ (4)	0·08 (4)
80	4	$2{\cdot}8^{c}$	$0{\cdot}23^{abc}$	$2{\cdot}7^{a}$	$0{\cdot}12^{c}$	$4{\cdot}6^{a}$ (5)	0·13 (4)
145	5	—	$0{\cdot}07^{d}$	—	$0{\cdot}01^{c}$	—	0·06 (5)
Error mean square	—	0·37	0·0028	0·38	0·1140	0·42	0·0013
Degrees of freedom	—	40	48	40	48	26	24
		(*b*) Alkaline phosphatase activity					
10	4	4·6	$24{\cdot}7^{a}$*	3·4	$9{\cdot}5^{a}$	4·8 (6)	$3{\cdot}3^{a}$ (5)
15	4	5·0	$6{\cdot}3^{b}$*	4·3	$5{\cdot}0^{b}$*	4·4 (7)	$1{\cdot}4^{b}$ (5)
20	5	4·7	$4{\cdot}0^{bc}$	3·6	$3{\cdot}5^{b}$	4·5 (4)	$1{\cdot}7^{b}$ (5)
25	5	4·4	$3{\cdot}6^{bc}$	4·1	$4{\cdot}9^{b}$	4·8 (5)	$1{\cdot}7^{b}$ (5)
30	4	4·4	$3{\cdot}2^{bc}$	4·4	$5{\cdot}1^{b}$	4·6 (5)	$2{\cdot}0^{b}$ (5)
80	4	4·2	$2{\cdot}6^{bc}$	4·5	$8{\cdot}9^{a}$	5·0 (5)	$3{\cdot}4^{a}$ (5)
145	5	—	$0{\cdot}6^{c}$	—	$0{\cdot}8^{c}$	—	$0{\cdot}4^{c}$ (5)
Error mean square	—	0·33	6·88	0·45	3·04	0·21	0·51
Degrees of freedom	—	40	48	40	48	26	28

[A] Mean activity for the gravid and non-gravid uterine horns.
[B] Numbers in parentheses are the number of maternal caruncles for each day of pregnancy.

Alkaline Phosphatase

(*a*) *Histochemical*

Activity (mean score) of ALP in the luminal epithelium, uterine glands and caruncles was very high and did not change during pregnancy (Table 1*b*). No ALP activity was found in the longitudinal layer of the myometrium and the blood vessels. The activity was higher ($P < 0{\cdot}05$) in the circular myometrium on day 10 ($3{\cdot}5 \pm 0{\cdot}4$) compared with other stages ($2{\cdot}2 \pm 0{\cdot}4$). In the stroma, the activity was constant between days 10–30 ($3{\cdot}0 \pm 0{\cdot}3$), but it decreased significantly ($P < 0{\cdot}05$) on day 80 ($2{\cdot}1 \pm 0{\cdot}3$).

(*b*) *Microfluorometric analysis*

On day 10 post-mating, activity of ALP in the luminal epithelium was higher in the gravid horn than in the non-gravid horn ($P<0\cdot05$). Between days 10 and 15, the activity decreased in both uterine horns, but relatively less in the non-gravid horn, so that by day 15, ALP activity was higher in the non-gravid horn compared with the gravid horn ($P<0\cdot05$). The activity did not change between days 20–80, but it had decreased significantly on day 145 compared with the activity on days 10 and 15 (Table 1*b*).

On day 15 post-mating, ALP activity in the uterine glands was higher in the gravid horn than in the non-gravid horn ($P<0\cdot05$). Between days 10–15 ALP activity decreased in the uterine glands as well as the maternal caruncles, but there was an increased activity in both tissues on day 80, approaching day 10 values, followed by a fall on day 145 (Table 1*b*). No activity was found in the trophoblast and foetal cotyledons on days 80 and 145.

Discussion

In a histochemical study of placentome formation in the ewe, Boshier (1969) found a high level of ACP activity in the luminal epithelium from days 14 to 16 of pregnancy which persisted in the caruncular epithelium until the latter part of the third week but decreased during the fourth week. In the present work, a high level of ACP was demonstrated in the caruncles up to day 20, decreasing significantly at day 25 and then increasing to day 80. In the luminal epithelium, ACP activity was very high on days 10 and 15, but decreased on days 25 and 80.

The luminal epithelium, uterine glands and caruncles showed high levels of ALP irrespective of the stage of pregnancy. Boshier (1969) reported the presence of ALP at the junction of the trophoblast and the maternal epithelium between the third to fourth week of ovine pregnancy. In contrast to Boshier (1969), who reported high levels of ALP in the subepithelial capillaries, no ALP was found in blood vessels in the present investigation. In studies by Murdoch (1970*b*) of the ovine uterus during early pregnancy ALP was localized mainly in the luminal epithelium and no activity was seen in the endometrial blood vessels and myometrium. Foley *et al.* (1954), using a method similar to Boshier's (1969), reported strong staining of capillaries in the endometrium of the cow during pregnancy.

In the placentomes ACP and ALP were mainly localized in the maternal caruncles and the trophoblast; whereas in the foetal cotyledons the activity was much lower, a finding similar to that reported by Fahmy (1957) and Christie (1967, 1968).

The substrate used for the enzymic reaction was the same in both the histochemical and microfluorometric methods, and there appears to be no explanation for the discrepancies observed between these methods. Some studies on ACP have indicated that enzymic activity demonstrated histochemically by azo-dye techniques was different from that characterized biochemically, although it is not known if the same substrate was used in these experiments (Chayen *et al.* 1969).

Although the luminal epithelium is a narrow structure and difficult to dissect into large pieces completely free of stroma, it is unlikely that the biochemical activity was influenced by such contamination as several small, clean (checked at $\times80$ magnification) pieces of epithelium were used in each tube. Furthermore, subepithelial stroma shows similar enzymic activities to the luminal epithelium (Zamiri, unpublished data).

Diffusion of enzymes into the incubation medium is one of the limitations in the histochemistry of soluble enzymes such as ALP, and if considerable diffusion of ALP had occurred in the present work, this could explain the constant activity noticed during pregnancy and thus the lack of correlation between histochemical and fluorometric data.

The use of semipermeable membranes has to some extent alleviated the problem of enzyme diffusion into the incubation medium (Meijer 1973) and it could improve ALP histochemistry. Alternatively, because ALP activity on days 10–80 of pregnancy was generally high in the ute ine structures studied (fluorometric data), if the incubation time (45 min) were to be shortened this would allow changes in activity to be detected histochemically.

When assayed fluorometrically, ACP activity in the maternal caruncles remained unchanged between days 10 and 145 of gestation; similarly, Hafez and White (1968) and Murdoch (1972) found a constant activity during the first 6 weeks of gestation. ACP activity in the uterine glands decreased during pregnancy, but in the luminal epithelium maximum activity was recorded on days 15–25 and day 80. In homogenates of the whole endometrium maximum activities were found on day 8 (Murdoch 1970*a*) and on days 20–30 (Hafez and White 1968; Murdoch 1970*a*).

Activity of ALP in all uterine structures was highest on day 10 and decreased between days 10 and 15. This considerably lower level of activity was maintained until day 80 in the luminal epithelium, and until day 30 in the maternal caruncles and glands. On day 80, ALP activity in the maternal caruncles and uterine glands increased to day 10 levels; lowest activity was recorded on day 145 of gestation. The results confirmed the findings of Murdoch (1970*a*, 1972) who reported high levels of ALP in the caruncles and endometrium on day 8 of pregnancy followed by a decreased activity on day 14. By contrast, Hafez and White (1968) found maximum levels of ALP on days 13–17 of gestation in sheep.

ACP results are in agreement with those suggesting a role for lysosomes in implantation, placentation (Boshier 1969; Contractor *et al.* 1977) and transfer of iron to the foetus (Seal *et al.* 1972; Myagkaya *et al.* 1979). High concentrations of fructose have been found in blood of the foetal sheep (Ainsworth *et al.* 1950), and Huggett *et al.* (1951) showed that fructose was formed from glucose in the ovine placenta. Fahmy (1957) suggested that this transformation occurred in the maternal part of the placenta and possibly was regulated by ALP, although according to Britton *et al.* (1967) fructose could also be formed from glucose via the sorbitol pathway. However, the present ALP data do not indicate any significant role for ALP in fructose formation by the sheep placenta, since in the maternal caruncles as well as the luminal epithelium and glands ALP activity decreased between days 80–145, the period when a large amount of fructose is found in the blood of foetal sheep.

Acknowledgment

I would like to thank Dr Alan Blackshaw for encouragement and criticism of the manuscript.

References

Ainsworth, I. B., Parr, C. W., and Warren, F. L. (1950). Fructose formation in the placenta. *J. Endocrinol.* **7**, lxiii–lxv.

Boshier, D. P. (1969). A histological and histochemical examination of implantation and early placentome formation in sheep. *J. Reprod. Fertil.* **19**, 51–61.

Britton, H. G., Huggett, A. St. G., and Nixon, D. A. (1967). Carbohydrate metabolism in the sheep placenta. *Biochim. Biophys. Acta* **136**, 426–40.

Chayen, J., Bitensky, L., Butcher, R. J., and Poulter, L. W. (1969). 'A Guide to Placental Histochemistry. (Oliver and Boyd: Edinburgh.)

Christie, G. A. (1967). Comparative histochemical distribution of glycogen and alkaline phosphatases in the placenta'. *Histochemie* **9**, 93–116.

Christie, G. A. (1968). Comparative histochemical distribution of acid phosphatase, non-specific esterase, and β-glucuronidase in the placenta and foetal membranes. *Histochemie* **12**, 189–207.

Contractor, S. F., Banks, R. W., Jones, C. J. P., and Fox, H. (1977). A possible role for placental lysosomes in the formation of villous syncytiotrophoblast. *Cell Tiss. Res.* **178**, 411–19.

Fahmy, A. N. (1957). Site and origin of fructose in sheep placenta. *Gaz. Egypt. Soc. Gynaec. Obstet.* **5**, 54–67.

Foley, R. C., Reece, R. P., and Leathem, J. H. (1954). Histochemical observations of the bovine uterus, placenta, and corpus luteum during early pregnancy. *J. Anim. Sci.* **13**, 131–7.

Hafez, E. S. E., and Jainudeen, M. R. (1974). Gestation, prenatal physiology and parturition. In 'Reproduction in Farm Animals'. (Ed. E. S. E. Hafez.) 3rd edn. pp. 166–202. (Lea and Febiger: Philadelphia.)

Hafez, E. S. E., and White, I. G. (1968). Endometrial and embryonic enzyme activities in relation to implantation in the ewe. *J. Reprod. Fertil.* **16**, 59–67.

Huggett, A. St. G., Warren, F. L., and Warren, N. V. (1951). The origin of the blood fructose of the foetal sheep. *J. Physiol. (London)* **113**, 258–75.

Lowry, O. H., and Passonneau, J. V. (1972). 'A Flexible System of Enzymatic Analysis.' (Academic Press: New York.)

Lowry, O. H., Roberts, N. R., Wu, M. L., Hixon, W. S., and Crawford, E. J. (1954). The quantitative histochemistry of brain. II. Enzyme histochemistry. *J. Biol. Chem.* **207**, 19–37.

Matschinsky, F. M., Passonneau, J. V., and Lowry, O. H. (1968). Quantitative histochemical analysis of glycolytic intermediates and cofactors with an oil well technique. *J. Histochem. Cytochem.* **16**, 29–39.

Meijer, A. E. F. (1973). Semipermeable membranes for improving the histochemical demonstration of enzyme activities in tissue sections. III. Lactate dehydrogenase. *Histochemie* **35**, 165–72.

Moulton, B. C., Koenig, B. B., and Borkan, S. C. (1978). Uterine lysosomal enzyme activity during ovum implantation and early decidualization. *Biol. Reprod.* **19**, 167–70.

Murdoch, R. N. (1970*a*). Uterine endometrial phosphomonoesterases in relation to implantation in the ewe and rabbit doe. *Aust. J. Biol. Sci.* **23**, 1089–97.

Murdoch, R. N. (1970*b*). Glycogen, glycogen-metabolizing enzymes, and acid and alkaline phosphatases in the endometrium of the ewe during early pregnancy. *Aust. J. Biol. Sci.* **23**, 1289–96.

Murdoch, R. N. (1972). Phosphomonoesterases and histamine in the uterus of the ewe during early pregnancy. *Aust. J. Biol. Sci.* **25**, 1289–97.

Myagkaya, G., Schellens, J. P. M., and Vreeling-Sindelarova, H. (1979). Lysosomal breakdown of erythrocytes in the sheep placenta. An ultrastructural study. *Cell Tiss. Res.* **197**, 79–94.

Seal, U. S., Sinha, A. A., and Doe, R. P. (1972). Placental iron transfer: Relationship to placental anatomy and phylogeny of the mammals. *Am. J. Anat.* **134**, 263–9.

Sokal, R. R., and Rohlf, F. J. (1969). 'Biometry.' (W. H. Freeman and Co.: San Fransisco.)

Zamiri, M. J., and Blackshaw, A. W. (1979*a*). Microfluorometric study of glycolytic enzymes in histologically defined areas of the sheep uterus and placentomes during pregnancy'. *Biol. Reprod.* **21**, 1257–61.

Zamiri, M. J., and Blackshaw, A. W. (1979*b*). Enzyme histochemistry of the sheep uterus during the oestrous cycle. *Aust. J. Biol. Sci.* **32**, 409–14.

Manuscript received 20 November 1979, accepted 18 July 1980