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Metabolic Properties of Mouse Uterine Endometrial Cells after Isolation with Collagenase

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Abstract

Uterine horns from unmated, pseudopregnant (days 1–5) and pregnant (days 4 and 5) mice were dissected and treated with collagenase (5 mg/ml) in a Ringer solution to isolate viable endometrial cells with maximal recovery of the luminal epithelium.

The oxidative metabolism of the cells incubated with radioactive glucose significantly increased on day 4 of pregnancy, was stimulated during pseudopregnancy by the addition of either concanavalin A (Con A, 100 μg) or insulin (0·1 i.u.), and responded to a variety of traditionally used metabolic inhibitors in a manner consistent with that of cells possessing normal transport and oxidative functions. The metabolism of endogenous materials was an important feature of the cells and no evidence of a Crabtree effect was detected in the presence of various exogenous substrates. The cells also failed to alter their capacity to oxidize radioactive glucose after a 3-h pre-incubation period *in vitro*. Although the cells agglutinated in the presence of Con A at all reproductive stages studied, the agglutination indices significantly increased both on day 1 and on days 4 and 5 *post coitum*.

The results indicated that metabolic changes and membrane modifications occurring in the cells at the time of implantation may have important roles in facilitating the induction of the decidual cell reaction. It was concluded that, if further technological improvements to the collagenase procedure succeed in producing pure epithelial cell preparations, the cells should be suitable for use in studies designed to elucidate these roles.

Introduction

The intimate association established between the blastocyst and uterine epithelium during early pregnancy in rodents has led to the widely held belief that luminal epithelial cells are involved in the transmission of a stimulus or signal from the embryo to the underlying stromal tissue for induction of the decidual cell reaction (DCR) (Finn and Porter 1975; Jonsson *et al.* 1979; Lundkvist *et al.* 1979). This proposal is supported by the recent experimental findings of Lejeune *et al.* (1981) which more directly indicate that the luminal epithelium is an obligatory transmitter of the deciduogenic stimulus in rats.

The association between the blastocyst and the luminal epithelium at the time of decidual cell induction may involve cell surface alterations (Johnson and Calarco 1980) that facilitate interactions between glycoprotein residues (Buxton and Murdoch 1982) with the subsequent production by the epithelial cells of the transmitter material. Although the identity of this putative transmitter substance remains obscure, the available evidence suggests that prostaglandins may be contenders for the role (Tobert 1976; Kennedy 1977; Rankin *et al.* 1979; Lundkvist and Nilsson 1980) and may include prostacyclin (PGI_2) (Jonsson *et al.* 1979).

One experimental approach that could help to elucidate both the nature of the blastocyst stimulus and the transmitter role of the uterine luminal epithelium is to study the *in vitro* metabolism of isolated epithelial cells in the presence of various stimuli. However, most procedures adopted for the isolation of epithelial cells from the uterus and other tissues rely to varying extents on the use of potentially damaging physical forces and/or treatment with proteolytic enzymes, such as collagenase or trypsin (Alberga and Baulieu 1968; Smith *et al.* 1970; Boshier and Katz 1975; Heald *et al.* 1975; Kimmich 1975; Guy *et al.* 1977; Kirk *et al.* 1978; Fagg *et al.* 1979). Although the enzyme-based procedures are disadvantaged by causing cell-surface alterations (Inbar and Sachs 1969; Kraemar 1971), those using collagenase have found wide application because many of the adverse effects of trypsin are avoided and they gently disintegrate tissues into single-cell populations suitable for culture. In this context, Pietras and Szego (1979) indicated that endometrial cells isolated from the rat uterus with collagenase were capable of hormone-evoked growth in primary culture.

It has recently been reported that the cell-surface effects of collagenase are reversible (Moskalewski and Thyberg 1981). It is therefore likely that collagenase-based procedures may be increasingly exploited in future attempts to obtain epithelial cell preparations for study, particularly if technological improvements can be achieved to maximize cell yields and purity. In view of these considerations, the metabolic properties and viability of endometrial cells isolated from the mouse uterus with collagenase were investigated in the present study. Uterine horns from pseudo-pregnant and pregnant animals were treated with the enzyme to recover the maximum number of luminal epithelial cells. The viability of the cells and their retention of metabolic functions were assessed using dye-exclusion techniques and metabolic inhibitors. In addition, the response of the cells to concanavalin A (Con A), insulin, and various substrates, and their ability to oxidize radioactive glucose at different reproductive stages were also investigated.

Materials and Methods

Materials

Biochemicals and other materials were purchased from the following sources: *Clostridium histolyticum* collagenase (EC 3.4.24.3) and sodium pyruvate from Boehringer Mannheim Australia Pty Ltd, Mount Waverley, Vic.; iodoacetic acid (lithium salt), ouabain (Strophanthin G) and insulin (from bovine pancreas) from Calbiochem-Behring Corp. (Australia), Carlingford, N.S.W.; concanavalin A (Con A) and Ficoll-Paque from Pharmacia South Seas Pty Ltd, North Ryde, N.S.W.; sodium acetate, sodium chloride, potassium chloride and disodium hydrogen orthophosphate from Ajax Chemicals, Sydney, N.S.W.; L(+)-lactic acid and 2,4-dinitrophenol from Sigma Chemical Co., St Louis, Mo., U.S.A.; D-glucose and potassium dihydrogen orthophosphate from BDH Chemicals (Australia) Pty Ltd, Port Fairy, Vic.; phlorizin dihydrate from Fluka A.G., Buchs, Switzerland; bovine serum albumin (Fraction V) from Commonwealth Serum Laboratories, Melbourne, Vic.; D-[U-^{14}C]glucose from The Radiochemical Centre (Australia) Pty Ltd, Sydney, N.S.W.; methyl-α-D-mannopyranoside from P. L. Biochemicals Inc., Milwaukee, Wis., U.S.A.; nigrosin from Hopkin and Williams, Chadwell Heath, Essex, England; Spectramesh N (Nylon with 70-μm mesh openings) from Solandra Scientific Pty Ltd, Revesby, N.S.W.

Animals

Female Quackenbush strain mice, aged 8–12 weeks, were used in all experiments and were housed as previously described (Murdoch *et al.* 1978). Pregnancy or pseudopregnancy was induced by pairing the females with intact or vasectomized males, respectively. The females were examined

for copulation plugs each morning and the day of finding a plug was designated as day 1 or the first day of pregnancy or pseudopregnancy. Mice were killed by cervical dislocation at 0900 h on the required day of pseudopregnancy or pregnancy.

Isolation of Endometrial Cells

At the time of autopsy, mice were quickly dissected to expose the uterine horns which were immediately opened *in situ* along the anti-mesometrial axis using heat-sterilized fine scissors. Each horn was then individually excised and placed in a Petri dish containing about 5 ml of Ca^{2+}- and Mg^{2+}-free (incomplete) Ringer solution at 37°C. The Ringer solution (complete) used in experiments was similar to that described by Pietras and Szego (1979). It contained 136·9 mM NaCl, 2·7 mM KCl, 0·5 mM $CaCl_2$, 0·5 mM $MgCl_2$ and 1 mM sodium pyruvate and was buffered at pH 7·4 with 8·1 mM Na_2HPO_4 and 1·5 mM KH_2PO_4. The solution was supplemented with 1% (w/v) rather than 0·1% (w/v) bovine serum albumin to minimize cell surface effects due to proteolytic enzymes contained in the collagenase preparation described below.

Each uterine horn was trimmed free of connective and fatty tissue under a dissecting microscope and divided into two pieces by cutting longitudinally along the mesometrial axis while still immersed in solution. The dissected uterine horns were then incubated for 30 min at 37°C in a conical flask containing fresh incomplete Ringer solution and collagenase at a concentration of 5 mg/ml, with gentle agitation. The results of preliminary studies indicated that epithelial cells were successfully liberated from the uterus by this method only when there were no more than four pieces of dissected uterine tissue (equivalent to one whole uterus) per millilitre of collagenase solution. In all experiments, therefore, care was taken not to exceed this ratio when dissected uterine horns were treated with collagenase.

At the end of the incubation period the uterine horns were removed from the collagenase solution with fine forceps and the remaining cell suspension was filtered through Spectramesh to remove any large cellular aggregates. The filtered suspension was then centrifuged at 360 *g* for 5 min and the pelleted cells were washed once in 5 ml of incomplete Ringer solution to remove any remaining collagenase. Dead endometrial cells and red blood cells were then removed from the cell suspension (0·5 ml) by centrifuging at 1500 *g* for 15 min on 3 ml of Ficoll-Paque. The endometrial cells remaining at the interface of centrifuged preparations were carefully removed and were washed twice in 5 ml of incomplete Ringer solution. Cells were finally dispersed in a volume of pyruvate-free complete Ringer solution convenient for study, and their viability was immediately evaluated by the dye-exclusion method described by Pietras and Szego (1979) using 0·05% (w/v) nigrosin. Cell numbers were estimated using a haemocytometer.

Microscopic Examination of Uteri

Uteri incubated in the presence and absence of collagenase as described above were placed in Bouin's fixative and embedded in paraffin-wax. Sections (5 μm) cut from the mid-region, uterotubal junction and cervical ends of the uterus were stained with Cole's haematoxylin and eosin and examined under the light microscope.

Metabolic Studies

The metabolism of endometrial cells isolated from the uteri of pregnant (days 4 and 5), pseudopregnant (days 1–5), and unmated, cycling mice (selected without regard for oestrous cycle activity) was assessed using Warburg manometric techniques as described by Umbreit *et al.* (1972). Unless otherwise stated, endometrial cell suspensions (0·5 ml, 2×10^6–4×10^6 cells per flask) were incubated with 0·5 ml of pyruvate-free complete Ringer solution containing D-[U-^{14}C]glucose (2·0 μmol; 500 nCi) in single side-arm Warburg flasks of 6 ml capacity at 37°C for 90 min. The flasks also contained CO_2-free 20% (w/v) KOH (0·1 ml) in the centre well and 0·05 M H_2SO_4 (0·2 ml) in the side-arm. Oxygen consumption was measured at 30-min intervals by using air as the gas phase. At the end of the incubation period, the contents of the side-arm were tipped into the main compartment of the flask and incubation was continued for a further 30 min. Glucose oxidation was determined by the measurement of $^{14}CO_2$ trapped in the KOH of the centre well. The KOH was washed with 0·7 ml of distilled water into a scintillation vial containing 10 ml scintillation fluid and counted by liquid scintillation techniques.

Control flasks not containing endometrial cells but identical to the experimental flasks in all other respects, were included in all experiments to ensure that estimates of oxygen uptake and oxidative capacity were attributable solely to the metabolic functions of the cells.

The extent to which the cells retained their metabolic functions after isolation was assessed by incubating cell suspensions as described above in the presence of various inhibitors, including ouabain (200 μm), phlorizin (2 mM), 2,4-dinitrophenol (200 μm), and iodoacetate (5 mM) (Kimmich 1975). The metabolic responses of the cells to Con A (100 μg/ml) and insulin (0·1 i.u./ml) in the presence and absence of methyl-α-D-mannopyranoside (50 mM) were also studied in view of previous reports that the lectin exerts insulin-like activities in other cell systems (Cuatrecasas and Tell 1973; Czech and Lynn 1973; Katzen and Mumford 1976). Finally, the rate of oxygen consumption by the cells in the presence of various exogenous substrates was measured to test for evidence of a Crabtree effect (Martinez and Núñez de Castro 1980) and to gauge the extent to which the metabolism of endogenous substrates contributes to estimates of respiration.

Lectin-induced Agglutination of Endometrial Cells

Since Con A binds to uterine epithelial cells (Enders and Schlafke 1974) and induces a DCR in pseudopregnant mice through an interaction with the epithelium (Buxton and Murdoch 1982), experiments were conducted to determine the susceptibility of isolated endometrial cells to agglutination in the presence of the lectin. Cell suspensions from unmated, cycling mice and from pseudopregnant and pregnant mice were incubated with Con A (100 μg) in a total volume of 1 ml of complete Ringer solution at 37°C for 30 min in the presence and absence of 50 mM methyl-α-D-mannopyranoside (hapten). Cell numbers were counted using a haemocytometer and agglutinability was determined using the following relationship:

$$I = (N_0 - N_t)/N_0,$$

where I is the index of agglutination and N_0 and N_t are the numbers of single cells in the suspension at zero time and after 30 min incubation, respectively. On this basis, index of agglutination values range from 0 (no agglutination) to 1 (all cells agglutinated).

Replication of Experiments and Statistical Methods

Unless stated otherwise, experiments were replicated six times using different cell preparations on each occasion. The significance of results was assessed by analysis of variance after converting the primary data to logarithms. Index of agglutination values were converted to angles prior to statistical analysis. Comparisons between treatments were made using Student's t-test. Values in the tables are means ± standard error of the mean of unconverted data.

Results

Isolation of Cells

Figs 1*a* and 1*b* show transverse sections of dissected uterine horns from unmated, cycling mice incubated for 30 min at 37°C in incomplete Ringer solution containing or not containing collagenase. In the absence of collagenase, the luminal epithelium remained intact and the only cells liberated from the uterus under these conditions were a small number of red blood cells and leucocytes. Treatment with collagenase, caused the total removal of the luminal epithelial cells, but also caused some damage to the underlying stromal tissue. At the end of the incubation period the cells were present in the enzyme-containing medium as single entities and little evidence was found for the presence of aggregated cellular material. When cell aggregates did occur they were effectively removed from suspensions by filtration through Spectramesh N. Contaminating red blood cells and most dead cells were also successfully removed by centrifugation on Ficoll-Paque.

Although the sections illustrated in Figs 1*a* and 1*b* were taken from the mid-region of the uterus, they are typical of those prepared from other parts of the

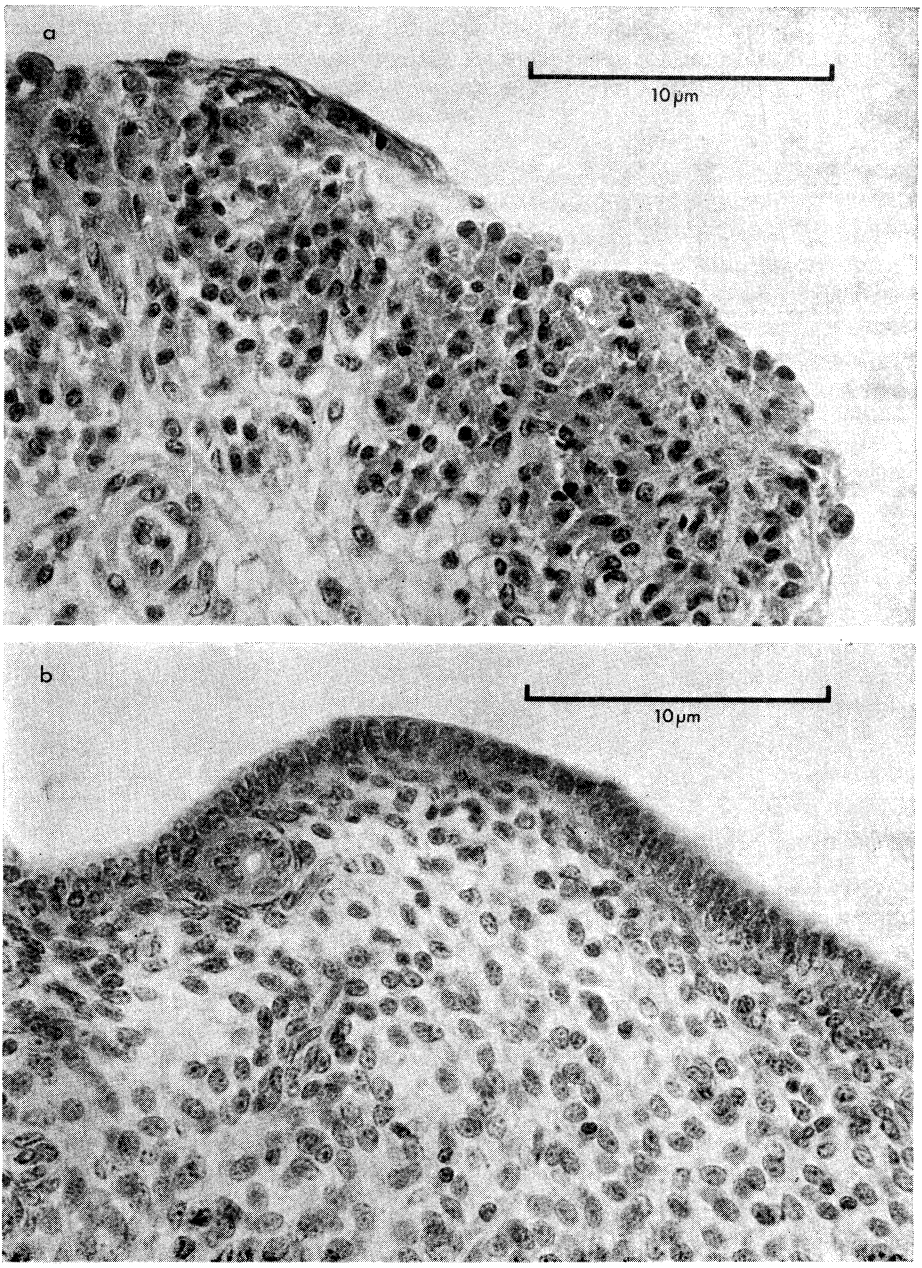

Fig. 1. Transverse section of a uterine horn incubated (*a*) in Ringer solution containing collagenase at a concentration of 5 mg/ml; (*b*) in collagenase-free Ringer solution.

uterus, including the uterotubal junction and cervical ends of the uterus. Similar sections and endometrial cell preparations were also obtained from pseudopregnant (days 1–5) and pregnant (days 4 and 5) mice.

Haemocytometer counts of 14 cell preparations indicated that $(1{\cdot}32 \pm 0{\cdot}08) \times 10^6$ cells were collected from each uterine horn. Nigrosin dye-exclusion tests showed that no less than 98% of the cells in each preparation were viable.

An important finding was that uterine horns, when opened only along the anti-meso-metrial axis and not subsequently dissected into two pieces, released epithelial cells from discrete areas rather than from the whole uterus when treated with collagenase.

Table 1. Metabolism of uniformly labelled glucose by and Con A-mediated agglutinability of uterine endometrial cells from unmated, pseudopregnant and pregnant mice

Values are the means ± standard errors for six replicates. Oxygen uptake and glucose oxidation values were calculated per 10^6 cells per 90 min. *, ** Significantly different from unmated mice, $P < 0{\cdot}05$, $P < 0{\cdot}01$, respectively

Day of pseudopregnancy or pregnancy	Oxygen uptake (μl)	Glucose oxidized (nmol)	Agglutination index with: 100 μg Con A	100 μg Con A + 50 mM hapten
		Unmated mice		
—	2·54±0·21	2·42±0·22	0·46±0·06	0·00
		Pseudopregnant mice		
1	2·09±0·15	2·48±0·27	0·69±0·04*	0·00
2	2·37±0·32	3·08±0·51	0·52±0·04	0·00
3	2·01±0·24	1·93±0·53	0·53±0·02	0·00
4	2·20±0·14	1·53±0·15	0·76±0·03**	0·00
5	2·58±0·29	2·60±0·75	0·87±0·04**	0·00
		Pregnant mice		
4	7·14±0·09**	4·20±0·40*	0·77±0·02**	0·00
5	3·26±0·05	1·90±0·18	0·89±0·05**	0·00

Metabolic and Agglutination Studies

Table 1 shows that isolated uterine endometrial cells from unmated, pseudopregnant and pregnant mice incubated with D-[U-^{14}C]glucose for 90 min at 37°C consumed oxygen and oxidized the exogenous substrate to $^{14}CO_2$ at rates sufficient to permit the measurement of these metabolic parameters by Warburg manometric techniques. The rates of oxygen uptake and glucose oxidation by the cells remained essentially unaltered during early pseudopregnancy, but both parameters significantly increased on day 4 of pregnancy. Values recorded on day 5 of pregnancy were, however, not significantly different from those obtained with unmated, control mice. The metabolism of endogenous materials appeared to make a substantial contribution to the oxygen consumption of the cells. Thus, the rate of oxygen uptake at all reproductive stages studied was considerably greater than that required merely to support the oxidation of the exogenous radioactive glucose.

The endometrial cells from uteri at all reproductive stages described in Table 1 agglutinated when incubated with Con A. However, the agglutination index was significantly increased on days 1, 4 and 5 of pseudopregnancy, and on days 4 and 5 of pregnancy when compared with cells from unmated, control animals. The addition of 50 mM methyl-α-D-mannopyranoside inhibited the Con A-mediated agglutination of the cells in all cases.

The results of experiments conducted to assess the oxidative capacity of isolated uterine endometrial cells in response to various metabolic inhibitors, Con A and insulin, are shown in Table 2. Uteri from day-4 pseudopregnant mice were used as a source of endometrial cells in this study, and D-[U-^{14}C]glucose was again employed as the exogenous substrate. The addition of ouabain, phlorizin and iodoacetate significantly ($P < 0{\cdot}01$) depressed the rate of oxidation of radioactive glucose, while 2,4-dinitrophenol, Con A and insulin significantly ($P < 0{\cdot}01$) increased this metabolic parameter. When 50 mM methyl-α-D-mannopyranoside was included in the incubation mixture, the stimulatory effects of Con A and insulin were either reduced or unaltered, respectively.

Table 2. Effects of metabolic inhibitors, Con A, and insulin, on the oxidation of uniformly labelled glucose by uterine endometrial cells from day-4 pseudopregnant mice

Values are the means ± standard errors for six replicates. Glucose oxidation values were calculated per 10^6 cells per 90 min. *, ** Significantly different from the control group, $P < 0{\cdot}05$, $P < 0{\cdot}001$, respectively

Compound	Glucose oxidized (nmol)	Compound	Glucose oxidized (nmol)
Control	1·93±0·19	Con A (100 μg)	3·85±0·58**
Ouabain (200 μm)	0·53±0·04**	Con A (100 μg)+50 mM hapten	2·80±0·10*
Phlorizin (2 mM)	0·42±0·07**	Insulin (0·1 i.u.)	3·27±0·43**
2,4-Dinitrophenol (200 μm)	6·58±0·63**	Insulin (0·1 i.u.)+50 mM hapten	3·31±0·30**
Iodoacetate (5 mM)	0·17±0·01**		

The rate of oxygen consumption of cells from pseudopregnant mice incubated in the presence of either glucose (2 mM and 10 mM), lactate (4 mM), pyruvate (4 mM) or acetate (6 mM) was not significantly different from that observed in the absence of any added substrate (results not shown). Thus, the addition of exogenous substrates failed to alter the rate of oxygen uptake from that attributable to the metabolism of endogenous sources and no evidence of a Crabtree effect was detected. In these experiments, cells consumed 2·16±0·37 μl of oxygen over 90 min at 37°C. This value represents the mean ± standard error of 36 observations.

Riggs and Pan (1972) showed that rat uterine tissue preincubated *in vitro* under physiological conditions undergoes metabolic changes that lead to an increase in its ability to transport various amino acids and other amine-containing compounds. In order to determine whether similar changes occur in mouse uterine endometrial cells after isolation to influence the transport of carbohydrates, and thus the metabolic values reported above, cells from day-4 pseudopregnant mice were preincubated in the present study for 3 h at 37°C in complete Ringer solution containing or not containing 1 mM pyruvate before assessing their capacity to oxidize radioactive glucose. The results of these experiments (not shown) indicated that a 3-h preincubation period did not significantly alter the rate at which the cells either consumed oxygen or oxidized radioactive glucose to $^{14}CO_2$. The addition of pyruvate during preincubation failed to exert any beneficial effects on the subsequent metabolism of the cells. Oxygen uptake and glucose oxidation values, both before and after preincubation, were similar to those obtained from pseudopregnant mice in Table 1. The dye-exclusion procedure also indicated that the cells remained viable over the entire experimental period.

Discussion

The present results leave little doubt that mouse uterine endometrial cells, isolated from suitably dissected uterine horns to achieve the maximal recovery of luminal epithelial cells, retain their oxidative capacities and remain viable after treatment with collagenase. The cells consumed oxygen in a linear fashion over a 90-min period, oxidized radioactive glucose to $^{14}CO_2$, and responded to various metabolic inhibitors in a manner consistent with that of cells possessing normal transport and oxidative functions (see Kimmich 1975). The metabolism of endogenous materials may be an important factor for survival after isolation since the addition of exogenous energy sources failed to cause any direct effect on the rate of oxygen consumption or influence the subsequent metabolism and viability of the cells after a 3-h incubation period *in vitro*. It is possible, however, that the bovine serum albumin in the incubation medium may have contributed to the metabolism and survival of the cells by preventing the early onset of degenerative changes that lead ultimately to disintegration (Kimmich 1975).

The increased oxidative metabolism of the cells on day 4 of pregnancy, but not on day 4 of pseudopregnancy, may reflect a relatively short-lived response to stimulation by blastocysts in the uterus in combination with differences in the secretion rates of ovarian steroids (Forcelledo *et al.* 1981). Thus, these results indicate that major changes in the rates of oxygen uptake and oxidation of glucose do not occur during pregnancy until such time as the endometrial tissue is appropriately sensitized by ovarian hormones and stimulated to participate in the DCR. If it is true that the metabolically unstable compound, PGI_2, is involved in this process (Jonsson *et al.* 1979), and that the metabolic changes reported in the present study facilitate its production by the epithelial cells, then the above proposal represents a plausible mechanism for the conservation of metabolic processes required for the timely production of transmitter material. In this context, the deciduogenic action of Con A in mice (Buxton and Murdoch 1982) may be explained by its ability to stimulate the oxidative metabolism of endometrial cells from pseudopregnant animals (present study). Because the collagen content of the uterine endometrium varies with reproductive status (see Finn and Porter 1975), it is possible that alterations in the susceptibility of the uterine tissue to disruption by collagenase could occur with advancing pregnancy and pseudopregnancy and thereby influence metabolism through changing proportions of different cell types in the isolate. However, there was no significant change in the total number of cells isolated from uterine horns during early pregnancy or pseudopregnancy in the present study.

The increased Con A-mediated agglutinability of the uterine cells observed on day 1 and on days 4 and 5 *post coitum*, when pro-oestrous and nidatory oestrogens are produced, respectively (Finn and Porter 1975), is consistent with the findings of Pietras and Szego (1979) that 17β-oestradiol elicits membrane modifications in isolated endometrial cells. If these results reflect true membrane modifications that occur under normal physiological conditions and are not simply artefacts of the isolation procedure, the membrane changes occurring on day 4 *post coitum* in particular may be key factors in allowing stimulation of the epithelium by blastocysts (Johnson and Calarco 1980) or Con A (Buxton and Murdoch 1982) to induce a DCR and increase the oxidative metabolism of glucose. Similar stimulatory effects of Con A on the oxidative metabolism of isolated white fat cells have been reported

by Czech and Lynn (1973). In addition, the ability of this lectin to mimic the action of insulin on the oxidative metabolism of glucose by the endometrial cells used in the present study confirms the observations of other investigators (Cuatrecasas and Tell 1973; Czech and Lynn 1973; Katzen and Mumford 1976) and indicates that the isolation procedure does not seriously disrupt membrane receptors for insulin (Katzen *et al.* 1981).

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References

Alberga, A., and Baulieu, E. E. (1968). Binding of estradiol in castrated rat endometrium *in vivo* and *in vitro*. *Molec. Pharm.* **4**, 311–21.

Boshier, D. P., and Katz, J. M. (1975). Effects of ovarian steroid hormones on esterases in the rat endometrium. *J. Endocrinol.* **64**, 465–74.

Buxton, L. E., and Murdoch, R. N. (1982). Lectins, calcium ionophore A23187, and peanut oil as deciduogenic agents in the uterus of pseudopregnant mice: effects of tranylcypromine, indomethacin, iproniazid, and propranolol. *Aust. J. Biol. Sci.* **35**, 63–72.

Cuatrecasas, P., and Tell, G. P. E. (1973). Insulin-like activity of concanavalin A and wheat germ agglutinin. Direct interactions with insulin receptors. *Proc. Natl Acad. Sci. U.S.A.* **70**, 485–9.

Czech, M. P., and Lynn, W. S. (1973). Stimulation of glucose metabolism by lectins in isolated white fat cells. *Biochim. Biophys. Acta* **297**, 368–77.

Enders, A. C., and Schlafke, S. (1974). Surface coats of the mouse blastocyst and uterus during the preimplantation period. *Anat. Rec.* **180**, 31–46.

Fagg, B., Martin, L., Rogers, L., Clark, B., and Quarmby, V. E. (1979). A simple method for removing the luminal epithelium of the mouse uterus for biochemical studies. *J. Reprod. Fertil.* **57**, 335–9.

Finn, C. A., and Porter, D. G. (1975). The decidual cell reaction. In 'The Uterus'. (Ed. C. A. Finn.) pp. 74–85. (Elek Science: London.)

Forcelledo, M. L., Vera, R., and Croxatto, H. B. (1981). Ovum transport in pregnant, pseudopregnant, and cyclic rats and its relationship to estradiol and progesterone blood levels. *Biol. Reprod.* **24**, 760–5.

Guy, D., Latner, A. L., and Turner, G. A. (1977). Radioiodination studies of tumour cell-surface proteins after different disaggregation procedures. *Br. J. Cancer* **36**, 166–72.

Heald, P. J., Govan, A. D. T., and O'Grady, J. E. (1975). A simple method for the preparation of luminal epithelium and stromal cells from rat uterus. *J. Reprod. Fertil.* **42**, 593–5.

Inbar, M., and Sachs, L. (1969). Interaction of the carbohydrate-binding protein concanavalin A with normal and transformed cells. *Proc. Natl Acad. Sci. U.S.A.* **63**, 1418–25.

Johnson, L. V., and Calarco, P. G. (1980). Mammalian preimplantation development: the cell surface. *Anat. Rec.* **196**, 201–19.

Jonsson, H. T., Rankin, J. C., Ledford, B. E., and Baggett, B. (1979). Uterine prostaglandin levels following stimulation of the decidual cell reaction: effects of indomethacin and tranylcypromine. *Prostaglandins* **18**, 847–57.

Katzen, H. M., and Mumford, R. A. (1976). Evidence for insulin receptors mediating the insulin-like activities of concanavalin A. *Fed. Proc.* **35**, 1719.

Katzen, H. M., Soderman, D. D., and Green, B. G. (1981). Evidence that insulin and concanavalin A can co-bind to solubilized insulin receptors without inhibiting each other. *Biochem. Biophys. Res. Commun.* **98**, 410–16.

Kennedy, T. G. (1977). Evidence for a role for prostaglandins in the initiation of blastocyst implantation in the rat. *Biol. Reprod.* **16**, 286–91.

Kimmich, G. A. (1975). Preparation and characterization of isolated intestinal epithelial cells and their use in studying intestinal transport. In: 'Methods in Membrane Biology'. Vol. 5. (Ed. E. D. Korn.) pp. 51–115. (Plenum Press: New York.)

Kirk, D., King, R. J. B., Heyes, J., Peachey, L., and Hirsch, P. J. (1978). Normal human endometrium in cell culture. I. Separation and characterization of epithelial and stromal components *in vitro*. *In Vitro* **14**, 651–62.

Kraemer, P. M. (1971). Heparan sulfates of cultured cells. I. Membrane-associated and cell-sap species in Chinese hamster cells. *Biochemistry* **10**, 1437–45.

Lejeune, B., Van Hoeck, J., and Leroy, F. (1981). Transmitter role of the luminal epithelium in the induction of decidualization in rats. *J. Reprod. Fertil.* **61**, 235–40.

Lundkvist, Ö., and Nilsson, B. O. (1980). Ultrastructural changes of the trophoblast–epithelial complex in mice subjected to implantation blocking treatment with indomethacin. *Biol. Reprod.* **22**, 719–26.

Lundkvist, Ö., Nilsson, O., and Bergstrom, S. (1979). Studies on the trophoblast–epithelial complex during decidual induction in rats. *Am. J. Anat.* **154**, 211–30.

Martinez, P., and Núñez de Castro, I. (1980). Crabtree effect induced by fructose in hepatocytes isolated from regenerating liver. *Enzyme* **25**, 377–81.

Moskalewski, S., and Thyberg, J. (1981). Reversible changes in nuclear and cell surface topography in cells exposed to collagenase and EDTA. *Cell Tiss. Res.* **220**, 51–60.

Murdoch, R. N., Kay, D. J., and Cross, M. (1978). Activity and subcellular distribution of mouse uterine alkaline phosphatase during pregnancy and pseudopregnancy. *J. Reprod. Fertil.* **54**, 293–300.

Pietras, R. J., and Szego, C. M. (1979). Estrogen-induced membrane alterations and growth associated with proteinase activity in endometrial cells. *J. Cell Biol.* **81**, 649–63.

Rankin, J. C., Ledford, B. E., Jonsson, H. T., and Baggett, B. (1979). Prostaglandins, indomethacin and the decidual cell reaction in the mouse uterus. *Biol. Reprod.* **20**, 399–404.

Riggs, T. R., and Pan, M. W. (1972). Transport of amino acids into the oestrogen-primed uterus: enhancement of the uptake by a preliminary incubation. *Biochem. J.* **128**, 19–27.

Smith, J. A., Martin, L., King, R. J. B., and Vertes, M. (1970). Effects of oestradiol-17β and progesterone on total and nuclear protein synthesis in epithelial and stromal tissues of the mouse uterus, and of progesterone on the ability of these tissues to bind oestradiol-17β. *Biochem. J.* **119**, 773–85.

Tobert, J. A. (1976). A study of the possible role of prostaglandins in decidualization using a nonsurgical method for the instillation of fluids into the rat uterine lumen. *J. Reprod. Fertil.* **47**, 391–3.

Umbreit, W. W., Burris, R. H., and Stauffer, J. F. (1972). 'Manometric and Biochemical Techniques.' 5th Edn. p. 146. (Burgess Publ. Co: Minneapolis.)

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