Alterations in uterine calcium ions during induction of the decidual cell reaction in pseudopregnant mice

J. A. Sakoff and R. N. Murdoch

Department of Biological Sciences, The University of Newcastle, University Drive, Callaghan, NSW 2308, Australia

The present study exploited the decidualogenic actions of concanavalin A (Con A) in pseudopregnant Quackenbush strain mice to assess whether alterations in uterine Ca^{2+} status accompany stromal cell transformations that culminate in the decidual cell reaction and the establishment of pregnancy. It was found that the introduction of 15 blastocyst-size Con-A-coated Sepharose beads, but not lectin-free Sepharose beads, into the lumen of the left uterine horn of pseudopregnant mice on day 3 or 4 induced a decidual response that was virtually indistinguishable from that produced by blastocysts during normal pregnancy. The right uterine horn received no treatment and served as a control. Although the simultaneous administration of $^{45}\text{CaCl}_2$ (1 mmol l^{-1} , $200 \text{ mCi mmol}^{-1}$) on day 3 of pseudopregnancy impeded this response, the decidualogenic potential of the Con-A-coated beads was not disrupted when these agents were simultaneously administered on day 4. This experiment with $^{45}\text{CaCl}_2$ produced discrete areas of decidualization, as shown by the pontamine sky blue reaction on day 5 of pseudopregnancy. The decidualized areas contained significantly greater amounts of $^{45}\text{Ca}^{2+}$ than did the non-decidualized interjacent areas, indicating that the beads preferentially stimulated the rate of influx of the cation into these sites. Much of this cellular $^{45}\text{Ca}^{2+}$ content was lost in the early afternoon of day 5 of pseudopregnancy at a time when the luminal epithelium undergoes autolytic breakdown. The intraluminal introduction of $125 \mu\text{g}$ of Con A in solution ($5 \mu\text{l}$) was also decidualogenic but stimulated a larger uterine response than did the Con-A-coated beads and promoted a significantly greater uptake and retention of $^{45}\text{Ca}^{2+}$ than did the intraluminal injection of other agents. The results suggest that the embryonic signal responsible for induction of the decidual cell reaction in mice involves surface interactions between the embryo and uterine luminal epithelium to promote the influx of Ca^{2+} from the lumen into the epithelial cells and to modify their metabolism to stimulate the stromal cell transformations.

Introduction

The establishment of pregnancy in many animals involves the successful relay of blastocyst signals via the uterine epithelium to adjacent areas of the stroma where a decidual cell reaction is induced. This response occurs only in suitably hormone-primed uteri and is accompanied by an increase in the permeability of the endometrial vasculature (Psychoyos, 1973). Thus, macromolecular dyes injected i.v. into animals either naturally or artificially stimulated for decidualization (DeFeo, 1967; Finn and Porter, 1975) will leave the circulation in the areas of increased vascular permeability, producing discrete bands along the uterus coincident with implantation or decidualization sites.

Work conducted in our laboratory has implicated a role for Ca^{2+} -dependent signal transduction mechanisms in the decidualization of the mouse uterus. In this context, it was found that compounds that influence the Ca^{2+} status of cells, such as the lectin Concanavalin A (Con A) and ionophore

A23187, act as potent decidualogenic agents in this species (Buxton and Murdoch, 1982; Kyd and Murdoch, 1992). Con A has a specific affinity for various carbohydrate residues in glycoproteins (Goldstein and Hayes, 1978) and causes alterations in cellular metabolism on binding to membrane receptors (Czech and Lynn, 1973). These metabolic alterations may be related to the role that Con A plays in promoting both the release of Ca^{2+} from intracellular stores and the transport of Ca^{2+} across the plasma membrane (Larner *et al.*, 1980; Isakov *et al.*, 1986; Gukovskaya *et al.*, 1990), since it is widely recognized that Ca^{2+} ions function as important cell regulators (Cheung, 1982, 1987).

In the present study the decidualogenic ability of Con A covalently bound to blastocyst-size Sepharose 4B beads ($100\text{--}150 \mu\text{m}$ diameter) (Buxton and Murdoch, 1982; Hogan *et al.*, 1986) was exploited using $^{45}\text{CaCl}_2$ and pseudopregnant mice to test further the hypothesis that alterations in uterine Ca^{2+} status facilitate the induction of the decidual cell reaction. These lectin-coated beads, as opposed to lectin-free beads of similar size, mimic the ability of the blastocyst to stimulate a

decidual cell reaction and induce a morphological response in pseudopregnant mice that is indistinguishable from that induced during normal pregnancy (Buxton and Murdoch, 1982). We used the lectin-coated beads and solutions of the lectin rather than preimplantation embryos to induce the decidual cell reaction, to avoid the possibility of introducing confounding effects into determinations of $^{45}\text{Ca}^{2+}$ uptake by the uterus that could be generated as a consequence of either transfer-induced embryonic loss (Yovich, 1988) or embryonic metabolism.

Materials and Methods

Animals

Female Quackenbush strain (QS) mice (aged 8–12 weeks, 20–30 g) were used in all experiments and were housed under controlled conditions as described by Murdoch and Simm (1992). 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 a plug was found was designated as day 1 of pregnancy or pseudopregnancy.

Materials

Con A, Sepharose 4B beads, and Con-A-Sepharose beads were obtained from Pharmacia South Seas Pty Ltd (North Ryde). Other materials were obtained from the following sources: $^{45}\text{CaCl}_2$ from Amersham Australia Pty Ltd (Sydney), 2,2,2-tribromoethanol (Avertin) from Aldrich Chemical Company, Inc. (Milwaukee, WI), lanthanum chloride (LaCl) from Ajax Chemicals (Sydney), and pontamine sky blue 5BX from BDH Chemicals Ltd (Poole). Other chemicals were of analytical grade and experiments were carried out using demineralized and twice distilled water.

Induction of deciduomata

Deciduogenic agents in 5 μl of saline (0.9% NaCl, w/v) were injected into the lumen at the uterotubal end of the left uterine horn of pseudopregnant mice following anaesthesia with Avertin (i.p., 200 mg kg^{-1} body mass administered as a 1% solution in saline, v/v). Con A (125 μg per uterine horn), selected blastocyst-size Con-A-coated Sepharose 4B beads and lectin-free Sepharose beads (8–15 per horn) were either dissolved or suspended in saline and, unless otherwise stated, separately introduced into the lumen of the uterus together with 5 μl of $^{45}\text{CaCl}_2$ (1 mmol l^{-1} , 200 mCi mmol $^{-1}$) between 13:00 and 15:00 h on day 3 or 4 of pseudopregnancy. Administrations on day 3 were performed to provide ample time for the Sepharose beads to space evenly along the uterus before initiating the decidual cell reaction and to mimic conditions that relate to the artificial transfer of embryos (McLaren and Michie, 1956). Alternatively, administrations on day 4 of pseudopregnancy correspond to the time that the uterus is sensitized for blastocyst implantation and induction of the decidual cell reaction (Yochim and De Feo, 1963;

Psychoyos, 1973). The specific activity of $^{45}\text{CaCl}_2$ indicated above was the minimum amount that could be introduced into the uterus and subsequently detected in the tissues. All soluble agents (i.e. Con A and NaCl) were administered using a microsyringe (Scientific Glass Engineering model 100A-RN), while non-soluble agents (i.e. Sepharose beads) were deposited in the uterine lumen with finely drawn siliconized Pasteur pipettes using the embryo transfer technique described by Mullen and Carter (1973). The ovaries of all pseudopregnant mice were examined under a dissecting microscope before the intrauterine injections were administered, and animals were used in experiments only when distinct corpora lutea were apparent. The right uterine horn in all experiments received no treatment and acted as a control in the experiments. Changes in uterine mass and morphology from day 5 to 10 of pseudopregnancy were the main criteria used to gauge the decidual cell reaction in stimulated horns.

Preparation of uterine tissue for determination of Ca^{2+} uptake

At appropriate times following the simultaneous introduction of either Con A, beads or NaCl and $^{45}\text{Ca}^{2+}$, the mice were killed by cervical dislocation, the uteri were removed, dissected free of connective and fatty tissue and of the attached oviducts and cervix, and then weighed. The uteri were placed in ice-cold lanthanum solution composed of 10 mmol LaCl l^{-1} , 10 mmol Hepes l^{-1} , 127.5 mmol NaCl l^{-1} , 4.6 mmol KCl l^{-1} , 1.2 mmol MgCl_2 l^{-1} and 11 mmol glucose l^{-1} , pH 7.4 (Batra, 1982), and were carefully opened down the mesometrial side to expose the endometrium. The uteri were washed three times (20 min per wash at 4°C) in the lanthanum solution to minimize the efflux of cellular $^{45}\text{Ca}^{2+}$ (Batra, 1982) before being digested in 0.5 ml 4 mol NaOH l^{-1} at 50°C. A 50 μl aliquot of the mixture was then neutralized with 50 μl 4 mol HCl l^{-1} and its $^{45}\text{Ca}^{2+}$ content assayed by liquid scintillation counting in a Packard Tri-Carb Liquid Scintillation Spectrometer.

In other experiments in which animals were similarly treated as above with Con-A-coated beads and $^{45}\text{Ca}^{2+}$, autopsy was performed 15 min after an i.v. tail injection of 0.25 ml pontamine sky blue solution (1% solution in saline, w/v), to detect specific sites of the uterus undergoing the decidual cell reaction. The blue-banded and interjacent areas of the uterus discriminated by this technique were separately pooled, weighed, washed, digested and analysed for $^{45}\text{Ca}^{2+}$ content as described above.

All Ca^{2+} uptake data were expressed as pmol per gram of tissue protein. Protein concentrations were determined by the method of Lowry *et al.* (1951) using standards of BSA.

Statistical analysis

The significance of results was assessed by analysis of variance after the data were logarithmically transformed to ensure homogeneity of variance (Snedecor, 1957). All main effects and their first order interactions were isolated and tested for significance using the within-group error mean square to calculate variance ratios. Comparisons between treatments were made using Student's *t* test or Duncan's multiple range

Fig. 1. Photographs of uteri from mice on (a) day 5 and (b) day 10 of normal pregnancy, and (c) day 5 and (d) day 10 of pseudopregnancy following the administration of 15 concanavalin-A-coated beads into the left uterine horn on day 4. Parts (a) and (c) represent uteri from mice injected i.v. 15 min before necropsy with pontamine sky blue. Scale bars represent 1 cm.

test (Snedecor, 1957). All values presented in the figures are means $\pm$ SEM of the non-transformed data.

Results

Deciduogenicity of Con-A-coated Sepharose beads

Figure 1 illustrates changes in the morphology of uteri from mice in which Con-A-coated beads were deposited into the left uterine horn on day 4 of pseudopregnancy. For comparative purposes, uteri from mice at corresponding stages of pregnancy are also shown. It is evident that the Con-A-coated Sepharose beads were decidualogenic in pseudopregnant mice and induced a morphological response on days 5 and 10 of pseudopreg-

nancy similar to that naturally induced by embryos during pregnancy. Discrete areas of blue banding due to the vascular release of pontamine sky blue were also discerned in stimulated horns, but not in untreated horns.

The extent to which the decidual cell reaction was successfully induced in pseudopregnant mice by the Con-A-coated beads varied with the number of beads deposited in the left uterine horn on day 4. When eight Con-A-coated beads were introduced into the left uterine horn an effective stimulation threshold was not reached and all five mice tested failed to produce any detectable decidual cell reaction. With the introduction of 12 Con-A-coated beads, three of five mice tested failed to produce evidence of the decidual cell reaction. However, when 15 Con-A-coated beads were introduced into the lumen on day 4 of pseudopregnancy, all 15 mice tested

displayed a decidual cell reaction and showed multiple blue banding corresponding to sites of increased permeability (up to eight sites) in the left horn. The administration of 15 Con-A-coated beads into the lumen on day 3 of pseudopregnancy ($n = 5$) also produced decidual responses with multiple blue banding. However, these sites were more evenly spaced along the uterus than those produced when the beads were introduced on day 4 (data not shown). Only one mouse showed evidence of a decidual cell reaction occurring in the right untreated uterine horn when beads were introduced into the left horn on day 4 of pseudopregnancy, indicating that the beads very rarely migrate from the left horn of the uterus to the right horn under these conditions. In view of these results, 15 Con-A-coated beads were consistently used for administration into the uterus in all subsequent experiments. In addition, the administration of Con-A-free beads on days 3 ($n = 6$) or 4 ($n = 7$) of pseudopregnancy produced no evidence of the decidual cell reaction or increased permeability response. These lectin-free beads were therefore used as controls in other experiments.

When $^{45}\text{CaCl}_2$ was simultaneously administered with 15 lectin-coated beads on day 3 of pseudopregnancy, the decidual effects of the beads were completely suppressed. However, no disruptive effects of the exogenous $^{45}\text{CaCl}_2$ were detected when it was administered with the beads on day 4 of pseudopregnancy (data not shown). In view of this, all further investigations were undertaken using pseudopregnant animals at day 4 in which beads and $^{45}\text{Ca}^{2+}$ were simultaneously introduced into the left uterine horn.

Incorporation of $^{45}\text{Ca}^{2+}$ into the uterus with decidual stimulation

A time-course experiment was designed to examine the pattern of $^{45}\text{Ca}^{2+}$ labelling in the uterus 15–120 min after the simultaneous introduction of $^{45}\text{Ca}^{2+}$ and Con A into the left uterine horns of mice during the hormone-sensitive 'window' on day 4 of pseudopregnancy. Since Con A, when either covalently bound to the Sepharose beads or dissolved in solution is decidualogenic in pseudopregnant mice (Buxton and Murdoch, 1982), both forms of the lectin were used in the present experiment as decidualogenic agents for comparative purposes. Non-decidualogenic Con-A-free Sepharose 4B beads and saline solution were used as controls. The results (Fig. 2) showed that the Con A solution promoted a significantly greater ($P < 0.01$) uptake of Ca^{2+} than did any of the other agents introduced into the lumen of the left uterine horn, and maximal values were reached 30 min after administration. However, within 15 min of administering $^{45}\text{Ca}^{2+}$ with or without a decidualogenic stimulus, a large initial uptake of the cation into the cellular components of the left uterine horn also occurred independently of the lectin stimulus. With the exception of uteri treated with the Con A solution, this initial uptake of Ca^{2+} was rapidly lost over the next 15 min, and 60 min after administration, the $^{45}\text{Ca}^{2+}$ content was similar to that detected in untreated right uterine horns. The right uterine horns in all cases showed no significant differences between treatments with respect to $^{45}\text{Ca}^{2+}$ content; the data relating to this horn were therefore pooled and recorded as a single response with time.

Fig. 2. Ca^{2+} uptake in the mouse uterus 15–120 min after the simultaneous introduction of $^{45}\text{CaCl}_2$ and (●) concanavalin (Con A)-coated Sepharose 4B beads, (■) Con A solution, (○) Sepharose 4B beads or (□) saline into the left uterine horn on day 4 of pseudopregnancy. Data for the right untreated horn were pooled and recorded as a single response with time (△). Values are means $\pm$ SEM for four mice.

It was not apparent from the results of the above experiment whether Con-A-coated Sepharose beads promote an influx of Ca^{2+} preferentially into adjacent uterine cellular sites that will ultimately be implicated in decidualization or a more general influx that involves the whole uterine horn. It is feasible that the Ca^{2+} influxes recorded above may be confined only to those sites adjacent to the beads and may therefore be substantially 'diluted' when the whole uterus is used for the assay of the Ca^{2+} isotope. In view of these considerations, a further experiment was undertaken to address this problem. $^{45}\text{CaCl}_2$ and Con-A-coated Sepharose beads were simultaneously introduced into the left uterine horn of another group of mice on day 4 of pseudopregnancy. Decidualized and non-decidualized areas of the uterus were subsequently isolated (using pontamine sky blue to detect the permeability response) and prepared for the assay of $^{45}\text{Ca}^{2+}$ at two separate times on day 5 of pseudopregnancy. The results (Fig. 3) show that Ca^{2+} was preferentially incorporated into uterine sites that were stimulated by Con-A-coated beads to undergo decidualization compared with non-decidualizing sites that were not stimulated by the beads. It was also evident that the amount of labelled $^{45}\text{Ca}^{2+}$ taken up in the uterus of mice during the earlier part of day 5 was significantly greater ($P < 0.05$) than that taken up in the uterus of mice during the later stages of the same day. In all cases, the right uterine horn, which was untreated on day 4 of pseudopregnancy, contained only negligible amounts of $^{45}\text{Ca}^{2+}$ when assayed on day 5.

Discussion

The results of the present study confirm previous observations (Buxton and Murdoch, 1982) that blastocyst-size Con-A-coated Sepharose 4B beads, but not lectin-free beads, are decidualogenic in pseudopregnant mice and induce deciduomata when introduced into the lumen of the uterus either on day 3 or 4 of pseudopregnancy. This finding is consistent with

Fig. 3. Ca^{2+} uptake in decidualized (▨) and non-decidualized (■) sites of the left uterine horn on day 5 pseudopregnancy in mice following the simultaneous introduction of $^{45}\text{CaCl}_2$ and 15 concanavalin-A-coated beads on day 4. The untreated right horn was used as a control (□). Values are means $\pm$ SEM for seven mice.

phenomena associated both with normal pregnancy and embryo transfer experiments in which embryos, naturally or artificially transferred into the uterus on day 3 of pregnancy or pseudopregnancy, respectively, initiate the implantation response on day 4.5 and effect the establishment of pregnancy (McLaren and Michie, 1956; Hogan *et al.*, 1986). In addition, the Con-A-coated Sepharose beads, again like embryos in normal pregnancy, induced more evenly spaced decidualization along the uterus when they were intraluminally administered on day 3 of pseudopregnancy than on day 4. Notwithstanding this, it was feasible to exploit their decidualogenic capacity only on day 4 of pseudopregnancy, because Ca^{2+} negated this action when administered simultaneously with the beads on day 3. It was necessary to introduce 15 Con-A-coated beads into the uterine lumen to imitate optimally the decidualogenic response induced during normal pregnancy in this strain of mouse (that is, 6–8 stimulated sites per horn). With fewer than 15 beads, the 'decidualogenic threshold' was not always reached. It appears, therefore, that not all of the lectin-coated beads could individually induce the decidual response; otherwise, 15 separately stimulated sites would have been detected.

Although, as suggested above, the inclusion of $^{45}\text{CaCl}_2$ solution with beads on day 4 of pseudopregnancy had no effect on the ability of Con A to induce the decidual cell reaction, their simultaneous administration on day 3 completely blocked the lectin-induced response. This inhibitory effect of the exogenous Ca^{2+} introduced into the uterus approximately 24 h before the hormone-sensitive induction 'window' may be due to premature Ca^{2+} -mediated modifications in uterine metabolism that interrupt the hormonally orchestrated sequence of events needed to prepare the uterus to receive and respond optimally to the blastocyst signal on day 4.5. Kyd and Murdoch (1992) also reported that premature alterations in the Ca^{2+} status of uterine cells blocked the artificial induction of the decidual cell reaction in pseudopregnant mice. Nonetheless, decidualogenic quantities of Con A did further alter the Ca^{2+} status of the uterine cells, with solutions of the lectin producing both a larger effect in this respect and a greater decidual cell

reaction than when bound to Sepharose beads. The greater effectiveness of the Con A solutions compared with beads is due to a concentration effect and to the ability of the solutions to gain access to a larger surface area of the uterine epithelium for stimulation than did the blastocyst-size beads.

It is evident that the Con-A-coated Sepharose beads failed to promote a significantly increased rate of intracellular influx of $^{45}\text{Ca}^{2+}$ when the entire uterus was assayed in the first 2 h of stimulation. As these beads interact only with a very small area of the uterus, like blastocysts, which could not be discriminated at these early times by the pontamine sky blue reaction, any accelerated influx of $^{45}\text{Ca}^{2+}$ into the sites would have been masked by 'dilution' effects when the whole uterus was taken for assay. This proposal is supported by assays of the tissue on day 5 of pseudopregnancy when the stimulated and non-stimulated sites of the uterus could be discriminated by pontamine sky blue. Thus, when stimulated and non-stimulated uterine sites were independently isolated and assayed for $^{45}\text{Ca}^{2+}$ on day 5 after introducing the isotope and Con-A-coated beads together on day 4, significantly greater amounts of the labelled cation were found in sites undergoing decidualization than in the interjacent areas. However, the concentration of $^{45}\text{Ca}^{2+}$ in these samples fell rapidly between the late morning and early afternoon on day 5. At this later stage on day 5, the uterine luminal epithelium begins to break down owing to autolytic processes inherent in the decidual cell reaction (Finn and Hinchliffe, 1964; Finn, 1971). If the $^{45}\text{Ca}^{2+}$ ions introduced into the uterine lumen were taken up only by and confined to the cells of the luminal epithelium, then the degradative changes in these cells on the afternoon of day 5 of pseudopregnancy would account for the loss of uterine $^{45}\text{Ca}^{2+}$ observed at this time.

The enhanced uptake of Ca^{2+} induced by decidualogenic concentrations of Con A may be an indispensable condition for decidualization. This view is supported by recent findings indicating that Ca^{2+} -dependent signal transduction pathways in the uterus play important roles in the induction of the decidual cell reaction in mice (Kyd and Murdoch, 1992). Indeed, the various agents commonly used in mice to induce the decidual cell reaction artificially are, in many cases, known to cause alterations in the Ca^{2+} status of various cellular systems (Larner *et al.*, 1980; Kyd and Murdoch, 1992). Thus, the available evidence favours the hypothesis that alterations in uterine cellular Ca^{2+} status play an obligatory role in the induction of the decidual cell reaction in mice. Further studies are required to ascertain whether this phenomena is confined only to the luminal epithelium or involves the stromal tissue as well.

In conclusion, the results of the present study demonstrate that Con A is decidualogenic in pseudopregnant mice and can promote a uterine morphology that resembles early pregnancy when attached to blastocyst-size Sepharose beads. The results lend credence to the proposal that changes in Ca^{2+} status regulate metabolic processes in the uterus that ultimately lead to the production of specific factors responsible for signalling the stroma to undergo the decidual cell reaction. In this context, a number of Ca^{2+} -dependent mechanisms have already been associated with decidualization, including the intracellular signal transduction pathways involving phosphatidylinositol (Kyd and Murdoch, 1992), cAMP (Villar-Rojas

et al., 1982) and prostaglandins (Kennedy, 1986; Poyser, 1987).

The authors wish to acknowledge the financial support of the Research Management Committee of The University of Newcastle. All animal experimentation was carried out under approved protocols of the Animal Care and Ethics Committee of The University of Newcastle.

References

- Batra S (1982) Uptake and energy-dependent extrusion of calcium in the rat uterus *Acta Physiologica Scandinavica* **114** 447–452
- Buxton LE and Murdoch RN (1982) Lectins, calcium ionophore A23187 and peanut oil as decidual agents in the uterus of pseudopregnant mice: effects of tranlycypromine, indomethacin, iproniazid and propranolol *Australian Journal of Biological Sciences* **35** 63–72
- Cheung WY (1982) Calmodulin *Scientific American* **246** 48–56
- Cheung WY (1987) *Calcium and Cell Function* Vol. VII. Ed. WY Cheung. Academic Press, Orlando
- Czech MP and Lynn WS (1973) Stimulation of glucose metabolism by lectins in isolated white fat cells *Biochimica et Biophysica Acta* **297** 368–377
- De Feo VJ (1967) Decidualization. In *Cellular Biology of the Uterus*, pp 191–291 Ed. RM Wynn. Appleton–Century–Crofts, New York
- Finn CA (1971) Biology of decidual cells. In *Advances in Reproductive Physiology* **5**, pp 1–26 Ed. MWH Bishop. Elek, London
- Finn CA and Hinchliffe JR (1964) The reaction of the mouse uterus during implantation and decidualoma formation as demonstrated by changes in the distribution of alkaline phosphatase *Journal of Reproduction and Fertility* **8** 331–338
- Finn CA and Porter DG (1975) The Endometrium. In *The Uterus*, pp 74–85 Ed. CA Finn. Elek Science, London
- Goldstein IJ and Hayes CE (1978) The lectins: carbohydrate binding proteins of plants and animals. In *Advances in Carbohydrate Chemistry and Biochemistry*, pp 127–340 Eds RS Tipson and D Horton. Academic Press, New York
- Gukovskaya AS, Arias Pulido H, Petrunyaka VV, Zinchenko VP and Bezuglov VV (1990) Lipoxigenase inhibitors suppress intracellular calcium rise induced by ionomycin in rat thymocytes *Cell Calcium* **11** 539–546
- Hogan B, Costantini F and Lacy E (1986) Recovery, culture and transfer of embryos. In *Manipulating the Mouse Embryo*, pp 135–145. Cold Spring Harbor Laboratory, New York
- Isakov N, Scholz W and Altman A (1986) Signal transduction and intracellular events in T-lymphocyte activation *Immunology Today* **7** 271–277
- Kennedy TG (1986) Prostaglandins and uterine sensitization for the decidual cell reaction *Annals of the New York Academy of Sciences* **476** 43–48
- Kyd JM and Murdoch RN (1992) Decidual effects of mediators of the polyphosphatidylinositol pathway in pseudopregnant mice *Experientia* **48** 741–744
- Larner A, Rebhum LI, Larner S and Oron Y (1980) Concanavalin A-stimulated Ca^{2+} uptake in rat splenocytes *Molecular and Cell Biochemistry* **32** 123–130
- Lowry OH, Rosebrough NJ, Farr AL and Randall RJ (1951) Protein measurements with the folin-phenol reagent *Journal of Biological Chemistry* **193** 265–275
- McLaren A and Michie D (1956) Studies on the transfer of fertilized mouse eggs to uterine foster-mothers. I. Factors affecting the implantation and survival of native and transferred eggs *Journal of Experimental Biology* **33** 394–416
- Mullen RJ and Carter SC (1973) Efficiency of transplanting normal, zona-free, and chimeric embryos to one and both uterine horns of inbred and hybrid mice *Biology of Reproduction* **9** 111–115
- Murdoch RN and Simm B (1992) Impaired glucose homeostasis during post-implantation pregnancy in the mouse following acute exposure to ethanol, with particular reference to the uterus and embryo *Biochemical Medicine and Metabolic Biology* **47** 54–65
- Poyser NL (1987) Effects of various factors on prostaglandin synthesis by the guinea-pig uterus *Journal of Reproduction and Fertility* **81** 269–276
- Psychoyos A (1973) Endocrine control of egg implantation. In *Handbook of Physiology* (Section 7, Vol. II, Part 2), pp 187–215. Eds RO Greep, EB Astwood and SR Geiger. Bethesda American Physiology Society, Bethesda
- Snedecor GW (1957) *Statistical Methods*. Iowa State University Press, Ames
- Villar-Rojas C, Castio-Osuna G and Hicks JJ (1982) Cyclic AMP and cyclic GMP in the implantation site of the rat *International Journal of Fertility* **27** 56–59
- Yochim JM and De Feo VJ (1963) Hormonal control of the onset, magnitude and duration of uterine sensitivity in the rat by steroid hormones of the ovary *Endocrinology* **72** 317–326
- Yovich JL (1988) Treatments to enhance implantation. In *Implantation*, pp 239–255 Eds MC Chapman, JG Grudginskas and T Chard. Springer Verlag, London, New York