Maternal Recognition of Pregnancy

Ciba Foundation Symposium 64

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Ciba Foundation Symposium 64 (new series)

1979

Excerpta Medica

Amsterdam · Oxford · New York

ISBN Excerpta Medica 90 219 4070 1
ISBN Elsevier/North-Holland 0 444 90061 6

Published in May 1979 by Excerpta Medica, P.O. 211, Amsterdam
and Elsevier/North-Holland, Inc., 52 Vanderbilt Avenue, New York, N.Y. 10017.

Suggested series entry for library catalogues: Ciba Foundation Symposia.
Suggested publisher's entry for library catalogues: Excerpta Medica

Ciba Foundation Symposium 64 (new series)
435 pages, 108 figures, 33 tables

Library of Congress Cataloging in Publication Data

Symposium on Maternal Recognition of Pregnancy, London, 1978.
Maternal recognition of pregnancy.

(Ciba Foundation symposium; 64 (new ser.))
Bibliography: p.
Includes indexes.
1. Pregnancy–Immunological aspects–Congresses. 2. Obstetrical endocrinology–Congresses. 3. Ovum implantation–Congresses. I. Series: Ciba Foundation. Symposium; new ser., 64.
RG557.S95 1978 599'.01'6 79-4137
ISBN 0-444-90061-6

Printed in The Netherlands by Casparie, Amsterdam

The Ciba Foundation for the promotion of international cooperation in medical and chemical research is a scientific and educational charity established by CIBA Limited–now CIBA-GEIGY Limited–of Basle. The Foundation operates independently in London under English trust law.

Ciba Foundation Symposia are published in collaboration with Excerpta Medica in Amsterdam.

Excerpta Medica, P.O.Box 211, Amsterdam

Participants

Symposium on Maternal Recognition of Pregnancy, held at the Ciba Foundation, London, 9th–11th May, 1978

Chairman: R. B. HEAP ARC Institute of Animal Physiology, Babraham, Cambridge CB2 4AT, UK

R. J. AITKEN MRC Reproductive Biology Unit, 2 Forrest Road, Edinburgh EH1 2QW, UK

W. R. ALLEN ARC Institute of Animal Physiology, Animal Research Station, 307 Huntingdon Road, Cambridge CB3 0JQ, UK

E. C. AMOROSO ARC Institute of Animal Physiology, Babraham, Cambridge CB2 4AT, UK

F. W. BAZER Animal Science Department, Livestock Pavilion, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, Florida 32611, USA

A. E. BEER * Department of Cell Biology, Southwestern Medical School, The University of Texas Health Science Center at Dallas, 5323 Harry Hines Boulevard, Dallas, Texas 75235, USA

H. M. BEIER Department of Anatomy and Reproductive Biology, Medical Faculty, Rhein.-Westf. Technische Hochschule, Med. Theor. Institute, Melatener Strasse 211, D-5100 Aachen, Federal Republic of Germany

W. D. BILLINGTON Reproductive Immunology Group, Department of Pathology, University of Bristol, Bristol Royal Infirmary, Marlborough Street, Bristol BS2 8HW, UK

**Address from 1 May 1979:* Department of Obstetrics and Gynecology, University of Michigan, Ann Arbor, Michigan 48109, USA.

A. C. ENDERS Department of Human Anatomy, University of California, Davis, California 95616, USA

J. K. FINDLAY Department of Physiology, University of Melbourne, Animal Research Institute, Department of Agriculture, Werribee, 3030 Australia

C. A. FINN Department of Veterinary Physiology, University of Liverpool, University Veterinary Field Station, Leahurst, Neston, Wirral L64 7TE, UK

A. P. F. FLINT ARC Institute of Animal Physiology, Babraham, Cambridge CB2 4AT, UK

P. J. HEALD Department of Biochemistry, University of Strathclyde, The Todd Centre, 31 Taylor Street, Glasgow G4 ONR, UK *

J. P. HEARN MRC Reproductive Biology Unit, 2 Forrest Road, Edinburgh EH1 2QW, UK

M. H. JOHNSON Department of Anatomy, University of Cambridge, Downing Street, Cambridge CB2 3DY, UK

H. R. LINDNER Department of Hormone Research, The Weizmann Institute of Science, Rehovot, Israel

ANNE MCLAREN MRC Mammalian Development Unit, Wolfson House (University College London), 4 Stephenson Way, London NW1 2HE

O. NILSSON Department of Anatomy, University of Uppsala, Biomedicum, Box 571, S-751 Uppsala, Sweden

N. L. POYSER Department of Pharmacology, University of Edinburgh, 1 George Street, Edinburgh EH8 9JZ, UK

A. PSYCHOYOS Centre Nationale de la Recherche Scientifique, E.T. 122 Physiologie de la Reproduction, Hôpital de Bicêtre, 78 Avenue du Général Leclerc, 94270 Bicêtre, France

**Present address:* Office of the Dean, Faculty of Science, Memorial University of Newfoundland, St. John's, Newfoundland, Canada AIB 3X7.

G. T. ROSS Clinical Center, National Institutes of Health, Bethesda, Maryland 20014, USA

M. I. SHERMAN Department of Cell Biology, Roche Institute of Molecular Biology, Nutley, New Jersey 07110, USA

R. V. SHORT MRC Reproductive Biology Unit, 2 Forrest Road, Edinburgh EH1 2QW, UK

M. A. H. SURANI The Physiological Laboratory, University of Cambridge, Downing Street, Cambridge, CB2 3EG, UK

C. H. TYNDALE-BISCOE CSIRO Division of Wildlife Research, P.O. Box 84, Lyneham, Canberra, A.C.T. 2602, Australia

J. VAN BLERKOM Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado 80302, USA

A. WALLACE CSIRO Division of Animal Production, Ian Clunies Ross Animal Research Laboratory—Prospect, P.O. Box 239, Blacktown, NSW 2148, Australia

K. YOSHINAGA * Laboratory of Human Reproduction and Reproductive Biology, Harvard Medical School, 45 Shattuck Street, Boston, Massachusetts 02115, USA

Editor: JULIE WHELAN

**Present address:* Center for Population Research, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Md. 20014, USA.

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Introduction

R. B. HEAP

ARC Institute of Animal Physiology, Babraham, Cambridge

Almost 10 years ago a Ciba Foundation Symposium was held entitled 'Foetal Autonomy' in which the opening paper by Professor R. V. Short (1969) focused our attention on a simple question: how does an animal know that it is pregnant? Emphasis was given to the ways by which the lifespan and function of the corpus luteum is prolonged by the presence of an embryo, a topic of conjecture ever since Fraenkel demonstrated at the turn of the century that corpora lutea were essential for the maintenance of pregnancy in the rabbit. During the last decade this 'maternal recognition of pregnancy', as it was described by Roger Short, has been investigated in diverse ways, for the term has different implications for different disciplines. The fact that it is now the subject of a symposium in its own right results from advances in knowledge of the morphology and physico-chemical characteristics of embryo–maternal interactions, the local responses of the uterus to the fertilized egg, the developmental changes in the embryo by which its presence is proclaimed, and the maternal adjustments to a resident allogeneic embryo that allow its retention in the uterus rather than its rejection as a foreign tissue.

The symposium will be concerned principally with the recognition of pregnancy in mammals since it is among this class that viviparity has been adopted, almost without exception, as a preferred mode of reproduction. This habit of giving birth to living young has been adopted as a reproductive stratagem by representatives of all classes of vertebrates except for the birds, and by many groups of invertebrates. The patterns of the occurrence of viviparity among members of distantly related genera, and in some, but not other species of a genus, as seen in fishes and reptiles in particular, leave one in no doubt that it has arisen many times in widely different groups of animals. However, although the role of the corpus luteum in the regulation of gestation is only rudimentary in non-mammalian vertebrates, it would be a mistake to assume

that a form of maternal recognition of pregnancy is therefore absent. In the worm-like arthropod, *Onycophora*, a placenta-like relationship develops which is functionally analogous to that of the yolk sac in mammals. Among teleost fishes the young develop within the ovarian cavity in certain species and even within the follicle in others; in the latter, two or more broods at different stages of maturation are harboured simultaneously, which implies that each follicle has some autonomy in regulating its own ovulation. Among the reptiles advanced forms of placentation are found where the allantoic blood supply is apposed to the much folded glandular maternal tissues immediately overlying the main uterine blood vessels.

During this meeting our purpose will be to explore the nature and interplay of mechanisms that are indispensable for the successful establishment of pregnancy in mammals. We shall address ourselves to the diversity of these maternal recognition mechanisms and to the special case of delayed implantation, or embryonic diapause, an experiment of nature (and of enquiring biologists) which may yet prove to have singular value in elucidating the role of the uterine environment in the control of embryonic development. Outstanding questions remain about whether the growth of the embryo is arrested by inhibitory factors produced by the uterus, or by the lack of a maternal stimulus; whether the mother recognizes the presence of an embryo during delay, or whether the embryo withholds evidence of its existence during diapause.

We shall finally examine the biological puzzle of immunological coexistence between mother and embryo with its wider implications in other branches of the natural sciences, and discuss the results of procedures designed to regulate fertility by the immunological neutralization of specific signals of embryonic origin. While the application of these latter techniques for regulating human fertility is a long way off, current research promises to supplement knowledge of the interplay between structural, endocrinological and immunological events in gestation.

Reference

SHORT, R. V. (1969) Implantation and the maternal recognition of pregnancy, in *Foetal Autonomy (Ciba Found. Symp.)*, pp. 2–26, Churchill, London

Comparative aspects of blastocyst–endometrial interactions at implantation

A. C. ENDERS and S. SCHLAFKE

Department of Human Anatomy, University of California, Davis

Abstract Since the trophoblast–uterine adhesion is as nearly a universal phenomenon in implantation as can be found, an attempt was made to determine whether or not there was a reduction in cell surface glycoproteins in the rat, as can be observed in the ferret. Neither colloidal iron nor cationized ferritin revealed the type of pattern anticipated for a localized reduction in surface negativity in the imprint of the blastocyst in the implantation chamber. The use of lectin-coated latex beads also proved disappointing in defining regional differences in adhesiveness. However, a number of observations on the changing shape of the implantation chamber, the secretion of periluminal material by decidual cells, and the penetration of the residual basal lamina of the luminal epithelium by the decidual cells were made in the course of these studies. The implantation chamber of the rabbit, in which the blastocyst does not make an imprint, was contrasted with that of the rat. The areas of fusion of trophoblast knobs with uterine epithelial cells were shown to be visible by scanning electron microscopy. Finally, some observations on the hypertrophy of maternal epithelial cells to form the uterine plaque in the rhesus monkey are described, and the hypertrophy of endothelial cells to form cells admirably suited to protein secretion is presented.

Comparative aspects of implantation such as the orientation of the blastocyst, position of the blastocyst in the uterus, relative penetration of the uterine epithelium and other variable parameters have been reviewed by Wimsatt (1975). In addition we have reviewed common features associated with the events of implantation, such as apposition, adhesion and uterine epithelial penetration (Schlafke & Enders 1975; Enders 1976*a*). Many aspects of alteration of the luminal epithelium, trophoblast surface and adhesiveness have been discussed in broadly based articles by Sherman (Sherman & Salomon 1975; Sherman & Wudl 1976). The role of proteases, especially in the rabbit, has been clarified by the monograph of Denker (1977).

Rather than reviewing the field of comparative implantation it is our intention here to present some aspects of our current investigation of implantation, especially as they relate to the endometrial response to the blastocyst during the progress of implantation.

MATERIALS AND METHODS

Implantation sites used in this study were prepared for light microscopy and for transmission and scanning electron microscopy by the following procedures. Animals at the selected stage of gestation were infused, via the abdominal aorta, with an aldehyde fixative containing 2% glutaraldehyde and 2% formaldehyde, freshly prepared from paraformaldehyde, in 0.1 M-phosphate buffer, pH 7.3. Uteri were then rapidly removed and the implantation sites sliced or split. After fixation times of 1–2 hours, tissues were rinsed in 0.1 M-phosphate buffer, treated for alternative procedures if required, postfixed with 1% osmium tetroxide in phosphate buffer for one hour, dehydrated in alcohol, and embedded in Araldite epoxy resin. Generally, serial one-micrometre sections of the implantation sites were stained with 2% Azure B, and carefully examined until the proper stage or orientation was found. If the material was to be observed with scanning electron microscopy, tissues were placed in acetone, then critical-point dried with CO_2 and sputter-coated using gold. Tissues were examined in an AEI 801 transmission electron microscope or Cambridge Stereoscan or Philips 501 scanning electron microscopes.

Rhesus monkey

Rhesus monkey conceptuses have been obtained on Days 13–16 of gestation. Optimal time of mating was determined by identification of the oestrogen surge associated with ovulation; rapid elevations in plasma oestrogen and progesterone concentrations on Days 10–13 identified a developing conceptus. Peri-implantation areas were examined with light, transmission and scanning electron microscopy.

Rabbits

Implantation stages of Dutch-belted rabbits were collected at seven days 0, 4 and 6 hours *post coitus*. After critical-point drying, slices of the implantation sites were carefully examined and dissected to reveal attachment areas before being placed on stubs for examination in the scanning electron microscope.

Rats

Implantation stages in the rat were studied on Days 6 and 7 of gestation (Day 1 = day sperm found in vaginal smear). In experiments concerning anionic binding, cationized ferritin (Miles-Yeda Ltd; 10.6 mg/ml) was used at a dilution of approximately 1 mg/ml in phosphate-buffered saline. Tissues were treated for 10 or 30 minutes with agitation, rinsed in buffered saline, post-fixed and processed for transmission electron microscopy. Colloidal iron hydroxide, freshly prepared according to the method of Nicolson (1972), was used, at pH 1.6, with an iron concentration of 5.45 g/l.. Tissues were stained for 10 minutes. More extensive studies involving these experimental procedures will appear elsewhere (Enders & Schlafke, in preparation).

ADHESION STAGE OF IMPLANTATION

The first surface of the maternal system encountered by the trophoblast at the initiation of implantation is the apical surface of the uterine luminal epithelium. Transmission electron microscopy revealed that interdigitation of microvilli could occur before adhesion and that broad areas of apposition of membrane could be discerned once adhesion was initiated in the rat and mouse (Enders & Schlafke 1967, 1969; Potts 1968). Scanning electron microscopy confirmed the blunting of uterine microvilli adjacent to the blastocyst (Enders 1975). In addition, this method demonstrated the shape of the blastocyst *in situ*, in particular the indentation of the abembryonic trophoblast by protruding luminal epithelial cells in the rat and mouse (Bergström 1972; Enders 1975).

Since it has been known for a number of years that relative adhesiveness can affect cell sorting and that such adhesiveness appears to be a property of glycoproteins of the cell membrane (Moscona 1971), it was natural that a number of investigators turned towards an examination of the nature of the cell membranes at the time of implantation (Holmes & Dickson 1973; Enders & Schlafke 1974; Nilsson *et al.* 1973). Unfortunately, the variety of possible changes that could result in greater affinity between the two surfaces is great: synthesis of new constituents by one or both surfaces, partial removal of a portion of the glycoproteins resulting in exposure of different constituents, or alteration in lateral mobility of membrane constituents are three different types of possible alteration.

Cytological studies of implantation demonstrated that, in the ferret, the uterine epithelial glycocalyx is so thick that it can be readily demonstrated without the use of cytochemical methods (Enders & Schlafke 1972). There is

an apparent removal of this glycocalyx at specific regions of the adjacent trophoblast. The possibility that such a reduction in uterine glycocalyx could be a common prerequisite for adhesion led us to attempt to investigate it further.

Our initial cytochemical studies of the mouse uterus and trophoblast at implantation indicated that both surface membranes contained negatively charged glycoproteins, and did not show marked reduction in negativity of either trophoblast or uterus at implantation (Enders & Schlafke 1974). Some investigators, however, have suggested a loss of charge on trophoblast at implantation (Jenkinson & Searle 1977). Even if there is no reduction in surface negativity of the blastocyst or the general uterine luminal epithelium, there remains the possibility of local alteration in the constituents of the uterine surface adjacent to the adhering trophoblast. For example, removal of sialic acid could result in both exposure of different glycosyl groups and reduction in charge.

Two principal approaches were used in an attempt to demonstrate alterations at the site of implantation in the rat. The first was to directly visualize implantation sites split to expose the blastocyst and its 'imprint' (Fig. 1), and to make a subsequent cytochemical demonstration of charge. The second procedure was to visualize differential adhesiveness through exposure of the blastocyst and imprint to appropriately treated beads.

In the vast majority of our observations, no reduction in binding of positively charged colloidal iron could be demonstrated on the uterine surface, and in no instance was there a localized depletion as though in response to a single adjacent trophoblast cell. When cationized ferritin (CF) was used as an indicator of surface negativity, a finer distribution of particles was found. This method clearly demonstrated thick, highly uniform binding on the trophoblast, and relatively thin, more irregular binding to uterine epithelium (Fig. 2). However, there appeared to be little difference in the binding of CF in the imprint as opposed to outside the imprint in the implantation chamber (Fig. 3). Some of the extracellular material in the uterine lumen bound more CF than did the uterine surface.

In studies of relative adhesiveness of the imprint, we selected the lectin concanavalin A, since we had previously shown that this lectin will bind to the apical surface of uterine epithelium. When a graded series of agarose beads that contained con A (ranging in size from a few micrometres to over 100 micrometres) were exposed to the implantation chamber and imprints, the beads adhered only to the stromal surface. A second series of experiments were done using con A bound to latex beads of relatively small size (about three times the diameter of a normal uterine luminal microvillus).

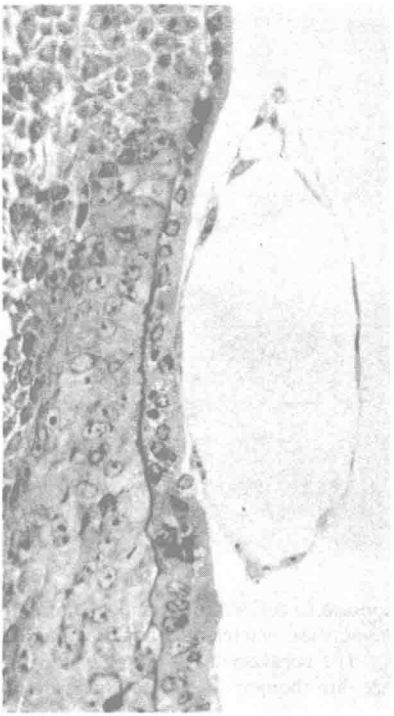

FIG. 1. Transverse section of a rat blastocyst from a split implantation site. The surfaces (shown here in section) and the imprint of the blastocyst on the contralateral surface are made available to exposure to marker materials by this method. Day 6, 14.00 h.

In analysing the results, a number of features of the physical nature of the surfaces had to be considered. The implantation chamber has digitiform microvilli, the imprint itself has blunt microvilli, the blastocyst has fewer microvilli than the uterine surface, and a split blastocyst has a flat exposed basal lamina. The physical properties of smooth undulating surfaces should allow more surface to be exposed to the small-sized beads than a microvillous surface. Blunt microvilli should provide greater exposure than tall microvilli. If these factors are taken into consideration, the latex spheres should bind best to the basal lamina of the blastocyst, next to the blastocyst surface, then to an imprint, and lastly to the uterine chamber. In examining the preparations, we found that this was the order appearing, with the exception that there was only moderate adherence to the blastocyst surface (the aggregation of beads made the actual count difficult to assess in most cases) (Figs. 4 and 5). At any rate, we could not demonstrate a large increase in the number of beads adhering to the imprint. Therefore, in relation to the idea that a reduction

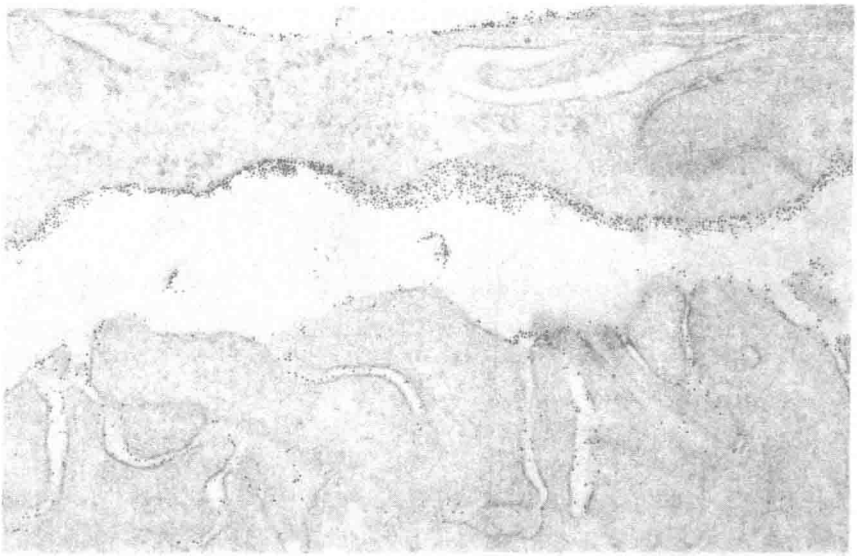

FIG. 2. Rat trophoblast (above) and uterus after exposure to cationized ferritin. Note that the trophoblast abundantly binds this tracer of anionic sites, whereas the adjacent uterine surface contains only irregularly scattered particles. The occasional aggregates of ferritin molecules, loosely associated with the uterine surface, are thought to be marking secreted material. Day 6, 14.00 h.

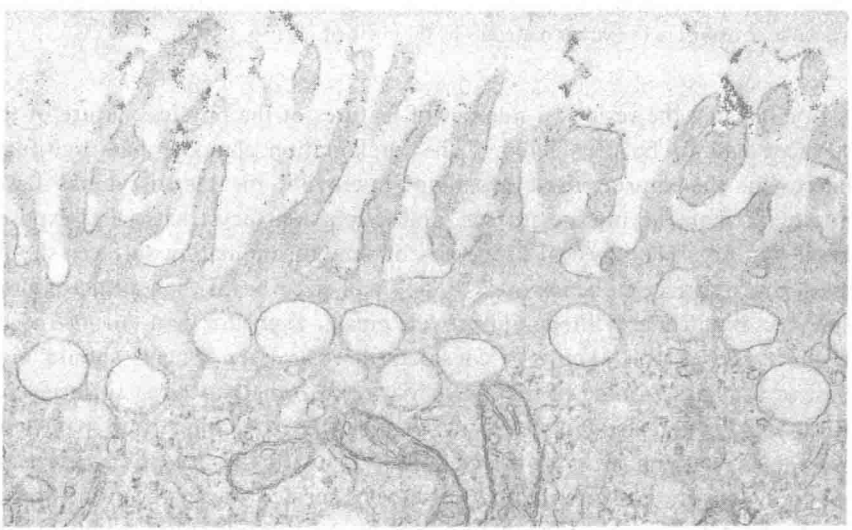

Fig. 3. Rat uterine surface within the implantation chamber outside the imprint. Microvilli are less blunt than in the imprint, and have similarly distributed ferritin particles. Day 6, 14.00 h.