Uterine vascular permeability, blood flow and extracellular fluid space during implantation in rats

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Summary. Vascular permeability to plasma proteins in uterine implantation and non-implantation sites (i.e. dye sites and non-dye sites) was assessed quantitatively by a method which accounts for steady-state volumes of distribution. Extracellular fluid volume and uterine blood flow were also determined. On both the evening of Day 5 and the morning of Day 6, vascular permeability to $^{125}$I-labelled human serum albumin, extracellular fluid volume and blood flow were significantly increased in implantation sites compared to non-implantation sites. Vascular permeability in implantation sites was increased significantly between Days 5 and 6, whereas that in non-implantation sites was unchanged. This increase in vascular permeability between Days 5 and 6 was not accompanied by further increases in extracellular fluid volume and blood flow. This result shows a dissociation between vascular permeability and extracellular fluid volume immediately after the onset of implantation and raises important questions as to whether the rat uterus undergoes a truly oedematous response at implantation as has been generally accepted.

Keywords: implantation; uterus; vascular permeability; blood flow; oedema

Introduction

The uterine luminal environment is separated from the surrounding uterine tissue by a permeability barrier which exhibits complex behaviour towards blood-borne substances (McRae & Kennedy, 1981, 1983a, b) and seems to function more broadly than the simple exclusion of certain substances from the uterine lumen (see McRae, 1984, 1988, for reviews). The composition of this environment at any given time will depend upon a balance between the influx and efflux of material across the luminal and glandular epithelia. For substances not synthesized or metabolized locally in the uterus, bi-directional fluxes will be greatly influenced by the uterine vascular system through its effects upon concentrations in interstitial fluid. Interstitial concentrations are important for substances which primarily diffuse across the barrier and for substances which are transported by specific mechanisms, the kinetics of which are related to ambient concentrations. Thus, changes in the perfusion rate and permeability of the uterine vasculature during implantation are likely to influence the uterine luminal environment.

The uterine vasculature at the time and in the location of implantation in several rodent species is considered to exhibit an increase in vascular permeability. This conclusion is based upon repeatedly consistent observations that plasma proteins, labelled with a vital dye such as Pontamine Sky Blue or radiolabelled, accumulate in the vicinity of the implanting blastocyst by 15–30 min after intravenous injection of the marker substance (Psychoyos, 1960; Orsini, 1963; Finn & McLaren, 1967). This accumulation is usually quantitatively estimated by injecting radiolabelled human or bovine

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serum albumin intravenously and measuring tissue levels of radioactivity, expressed per milligram of tissue, 15–30 min after injection (Psychoyos, 1961, 1973). This method is limited because the volume into which the marker protein diffuses is not taken into account and so the concentration of the radiolabelled protein is only approximated. This is an important consideration since this variable may also be affected locally by the implanting blastocyst. Indeed, a localized dispersal of the cellular components of the stroma, commonly considered to be oedema, is apparent histologically (Finn & McLaren, 1967; Lundkvist & Ljunckvist, 1977; Rogers et al., 1982). Therefore, the work described in this paper was carried out to determine quantitatively whether vascular permeability is increased in the implantation site of the rat and, if so, to what extent it is correlated with uterine blood flow and interstitial fluid volume. These measurements were made in rats during the evening of Day 5 of pregnancy when implantation is beginning and during the following morning by which time the blastocyst has become relatively firmly attached to the uterine wall.

Materials and Methods

Animals. Mature Porton-Wistar female (175–250 g) and male (≥300 g) rats from the Institute’s colony were housed under controlled light (lights on 05:00–19:00 h) conditions with free access to food and water. Vaginal smears were taken from female rats each morning and those exhibiting a pro-oestrus smear were placed in the afternoon with males of proven fertility. The following day was taken as Day 1 of pregnancy for female rats bearing a copulation plug or with spermatozoa in the smear. Implantation sites were identified on the evening of Day 5 (between 20:00 and 24:00 h) or the morning of Day 6 (between 10:30 and 12:00 h) of pregnancy by localized accumulation of Evans Blue dye given in a 0.5 ml volume of a 2% solution into a femoral or lateral tail vein 15 min before being killed and under pentobarbital sodium anaesthesia (Sagatal; May and Baker, Dagenham; 50 mg/kg injected intraperitoneally). In one experiment nephrectomized animals were used. These animals were anaesthetized with pentobarbital sodium and both kidneys of each animal were exposed by midventral incisions. To minimize blood loss, the renal pedicles were tightly ligated before removal of the kidneys. This surgery was completed within 1 h before the animals were killed.

Estimation of vascular permeability. The method used to assess vascular permeability was that of Satchell & Sharpe (1981) modified as necessary. With this method, permeability is assessed from the rate at which the volume of distribution or space of the marker protein approaches its steady state value. Animals were given intravenous injections of the marker protein, 125I-labelled human serum albumin (Amersham International plc, Aylesbury, Bucks, U.K.), and killed at predetermined times thereafter. The volume of distribution for albumin, in a given tissue, was calculated by dividing the tissue radioactivity (d.p.m./mg wet tissue) by the plasma water radioactivity (d.p.m./μl), giving an estimate of volume as μl/mg tissue.

Albumin volumes of distribution were determined in implantation and non-implantation sites in two separate experiments. The first experiment was carried out to estimate volumes of distribution at steady state. Therefore, in this experiment animals were killed 6, 12 or 24 h (N = 4) after single injections of 125I-labelled human serum albumin into a lateral tail vein. The second experiment was carried out to assess the time course for volumes of distribution for times shortly after injection. Therefore, in this experiment, the times of death were 5 min or 1, 2 or 4 h (N = 4) after injection. The results from these two experiments are used to define the function of log [1 - (albumin space/steady state albumin space)] versus time.

To assess the degree of enzymic degradation of the injected 125I-labelled human serum albumin into lower molecular weight, radiolabelled fragments, plasma obtained 24 h after injection from one animal was separated by gel filtration on a Sephadex G-25 column. Approximately 85% of the total radioactivity eluted as high molecular weight material.

Extracellular fluid volume and tissue water. Extracellular fluid volume was estimated from the volume of distribution of 51Cr-labelled ethylenediaminetetra-acetate (EDTA) (Larsson et al., 1980) at steady state. This method assumes that the concentration in the extracellular fluid of any substance such as EDTA, which is able to diffuse rapidly out of the vasculature but unable to penetrate cell membranes, will be equal to that in blood plasma at steady state. 51Cr-labelled EDTA is normally cleared very rapidly from the blood by the kidneys. Therefore nephrectomized animals were used to ensure that volumes of distribution for EDTA were measured under conditions appropriate for achieving equilibrium (that is, relatively constant plasma levels). In this experiment animals were killed 15, 30 or 60 min (N = 4) after single intravenous injections of 51Cr-labelled EDTA. Volumes of distribution in implantation and non-implantation site tissue were estimated as described above.

Total tissue water was calculated as the percentage difference of the wet tissue weight and dry tissue weight. The uterine samples collected to determine extracellular fluid volume were dried at 160°C for 48 h or until no further weight change occurred after further heating.

Uterine blood flow. Uterine blood flow was estimated by the radioactively-labelled microsphere technique (Rudolph & Heymann, 1967). In this experiment the right carotid and right femoral arteries were cannulated with
polyvinylchloride tubing (Dural Plastics, Dural, New South Wales, Australia) under pentobarbitone sodium anaesthesia. The carotid cannula (0.8 mm o.d., 0.5 mm i.d.) was attached to a pressure transducer and recording apparatus, whereas the femoral cannula (0.5 mm o.d., 0.2 mm i.d. connected to 0.8 mm o.d., 0.5 mm i.d. tubing and inserted into artery) was connected to a Harvard syringe pump. The saline (0.154 M-NaCl) filling these cannulae contained heparin in an attempt to ensure that flow would not be impeded by blood clots. The carotid cannula contained 20 i.u. heparin/ml saline whereas the femoral cannula contained 200 i.u. heparin/ml saline as there was a greater likelihood that blood clots would otherwise form and block this smaller cannula. The following procedures were used in the actual measurement of blood flow. First, about 20 min before administration of the microspheres, Evans Blue dye was injected into the left femoral vein. Next, the carotid cannula was advanced until the tip entered the left ventricle, indicated by a shift in the blood pressure recording from an arterial to ventricular pattern. The withdrawal pump was then turned on and blood was collected at the rate of 167 μl/min. The carotid cannula was then connected to a 2 ml syringe containing 10 μCi 57Co-labelled microspheres having a mean diameter of 15 μm and suspended in saline with 20% Dextran, 0.01% Tween 80 (New England Nuclear Research Products, Stevenage, Herts, U.K.) in 0.2 ml saline. This syringe was vigorously agitated on a vortex mixer and the well mixed contents were flushed into the left ventricle of the heart via the carotid cannula. Blood withdrawal was continued for about 3 min and then the animal was killed by cervical dislocation and the uterus, the ovaries and the kidneys were removed. Blood flow was calculated as

\[ F = Q_{\text{ref}} \times \left( \frac{N_{\text{org}}}{N_{\text{ref}}} \right) \]

where \( F \) = flow, \( Q_{\text{ref}} \) = rate of withdrawal of reference sample, \( N_{\text{org}} \) = number of microspheres present in organ and \( N_{\text{ref}} \) = number of spheres present in reference sample.

Blood flows were estimated on Days 5 and 6 of pregnancy in uterine (dye site and non-dye site) tissue as well as in the ovaries and kidneys of each animal (\( N = 5 \)).

Sample collection. In all experiments the animals were anaesthetized at the time of autopsy. Typically, the abdominal cavity was opened and a blood sample was collected into a heparinized syringe from the abdominal aorta. The animal was then killed by cervical dislocation. The uterus and other organs as specified above were then removed and trimmed of mesentery. The uterus was dissected into dye sites and non-dye sites. These were weighed separately. Plasma was separated from blood by centrifugation. Blood water content was determined in the same manner as tissue water described above. When appropriate, radioactivities in plasma (0-1 ml samples in duplicate) and tissue samples were determined with a gamma-spectrometer (LKB-Wallac, Turku, Finland).

Statistical analysis. All data from each experiment were tested for heterogeneity of variance by Bartlett’s test (Snedecor & Cochran, 1967). Logarithmic transformations of data were carried out to eliminate heterogeneity when appropriate. The significance of the effects of time of death after injection, day of pregnancy and site of sampling on the calculated value of \( \log [1 - (\text{albumin space/steady state albumin space})] \) and EDTA volumes of distribution was determined by analysis of variance with repeated measures (site of sampling) (Winer, 1971). The significance of the effects of day of pregnancy and site of sampling on tissue water was determined similarly. When significant interactions were indicated the data were appropriately partitioned and re-analysed to identify the source of the interaction. In the event that significant interactions persisted, Duncan’s New Multiple Range Test (Steel & Torrie, 1960) was used for group comparisons. Regression analysis was used to define the line of best fit for the function of \( \log [1 - (\text{albumin space/steady state albumin space})] \) versus time. From the slope and \( y \)-intercept of the line the time required for the ratio of albumin space/steady state space to equal 0.5 (\( t_{50} \)) was calculated. The antilogarithm of this value was then found to convert it to linear time. Blood flow data for animals in which the right and left kidney values differed by more than 15% were eliminated on the assumption that intraventricular mixing of microspheres was inadequate. The significance of the effect of site of sampling on uterine blood flow (logarithmically transformed data) for each day of pregnancy was determined by the Wilcoxon Signed Rank Test (Snedecor & Cochran, 1967) since the considerable variation between animals precluded analysis of variance. The ratio of blood flow in implantation site to non-implantation site tissue for each day of pregnancy was tested for significance by the Mann–Whitney test (Snedecor & Cochran, 1967).

Results

Vascular permeability

The volumes of distribution of 125I-labelled human serum albumin were significantly \((P < 0.05)\) greater at 24 h after intravenous injection than at 12 or 6 h, in both tissue sites and on both days of pregnancy, with the exception of non-implantation tissue on Day 5 (Table 1, upper panel). Although the difference between 12 and 24 h was statistically significant, the mean values at 24 h were on average only 6.4% (range, 1.5–11.6%) larger than those at 12 h. Therefore, the mean volume of distribution at 24 h for each tissue type was taken to be a reasonable estimate of the albumin space at steady state. Albumin volumes of distribution for earlier times after injection (<6 h) are shown in the lower panel of Table 1. These results were used to generate the time functions of \( \log [1 - (\text{albumin space/steady state albumin space})] \) shown in Fig. 1.
Table 1. Comparison between days of pregnancy and between tissue surrounding and adjacent to implanting rat blastocysts for albumin volumes of distribution or spaces (µl/mg) in uterine tissues as functions of time after intravenous injection with $^{125}$I labelled human serum albumin

<table>
<thead>
<tr>
<th>Exp.*</th>
<th>Time (h)</th>
<th>Implantation tissue</th>
<th>Non-implantation tissue</th>
<th>Implantation tissue</th>
<th>Non-implantation tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>0.447 ± 0.007</td>
<td>0.306 ± 0.013</td>
<td>0.429 ± 0.007</td>
<td>0.337 ± 0.011</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0.477 ± 0.004</td>
<td>0.422 ± 0.008</td>
<td>0.447 ± 0.006</td>
<td>0.406 ± 0.018</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0.484 ± 0.005</td>
<td>0.404 ± 0.014</td>
<td>0.484 ± 0.007</td>
<td>0.453 ± 0.005</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0.036 ± 0.004</td>
<td>0.020 ± 0.001</td>
<td>0.047 ± 0.004</td>
<td>0.022 ± 0.001</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.167 ± 0.006</td>
<td>0.065 ± 0.003</td>
<td>0.239 ± 0.008</td>
<td>0.076 ± 0.008</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.297 ± 0.009</td>
<td>0.134 ± 0.003</td>
<td>0.361 ± 0.010</td>
<td>0.159 ± 0.015</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.358 ± 0.005</td>
<td>0.178 ± 0.007</td>
<td>0.415 ± 0.016</td>
<td>0.193 ± 0.014</td>
</tr>
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Values are mean ± s.e.m. for N = 4.

*Exp. 1 estimated volumes of distribution at steady state and Exp. 2 assessed the early time course for volume of distribution.

Fig. 1. Calculated values of log[1 - (albumin space/steady state albumin space)] (see text for derivation) plotted against time after intravenous injections of $^{125}$I-labelled human serum albumin to rats on the evening of Day 5 or the morning of Day 6 of pregnancy. Values are mean ± s.e.m. (N = 4) for tissue surrounding implanting blastocysts (dye sites, •) or adjacent to implanting blastocysts (non-dye sites, ■); $t_{1/2}$ is the time required for the ratio of albumin space/steady state albumin space to equal 0.5.

The relative rates at which albumin leaks out of the uterine vasculature are represented by the slopes of the curves in the figure and the half times (i.e. the time required for the ratio of albumin space to steady state space to equal 0.5) in Table 2. On either day of pregnancy these slopes were...
Table 2. Comparison between uterine tissue surrounding and adjacent to implanting blastocysts for vascular and tissue fluid responses to implantation on Days 5 and 6 of pregnancy in the rat

<table>
<thead>
<tr>
<th></th>
<th>Day 5</th>
<th>Day 6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of animals</td>
<td>Implantation tissue</td>
</tr>
<tr>
<td>Vascular permeability (t,h)</td>
<td>16</td>
<td>1.5</td>
</tr>
<tr>
<td>Extracellular fluid volume (µl/mg)</td>
<td>12</td>
<td>0.619 ± 0.009a</td>
</tr>
<tr>
<td>Total tissue water (%)</td>
<td>12</td>
<td>85.2 ± 0.32</td>
</tr>
<tr>
<td>Blood flow† (µl/min/mg)</td>
<td>5</td>
<td>0.509 ± 0.049a</td>
</tr>
</tbody>
</table>

Values except those for vascular permeability are mean ± s.e.m.
†Values are geometric mean ± s.e.m.
Values with different superscripts are significantly (P < 0.05) different.

significantly steeper in implantation tissue than non-implantation tissue (time × site of sampling interaction: 0.005 < P < 0.01). Furthermore, these slopes were significantly steeper on Day 6 than on Day 5 in implantation tissue, but did not differ between days in non-implantation site tissue (day × site of sampling interaction: 0.01 < P < 0.05). (However, significant (P < 0.05) heterogeneity of variance was present in the data and was not eliminated by logarithmic transformation.) Albumin spaces therefore approached steady state at measurably faster rates in implantation sites than non-implantation sites on both days and on Day 6 than on Day 5 in implantation sites.

Extracellular fluid volume

Volumes of distribution for 51Cr-labelled EDTA did not differ significantly (P > 0.05) at 15, 30 or 60 min after injection regardless of day of pregnancy or tissue site sampled (data by time after injection not shown). This absence of a time effect suggested that values for the volumes of distribution for EDTA rapidly reached a maximum after injection. Therefore, the data were pooled over time and the mean values are presented in Table 2. Volumes of distribution for 51Cr-labelled EDTA were significantly (0.001 < P < 0.005) larger in implantation site tissue than non-implantation site tissue on both days of pregnancy. There was however no significant (P > 0.05) difference between days of pregnancy. Total tissue water as a percentage of wet weight exhibited a parallel pattern of differences (Table 2).

Uterine blood flow

Uterine blood flows varied considerably between animals, as indicated by coefficients of variation between 15.2% and 31.1%. Nevertheless, blood flows were significantly (0.01 < P < 0.05) greater in implantation sites than in non-implantation site tissue on each day studied. Blood flows within each tissue type did not differ significantly (P > 0.05) between days of pregnancy.

Discussion

The present results confirm that the vascular beds (i.e. venous capillaries and post-capillary venules; Abrahamsohn et al., 1983) underlying implanting blastocysts are measurably more permeable to albumin than are beds located adjacent to implantation sites. Furthermore, these results clearly demonstrate that the vascular beds in implantation sites become more permeable to albumin as
implantation proceeds to the morning of Day 6 of pregnancy as indicated by estimates of the relative rate of albumin leakage from uterine vasculature. The mechanisms involved in this amplification of response between Days 5 and 6 of pregnancy are largely speculative because little is known about the mechanisms through which blastocysts, separated from the stroma by an intact epithelium, induce increased vascular permeability and connective tissue decidualization. Chemical mediators originating either from the blastocyst or within the endometrium (epithelium or stroma) are likely to be involved (see below). This amplification of response could therefore result from the availability of increasing amounts of mediator, perhaps because production involves a sequential cascade of enzymes which allows amplification. Alternatively, the increased permeability to Day 6 could result from increased responsiveness of the vascular endothelial cells to these mediators. However, a role for the blastocyst itself, which is growing and differentiating during this period, or for the epithelium which appears to be obligatory in inducing these endometrial responses (Leroy & Lejeune, 1981) should also be considered. This temporal amplification of the vascular permeability response to implanting blastocysts may provide an additional experimental approach to the mechanisms by which the blastocyst induces this and other endometrial responses.

In addition to increased vascular permeability the present results demonstrate that the vascular beds underlying implanting blastocysts are perfused at significantly higher flow rates than are beds located adjacent to implantation sites, on both days of pregnancy studied. Although the Day-6 flows measured by microspheres are slightly lower than those reported using the $^{86}$Rb fractional distribution method (Mitchell & Hammer, 1983), the relative differences between implantation and non-implantation sites are quite similar for both techniques. Angiogenesis first appears in implantation sites on Day 7, as indicated by increased volume and density in the capillary beds or resin vascular casts (Takemori et al., 1984), suggesting that the elevated blood flows reported here represent, at least in part, true increases in flow rather than sprouting of new blood vessels. Furthermore, the diameters of blood vessels measured in resin casts of the uterine vasculature on the morning of Day 6 were significantly larger in implantation sites than in non-implantation sites (Rogers et al., 1982), again suggesting hyperaemia and not angiogenesis alone. However, uterine blood flows in the present study were only 10% of those obtained in ovariectomized animals 60 min after an injection of oestradiol at a dose of 0.5 μg/kg body weight (Phaily & Senior, 1978). In fact, the uterine blood flows determined in this study are in good agreement with those reported by Phaily & Senior (1978) for ovariectomized rats. Thus, the uterine vasculature appears to have a far greater capacity to respond to vasodilatory stimuli than is found during implantation.

Non-uterine blood flows in general agreed well with those reported elsewhere. For example, renal blood flow was measured to be 3.27 μl/min/mg (s.d. 1.52; N = 10) in these animals. These values compare favourably to 3.39 ± 0.15 μl/min/mg reported by Phaily & Senior (1978) using microspheres with a similar size and are slightly higher than those reported by Bruce (1976) and slightly lower than those reported by Harvey & Owen (1976) using larger (25 μm) microspheres. In addition, ovarian blood flows were 3.64 μl/min/mg (s.d. 0.59) on the evening of Day 5 and 2.66 μl/min/mg (s.d. 0.41) on Day 6 ($P < 0.05$; t test, d.f. = 8). Both values agree well with those reported by Harvey & Owen (1976) and the Day-6 flows agree particularly well with those reported by Bruce (1976) for non-pregnant (met- or dioestrous) animals.

Prostaglandins, particularly of the E series, are likely to be important mediators in the vascular permeability response of the uterus to a deciduogenic stimulus (Kennedy, 1979, 1980). Kennedy & Armstrong (1981) suggested that, as in other tissues, prostaglandins in the uterus may have a primary function as vasodilators and as such they act by potentiating the permeability-inducing effects of other mediators such as bradykinin or histamine and have only minor effects on vascular permeability per se. The view that prostaglandins during implantation are primarily vasodilatory is supported by the direct demonstration here that uterine blood flow was greater in the implantation site compared with that in the non-implantation site. The possibility that prostaglandins may not be solely responsible for this localized hyperaemia is raised by histamine and kinins being implicated in oestrogen-induced hyperaemia (Phaily & Senior, 1978). Direct effects upon the vasculature of
steroids, possibly of blastocyst origin (Logeat et al., 1980; DeHertogh et al., 1986) also cannot be ignored. As noted above, the mechanisms and mediators involved in the endometrial responses to implanting blastocysts are poorly understood.

Marked expansions of the extracellular fluid volume and tissue water contents were localized to implantation sites and occurred coincidentally with increased vascular permeability to albumin and increased blood flow. The steady state albumin spaces in implantation and non-implantation site tissue were in the range of 25–35% smaller than the 51Cr-labelled EDTA volumes of distribution. This discrepancy, known as the excluded volume fraction for albumin (Parker et al., 1979), has been observed in tissues such as heart, lung, muscle and intestine (Kwong & Egan, 1985) but apparently not in testis (Sitchell & Sharpe, 1981). In addition, the extracellular fluid volume measured in this study in both regions of uterine tissue are in the range of 1.5–4-fold larger than those measured in other tissues such as lung (0.4 μl/mg), skin (0.3 μl/mg) and skeletal muscle (0.13 μl/mg) (Larsson et al., 1980). The size of these volumes in uterine tissue is consistent with the general morphological appearance of the stroma as a loose connective tissue with relatively large extracellular spaces (Lundkvist & Ljungkvist, 1977). Primarily on the basis of this morphological appearance, the stroma has been considered oedematous during the preimplantation period in mice (McLaren, 1970), after oestrogen treatment of progesterone-treated rats (Lundkvist, 1979) and mice (Martin et al., 1973), in the vicinity of an implanting blastocyst (Finn & McLaren, 1967; Lundkvist & Ljungkvist, 1977) and after an experimental deciduogenic stimulus (DeFeo, 1963; Lundkvist, 1978). Furthermore, this oedema is thought to result directly from increased vascular permeability (Rogers et al., 1982; Milligan & Mirembe, 1984), although other explanations have been presented (Lundkvist, 1979). However, oedema in the clinical sense is the presence of excess interstitial fluid in the tissue and occurs when the tissue fluid balance regulatory mechanisms have been overridden due to a uncompensated loss of negative interstitial pressure. It follows then that increased vascular perfusion does not invariably cause oedema. Therefore, the dispersed appearance of the cellular components of uterine stromal connective tissue observed during implantation in rats and mice need not result directly from the observed increase in vascular permeability. That the two events may be dissociated functionally is supported by the present finding that the marked increase in vascular permeability in implantation sites on the morning of Day 6 over that on the evening of Day 5 (t1 1.0 h vs 1.5 h) was not associated with a significant increase in the extracellular fluid volume or tissue water. This dissociation may indicate that vascular responses to implantation develop sufficiently slowly to allow fluid balance regulatory mechanisms to maintain negative interstitial pressure, thus preventing oedema. Additional evidence of a discrepancy between uterine vascular changes and uterine tissue water has been reported by Martin et al. (1973): a second oestrogen injection to progesterone-treated mice resulted in a marked increase in the proportion of capillaries and venules exhibiting fenestrations and a marked reduction in the water imbibition response to oestrogen. Furthermore, during the decidual cell reaction increases in the extravascular albumin volumes of distribution (Milligan & Mirembe, 1984) and the histological looseness of the stroma (Lundkvist & Ljungkvist, 1977) occur several hours before the appearance of the Pontamine blue response (Lundkvist et al., 1977). Thus, the morphological appearance of the stroma at the time of implantation which is widely held to be oedematous in fact may not be oedema according to the classical clinical definition.

An alternative explanation for the histologically apparent loosen of the stroma during implantation is that there are changes in both the composition of the stromal extracellular matrix and the vasculature which are induced by the presence of an implanting blastocyst. The observations that the dense collagen bundles which dominate distal areas of the endometrial stroma are absent from areas of decidual reaction in rats (Fainstat, 1963) and mice (Martello & Abrahamsohn, 1986) support this explanation. However, a relationship between extravasation of plasma protein and the volume of extracellular space is not precluded by this explanation. Expansion of the extracellular fluid volume may require both a structural loosening of the extracellular matrix and exudation of plasma water. In turn, an expansion of the extracellular fluid volume may increase the rate at which plasma
protein enters the interstitial fluid by increasing the distribution volume for proteins thus lowering their concentrations and increasing the difference in osmotic pressure due to proteins between plasma and tissue. Complex relationships between uterine vascular dynamics and extracellular fluid volume are likely to exist.

The functional significance of the uterine vascular and interstitial responses to an implanting blastocyst described in this paper and in many others remains uncertain although several consequences can be proposed. Recent evidence suggests that extravasation of plasma protein and extravascular clotting may be an important factor before modified tissue growth responses, including tumour growth and angiogenesis (Dvorak et al., 1985). Vascular changes may also influence the transfer of materials across the blood–uterine lumen barrier since non-saturated transfer across any such barrier is likely to be flow-limited, permeability-limited or a combination of both depending upon the physicochemical properties of the substance in question. Recent observations suggest that a blood–uterine lumen permeability barrier is formed by the primary decidual zone after sloughing of the epithelium (Rogers et al., 1983; Parr & Parr, 1986). A permeability barrier therefore appears to be a consistent feature and may be a prerequisite during implantation. Studies of the effects of vascular changes upon the transfer of materials across this barrier are relevant to our understanding of implantation.

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Uterine vascular dynamics during implantation


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