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Scanning electron microscopy study on corrosion cast of rat uterine vasculature during the first half of pregnancy

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INTRODUCTION

Uterine blood vessels undergo remarkable changes throughout the reproductive cycle. An increase in endometrial vascular permeability is thought to be essential if decidualisation is to occur (Psychoyos, 1973). As pregnancy progresses, further vascularisation takes place in the uterus to provide the placental circulation. In addition, the differential reactivity of the myometrial, endometrial, and placental vessels to vasoactive agents (Greiss, 1972; Rosenfeld, Barton & Meschia, 1976) suggests the existence of vascular structures which regulate blood flow in the uterus.

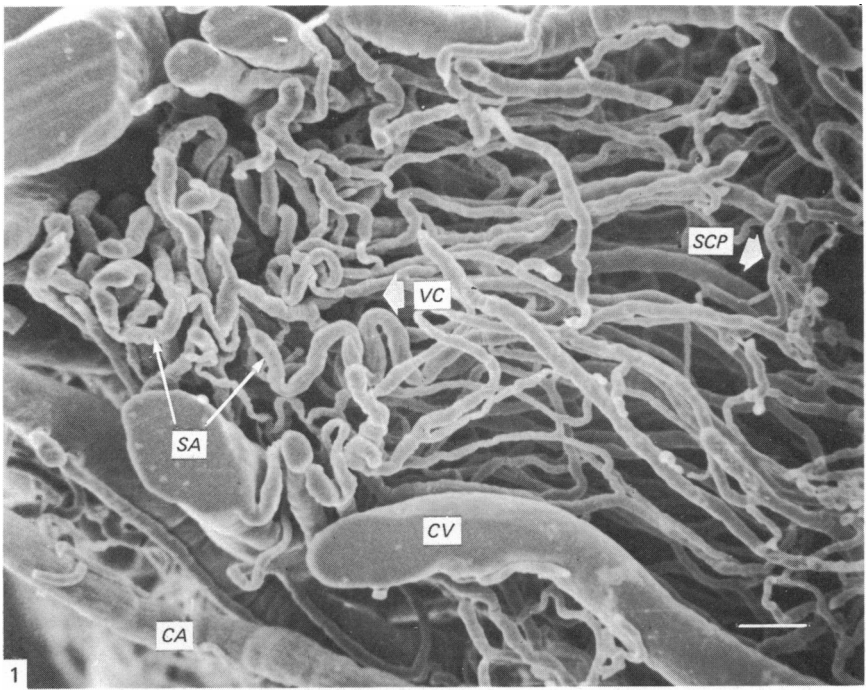
In spite of the important role of the uterine vascular system, little is as yet known of the changes in the uterine vasculature itself during pregnancy.

Recent improvements in the microcorrosion vascular casting/scanning electron microscopy method (Murakami, 1971; Nowell & Lohse, 1974) now make it possible to study microvascular architecture in three dimensions, and make it easier to understand the relationship between vascular architecture and its function. This technique has been employed in this investigation to study the fundamental uterine vasculature and its changes during the first half of pregnancy in the rat.

MATERIALS AND METHODS

Adult virgin Wistar rats weighing 210–250 g were kept at a room temperature of 23 ± 2 °C, with a period of 14 hours of light (0700–2100 hours per day). Vaginal smears were examined daily to determine the stage of the oestrous cycle. On the day of pro-oestrus females were housed one to two animals per cage with a male of proven fertility. The day on which spermatozoa were detected in the vaginal smears was taken as Day 1 of pregnancy. Forty rats were used to study the chronological vascular changes in the uterus during the first half of pregnancy (4 rats per day: Days 1–4; 6 rats per day: Days 5 and 6; 3 rats per day: Days 7–10).

Each rat was anaesthetised with ether, injected with 50 i.u. of heparin in the abdominal cavity, and laparotomy performed fifteen minutes after anaesthesia. In order to wash blood from the uterine blood vessels, heparinised Ringer's solution was infused through a cannula placed in the abdominal aorta, and the ovarian veins opened on both sides to provide outflow points. After the circulating blood had been drained from the uterus, Mercox CL resin mixed with the prescribed amount of



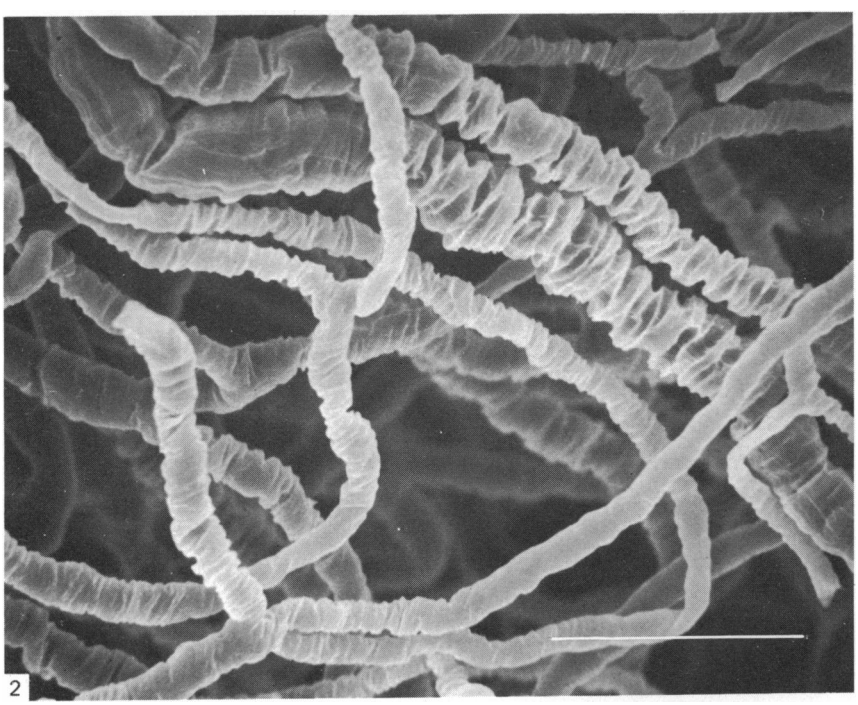
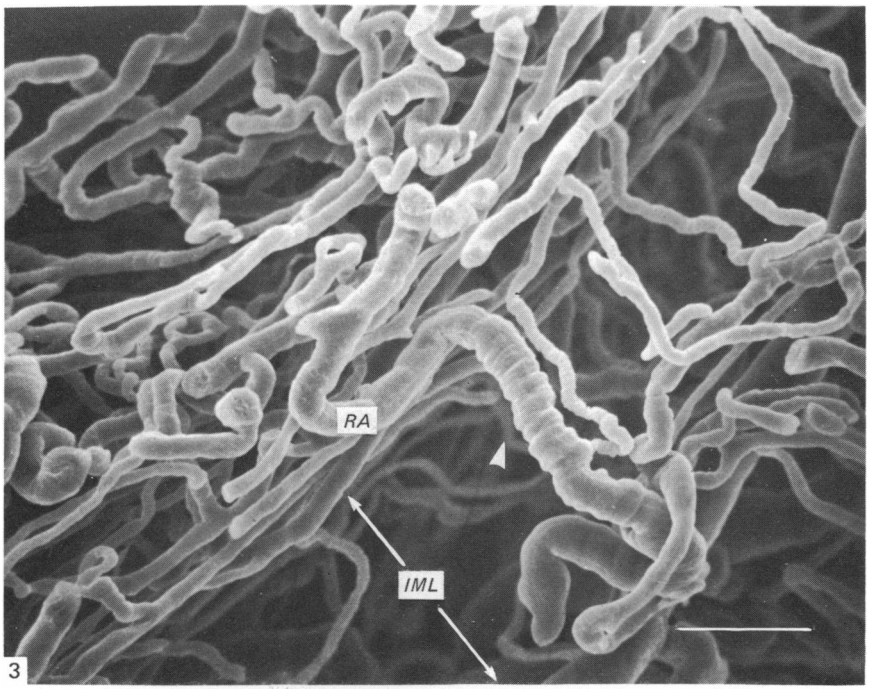


Fig. 1. Transverse section of the uterine vascular cast of a Day 1 pregnant rat showing the mesometrial triangle. Note the sigmoid arterioles (*SA*) composing the vascular conglomerate (*VC*). *CA*, circumferential artery; *CV*, circumferential vein; *SCP*, sub-epithelial capillary plexus. Bar = 50 μm.

Fig. 2. Uterine vascular cast of a Day 5 pregnant rat showing the vascular conglomerate. Circular impressions are seen around the cast of each sigmoid arteriole. Bar = 50 μm.



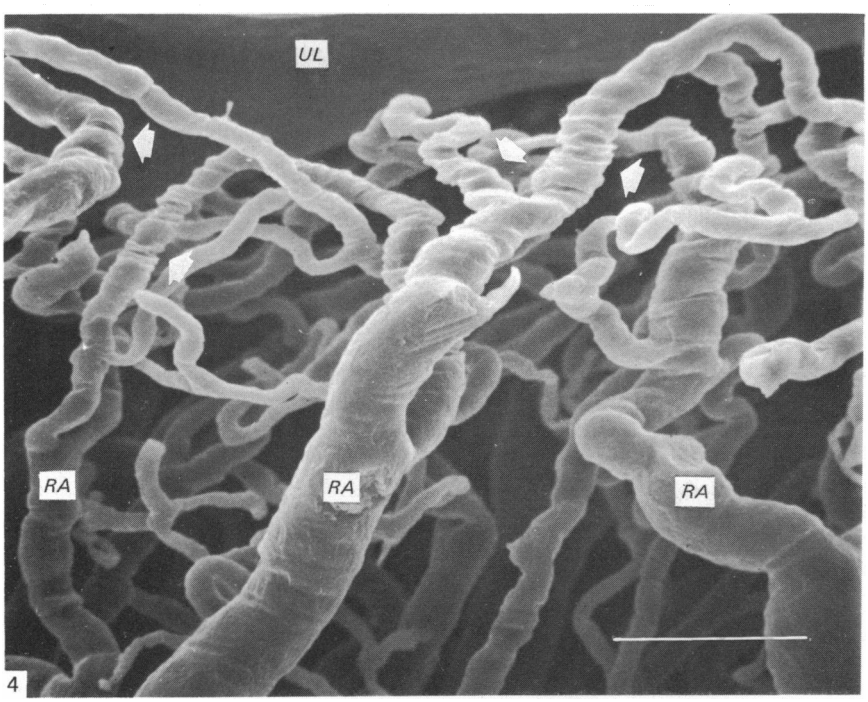


Fig. 3. Transverse section of the uterine vascular cast of a Day 9 pregnant rat. Circular impressions (arrowhead) are seen around the cast of a radial arteriole (*RA*) where it passes through the inner muscle layer (*IML*). Arrows indicate the width of the muscle layer. Bar = 50 μm.

Fig. 4. Transverse section of the uterine vascular cast of a Day 9 pregnant rat. Circular impressions (arrows) are seen at the terminal portion of each radial arteriole (*RA*). *UL*, uterine lumen. Bar = 50 μm.

polymeriser (Dainihon Ink & Chemicals, Inc., Japan) was injected under low pressure into the uterine vessels via the cannula. On the evening of Day 5 of pregnancy, while the implantation areas were still difficult to recognise, the pontamine blue reaction was employed to identify them. The uterus was removed *en bloc* after polymerisation of the resin, and each uterine horn was dissected longitudinally along the uterine lumen, or transversely either at the juxta- or inter-implantation areas. Some of these blocks were immersed in 20 % KOH solution for two days to dissolve the tissue, and were then rinsed in tap water to remove the residue from the casts. After being passed through a graded series of ethanol-isoamyl acetate solutions, the casts were dried using the critical point method, mounted with carbon paint on specimen holders, and finally spatter coated with gold giving a layer approximately 40 nm thick. These specimens were observed with a scanning electron microscope (Hitachi, S-430) at an accelerating voltage of 20 kV.

The remaining blocks were fixed in 10 % formalin, and paraffin-embedded sections 6 μm thick were stained with haematoxylin and eosin. They were examined with the light microscope to study the positional relationship between the vascular casts and the uterine tissues.

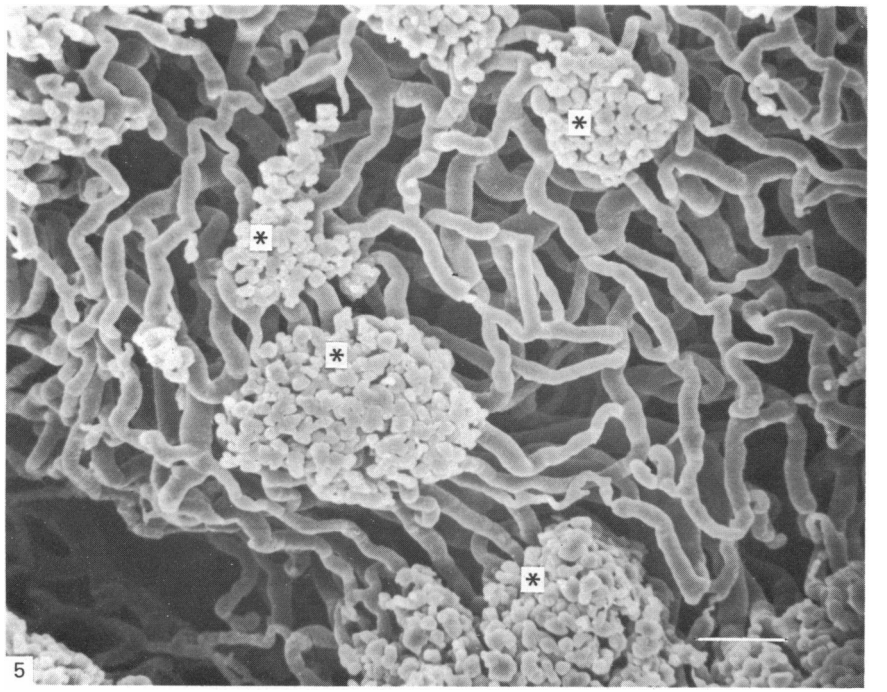
RESULTS

The fundamental vascular architecture of the rat uterus in early pregnancy was found to be similar to that observed in di- or met-oestrus (Takemori *et al.* 1982). The uterine artery gave off a series of segmental branches towards the uterine horn, each dividing into several sigmoid arterioles and a pair of circumferential arteries at the top of the mesometrial triangle. The sigmoid arterioles had a special mode of arrangement; each arteriole twisted, bifurcated, and the divisions anastomosed with each other, to form a vascular conglomerate along the uterine horn in the mesometrial triangle (Fig. 1). The arteriole then pierced the inner muscle layer as a straight segment to supply the mesometrial portion of the sub-epithelial capillary plexus. The circumferential arteries passed between the two muscle layers ventrally or dorsally to the antimesometrial border, with many radial arterioles branching inward to the rest of the sub-epithelial capillary plexus and to basket-like networks of capillaries around the endometrial glands. Capillary loops running among the muscle layers were supplied directly from these arteries.

Circular impressions were observed around the casts of the sigmoid arterioles in their course through the mesometrial triangle (Fig. 2), and around those of the radial arterioles where they passed through the inner layer of muscle (Fig. 3) just before they connected with the capillaries (Fig. 4). The circular impressions around the sigmoid arterioles were numerous and prominent on Days 5 and 6, whereas those around the radial arterioles were more noticeable after Day 7.

The capillary plexus and 'baskets' drained via several radial venules into circumferential veins which lay between the endometrium and the inner muscular layer. After piercing the muscle layer to enter the mesometrial triangle, the circumferential veins accompanied the circumferential arteries. Neither veins nor venules comparable to the characteristic sigmoid arterioles were observed.

The earliest vascular change observed in pregnancy was resin leakage from some of the capillaries composing the luminal surface of the sub-epithelial plexus (Fig. 5). This was first observed on Day 1, seemed to disappear on Day 3, but reappeared on Day 4. It extended deeply into the whole sub-epithelial plexus, together with leakage from capillaries composing the glandular 'baskets' (Fig. 6). Late on the evening of



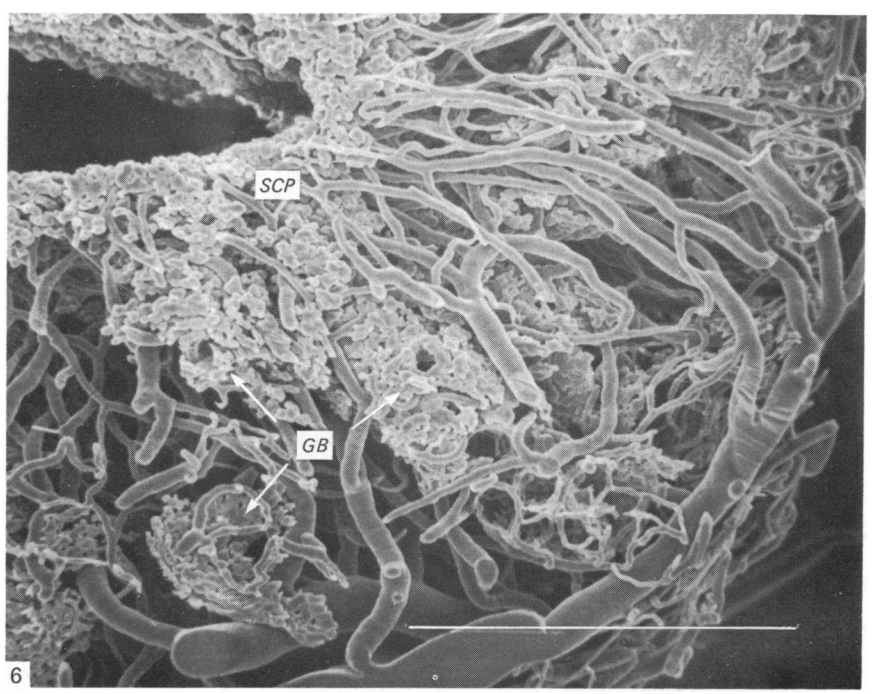
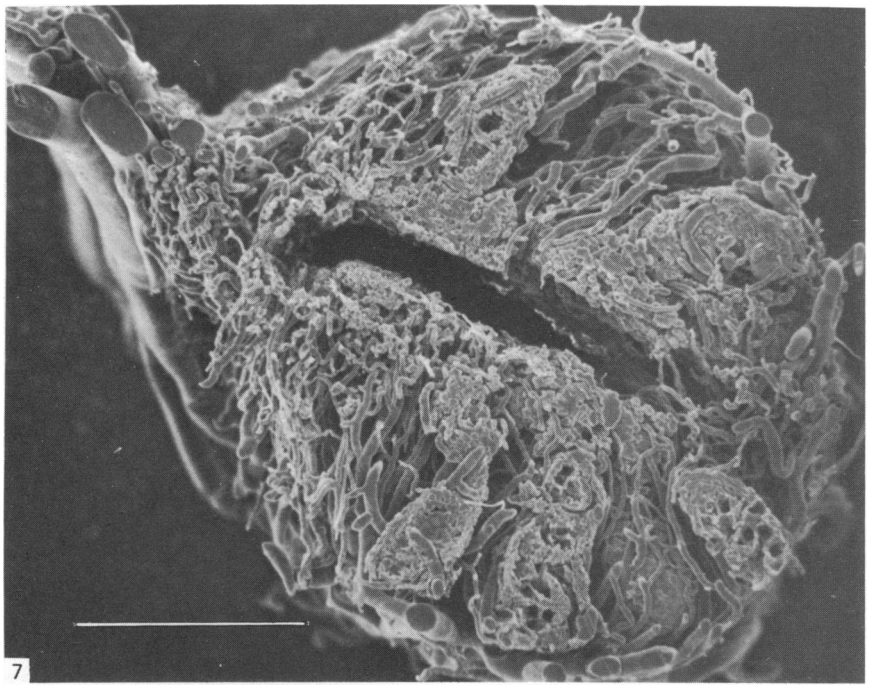


Fig. 5. Luminal view of the uterine vascular cast of a Day 1 pregnant rat. Resin leakages (*) are seen on the luminal surface of the sub-epithelial capillary plexus. Bar = 50 μm.

Fig. 6. Transverse section of the uterine vascular cast of a Day 4 pregnant rat. The intercapillary spaces of the sub-epithelial capillary plexus (*SCP*) and those of the glandular 'baskets' (*GB*) are filled with leaked resin. Bar = 500 μm.



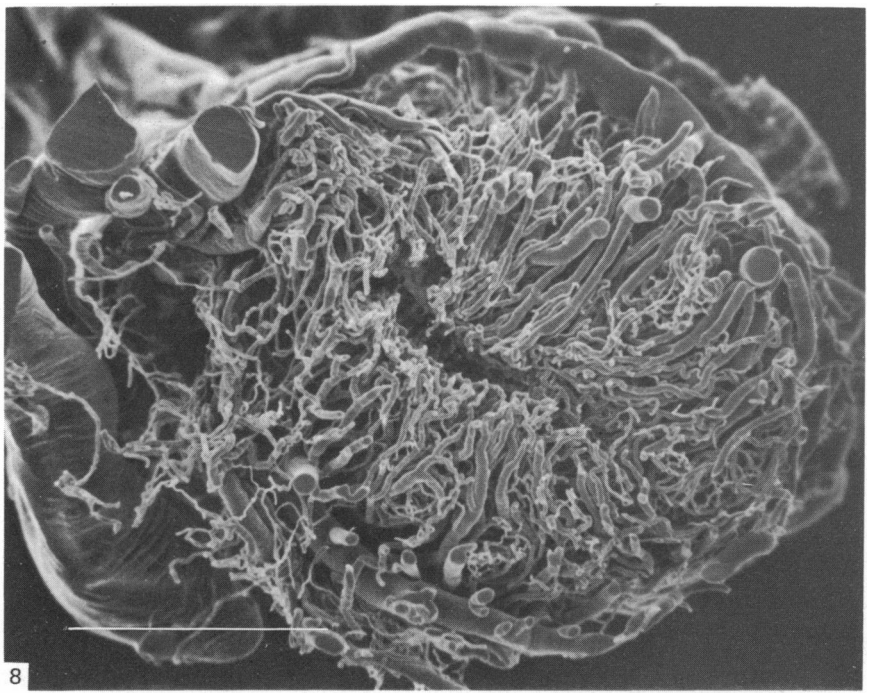


Fig. 7. Transverse section of the uterine vascular cast through an implantation site of a Day 6 pregnant rat. Prominent leaked resin is shown. Bar = 500 μm.

Fig. 8. Transverse section of the uterine vascular cast through the middle of an inter-implantation area of a Day 6 pregnant rat. Resin leakages are not seen in this section. Bar = 500 μm.

Day 5, concurrent with the appearance of a positive pontamine blue reaction, almost all capillaries in the implantation area were permeated by the leakage, whereas the leakage in the inter-implantation area was rather less. Thus, marked differences between these areas were present thereafter (Figs. 7, 8). The extent of the leakage in the implantation area reached a maximum on Day 6, and tended to decrease from Day 7.

The next phenomenon observed was a change in the volume and arrangement of the vascular bed, which started on Day 7 in the implantation area. In the immediate vicinity of the blastocyst, the capillary plexus rapidly increased in thickness and density within the antimesometrial decidual tissue, while capillaries composing the mesometrial half of the plexus began to dilate without increasing their density, and showed sinusoidal figures (Fig. 9). These changes in the implantation area were more conspicious on Day 8. The endometrial portion of the sigmoid arterioles, except for a few nearest the blastocyst, were pushed to the margin of the area by growing mesometrial decidual tissue and sinusoids. From Day 9, maternal blood sinuses which had formed in the ectoplacental cone were filled with resin from the selected sigmoid arterioles, and drained into the sinusoids (Fig. 10). The flourishing antimesometrial portion of the capillary plexus in the implantation area tended to decrease in thickness on Day 10 *pari passu* with the regression of the antimesometrial decidual tissue.

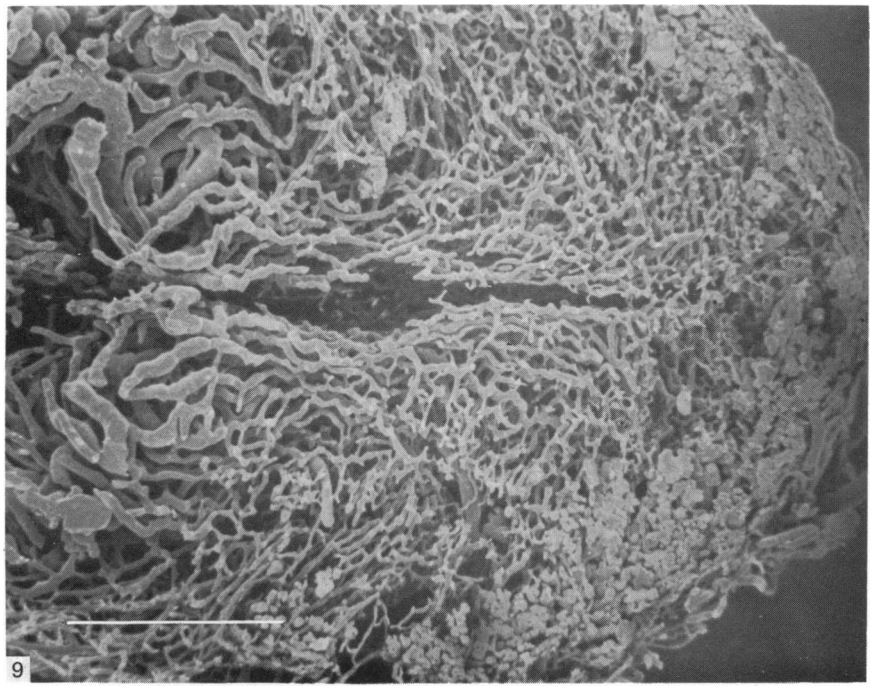
The vascular arrangement in the inter-implantation area remained almost unchanged throughout the period investigated.

DISCUSSION

In the present study, the microcorrosion vascular casting/scanning electron microscope method has provided three dimensional information on rat uterine vasculature during the first half of pregnancy.

The arteriolar system which supplies the endometrium can be divided into two types of blood vessels according to their shape and distribution: (i) the sigmoid arterioles compose the vascular conglomerate and supply only the mesometrial portion of the sub-epithelial capillary plexus; (ii) the radial arterioles supply the rest of the plexus and the glandular capillary 'baskets'. The vascular arrangement is unchanged during early pregnancy, but marked resin leakage is characteristic of this period. This phenomenon is unlikely to have been merely an artefact but rather seems to indicate an increase in capillary permeability, since it is observed only at certain times and in particular areas. Similar leakage suggestive of increased capillary permeability has been reported in ovarian follicles just before rupture (Kanzaki *et al.* 1982). On Day 1 of pregnancy, leakage occurs only from some of the capillaries composing the luminal surface of the sub-epithelial plexus. With the light microscope inflammatory changes are observed around the uterine lumen after copulation; many leucocytes, erythrocytes, and desquamated epithelial cells accumulate with the spermatozoa in the lumen. It is generally accepted that mediators of vascular permeability such as histamine, serotonin, and prostaglandins are released in inflamed tissue (Ward, 1974). Therefore, one possibility for the resin leakage appearing on Day 1 is the inflammatory change associated with the passage of the spermatozoa.

The resin leakage observed on Day 4 is more extensive than that on Day 1, and also emanates from the capillaries composing the glandular 'baskets' in all parts of the endometrium. Uterine capillary permeability and secretory activity of the luminal epithelial cells are increased after administration of oestrogens (Ham,



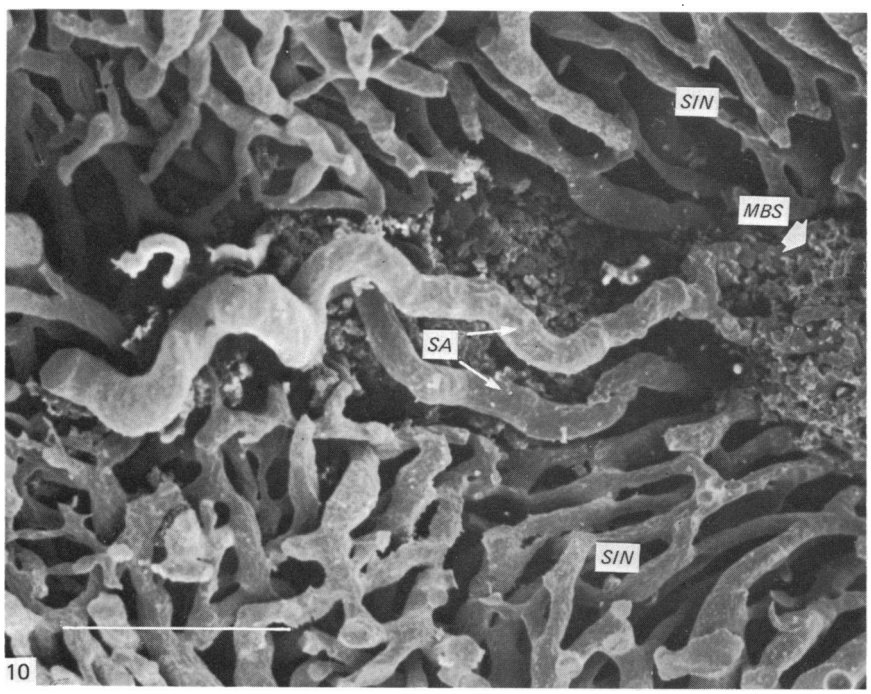


Fig. 9. Transverse section of the uterine vascular cast through an implantation site of a Day 7 pregnant rat. The capillaries are increased in the antimesometrial wall (right hand threequarters of Figure). Dilated capillaries are seen in the mesometrial wall (left hand quarter). Bar = 500 μm.

Fig. 10. Transverse section of the uterine vascular cast of a Day 10 pregnant rat showing the mesometrial wall beneath a placenta. *MBS*, maternal blood sinuses; *SA*, sigmoid arteriole; *SIN*, sinusoids. Bar = 500 μm.

Hurley, Lopata & Ryan, 1970), while secretion from the endometrial glands also increases after combined treatment with oestrogen and progesterone (Finn & Martin, 1976). Serum is one of the components of the luminal fluid, and is thought to be an important factor in development of the embryo (Surani, 1976). Therefore, it is reasonable to assume that the resin leakage observed on Day 4 indicates increased capillary permeability for materials reaching the glands, the epithelium, and/or the lumen, through the action of ovarian steroids known to increase in ovarian venous blood by Day 4 (Hashimoto, Henricks, Anderson & Melampy, 1968; Yoshinaga, Hawkins & Stocker, 1969).

In contrast with the wide ranging leakage of the earlier stages, the leakage after the onset of implantation shows a tendency to localise around the blastocyst. Although histamine has been proposed as one of the important factors influencing implantation (Finn, 1977), considerable evidence has accumulated recently to suggest that prostaglandins are responsible for mediating the increased vascular permeability and subsequent decidualisation around the blastocyst (Kennedy, 1980). The localisation of the leakage, therefore, may be explained by the increased concentration of prostaglandins in the endometrium adjacent to the blastocyst (Kennedy, 1977). The localisation suggests release of some signal (or signals) from the blastocyst to the endometrium which triggers an increase in capillary permeability. In support of this view, it has been suggested in the rat (Dickmann, SenGupta & Dey, 1977), and confirmed for some other species (Gadsby, Burton & Perry, 1976), that peri-implantation embryos synthesise oestrogen, which had been assumed to be one of the signals. Thus the resin leakage observed in the peri-implantation period would seem to suggest the presence of chemical substances passing between blastocyst and endometrial capillaries.

The antimesometrial capillary plexus rapidly increases in thickness and density within the preformed decidual tissue around the blastocyst, and then gradually regresses after the formation of the mesometrial decidual tissue in which placental blood vessels develop from the sigmoid arterioles and the mesometrial capillary plexus. The differential growth of these blood vessels is presumably due to the nature of rodent blastocysts which first implant in the antimesometrial endometrium and then later invade the mesometrial endometrium to form the placentae.

It is interesting to note the peculiar vascular arrangement of the sigmoid arterioles, the vascular conglomerate, in the mesometrial triangle. Orsini (1957) reports an analogue in the hamster uterus, and suggests that it is a mechanism for passively lowering both maternal blood pressure and pulse differential. However, the present results clearly reveal circular impressions, which indicate the presence of sphincter-like structures in the walls of the sigmoid arterioles. This implies that the vascular conglomerate in the uterus not only passively but also actively regulates the blood flow to the mesometrial portion of the endometrium, and later to the placentae.

In addition to the structures in the walls of the sigmoid arterioles, casts of some radial arterioles show two types of circular impressions. The proximal impressions, which lie within the inner layer of uterine muscle, are scarcely impressions of the muscle layer itself but are due rather to sphincter-like structures in the walls of the arterioles themselves, because they completely encircle the casts, and the circumference of each cast is itself almost circular. This concept is supported by reports on the uteri of primates, that contractions of the spiral arteries occur within the myometrium (Bartelmez, 1957), and that the spontaneous contractile activity of the spiral sections of the uterine arteries and that of the myometrium are different from

each other (Czekanowski, 1975). On the other hand, the distal circular impressions are found at the end of the arterioles just before they empty into the capillaries, and are similar to the circular impressions of precapillary sphincters reported in the brains of the dog, horse and ox (Anderson & Anderson, 1978), and also the ovary of the rabbit (Kanzaki *et al.* 1982). The larger arterioles and precapillary sphincters are generally under the control of vasomotor nerves and of circulating catecholamines respectively (Bevan, Bevan & Duckles, 1980); furthermore, the intramuscular blood vessels in the rat uterus are innervated by both adrenergic and cholinergic nerve fibres, while endometrial blood vessels are supplied by only a few cholinergic nerves (Adham & Schenk, 1969). Hence, it is probably reasonable to assume that these two types of vascular structure in the radial arterioles regulate blood flow in different ways.

Since the circular impressions around the sigmoid arterioles are numerous and prominent during antimesometrial decidualisation, while those of the radial arterioles become noticeable at the stage of placental formation and antimesometrial regression, it seems likely that the vascular structures indicated by these circular impressions contribute to the blood supply and differential development and regression of the uterine tissue.

The technique employed for this investigation is a useful method to comprehend the relationship between vascular architecture and associated phenomena. Future investigations using this technique with agents which influence vascular permeability or blood flow may lead to greater understanding of the role of the uterine blood vessels in reproduction.

SUMMARY

Vascular changes in the rat uterus during the first half of pregnancy were studied by the microcorrosion casting/scanning electron microscope method. Arterioles supplying the endometrium were divided into two groups according to differences in their shape, distribution, and development. Circular impressions indicative of the presence of vascular sphincters were observed around the casts of each group of arterioles. Resin leakage, suggesting an increase in vascular permeability, was observed from capillaries composing the sub-epithelial plexus and the glandular 'baskets'. Although leakage was noted throughout the endometrium before implantation, it tended to be localised around each blastocyst after the onset of implantation. The present results suggest the existence of a special control mechanism of the uterine blood flow, and that the uterine vasculature is affected by the conceptus from the beginning of implantation.

REFERENCES

ADHAM, N. & SCHENK, E. A. (1969). Autonomic innervation of the rat vagina, cervix, and uterus and its cyclic variation. *American Journal of Obstetrics and Gynecology* **104**, 508–516.

ANDERSON, B. G. & ANDERSON, W. D. (1978). Scanning electron microscopy of microcorrosion casts. Intracranial and abdominal microvasculature in domestic animals. *American Journal of Anatomy* **153**, 523–536.

BARTELMEZ, G. W. (1957). The form and the function of the uterine blood vessels in the rhesus monkey. *Contributions to Embryology* **36**, 153–182.

BEVAN, J. A., BEVAN, R. D. & DUCKLES, S. P. (1980). Adrenergic regulation of vascular smooth muscle. In *Handbook of Physiology*, Section 2 (ed. D. F. Bohr, A. P. Somlyo, H. S. Spanks & S. R. Geiger), pp. 515–566. Baltimore, Maryland: Williams & Wilkins Co.

CZEKANOWSKI, R. (1975). Investigations into the spontaneous contractile activity of isolated human arteries *in vitro*. *American Journal of Obstetrics & Gynecology* **121**, 718–722.

DICKMANN, Z., SENGUPTA, J. & DEY, S. K. (1977). Does "Blastocyst Estrogen" initiate implantation? *Science* **195**, 687–688.

FINN, C. A. & MARTIN, L. (1976). Hormonal control of the secretion of the endometrial glands in the mouse. *Journal of Endocrinology* **71**, 273–274.

FINN, C. A. (1977). The implantation reaction. In *Biology of the Uterus* (ed. R. M. Wynn), pp. 245–308. New York and London: Plenum Press.

GADSBY, M. A., BURTON, R. D. & PERRY, J. S. (1976). Steroid metabolism and synthesis in early embryonic tissue of the pig, sheep and cow. *Journal of Endocrinology* **71**, 45p–46p.

GREISS, F. C. (1972). Differential reactivity of the myometrial and placental vasculatures. Adrenergic responses. *American Journal of Obstetrics and Gynecology* **112**, 20–30.

HAM, K. H., HURLEY, J. V., LOPATA, A. & RYAN, G. B. (1970). A combined isotopic and electron microscopic study of the rat uterus to exogeneous oestradiol. *Journal of Endocrinology* **46**, 71–81.

HASHIMOTO, I., HENRICKS, D. M., ANDERSON, L. L. & MELAMPY, R. M. (1968). Progesterone and pregn-4-en-20α-ol-3-one in ovarian venous blood during various reproductive states in the rat. *Endocrinology* **82**, 333–341.

KANZAKI, H., OKAMURA, H., OKUDA, Y., TAKENAKA, A., MORIMOTO, K. & NISHIMURA, T. (1982). Scanning electron microscopic study of rabbit ovarian follicle microvasculature using resin injection–corrosion casts. *Journal of Anatomy* **134**, 697–704.

KENNEDY, T. G. (1977). Evidence of a role for prostaglandins in the initiation of blastocyst implantation in rat. *Biology of Reproduction* **16**, 286–291.

KENNEDY, T. G. (1980). Prostaglandins and the endometrial vascular permeability changes preceding blastocyst implantation. In *Progress in Reproductive Biology* (ed. F. Leroy, C. A. Finn, A. Psychoyos & P. O. Hubinont), pp. 234–243. Basel: S. Karger.

MURAKAMI, T. (1971). Application of the scanning electron microscope to the study of the fine distribution of the blood vessels. *Archivum histologicum japonicum* **32**, 445–454.

NOWELL, J. A. & LOHSE, C. L. (1974). Injection replication of the microvasculature for SEM. In *Scanning Electron Microscopy* (ed. O. Johari), pp. 267–274. Chicago, Illinois: IIT Research Institute.

ORSINI, M. A. (1957). The vascular knot of the hamster uterus. *Journal of Morphology* **100**, 565–599.

PSYCHOYOS, A. (1973). Endocrine control of egg implantation. In *Handbook of Physiology*, Section 7. part 2 (ed. R. D. Greep, E. B. Astwood & S. R. Geiger), pp. 187–215. Baltimore, Maryland: Williams & Wilkins Co.

ROSENFELD, C. R., BARTON, M. D. & MESCHIA, G. (1976). Effects of epinephrine on distribution of blood flow in the pregnant ewe. *American Journal of Obstetrics and Gynecology*, **124**, 156–163.

SURANI, M. A. H. (1976). Uterine luminal proteins at the time of implantation in rats. *Journal of Reproduction and Fertility* **48**, 141–145.

TAKEMORI, K., OKAMURA, H., KANZAKI, H., OKUDA, Y. & FUJII, S. (1982). Scanning electron microscopic observations of microcorrosion casts for non pregnant rat uterine vascular architecture. *Acta obstetrica et gynecologica (Japan)* **34**, 729–737.

WARD, P. A. (1974). The inflammatory mediators. *Annals of the New York Academy of Sciences* **221**, 290–298.

YOSHINAGA, K., HAWKINS, R. A. & STOCKER, J. F. (1969). Estrogen secretion by the rat ovary *in vivo* during the estrous cycle and pregnancy. *Endocrinology* **85**, 103–112.