J. Anat. (1985), **142**, pp. 21–31
With 12 *figures*
Printed in Great Britain

Scanning electron microscopy study on corrosion casts of rat uterine vasculature during the second half of pregnancy and post partum

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(*Accepted* 12 *December* 1984)

INTRODUCTION

As pregnancy progresses, considerable demands are placed on the uterine vasculature in order to meet the needs of a rapidly growing fetoplacental unit. In the rat, 70% of the uterine blood flow is directed to the placentae in late pregnancy (Bruce, 1976). In addition, total uterine blood flow and its distribution has been demonstrated to change after administration of vasoactive and uterotonic agents (Rosenfeld, Barton & Meschia, 1976; Novy, 1975). After delivery, uterine blood flow decreases abruptly (Assali, Dasgupta, Kolin & Holms, 1958), and blood loss from the uterus is generally slight despite the severing of the blood vessels. These phenomena indicate a disproportionate uterine vascular development in pregnancy and the existence of vascular structures controlling uterine blood flow. Yet, there is little structural evidence for this view.

We have already studied the changes in the rat uterine vasculature during the oestrous cycle (Takemori *et al.* 1982) and the first half of pregnancy (Takemori *et al.* 1984) by the method of microcorrosion vascular casting/scanning electron microscopy, which provides an effective means for investigating vascular architecture and its role. This technique has been employed in the present investigation to study the vascular changes in the rat uterus during the second half of pregnancy and after delivery.

MATERIALS AND METHODS

Adult virgin Wistar rats weighing 210–250 g were maintained under a controlled photoperiod (14 hours light/day; lights on at 07.00 hours) and temperature (23 ± 2 °C). Oriental rat chow and water were available *ad libitum*. On the day of pro-oestrus, as judged by vaginal smears, females were housed one to two animals per cage with a male of proven fertility. The day on which spermatozoa were detected was designated as Day 1 of pregnancy. Rats from this colony normally litter on Day 22 of pregnancy. Fifty three rats were used in this study (3 rats per day: Days 11–20; 5 rats per day: Day 21; 18 rats: 1–2 hours after delivery). Methods for experimental preparation and observations have been described previously (Takemori *et al.* 1984).

RESULTS

The maternal placental circulation had already formed by Day 11 of pregnancy (Fig. 1). The sigmoid arterioles (now sigmoid arteries) twisted, bifurcated and anastomosed with each other to form a vascular conglomerate along the uterine horn in the mesometrial triangle. Via this longitudinal anastomosing channel, usually one, occasionally two or three terminal segments of the sigmoid arteries led into each maternal blood space which was continuous laterally with a layer of extensive capillary networks in the giant cell layer of the decidua capsularis. The maternal blood space drained into many sinusoids formed in the decidua basalis. Several placental veins drained the sinusoids at the base of this layer and joined the circumferential veins just after traversing the inner layer of the uterine muscle. From this day onwards, the vascular conglomerate of sigmoid arteries continued to grow larger in each implantation site to form the so-called vascular knot, as first shown by Orsini (1957) in the pregnant hamster uterus. Arteries were dilated in the central portion of the knot and in the terminal segment emerged into the maternal blood space; the latter was designated the placental artery in this investigation. Circular impressions, as seen around the casts of the sigmoid arterioles in the vascular conglomerate during the first half of pregnancy, were no longer present around the casts of these dilated portions, but remained around those of both proximal and peripheral arteries of the knot throughout the second half of pregnancy (Figs. 2, 7). The placental artery showed tight twisting accompanied with narrowing at the segment where it passed through the inner muscle layer (Figs. 1, 3). From Day 13 onwards, the maternal blood space could be divided into three layers, according to the differentiation of the ectoplacental cone, into labyrinth, reticular layer and giant cell layer. At the fetal surface of the placenta, the placental artery radiated toward the margin of the placenta giving off a number of fine irregular anastomosing channels to the labyrinth (Fig. 4). These were continuous with the network of fewer and coarser vessels in the reticular layer, and a smaller number of vessels in the giant cell layer connected the network to the sinusoids of the decidua basalis (Fig. 5). The labyrinth continued to grow at the expense of other layers and the decidua basalis. Thus, on Day 15, the decidua basalis had become only a membranous layer occupied for the most part by the remaining sinusoids. The decidua capsularis was also degenerating, so that resin could not be injected to fill the capillaries of this layer completely except for the portion bordering the labyrinth. Due to the degeneration of the decidua capsularis and the stretching of the uterine muscle, the vascular architecture of the antimesometrial wall showed a lace-like appearance from Day 16 onwards (Fig. 6). The casts of arteries were relatively smooth in this portion, but there were circular impressions around those of the proximal segments of the circumferential arteries. In some specimens of Day 17, arteriovenous anastomoses were first recognised between the peripheral arteries of the knots and the circumferential veins (Fig. 7). Although the labyrinth and vascular knot tended to grow until the end of pregnancy, significant vascular changes were no longer seen after this stage.

The placenta separated at the thin fibrous layer outside the decidua basalis, but the placental artery was usually cut more distally with the end projecting into the uterine lumen. As resin did not enter the vascular knot after delivery, due to large thrombi formed in this region, 500 i.u. of heparin were administered intravenously per animal a few hours prior to delivery. In each specimen thus obtained, the central

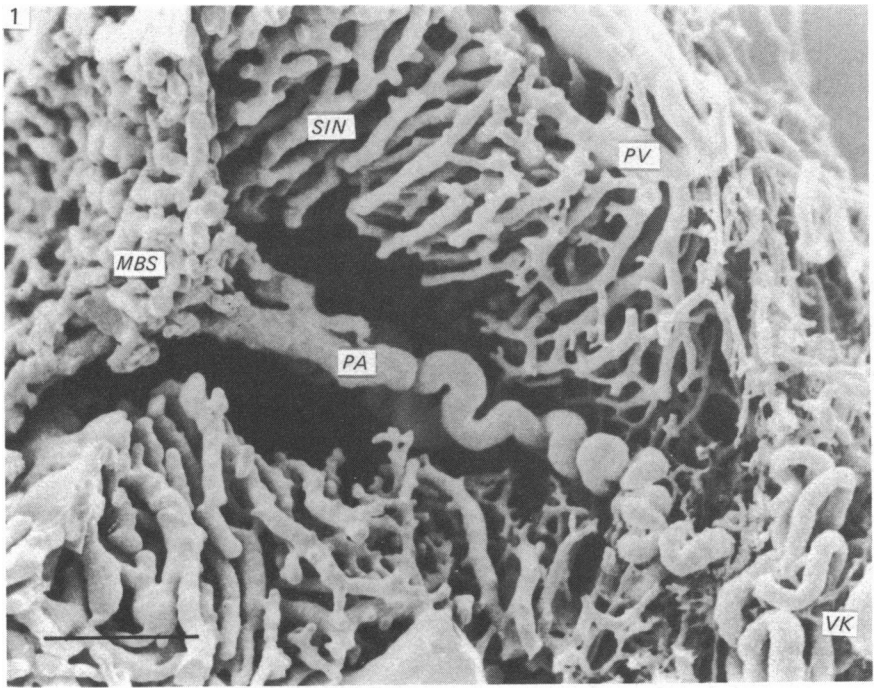

Fig. 1. Transverse section of a uterine vascular cast through an implantation site of a Day 11 pregnant rat, showing a part of the mesometrial wall. *VK*, vascular knot; *PA*, placental artery; *MBS*, maternal blood space; *SIN*; sinusoids; *PV*, placental vein. Bar, 500 μm.

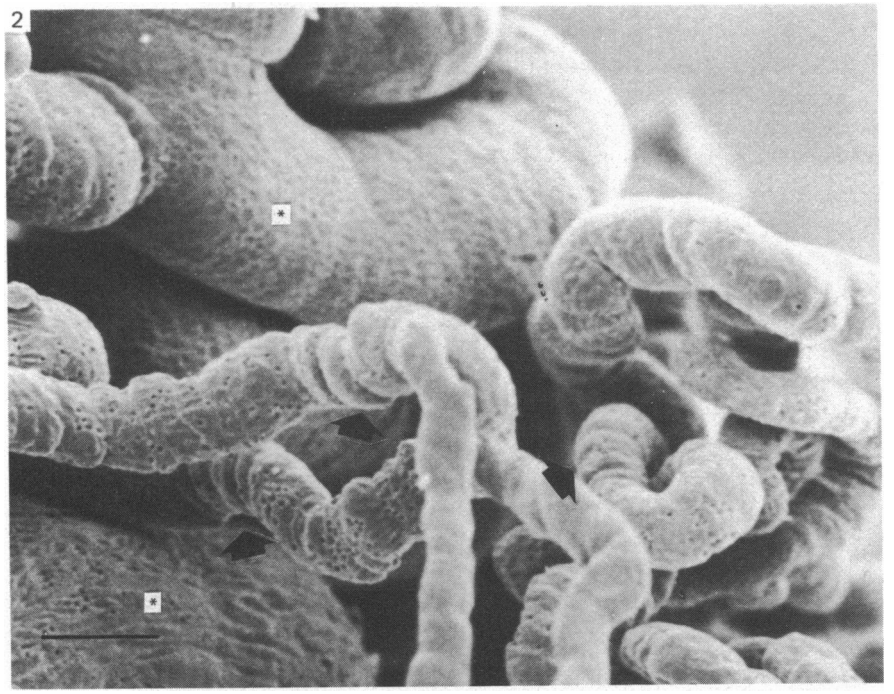

Fig. 2. Uterine vascular cast of a Day 21 pregnant rat showing a part of the vascular knot. Peripheral arteries of the knot show circular impressions (arrows). Central arteries of the knot (*) are dilated. Bar, 100 μm.

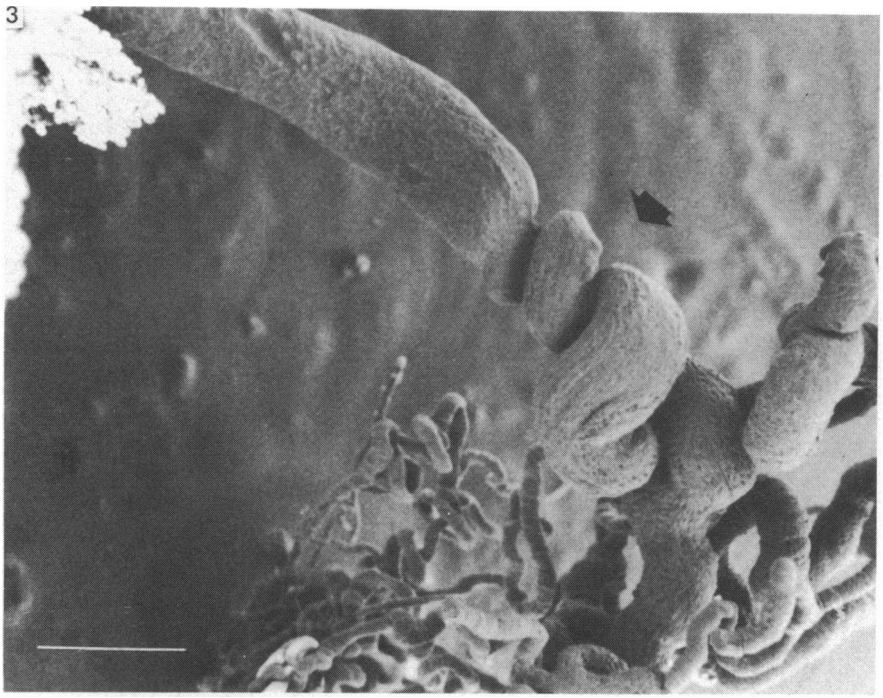

Fig. 3. Uterine vascular cast of a Day 19 pregnant rat. The placental artery shows twisting accompanied with narrowing at the portion where it traverses the inner muscle layer (arrow). Bar, 500 μm.

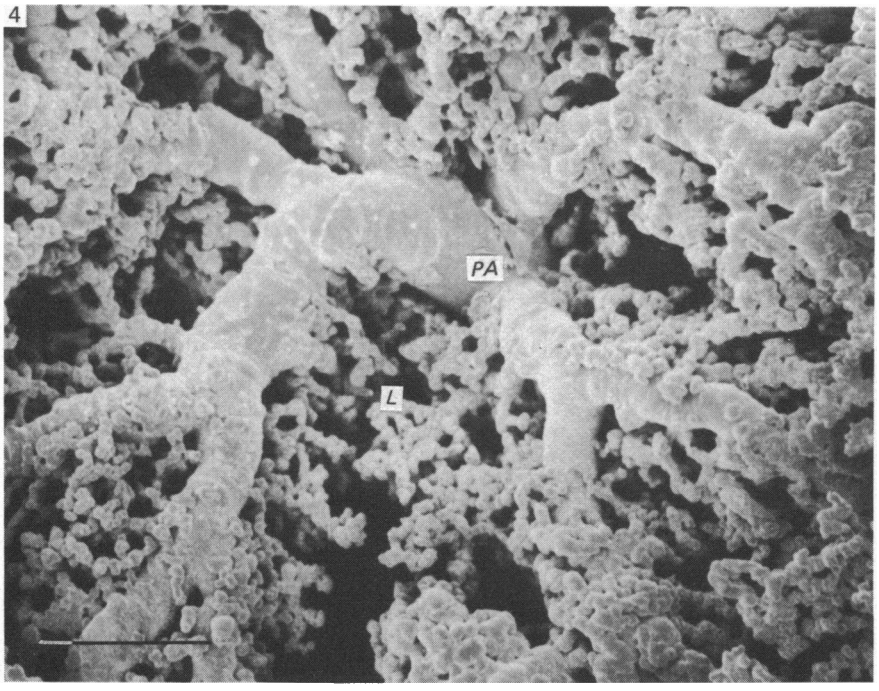

Fig. 4. The fetal surface of a placental vascular cast of a Day 16 pregnant rat. *PA*, placental artery; *L*, maternal channels in the labyrinth. Bar, 500 μm.

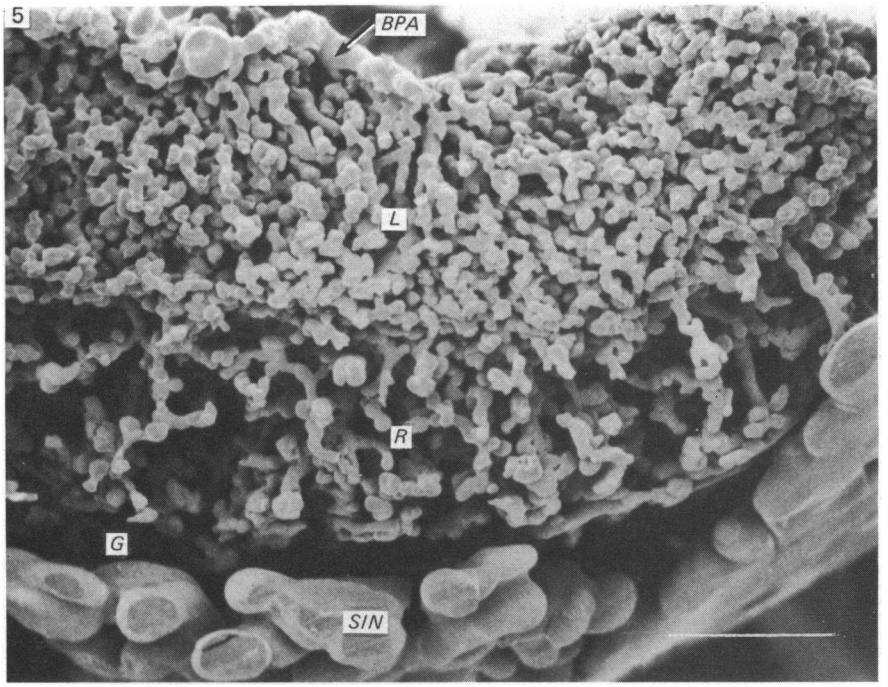

Fig. 5. Transverse section of a placental cast of a Day 15 pregnant rat. *BPA*, branch of the placental artery; *L*, maternal channels in the labyrinth; *R*, blood vessels in the reticular layer; *G*, blood vessels in the giant cell layer; *SIN*, sinusoids. Bar, 500 μm.

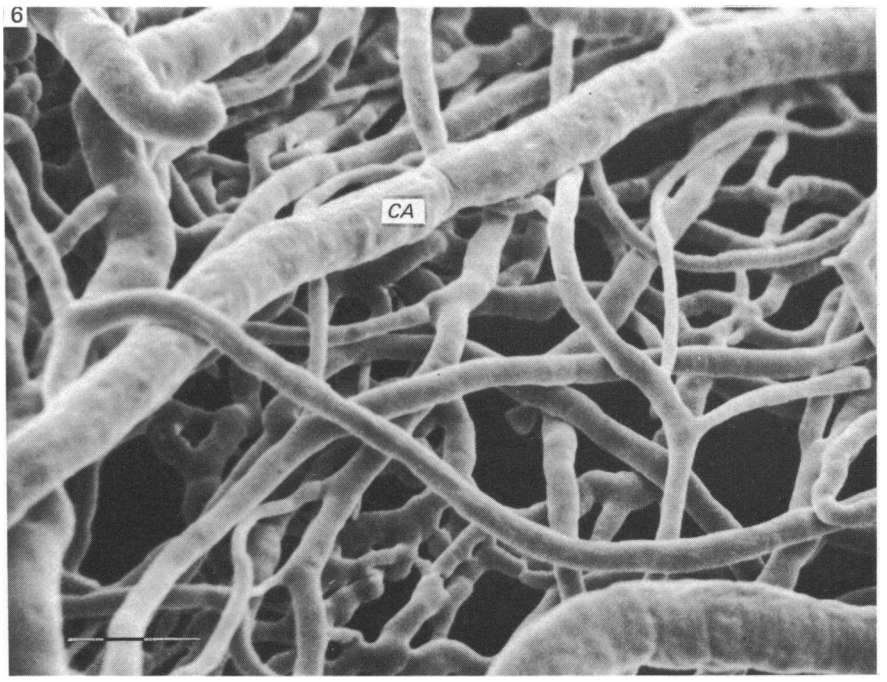

Fig. 6. The antimesometrial surface of a uterine vascular cast at a pregnancy site of a Day 16 pregnant rat. *CA*, circumferential artery. Bar, 100 μm.

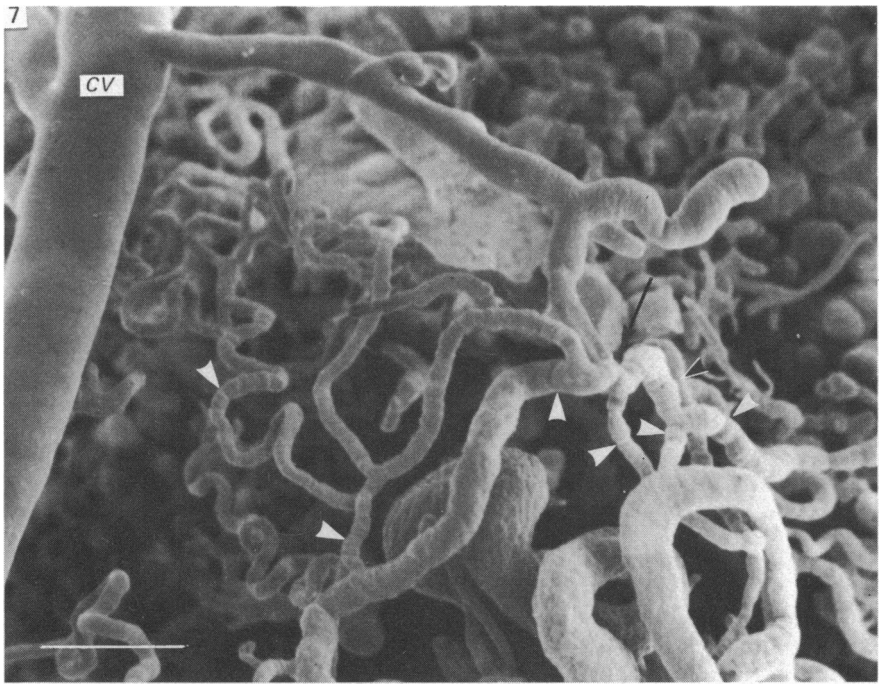

Fig. 7. Uterine vascular cast of a Day 17 pregnant rat. Arteriovenous anastomosis (arrow) is seen between the peripheral arteries of the knot and a branch of the circumferential vein (*CV*). Note the circular impressions (arrowheads) around the peripheral arteries of the knot. Bar, 500 μm.

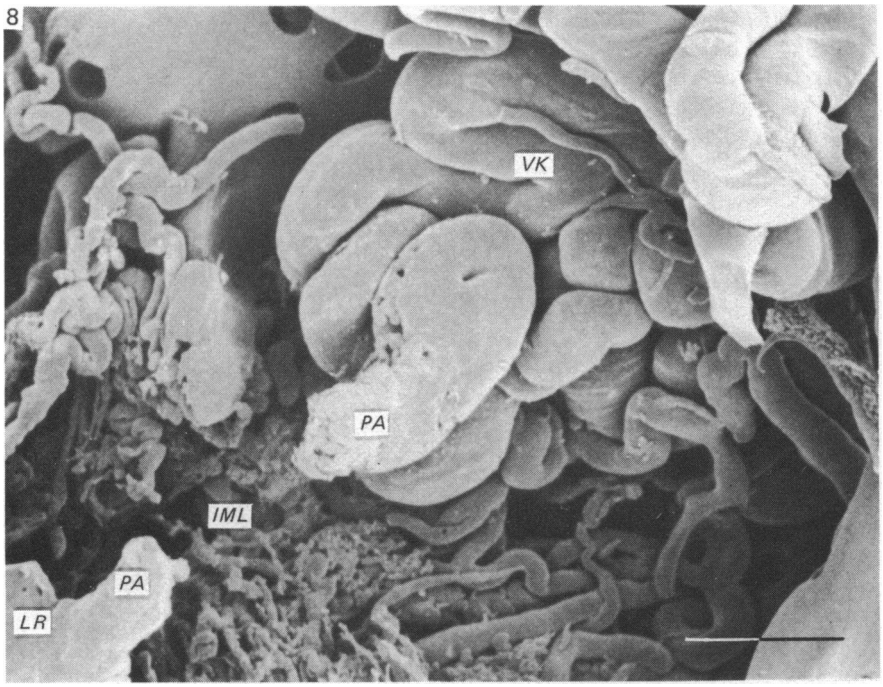

Fig. 8. Transverse section through a placentation site one hour after delivery. The cast of the placental artery (*PA*) is discontinuous in the inner muscle layer (*IML*). *VK*, vascular knot; *LR*, resin which has leaked into the uterine lumen. Bar, 500 μm.

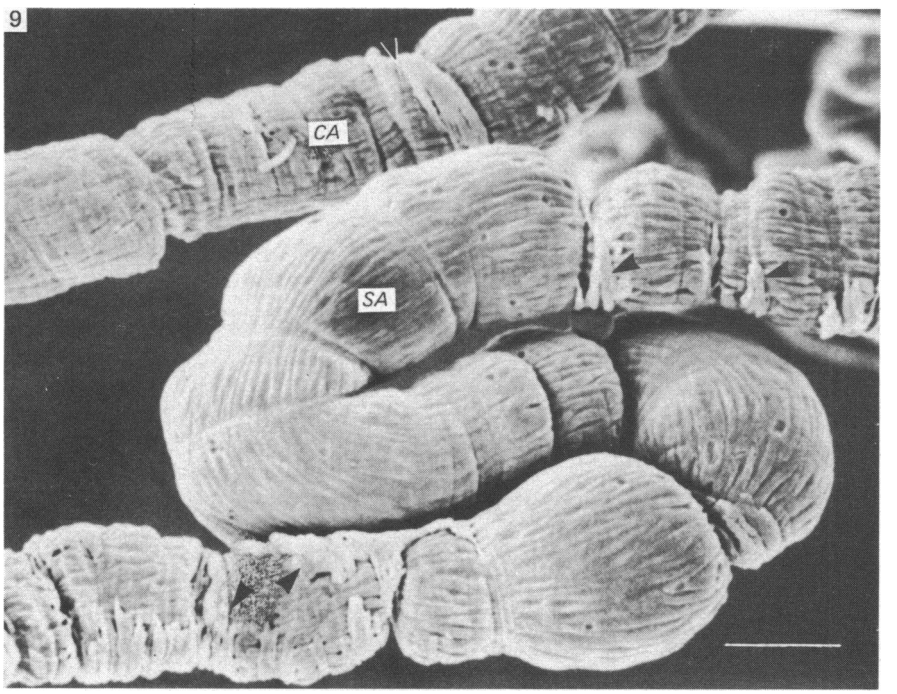

Fig. 9. Proximal segments of the sigmoid artery (*SA*) and circumferential artery (*CA*) at a placentation site one hour after delivery. Note the deep circular impressions. Undissolved tissues (arrowheads) remain around the casts. Bar, 50 μm.

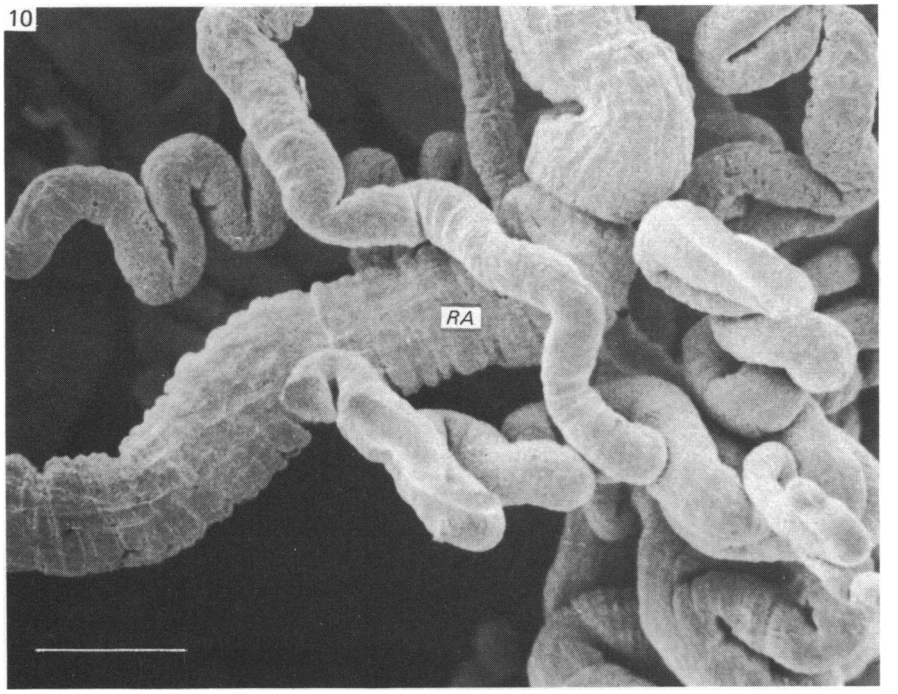

Fig. 10. Transverse section through a placentation site one hour after delivery showing a part of the antimesometrial wall. Circular impressions are seen around the cast of the radial arteriole (*RA*) at the segment where it traverses the inner muscle layer. Bar, 50 μm.

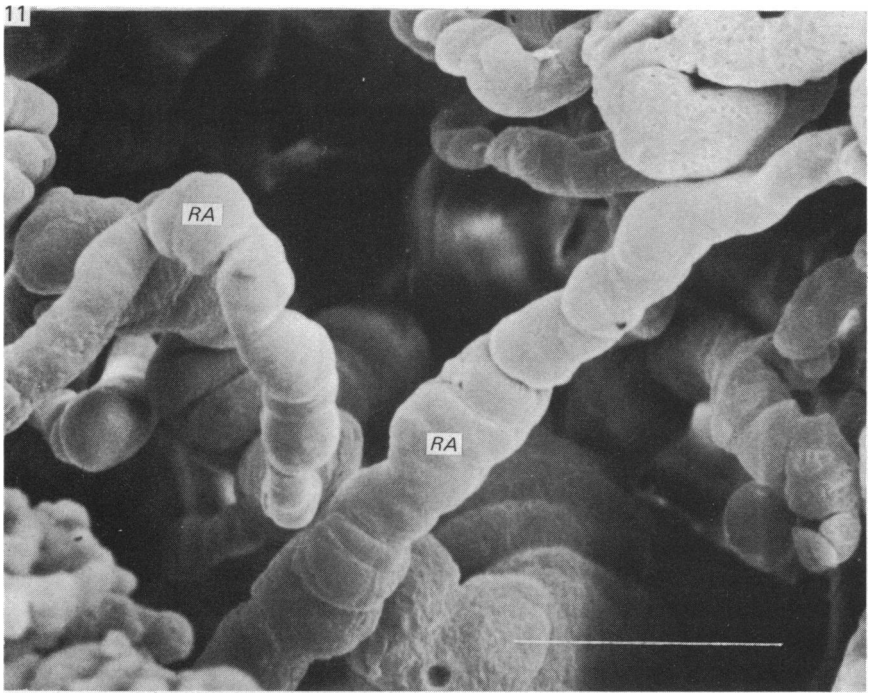

Fig. 11. Transverse section through a placentation site one hour after delivery showing a part of the antimesometrial wall. Circular impressions are seen around the casts of the terminal portions of the radial arterioles (*RA*). Bar, 50 μm.

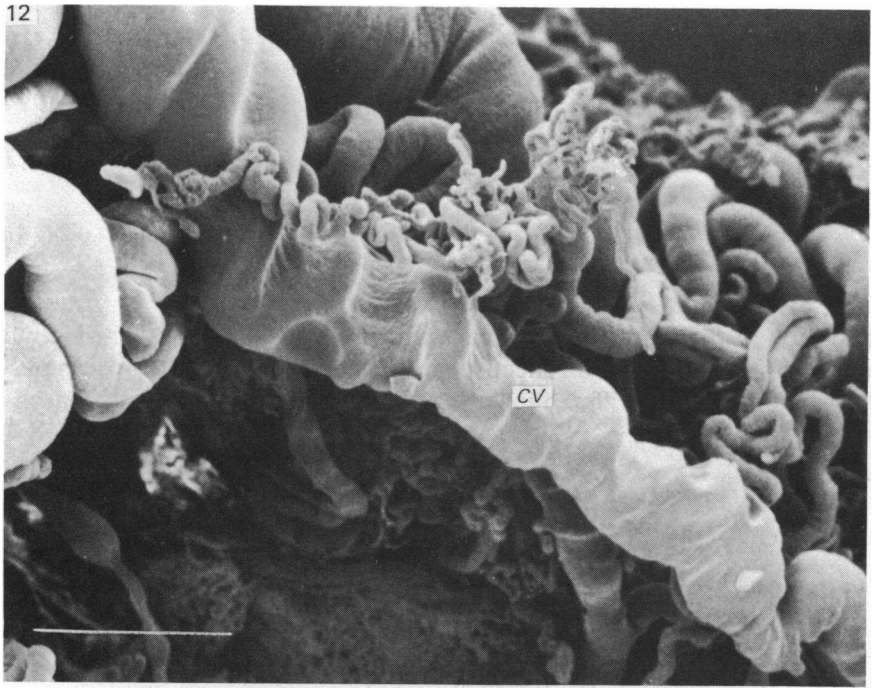

Fig. 12. Transverse section through a placentation site one hour after delivery showing a part of the side wall. Irregular impressions are seen around the cast of the circumferential vein (*CV*) at the segment where it passes through the inner muscle layer obliquely. Bar, 500 μm.

arteries of the vascular knot were still dilated, but the cast of the placental artery was discontinuous at the portion where the artery traversed the inner muscle layer (Fig. 8). The proximal portions of the circumferential and sigmoid arteries beneath the site of placental separation showed more and deeper circular impressions than seen before delivery (Fig. 9). In the contracted and therefore thicker uterine wall, the circumferential arteries and radial arterioles coiled closely, and again the latter vessels showed circular impressions, both in the portions in the inner muscle layer and just before they connected with the subepithelial capillary plexus (Figs. 10, 11). The circumferential veins showed irregular impressions at the segments where they passed through the inner muscle layer (Fig. 12).

The inter-implantation sites also became larger during the second half of pregnancy, but the fundamental vascular architecture did not alter greatly during the period investigated.

DISCUSSION

The present results on the developmental changes in the uterine vascular architecture are in general agreement with those of Young (1956), who employed radiography and other injection techniques with light microscopy, and further provide more detailed information regarding the state of the vascular wall in the uterus.

Marked dilatation and lack of circular impressions of the arteries supplying the placenta, i.e. the placental artery and its proximal branches which are the central component of the vascular knot, are characteristic of the second half of pregnancy. As seen by light microscopy, these arteries resemble veins and have few muscle fibres, indicating that they are not only organically but also functionally dilating in response to placental demands. The physiological study by Brinkman, Erkkola, Nuwayhid & Assali (1982) demonstrated that uterine arteries taken from the pregnant ewe at the level of the broad ligament contract less as the result of transmural nerve stimulation than do those from the non-pregnant animal; Brinkman *et al.* speculate that one of the reasons is the reduced sensitivity of the placental portion of the uterine vasculature to norepinephrine. In a histochemical study using the guinea-pig, Bell & Malcolm (1978) have demonstrated that loss of adrenergic fluorescence from the nerve supplying the uterine vasculature occurs initially near the site of implantation, possibly due to the effect of high concentrations of the placental progesterone localised to the uterus. These concepts accord well with the present authors' view. The dilatation of these arteries, together with the complicated vascular architecture of the knot itself, seems to be an effective means for passively lowering both placental blood pressure and pulse differential, as well as constituting a protective mechanism against placental ischaemia during maternal sympathetic activation.

In contrast with the dilated central arteries of the knot, the proximal and peripheral arteries are relatively narrow and show circular impressions around the casts, indicating the presence of vascular sphincters in the walls of the arteries. Even in the dilated placental arteries, narrowing and strong twisting indicative of strangulation by the uterine muscle are usually noticed at the portions where they pass through the inner muscle layer. It is likely, therefore, that these portions are the actual sites of the active control mechanism of maternal placental blood flow, as suggested from the results of haemodynamic studies using vasoactive and uterotonic agents (Rosenfeld *et al.* 1976; Novy, 1975).

The existence of arteriovenous anastomoses in the uterus has long been disputed. Knisely (1934) and Young (1956) suggested their presence in the mouse and rat

respectively, though the preponderance of opinion is that they are absent in various species, including the rat (Orsini, 1957; Ramsey, 1977). Such a vascular organisation could not be detected in the present authors' previous studies during the oestrous cycle and the first half of pregnancy (Takemori *et al.* 1982, 1984), but is clearly seen in the present experiment between the peripheral arteries of the knot and the circumferential veins. Although too few were noticed to determine the day of actual appearance, it appears that they are formed during the latter half of pregnancy. It should be noted that the arterial side of the anastomosis, i.e. the peripheral arteries of the knot, shows many circular impressions around the casts. In such portions, the blood flow to the arteriovenous anastomosis may be regulated and thus affect placental blood flow.

Close coiling of arteries and pronounced circular impressions around the casts after delivery are thought to be due to constriction of the uterine muscle and vascular sphincters respectively. These may play an essential role in the abrupt decrease in total uterine blood flow at this stage, by increasing vascular resistance. On the other hand, more marked constriction by the uterine muscle seems to occur specifically around the placental arteries, because their casts are discontinuous at the segments where they pass through the inner muscle layer, while those of the circumferential veins (of the same calibre as the placental arteries) show only irregular impressions at the corresponding segments. Concerning this phenomenon, it is of interest to note that Cupryn (1968) inferred from his tocological study that the essential factor for post partum haemostasis is contractility of the myometrium within the region of the placental site. Blood is thought to stagnate in the vascular knot because of simultaneous constrictions of the uterine muscle and the vascular sphincters. In general, thrombi are formed at sites of blood stagnation, and it is reported that blood coagulability is accelerated after delivery (Huse, 1984). Thus, together with the haematological changes, uterine muscle and vascular sphincters around the peripheral and proximal arteries of the knot seem to combine in an integrated mechanism for haemostasis at the site of placental separation, not only by compressing the blood vessels but also by promoting thrombus formation in the vascular knot.

SUMMARY

Uterine vascular changes in the rat during the second half of pregnancy and after delivery were studied by the method of microcorrosion vascular casting and scanning electron microscopy. The formative process of the maternal placental circulation and the state of each vascular wall were examined in detail.

The findings suggest that the so-called vascular knot, a structure formed along the course of the maternal blood vessels to the placenta, is an effective mechanism against placental ischaemia, and that contraction of the uterine muscle and vascular sphincters play important roles in the active control of the uterine blood flow and in haemostasis after the separation of the placenta.

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