THE PONTAMINE BLUE REACTION IN PREGNANT SHEEP UTERI

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The Pontamine Blue reaction (Finn & McLaren, 1967) reflects the increased vascular permeability at the site of blastocyst attachment (Psychoyos, 1960) which presages cellular changes in the uterus associated with implantation of the embryo (Finn & McLaren, 1967; Boshier, 1968, 1969). Leakage of this macromolecular dye, therefore, precedes any modifications in uterine histology that can be associated with attachment of the embryo. The increase in vascular permeability has been considered a *sine qua non* for decidua formation (Psychoyos, 1961).

In this study, the Pontamine Blue reaction was used (1) as confirmation of earlier observations (Boshier, 1968) that implantation in sheep occurs on the 16th day of pregnancy, and (2) to determine whether this reaction is present in sheep, a species in which there is no evidence of decidual cell formation preceding the uterine epithelial changes which occur at the implantation site (Boshier, 1968, 1969).

New Zealand Romney ewes of different ages were killed 15 min after an intravenous injection, lasting over 60 sec, of that volume of a 0.5% solution of Pontamine Sky Blue 5BX in normal saline which was equivalent to 10% of the recipient's blood volume [calculated as (lb body wt $\times$ 0.07 litres)/2.2]. Uteri containing extra-embryonic membranes were examined under cold normal saline, and the caruncular and inter-caruncular areas were compared for difference in colouration. Only those uteri in which there was a differentially heightened bluish colouration on the apical surface of the caruncles were regarded as showing increased vascular permeability.

Table 1 shows that a positive Pontamine Blue reaction in sheep was first seen and was most frequent on the 15th day of pregnancy, but might not develop until the 16th day.

These results parallel observations made in laboratory rodents (Finn & McLaren, 1967; Psychoyos, 1967). In sheep, cytological changes in the uterine epithelium associated with implantation are initiated by the blastocyst on the 16th day of gestation (Boshier, 1969). This demonstration of the Pontamine Blue reaction on the 15th day of gestation in sheep confirms (1) the observations of Psychoyos (1967) that microscopical evidence of implantation is apparent

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within 24 hr of the onset of the reaction and (2) the timing of embryonic attachment described in the previous study (Boshier, 1969). There is some variability in the length of oestrus and the time at which ovulation occurs in sheep (Asdell, 1965), and those ewes not showing the reaction on the 15th or 16th day are probably not sufficiently far into pregnancy for the changes in vascular permeability to have been initiated.

TABLE 1
TIME OF APPEARANCE OF PONTAMINE BLUE REACTIVITY

<i>Gestation length (days)</i>	<i>No. of pregnant ewes examined</i>	<i>No. of ewes showing no differences in staining</i>	<i>No. of ewes showing deeper stained caruncles</i>
14	3	3	—
15	8	3	5
16	4	1	3
17	3	—	3

The increased vascular permeability appears to be followed by stromal oedema in sheep (Boshier, 1969), as it does in laboratory rodents (Finn & McLaren, 1967; Psychoyos, 1967), but there is no concomitant decidual cell formation. Decidua formation, therefore, may be linked temporally, but not causally, with the increased vascular permeability demonstrated by the Pontamine Blue reaction.

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