INVASIVE BEHAVIOUR OF HAMSTER TROPHOBLAST TRANSPLANTED TO CHICK INTRAEMBRYONIC COELOM

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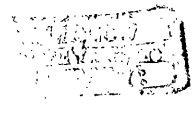

Dedicated with gratitude to **all** who contributed to its successful completion.

I hereby declare that this thesis embodies the results of my own original work, that it has been composed by myself and has not been submitted for consideration for any other degree in this or any other University.

Michael O. Babawale

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CONTENTS

VOLUME 1 - TEXT

	Page No.
TITLE PAGE	i
DEDICATION	ii
DECLARATION	iii
ACKNOWLEDGEMENTS	iv
ABSTRACT	vi
INTRODUCTION	1
1. Introduction	2
2. Early development of the hamster	5
3. Trophectoderm and origin of the trophoblastic giant cells	9
4. Role of the decidual tissue	14
5. Behaviour of trophoblast <u>in utero</u>	19
6. Behaviour of trophoblast grafted to ectopic sites	24
7. The mechanism of trophoblast invasion ...	43
8. Summary of the review of literature on the 'invasiveness' of trophoblast	52
MATERIALS AND METHODS	55
1. Preparation of the chick host for grafting: incubation and windowing of eggs	56
2. Preparation of the grafts	57
3. Preparation of controls	58
4. Grafting to the chick intraembryonic coelom	60
5. Recovery of grafts	62
6. Histological procedures	63
7. Summary of material prepared and studied .	63

Contd.

	Page No.
RESULTS	65
1. Preamble	66
2. 8-Hour group	71
3. 12-Hour group	83
4. 16-Hour group	93
5. 24-Hour group	108
6. Controls	116
DISCUSSION	133
I. The invasive capacity of hamster trophoblast	135
II. The mechanism of invasion	147
III. Behaviour of control tissues transplanted to the coelom	153
IV. Present findings related to previous studies	157
V. Intraarterial migration of trophoblast ...	167
CORRELATIONS	169
APPENDICES	173
REFERENCES	214-228
FIGURES (See VOLUME 2).	

ABSTRACT

1. Previous studies of the invasive and destructive behaviour of trophoblast when transplanted to a variety of ectopic sites have yielded conflicting results. Some workers (e.g. Kirby, 1965; Billington, 1966) have reported ectopic trophoblast as highly destructive, either by phagocytosis or cytolysis or both. Others (e.g. Fawcett, 1950; Porter, 1967) found trophoblast to be much less aggressive, with limited or absent powers of phagocytosis or cytolysis.

2. Interpretation of previous studies is complicated by two factors: (i) the possibility of species differences in behaviour of trophoblast and (ii) the fact that all ectopic sites previously used involve some damage to host tissues by the operative procedure.

3. For this study, the ectopic site chosen was the 3.5d chick's intraembryonic coelom (Dossel, 1954), in which grafts can be placed without causing any operative damage. The grafts used were: (a) 7-d hamster egg cylinder, or ectoplacental cone, chosen because hamster's trophoblast is generally regarded as highly invasive. The grafts, 37 in all, were studied, in situ, in wax serial sections at intervals of 8h, 12h, 16h and 24h. (b) Control grafts of 5.5d chick limb bud and 7-d hamster decidual tissue were studied at 48h and 72h (limb bud) and at 8h and 16h (decidua).

4. Grafts of egg cylinder/ectoplacental cone. At 8 hours, all 15 grafts were attached to host tissue, by 'erosion' of coelomic mesothelium by trophoblastic giant cells. The preferred site was the dorsal coelomic wall; this was thought to be a matter of chance in placement of the graft. The site of erosion was localized; adjacent mesothelium appeared normal. Mesothelium was undamaged by the close proximity of trophoblast (short of actual contact) and of yolk sac endoderm, (even when in contact). Spread of giant cells was mostly local, into the dorsal coelomic wall, and caused erosion of the aortic wall and bleeding in a few embryos. More distant spread of a very few individual giant cells, was seen in 4/15 embryos.

   At 12 hours there was modest progress of invasion, mostly local, with tongues of giant cells eroding the aortic wall (9/10) and ductus venosus (1/10). Despite erosion of major vessels, bleeding was temporarily limited by plugging of the vascular defect by giant cells. Each of 5/10 embryos showed more distant migration of small numbers of individual giant cells, mostly periaortic.

   At 16 hours, local invasion was again (6/9) most frequently into the dorsal coelomic wall and around the aorta, but with increased involvement of other tissues bounding the coelom. More distant migration of a few individual giant cells was limited to 4/9 embryos.

More giant cells had 'seeded' into veins, principally in the liver, and were adherent to the endothelium. Again bleeding from eroded aorta or large veins was limited by trophoblastic plugging, but several experiments were terminated prematurely because of bleeding.

At 24 hours, only 3 embryos survived, and the survival of 2 of them was probably due to placement of the graft away from the dorsal coelomic wall, so avoiding aortic erosion. In one embryo, the graft was attached to the ventral abdominal wall, allowing erosion of the right umbilical vein, with both anterograde and retrograde venous dissemination of larger numbers of individual giant cells.

The 8, 12 and 16 hour stages shared several common features in the pattern of further invasion after breaching of the mesothelium.

(i) Local invasion was by 'tongues' and individual giant cells. Invasion of the aorta (23/34 - 68%) was more frequent than of veins (18/34 - 53%) but not sufficiently so to support the idea of an "attraction" to arterial blood (as suggested by Billington (1966) of hamster's ectoplacental trophoblast transplanted beneath the tunica albuginea of the testis). The difference was probably a matter of chance in positioning of the EPC.

(ii) More distant (active) migration was surprisingly limited. Most cells seemed to have followed the outer aortic wall, possibly using the basement membrane as a migratory "cue". Distant migration was more commonly passive, by dissemination in veins.

(iii) The mechanisms of trophoblastic invasion remain unclear. Tongues of giant cells had clearly 'eroded' chick tissues, coelomic mesothelium, mesenchyme and aortic endothelium more readily than epithelial tissues such as liver and mesonephric duct. Individual giant cells had traversed chick mesenchyme without leaving traces of damage, and where they came into contact with surface ectoderm, notochord, hepatic sheets and sympathetic ganglion cells, these all appeared normal. No consistent evidence was seen of phagocytosis of healthy chick cells, nor of histolysis or cytolysis.

5. Control grafts

The two limb bud grafts were healthy, and had grown and developed at 48 and 72 hours after grafting. Each made only limited contact with the mesothelium which was locally discontinuous in the older graft. There was no invasion of host tissues by the surface ectoderm or mesenchyme.

The decidual grafts showed more or less extensive cell death. Surviving cells were confined to the centre of the grafts, making it unlikely that death was due to ischaemia,

which produces central necrosis. It is tentatively suggested that death represented "programmed cell death", such as occurs in experimental deciduomata (and, presumably, in the pregnant uterus).(Turnbull & Kent, 1963).

6. Normal implantation sites at 7d and 7.5 days

Trophoblast in utero appears to produce more extensive destruction of decidual tissue than it does when explanted to the chick's coelom. This reinforces the growing evidence that decidual degeneration in utero results largely from programmed cell death rather than active destruction by trophoblast. Intraarterial migration of giant cells was commonly seen at 7.5d unlike the situation in ectopic grafts in the chick.

7. Comparison of results with those of previous studies

i. of ectopic grafts

Although hamster trophoblast is obviously locally invasive in the chick, with some more distant spread of individual giant cells, its behaviour is much more like that described by Fawcett (1950) and Porter (1967) than that described by Kirby (1965) and Billington (1966).

The present results confirm and extend those of Barber (1989) who transplanted rat ectoplacental cone to the same site, although judging by the shorter survival in the present experiments, hamster trophoblast is more invasive than that of the rat.

ii. of trophoblast in utero

The present results are consistent with those obtained principally by Welsh and Enders (1987), using electron microscopy, which indicate that 'invasion' of uterine epithelium and maternal endothelium (and their basement membranes) is not entirely the result of activity of the trophoblast, but is assisted by the decidual cells themselves.

INTRODUCTION

1. INTRODUCTION

The word trophoblast, derived from the Greek word 'trophos' meaning 'to feed', was first conferred on the peripheral tissue of the hedgehog blastocyst by Hubrecht, a Dutch embryologist, in 1889, after recognising its nutritive function to the developing embryo following the establishment of an intimate contact with maternal sinusoids and tissues (Hubrecht, 1889). Since the time of Hubrecht, several other functions have been ascribed to the trophoblast, the outer covering of the blastocyst found in all viviparous mammals, including:

1. providing anchorage for the foetus;
2. invasion of the endometrium;
3. acting as an immunological 'buffer-zone' at the maternal-fetal tissue interface;
4. hormone production;
5. production of enzymes;
6. separation of the fetal placenta from the decidua at parturition (Amoroso, 1952; Jollie, 1960; Billington, 1975, Beer and Sio, 1982).

There is considerable species variation in the functions of the trophoblast. In some species, it is principally the nutritive function that is developed, whilst in others, secretory and invasive properties are more elaborated (see Amoroso, 1952, for review).

However, some of these functions are still strongly disputed, especially the role played by trophoblast in implantation (Fawcett et al., 1947). Early workers in this

field believed that trophoblast erodes the endometrial surface epithelium and elicits oedema, haemorrhage and decidual reactions so characteristic of mammalian implantation (Bryce, 1937; Hertig and Rock, 1945).

Blandau (1949), on the other hand, showed that all the aforementioned endometrial reactions could be duplicated by introducing small inert particles into the reproductive tract of pseudopregnant guinea pigs. Blandau's work (1949) raised again the question of whether the trophoblast played any essential role in the early phases of implantation, and made Boving (1959) re-echo the traditional controversy about the extent to which the trophoblast is aggressive and the uterus (particularly the decidual reaction) "defensive, passive, co-operative or lushly inviting".

The role of the trophoblast in establishing an intimate apposition of fetal and maternal tissues has for long been linked with its invasiveness which is variable among the different species (Steven, 1983). In those species bearing a haemochorial type of placenta (Grosser, 1927, cited by Mossman, 1937) e.g. rodents and humans, it has been reported that trophoblast rapidly penetrates the endometrium and erodes the maternal tissues by a mechanism thought to involve phagocytic and cytolytic destruction of cells (Billington, 1971; Pijnenborg, 1981), whereas, in the pig, which has an epitheliochorial type of placenta, trophoblast cells simply come into contact with an intact uterine epithelium.

One could therefore see that Grosser's histological classification of placentae (Grosser, 1927, cited by Mossman, 1937) based on the number of layers of tissue intervening between maternal and fetal circulations, reflects the role of trophoblast in placentation; the progressive invasive behaviour of the trophoblast has been presumed responsible for the successive loss of layers of maternal tissue at the feto-maternal junction in some species (Pijnenborg et al., 1985).

In an attempt to define more clearly the role of trophoblast at implantation, the behaviour of the trophoblast of various species in several ectopic sites has been the subject of intensive research, which the present review attempts to cover.

Of particular interest is the hamster trophoblast, which has been reported to be highly invasive and destructive (Billington, 1966) capable of migrating intravascularly (Orsini, 1954; Pijnenborg, 1981) and able to cause haemorrhage, for instance, into the chick's intraembryonic coelom, as a result of the erosion of walls of major blood vessels (Al-Janabi, 1988).

The behaviour of the golden hamster trophoblast explanted into the chick's intraembryonic coelom is thus the subject of this study. As it is essential to relate the behaviour of the trophoblast in the ectopic site to its behaviour in utero, I have therefore considered a brief account of the early development of the golden hamster, a logical starting point in the present review.

2. EARLY DEVELOPMENT OF THE HAMSTER

There are a handful of papers on the early development of the golden hamster, on which the following account is based. Samuel and Hamilton (1942) and Venable (1946) described the pre-implantation stages, while Graves (1945) studied in detail the development of both the embryo and its associated membranes through the first nine days. Ward (1948) described the implantation and the endometrial changes up to the fifth day of gestation, while Adams and Hillemann (1950) described the morphogenesis of the vitelline and allantoic placentae.

Two hours after copulation (p.c.), the first meiotic spindle is fully formed. Ovulation, which begins at about 5 hours p.c., is completed one hour later, which also marks the appearance of the second maturation spindle. Fertilisation of the hamster ova is accomplished eight hours p.c. (Graves, 1945; Boyer, 1953).

The first cleavage of the fertilized hamster ova is seen twenty-four hours p.c. and the 4-cell stage is attained at sixty hours p.c. and is located in the oviducts.

Early morulae are found in the uterus three days p.c. The uterine epithelium is made up of ciliated high columnar cells at this stage having a well vascularised stroma with some subepithelial cells beginning to differentiate into early decidual cells.

At 3.5 days p.c., the morula is hollowed out into an

early blastocyst, and the decidual reaction is slowly spreading in the stroma.

By the end of the 4th day p.c. the zona pellucida has disappeared and the blastocystic wall, otherwise known as the trophoblast, as well as the inner cell mass, become distinctly visible. (Fig. 1A).

At 5 days p.c. the blastocyst becomes ovoid in shape, attaches itself (implants) to the uterine epithelium at the antimesometrial border, with the inner cell mass pointing to the mesometrial side.

The proximal endodermal cells proliferate from the ventral aspect of the inner cell mass and distal endodermal cells migrate ventrolaterally from the inner cell mass along the inner surface of the trophoblast lining the blastocoele. The primary blastocoele thus becomes the yolk sac cavity. The ventral extension of the embryonic ectoderm and proximal endoderm into the primary blastocoele occurs, termed embolic invagination, which results in an inversion of the germ layers, with the entoderm covering the embryonic ectoderm. (Fig. 1B).

At 5.5 days, there appears in the central part of the mass of embryonic ectoderm a small cavity, termed the pro-amniotic cavity, which elongates and becomes separated by the fusion of two lateral primitive amniotic folds into a primitive ectoplacental and an amniotic part. (Fig. 1C).

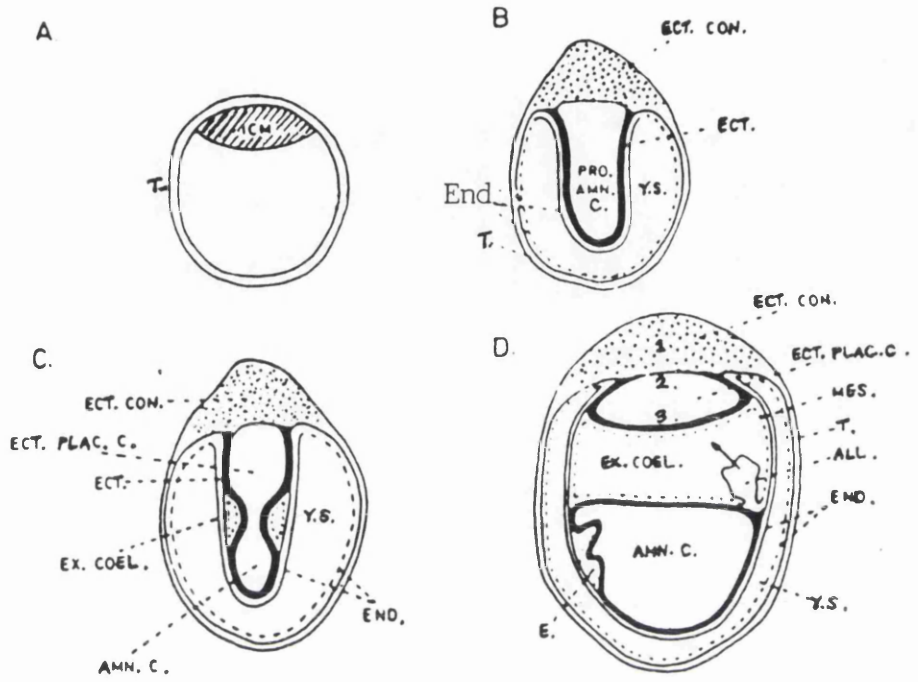

Fig. 1: Diagram summarizing the principal events of early hamster embryonic development.

A: Blastocyst; B: Egg-cylinder stage with inversion of the germ-layers; C: Appearance of mesoderm; D: Outgrowth of the allantois.

ALL.: allantois; AMN.C.: amniotic cavity; E: embryo; ECT: ectoderm; ECT. CON.: ectoplacental cone; ECT.PLAC. C: ectoplacental cavity; END: endoderm; EX. COEL.: exocoelome; ICM: inner cell mass; MES.: mesoderm; PRO. AMN. C.: pro-amniotic cavity; T.: trophoblast; Y.S.: yolk-sac; 1. cone cells; 2. basal lamina; 3. chorion.

(After Pijnenborg, R., 1975, with acknowledgement)

By the end of the 6th day, the hamster embryo is well implanted in the decidua. The polar trophoblast i.e. that covering the inner cell mass, continues to proliferate, leading to growth of the ectoplacental cone. In hamsters, the cone "sits like a wig" on top of the embryonic vesicle (Graves, 1945). This differs from the situation in the rat in which the cone grows tall and pointed. The mural trophoblastic cells increasingly transform into primary giant cells which migrate into the decidua.

At 6.5 days, the posterior amniotic fold starts to form, followed by the formation of the anterior amniotic fold about eight hours later. At this time, a cavity that becomes part of the extra-embryonic coelom first appears in the mesoderm of the posterior amniotic fold.

At 7 days p.c., the anterior and posterior amniotic folds fuse, leading to the formation of the three principal embryonic cavities, the amniotic cavity, the extra-embryonic coelom, and the ectoplacental cavity. (Fig. 1D). The formation of the primitive streak is observed at this stage, as well as the proliferation of the ectoplacental cone. By about eight days, the chorion becomes flattened against the base of the cone which is itself penetrated by the upgrowing tip of the allantois. In due course embryonic blood vessels grow along the allantoic stalk, and the ectoplacental cone, together with the chorion and allantois, becomes the definitive chorio-allantoic placenta.

3. TROPHECTODERM AND ORIGIN OF THE TROPHOBLASTIC GIANT CELLS

In the mouse, trophectoderm cells are first unequivocally identifiable at the 3.5 day blastocyst stage, where they form the outer cell layer enclosing the blastocoelic cavity (Rossant, 1986). At this stage, trophectodermal cells are morphologically distinguishable from the enclosed cells of the inner cell mass (ICM) both at light-microscope (LM) and electron-microscope (EM) levels. At LM level, trophectoderm cells are flattened epithelioid cells that stain differently from the smaller round ICM cells. At EM level, tight-junctional complexes have been reported between trophectoderm cells (Enders and Schlafke, 1965; Ducibella et al., 1975), whereas no similar junctional complexes have been reported in the case of ICM cells.

In an attempt to establish the normal fate of the various cell populations of the early embryo, Tarkowski and Wroblewska (1967) in their study on isolated and cultured 2-, 4-, and 8-cell stage mice blastomeres which formed either miniature blastocysts or trophectodermal vesicles, put forward the hypothesis which predicts that by altering the position of a blastomere from "outside" to "inside" or vice versa, it should be possible to alter its fate. In other words, it is the position that trophectoderm cells eventually occupy that determines their fate.

Evidence that neither the fertilizing sperm, nor the maternal environment during cleavage, are important decisive factors in trophectoderm cell differentiation was

produced by Tarkowski *et al.* (1970), who found that parthenogenetically activated eggs and fertilized eggs cultured *in vitro* from one-cell stage were able to develop into morphologically normal blastocysts capable of implanting in the uterus.

As time went by, it became necessary not only to demonstrate that blastomeres 'interiorised' or 'exteriorised' by manipulation during cleavage are subsequently found mainly in the ICM or trophectoderm layer of the blastocyst, but that they actually contribute cells to the differentiated tissues to which ICM and trophectoderm cells give rise. Hillman *et al.* (1972) using isozymes of glucose phosphate isomerase (GPI) as genetic markers, largely met this requirement. They analysed their results in fetal stages and postnatally and found that blastomeres placed on the outside of cleavage stages embryos contributed largely to trophoblast and yolk sac, whereas those placed inside, contributed to the fetus and other membranes.

The fate of genetically marked cells in chimeras was pursued further by Gardner *et al.* (1973), who also used isozymes of GPI as genetic markers. The results showed that the ectoplacental cone and trophoblast giant cells of the 7.5 day egg cylinder were solely derived from the trophectoderm layer of the blastocyst and that there was also a contribution from the trophectoderm to some other extraembryonic tissues.

Trophectoderm differentiation begins as early as the 2-4 cell stage in mice (Graham and Deussen, 1978), at the start of a process which involves several cleavage divisions, accompanied by blastomere compaction, tight-junction formation and cavitation (Smith and McLaren, 1977).

Mature trophectoderm cells appear following the formation of the blastocoele cavity (Gardner et al., 1973). The mouse blastocyst is composed of approximately 60 cells at this stage, three-quarters of which are trophectodermal while the remaining cells attached to the inner surface of the trophectoderm, constitute the ICM (Gardner, 1975). The ICM give rise to primitive ectoderm, mesoderm and endoderm of the embryo proper and the extraembryonic structures like the mesoderm of the allantois and yolk sac (Fig. 2).

The trophectoderm layer become separated into mural and polar components. The mural trophoblast cells surround the blastocoele, while the polar trophoblast cells overly the ICM.

In the rat, mural trophectoderm cells directly opposite the ICM stop dividing, undergo nuclear enlargement and transform into primary giant cells (Alden, 1948; Dickson, 1963, 1966). Transplantation experiments of Fawcett (1950), as well as the in vivo observations of Dickson and Bulmer (1960) and Jollie (1964), indicated the possibility of a limited life span or programmed death in these cells. In vitro studies by Dorgan and Schultz

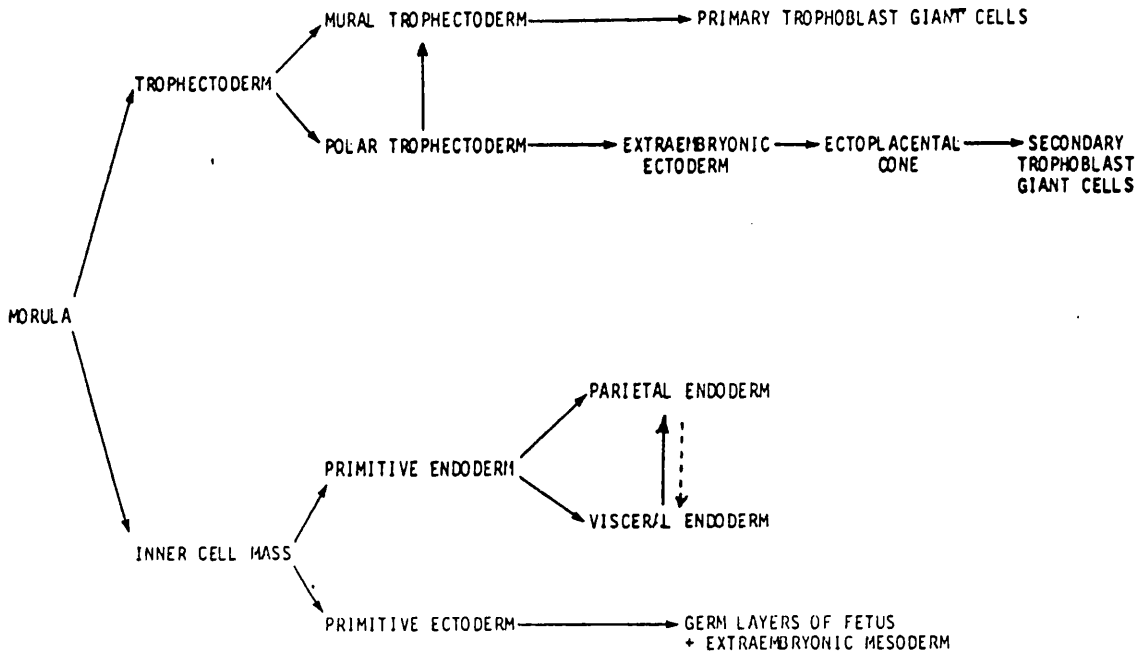

Fig. 2. Cell lineage restriction in the early mouse embryo.

(After Rossant, J., 1984, with acknowledgement)

(1971) of giant cells also demonstrated a limited life span or programmed death, comparable with that seen in in vivo studies. The giant cells were not observed to divide on or after the equivalent of day 13 of pregnancy but showed continuous deoxyribonucleic acid (DNA) synthesis up to day 16 with resultant endoreduplication of DNA.

Development of secondary giant cells, which are morphologically indistinguishable from primary ones, begins in the superficial cells of the ectoplacental cone at about day 6 of pregnancy in the hamster and spreads laterally and ventrally around the conceptus (Orsini, 1954). Tertiary giant cells and endovascular cells have also been described in the hamster (Orsini, 1954). See Figure 2 for the other derivatives of the trophectoderm.

Having considered the origin of trophoblastic giant cells, which are widely believed actively to invade maternal uterine epithelium in various species (see Steven, 1975 for review) and which are thought to be limited in their invasion by the decidua (Kirby and Cowell, 1968), a review of the literature on the decidual cell reaction (DCR) is necessary before considering the behaviour of trophoblast in uterine and ectopic sites.

4. ROLE OF THE DECIDUAL TISSUE

One of the most obvious early uterine changes in various species is the proliferation and differentiation of fibrocyte-like endometrial stromal cells into a characteristic type of cell, the decidual cell, a process known as decidualisation.

Decidual cells are large rounded cells which characteristically contain glycogen and lipid in their cytoplasm as well as large nuclei with prominent nucleoli (Selye and McKeown, 1935).

A predominance of smooth surfaced cisternae of endoplasmic reticulum has been reported in decidual cells (Jollie and Bencosme, 1965), cell structure that has previously been associated with glycogen synthesis (Millonig and Porter, 1960).

The normal stimulus for the decidual cell reaction (DCR) is the implanting blastocyst but the mechanism of initiation is still unknown (Finn, 1971).

Loeb (1908) demonstrated that the guinea pig endometrium could be induced to undergo decidualisation if it was traumatised at the appropriate time in the cycle. Later this was shown to be possible in the rat (Corner and Warren, 1919; Selye and McKeown, 1935), mouse (Parkes, 1929) and hamster (Kent and Atkins, 1959; Turnbull and Kent, 1963).

The DCR can be induced artificially by various stimuli such as passing a thread through the uterine wall or by the

introduction of beads, oil droplets or even air into the uterine lumen (see De Feo, 1967 for review). These artificial stimuli have been extensively investigated in the hope that they would throw light on the natural stimulus.

Shelesnyak (1952b) found that histamine instilled into the lumen of the uterus initiated the formation of a deciduoma in rats. He therefore suggested that histamine liberation was an important factor in the initiation of the DCR both by the blastocyst and by trauma. However, much of his evidence has been challenged by other workers, who have been unable to duplicate the experiments supporting the hypothesis (De Feo, 1963; Wrenn et al., 1964; Humphrey and Martin, 1968).

In an attempt to further investigate the nature of the stimulus for the DCR, Finn and Keen (1963) found that whilst oils and sulphated polysaccharides, e.g. agar, carragenin and heparin produced greater DCR than saline, none of the substances initiated the deciduoma.

Three main functions have been suggested for the decidua: first, a nutritive role for the embryo (Selye and McKeown, 1935; Amoroso, 1952); second, a role in the protection of the uterus during implantation (Billington, 1963; Kirby and Cowell, 1968; Ramsey et al., 1975) and third a role in the protection of the fetus from immunological rejection (Kirby et al., 1966).

Considerable passage of nutrient material between mother and offspring occurs during the rapid growth phase

of the embryo, prior to the development of the fetal circulation. Decidual cells are thought to be involved during this time, when nutritive substances must pass directly into the cells of the conceptus. Selye and McKeown (1935) ascribed a nutritive function to the decidua after finding that glycogen accumulates in large quantities in the decidual cells in the absence of an embryo. Orsini et al. (1970) also claimed to have seen more glycogen particles in EM preparations of deciduomata than in normal conceptuses, which was suggestive of a higher usage of glycogen in the conceptuses. However, chemical analysis of the decidual cell glycogen content did not reveal any difference of levels between deciduomata and normal conceptuses (Gregoire and Richardson, 1970).

The nutritive substances synthesised by the decidual cells might reach the embryo in two ways. Firstly they could be acquired as trophoblast phagocytosed decidual cells (Bridgman, 1948; Enders and Schlafke, 1967); El-Shershaby and Hinchliffe, 1975). The broken down epithelial and degenerating decidual cells are most likely acted upon by the lysosomal hydrolytic enzymes in the trophoblast cells, with the raw materials derived forming a pabulum for the developing embryo.

Diffusion of substances from the decidual cells to the trophoblast cells is the second route by which nutritive substances may reach the developing embryo prior to the formation of fetal circulation (Finn, 1971).

Turner (1876, cited by Finn, 1971) first suggested that the decidua might act as a barrier against the invasiveness of the trophoblast. Mossman (1937) also reported that the decidua may function as a "protective bulwark against active invasion of the maternal tissues by the trophoblast".

Trophoblastic growth in utero seems to occur in a controlled fashion, with the intact uterine epithelium initially acting as a barrier. It has been postulated that the presence of junctional complexes between adjacent decidual cells might present a physical barrier to the invading trophoblast (Jollie and Bencosme, 1965; Enders and Schlafke, 1967; Finn and Lawn, 1967). However, trophoblast cells behave differently when transplanted to extrauterine sites where they have been said to be "extremely destructive and invasive in some species": (in mice, Kirby, 1965; in hamster, Billington, 1966).

Kirby and Cowell (1968) produced evidence suggesting the decidua acts as a barrier to trophoblast invasion. They grafted pieces of ectoplacental cone from 7½-day mouse blastocysts into the uteri of cyclic and pseudopregnant mice. They found that in cases where the uteri became decidualised the trophoblast either failed to develop or invaded maternal tissues to a very limited extent, whereas in nondecidualised uteri, 'trophoblast invaded through the uterine epithelium, mesenchyme and, in some cases, the uterine musculature also'. Kirby and Cowell (1968) wrote "It seems then that the presence of decidual tissue

curtails the invasiveness of the trophoblast during normal pregnancy".

As the conceptus grows in utero, the implantation chamber enlarges to accommodate it. Billington (1963), in his studies on the regression of artificially-induced decidual tissue in the mouse, has suggested that the control of normal trophoblast is probably effected by the controlled degeneration of the decidual cells surrounding the blastocyst, which are then phagocytosed either by trophoblast or by maternal macrophages and thus enlarge the implantation chamber.

The demonstration of the spontaneous degeneration of decidual cells in the complete absence of trophoblast is thought to be an example of programmed cell death (apoptosis) (see Finn, 1982). Its occurrence suggests that, rather than serving a protective role, against trophoblastic invasion, decidua, by degenerating may, in fact, facilitate invasion.

The third function proposed for the decidua, that it may protect the trophoblast against immunological attack by the mother, was initially supported by the work of Kirby et al. (1966). They grafted blastocysts of C57BL mice beneath the kidney capsule and to the uteri of specifically hyperimmunized C3H mice. None of the grafts explanted to the kidney survived, while almost half of those transplanted to the uterus developed to term. Kirby et al. (1966) wrote "these results are interpreted as

demonstrating that immunological reactions are directed against the histocompatibility antigens present in the eggs transplanted to the kidney. Normal development of blastocysts in the uterus indicates a protective function of the surrounding decidua tissue. It is suggested that fibrinoid material which develops around each trophoblast cell protects the ectoplacental cone from immunological attack".

The possible relationship between antigenicity and invasive behaviour of trophoblast is not pursued further, since the question of the effect of immune responses does not arise in the chick embryo hosts used in the present study.

5. **BEHAVIOUR OF TROPHOBLAST IN UTERO**

A review of the literature on the behaviour of trophoblast in species representative of three groups of Grosser's classification (Grosser, 1927, cited by Mossman, 1937) follows, starting with representative species in the haemochorial (hamster, mouse, rat and human), then epitheliochorial (pig), and finally the endotheliochorial group (cat).

i. **HAMSTER**

Hamster mural trophoblast cells which had enlarged to become primary giant cells are first seen in intimate contact with the uterine epithelium throughout its

circumference between 3.3 and 4.5 days (p.c.) (Ward, 1948; Young et al., 1968). Thereafter, the cytoplasmic processes of the primary giant cells begin to insinuate themselves between the uterine epithelial cells to rest against their basement membrane. Some migrate further into the decidua tapping its blood sinuses, and releasing maternal blood which bathes Reichert's membrane. Several primary giant cells lose their connection with the ectoplacental cone, enlarge, become actively phagocytic by ingesting erythrocytes and degenerated epithelial cells and subsequently transform into secondary giant cells (Ward, 1948).

Ward (1948) also described the appearance of tertiary giant cells in the arteries at the outer circumference of the endometrium and in those of the intramyometrial connective tissue by 6½ days where they persist until the third post partum week. These, she distinguished from the endovascular cells which develop from the trophospongium during the 12th day of gestation to form the wall of the maternal arterial space through the placenta and later penetrate into the placental artery, replacing its walls and subsequently migrating into the maternal tissue. She noted the phagocytic nature of the giant cells which she thought might aid in implantation and enlargement of the decidual cavity.

ii. **MICE AND RAT**

In mice and rat, implantation occurs on about days 4

and 5 p.c. respectively (mice, Snell & Stevens (1966); rat, Enders & Schlafke (1967)). During the apposition stage, the trophectodermal cells are flattened and become directly apposed to the endometrial epithelium (Potts, 1968). Blastocyst adhesion to the endometrium is first noticeable in the mural trophectodermal region, and later spreads to the entire trophoblast-endometrial surface (Enders and Schlafke, 1969; Nilsson, 1974). A progressive increase in intimacy of association between the apposed cell membranes from the apposition to the adhesion stage has been reported (Enders and Schlafke, 1969).

In mice, the invasive phase of implantation is marked by trophoblast cell processes penetrating the uterine epithelial layer, initially up to the basement membrane. (Finn and Lawn, 1967; Potts, 1968). After phagocytosing the degenerated uterine epithelial cells, the giant cells subsequently penetrate the basement membrane, before migrating into the uterine stroma.

In the rat, trophoblast cell penetration of the endometrium is relatively passive, stopping at the basement membrane of the luminal epithelial cells after insinuating between underlying epithelial cells (Enders and Schlafke, 1969).

iii. **HUMAN**

In humans, there is an early differentiation of the wall of the blastocyst into an outer syncytial and an inner cytotrophoblastic layer, which starts proliferating to form

the precursors of the primary villi (Hertig and Rock, 1945). Subsequent to mesodermal growth leading to the formation of the secondary and tertiary villi, cytotrophoblastic cell columns extend through the peripheral syncytium, fan out and later fuse together forming the cytotrophoblastic shell which brings about the rapid expansion of the implantation area (Boyd and Hamilton, 1970). Although, at an early stage, erosion of the maternal tissues occurs under the influence of the syncytiotrophoblast, the establishment of the placenta involves a quite remarkable excursion of cytotrophoblast cells into the tissues of the future placental bed (Boyd and Hamilton, 1970; Pijnenborg et al., 1981).

Cytotrophoblastic cells start migrating into the lumina of maternal blood vessels as soon as the invading blastocyst encounters and "taps" the subepithelial capillary network (Ramsey et al., 1975). Sex chromatin and electron microscopy studies have provided evidence that the distinctive cells within the maternal capillaries and arteries communicating with the intervillous space are indeed cytotrophoblastic cells (Harris et al., 1971; Wynn et al., 1971).

The natural history of the cytotrophoblast cells is said to be divisible into three phases according to their relation to the vessels with which they are associated: (1) the intraluminal phase, in which the cytotrophoblastic cells are found within the lumen of the vessel; (2) the invasive phase, during which the cells penetrate the vessel

wall, and (3) the intramural phase when the cells constitute the wall of the vessel (Ramsey et al., 1975).

The quantitative morphometric studies of Pijnenborg et al. (1981) showed that maximal trophoblastic invasive activity occurred at the centre of the human placental bed myometrium, studied in 42 intact hysterectomy specimens ranging from 8 to 18 weeks gestation. The invasive cytotrophoblastic cells subsequently extended centrifugally to produce an annular pattern of invasion in the placental bed myometrium. They noted that cytotrophoblastic invasion into the myometrium appears to be restricted to the earlier stages of gestation and that morphological evidence indicated that subsequently clumps of cytotrophoblast fuse to form multinuclear syncytiotrophoblast, the characteristic placental bed giant cells.

iv. **PIG**

The pig placenta is epitheliochorial in its histological structure (Mossman, 1937; Wislocki and Dempsey, 1946b and Amoroso, 1952) and is a good example of a species in which the trophoblast appears to be completely non-invasive at all stages of pregnancy. The pig trophoblast is made up of a single layer of columnar cells, resting upon a well-defined basement membrane, which simply lies in close apposition to the uterine epithelium (Amoroso, 1952; Steven, 1983). Interdigitation of fetal and maternal microvilli occurs at all points where the two epithelia come into contact (Dantzer et al., 1981).

v. **DOMESTIC CAT**

The blastocyst of the domestic cat becomes attached to the uterine wall by means of solid tongue-like masses of trophoblast cells invading the endometrium between the thirteenth and fourteenth day of gestation (Amoroso, 1952). By so doing the epithelium is destroyed, leaving exposed a superficial layer of stroma which manifests the characteristic proliferation and enlargement of its cells (the decidual reaction).

The trophoblast cells, in addition to destroying the decidua while advancing into the maternal tissues, also destroy the glandular epithelium and the epithelial lining of the uterine crypts, but leave the maternal capillaries untouched. An endotheliochorial placentation is thereby established, as the fetal blood is separated from that in the maternal capillaries by the maternal endothelium, supported by a thin layer of cellular trophoblast, an irregular layer of mesenchymal cells and the delicate endothelial wall of the fetal blood vessels (Amoroso, 1952; Dempsey and Wislocki, 1956).

6. BEHAVIOUR OF TROPHOBLAST GRAFTED TO ECTOPIC SITES

Reports of ectopic pregnancies of varying duration in the early clinical literature generated the interest of many scientists in investigating whether the uterus was essential for normal embryonic development. This led to numerous transplantation studies involving eggs and blastocysts of various species being grafted to different

extra-uterine sites, testing the ability of such sites to support the growth of the transplants, and allowing study of the behaviour of such transplants.

The sites used included the peritoneum (Loeb, 1914; Fawcett et al., 1947; McLaren and Tarkowski, 1963) the anterior chamber of the eye (Fawcett et al., 1947; Runner, 1947; Grobstein, 1950), the kidney (Nicholas, 1942; Fawcett, 1950; Kirby, 1960; Porter, 1967; Michie, 1984), the testis (Kirby, 1963a; Billington, 1966) and lately the intraembryonic coelom of the chick embryo (De Souza, 1986; Al-Janabi, 1988; Barber, 1989).

Transplants of ectoplacental cone or blastocysts to extra-uterine sites have been carried out successfully in immature, adult virgin, pregnant and non-pregnant females, in normal cryptorchid and castrated male rodents, as well as in the chick embryo.

The recipient species have been mostly mice (Runner, 1947; Fawcett, 1950; Kirby 1960, 1962, 1968; Porter, 1967; Michie, 1984), others being the rat (Nicholas, 1942; Kirby 1962), rabbit (Shapiro and Harvey, 1957), guinea pig (Loeb, 1914; Bland & Donovan, 1965) and hamster (Billington, 1966).

The present review therefore deals with what is known of the behaviour of the trophoblast of various species in these ectopic sites.

6. A. **TRANSPLANTS OF MOUSE TROPHOBLAST TO:**

(i) **THE KIDNEY**

Fawcett (1950) transplanted 4-8 cell stage mouse ova under the capsule of the kidney in 32 mice. The transplantation, which was successful in about 60% of host mice, yielded abundant growth of trophoblast accompanied by extravasation of maternal blood. He found that the large haemorrhagic masses on the kidneys of the host mice were blood-filled cysts lined by large trophoblastic giant cells; this prompted the suggestion that the presence of viable trophoblastic giant cells appeared to have prevented the clotting and organization of the extravasated blood. He observed trophoblastic cell proliferation for upwards of 12-14 days which corresponded in duration with the growth period of the fetal placenta in normal intrauterine gestation in mice. In marked contrast with the infiltrative phagocytic and cytolytic proclivities associated with trophoblastic cells in utero, he observed that the kidney was relatively unaffected, as there was no interstitial haemorrhage within the kidney and the kidney parenchyma adjacent to active growths of trophoblastic giant cells showed no evidence of damage. He noted that the kidney cortex was always somewhat indented by the growth of the trophoblast which he thought "was largely due to compression". On the other hand, in a few cases, he found that the junctional zone between the two tissues was irregular, an observation he considered suggestive of

infiltrative and phagocytic activities by the trophoblast cells.

Kirby (1965) made the following observations on the behaviour of mouse blastocysts three days after transplantation under the capsule of the kidney:

. haemorrhagic vesicles which were often larger than the kidney formed beneath its capsule;

. trophoblastic giant cells infiltrated between the uriniferous tubules but caused very little damage at the early stages. At later stages however trophoblastic giant cells destroyed the renal tissue by phagocytosis. "Usually small clumps of renal cells are engulfed by the trophoblastic cytoplasm but not infrequently entire Bowman's capsules are ingested";

. although trophoblastic invasion occurred all around the periphery of the graft there was usually a 'spearhead' of attack where the trophoblast penetrated more deeply.

Porter (1967) in studies on the development of mouse blastocysts transplanted under the kidney capsule, grafted 66 blastocysts, of which 26 survived 5 days or more, in which he found:

. trophoblast proliferation was common in all successful transplants.

. trophoblastic giant cells actively invaded the surrounding parenchyma and lay closely bordered by renal tubules further from the locale of the transplant.

. the cell surfaces of the trophoblast cells were directly contiguous to the basement membrane of renal

tubule cells.

. the basement membrane of the tubule cells in contact with trophoblast was thickened by about 50% more than its normal thickness.

. "little necrosis occurred in the kidney and tubule cells with nearly normal cytoplasm were frequently found in contact with invading trophoblast cells".

. despite the disruption of renal tissue produced by transplants, there was no unequivocal evidence of phagocytosis by trophoblast cells, such as that described by Kirby (1960, 1962).

. "necrotic tubules may be explained more readily on the basis of surgical trauma plus interference with the vascular supply to the tubules".

7.5 day mouse ectoplacental cone transplanted under the capsule of the kidney by Michie (1984) yielded secondary trophoblastic giant cells which penetrated the cortex of the kidney, but no further inwards than the level of the outermost renal corpuscles. He found that in most cases the trophoblastic giant cells lay in contact with healthy tubular cells without causing any damage to them. A few isolated cases of tubular damage were found, which he attributed either to the pressure of accumulated extravasated blood or to trauma at operation. However, he found no evidence of cytolysis or phagocytosis of tubular cells. He found a gradual increase in the extravasated blood volume under the kidney capsule up to the 9th day

after transplantation which he considered might have exerted sufficient pressure on the kidney cortex to cause some of the tubular damage he observed.

(ii) **THE TESTIS**

Kirby (1963a) transplanted 3½-day mouse blastocysts beneath the tunica albuginea of scrotal and experimentally cryptorchid testes. At histology two days post-operatively, he found the blastocyst lay between the tunica albuginea and the seminiferous tubules, and trophoblastic giant cells insinuating between the seminiferous tubules, eroding the small blood vessels as they did so. He did not find any evidence of destruction by phagocytosis, but rather of what appeared to be a 'cytolytic agent' which was diffusing ahead of the advancing border of the trophoblast, causing lysis of the host tissue. Testicular tissue destruction occurred much earlier in the cryptorchid testis than in the scrotal testis, a difference which he attributed to the higher temperature in the cryptorchid testis.

Billington (1965) transplanted 3½-day mouse blastocysts and 7½-day ectoplacental cones beneath the tunica albuginea of the testis, within and between inbred strains of mice. He found that the amount of destruction of seminiferous tubules and interstitial tissue was directly proportional to the increase in weight of the testis, which made him suggest that since the weight increase provided an immediate measure of the

increase provided an immediate measure of the 'invasiveness' of the trophoblast, the need for continual histology no longer arose. He also found that transplants to genetically dissimilar hosts produced a greater increase in testicular weight, which made him conclude that trophoblast invasion appeared to be more extensive when there was antigenic difference between the trophoblast and the host.

In transplants of mouse blastocysts under the tunica albuginea of the testis, Porter (1967) observed the formation of a distinct fibrous capsule between the trophoblast cells and the surrounding seminiferous tubules He considered the fibrous capsule formation to be of host origin due to its rapid formation in the case of transplants to the testis and its absence in the case of transplants to the kidney. The transplants increased in volume despite the presence of a capsule, which suggested that such an enlargement could not be attributed to direct trophoblastic invasion as trophoblastic elements were rarely observed outside the capsule. Porter (1967) also made an important observation regarding damaged tubules. He noted that the necrotic tubules were not uniformly distributed about the periphery of the transplants, as normal tubules were seen contiguous to the transplant capsule as frequently as necrotic ones. He thought that this observation could be more readily explained as a result of surgical trauma, and of interference with the vascular supply to the tubules by the enlarging transplant

rather than of the elaboration of a cytolytic substance. He thus concluded that cytolytic substances were probably not elaborated by the trophoblast since minimal tissue necrosis was observed in the kidney as well, and "tubule cells with nearly normal cytology were frequently seen in contact with invading trophoblast cells".

(iii) **THE ANTERIOR CHAMBER OF THE EYE**

Runner (1947) reported the successful development of mouse ova, morulae and blastocysts of mouse transplanted to the anterior chamber of the eye. He reported that younger embryos were capable of developing and implanting in the eye, but did so less frequently than did blastocysts, and that 'implanting' blastocysts usually gave rise to a few trophoblastic giant cells and were associated with relatively small haemorrhagic areas.

Similarly Fawcett et al. (1947) transplanted 24 mouse ova to the anterior chamber of the eye through a small corneal incision, and examined their behaviour daily with the aid of a dissecting microscope until termination of the experiments. They found that all the transplants showed considerable growth of trophoblast, slight differentiation of inner cell mass, angiogenesis and connective tissue proliferation at the site of attachment of the blastocysts. They reported that "the growth of fetal tissue appeared to take place mainly in the aqueous humor... there was very little tendency for the trophoblast to invade the tissues of the eye" although at a late stage a few giant cells "had

quite evidently invaded the contiguous tissues". They attributed oedema and extravasation of blood to a 'chemical substance' secreted by the trophoblastic cells. They weighed the evidence that trophoblast causes extravasation of blood in extra-uterine sites against the well-documented similar responses of the endometrium which could be induced by mechanical or electrical stimulation (Krehbiel, 1935), and cautioned that the individual contributions of the conceptus and the uterine mucosa in the mechanism of implantation should not be thought of as mutually exclusive but mutually supporting and that neither is "chiefly" responsible for implantation.

Grobstein (1950) transplanted 7-day mouse ectoplacental cones into the anterior chamber of the eye, and confirmed the findings of Runner (1947) and of Fawcett et al. (1947) that trophoblastic giant cells produced such intra-ocular changes as local invasion, in the area of the blastocyst implantation and anterior synechea. Grobstein (1950) however failed to confirm the elaboration of a cytolytic enzyme purportedly secreted by trophoblastic giant cells as suggested by Fawcett et al. (1947).

(iv) **THE LUNG**

Carr and Feleki (1982) instilled 3.5 day mouse blastocysts into the lungs of 20 mice via the femoral vein and killed the animals 7-10 days later. They found that trophoblast cells proliferated in the lungs of mice but did not cause the extensive invasion, tissue destruction and

haemorrhage frequently observed with trophoblast growth in extra-uterine sites by Kirby (1960, 1963a & b) and Billington (1966).

B. TRANSPLANTS OF HAMSTER TROPHOBLAST

To the testis and kidney

Billington (1966) transplanted 3.5-day hamster blastocysts and 6.5-day ectoplacental cones beneath the tunica albuginea of the testis. Invasive trophoblastic giant cells grew from both transplant materials. He found that the transplants initially formed a nodule of trophoblast cells which soon began to open up blood vessels in the testis, producing haemorrhagic channels between the seminiferous tubules. 2-4 days post-operatively, trophoblastic giant cells had migrated away from the inoculation site to line the walls of the blood vessels of the testis. He found that the giant cells lay intimately apposed by their flattened cytoplasm to the endothelial lining of the testicular vessels. He postulated that since trophoblast had been reported by Kirby (1963a) to invade tissues by phagocytosis and cytolysis, that one or both of those mechanisms might be the method by which penetration of the vessel wall of the testis is effected.

Billington (1966) also noted that the migration pattern of hamster trophoblast cells appeared to be solely arterial, a situation similar to the intra-arterial trophoblast migration reported in utero by Orsini (1954)

and Pijnenborg et al. (1974) . This observation led him to suggest that the migratory pattern of the hamster trophoblast cells might be due to some sort of tropism towards higher oxygen tension in the testicular arteries.

Billington (1966) also reported the behaviour of the explanted hamster trophoblast under the kidney capsule which showed proliferation of trophoblast cells from the transplant nodule and their migration to the "endothelial wall of blood vessels in the boundary zone of the kidney". Large numbers of giant cells were found at the inoculation site 8 days post-operatively which suggested that invasive activity was minimal, perhaps because of the compact nature of the kidney tissue.

C. TRANSPLANTS OF RAT TROPHOBLAST

To the kidney

Nicholas (1942) and Tarkowski (1962) both reported trophoblast proliferation and formation of haemorrhagic nodules beneath the kidney capsule following rat blastocyst transplantation. Neither of them observed active invasion of the kidney cortex by giant cells.

D. TRANSPLANTS OF GUINEA PIG BLASTOCYST

(i) Intraperitoneal

Loeb (1914) first described the extrauterine development of a guinea pig 'ovum' which was thought to have escaped through a uterine incision made nearly 3 days after copulation. The young embryo was said to have been

superficially embedded in the connective tissue of the peritoneum, characterised by a somewhat distorted growth of the neural tube, coelom and enteric cavities. The implantation site was marked by trophoblastic giant cells forming a syncitium of cells. He also observed giant cells penetrating the uterine peritoneal coat, invasion of blood vessels and the replacement of the endothelial lining of the vessel walls. However, he did not observe any decidual tissue formation. He concluded that guinea pig blastocysts are able to develop at an extra-uterine site but unable to stimulate decidual tissue formation.

(ii) **To the kidney**

Bland and Donovan (1965) transplanted 5-day guinea pig blastocysts beneath the kidney capsule and to a pocket in contact with the abdominal muscle. The grafts implanted in 3 out of the 12 transplants to the kidney and in 1 out of 4 blastocysts inserted into the abdominal muscle. They found trophoblast proliferation in association with extravasated blood in all four successful grafts as well as phagocytosis of the extravasated blood. Bland and Donovan (1965) also transplanted six 9.5- to 12.5-day post implantation guinea pig embryos into the spleen, testes and anterior chamber of the eye; all the grafts survived. They found that trophoblast cells usually surrounded extravasated blood as well as a 'layering' effect on the part of the cells bordering the trophoblasts, which they claimed was indicative of lysis.

E. TRANSPLANT OF RABBIT EGGS AND BLASTOCYST

In 1957, Shapiro and Harvey reported their findings on transplants of rabbit eggs and blastocysts into the anterior chamber of the eye and the vitreous. Only 5 of the 53 transplants into the anterior chamber grew successfully whilst no growth was observed from the 19 transplants into the vitreous. Extensive vascularisation of the growth was observed but no mention was made of trophoblastic giant cell invasiveness.

F. TRANSPLANTS OF HUMAN TROPHOBLAST

Transplantation of the human trophoblast has been briefly described (Obata et al., 1976). Human trophoblast in early gestation was transplanted to the kidneys of 42 thymectomized, 33 prednisolone-treated and 55 untreated rats. The morphological 'hallmark' of host transplantation immunity, plasma cell infiltration, was observed in one third of the untreated group by day 3 after transplantation whereas in thymectomised and prednisolone-treated rats, infiltration by plasma cells was slight and found in only 20% of cases. They also observed trophoblastic cell proliferation and invasion with the formation of characteristic haemorrhagic vesicles. They noted that the "invasiveness" of the Langerhans cells - i.e. the cytotrophoblast-was more marked than that of the syncitiotrophoblast.

G. TRANSPLANTS OF PIG TROPHOBLAST

Samuel (1971) transplanted 6-7 day pig blastocysts to a variety of ectopic sites such as the ovarian stroma, ureteric lumen, under the splenic and kidney capsules as well as the outer wall of the uterus. In general, he observed proliferation of trophoblast and the transformation of the normally cellular trophoblastic cells (in the pregnant pig) into aggregates of trophoblast cells which subsequently coalesced into syncytial masses - the syncitiotrophoblast, a transformation never seen in normal pregnancy in pigs. He also reported that, in ectopic sites, pig trophoblast is invasive, phagocytic and cytolytic.

Samuel and Perry (1972) confirmed the syncitial transformation of pig trophoblast cells. They found, by electron microscopy, that the interaction between the syncytial trophoblast cells and the uterine tissues showed three kinds of extracellular materials in association with the trophoblast. First, collagen was seen in large quantities in the uterine stroma, which they thought might constitute a barrier to the further invasion of the trophoblast. Second, a dense flocculent and fibrous material which they thought might be fibrin was observed. Third, an electron-dense deposit, the nature of which was not determined, was also observed. They concluded that the observed uterine stromal reaction was not dissimilar to the initial stages of a foreign tissue reaction, hence the uterine stromal reaction they saw could not be called a decidual cell reaction.

Barber (1989) grafted 22-day pig allanto-chorionic tissue into the chick's intraembryonic coelom. He found that only the chorionic face of the grafts showed attachment to all the host tissues with which it came in contact. Fifteen of the 18 grafts he examined were found in contact with blood vessels but did not undermine their integrity. The graft cells were not found separately from the main body of the graft, neither could he find any evidence of phagocytosis, or of histolytic or cytolytic damage to the host tissues.

H. **TRANSPLANTS TO OTHER ECTOPIC SITES**

Kirby (1962) grafted mouse ectoplacental cones into a neoplastic growth, a mammary carcinoma. He observed proliferation of trophoblast cells, which penetrated deeply into the tumour mass and were found mostly in association with a cavity filled with extravasated blood and cellular debris. The trophoblast cells then "proceeded to destroy the neoplastic tissue by phagocytosis".

Kirby (1963b) transplanted 3½-day mouse blastocysts into the spleen of 70 mice of different ages, sex and condition of which he recorded 19 experimental 'takes'. In the first type of graft, he found a layer of trophoblast cells at the periphery of blood clots. In the second group, grafts which he noted were not characterised by excessive haemorrhage, produced a luxuriant growth of trophoblast.

He observed many trophoblastic giant cells with nuclear diameter of about 20-115μm growing in a reticular pattern with blood-filled interstitium. The trophoblast cells, he noted, destroyed the spleen by phagocytosis, as their short cytoplasmic projections infiltrated between the individual healthy host cells before they were engulfed. The invasive and phagocytic pattern of the trophoblast cells in the spleen was marked by an affinity for the vascularised red pulp which was said to be preferentially destroyed, before the lymphatic nodules. In the experiments which he allowed to continue for upward of 16-20 days, a marked reduction in the number of trophoblast cells was observed.

In a review Kirby (1965) described the behaviour of explanted blastocysts in the liver and the brain tissue. He found trophoblastic giant cell proliferation, invasion, haemorrhage and destruction of healthy host tissues by phagocytosis in both tissues.

The behaviour of a variety of benign and malignant tumours and of normal tissues, including trophoblast transplanted into the chick wing bud has been described (Tickle et al., 1978). The presence of single cells or small groups of cells away from the graft was used as the criterion for invasiveness. They found trophoblast (from ectoplacental cones of 7-day mouse embryos) to be the only invading normal tissue. In 15 out of 18 ectoplacental cone grafts, several individual trophoblast cells were found to have migrated away from the main graft into the chick

mesenchyme. They observed a variable pattern of invasion by the trophoblast cells, elongation of trophoblast cells along the interface between the ectoderm and the mesenchyme, trophoblast cells "streaking away from the graft under the ectoderm" i.e. a tendency to become aligned along the basal lamina of the ectoderm, a behaviour which had previously been termed contact guidance (Weiss, 1941).

They found trophoblast cells near blood vessels, although there was no indication that they 'homed' in to the vessels. Erosion of the host chick tissues seen was also associated with the invasion of trophoblast cells, and large blood blisters sometimes formed.

I. TRANSPLANTS OF ECTOPLACENTAL CONE TO THE CHICK INTRAEMBRYONIC COELOM

The behaviour of the explanted trophoblast of various species in the intraembryonic coelom of the chick embryo has been studied (in mouse, Michie, 1984; De Souza, 1986; in hamster, Al-Janabi, 1988; in rat and pig, Barber, 1989).

(i) Mouse

Michie (1984) found trophoblastic giant cells of mouse insinuating in the liver and in close relation to blood vessels which they did not penetrate. He also found evidence of phagocytosis of the host red blood cells characterised by the presence of haemosiderin in the cytoplasm of the trophoblast cells. Since only 2 out of 10

grafted chick embryos survived, in which the mouse trophoblast was found to be non-invasive, he found it unwise to draw conclusions on the invasiveness of the trophoblast in the mouse.

De Souza (1986) studied 24 grafted chick embryos which survived following intracoelomic transplantation and found that the mouse trophoblast cells had penetrated vessel walls in 75% of cases but had also plugged the vessel wall thereby preventing the host chick embryos from bleeding to death. He observed trophoblast cells in close apposition to healthy hepatocytes and to mesenchymal cells. He also found haemosiderin- like material in association with the cytoplasm of the trophoblast cells. He concluded that the mouse trophoblast is invasive, but not in a destructive manner. He found no evidence of cytolytic destruction of host tissues by trophoblastic cells. In a further 29 experiments the graft was not recovered and 37 chicks died at between 20 hours and 4 days post-operatively. The dead chick embryos were not studied histologically because of autolytic changes in them, hence he could not comment on the probable cause of their death.

(ii) Hamster

Al-Janabi (1988) transplanted ectoplacental cones of 7-day hamster embryos into the chick's intra-embryonic coelom. 22 out of the 75 chicks survived. Varying amounts of trophoblast cells grew from the grafts. Of the surviving hosts, the 16 chick embryos which survived beyond

48 hours were found carrying not more than one or two giant cells, which were in contact with the liver. The 6 chick embryos that were killed in the first 48 hours carried grafts which were located in the rostral part of the right intra-embryonic coelom. All showed proliferation of trophoblast cells at histology which:

1. caused local erosion of the tissues with which they had come in contact;
2. showed local migration into neighbouring structures like the liver and the body wall;
3. caused haemorrhage into the coelomic cavity due to the invasion of the walls of the large blood vessels.

He could not find any evidence of phagocytosis of normal host cells by the invading trophoblast cells, neither did he find any evidence of 'cytolysis' as previously reported (Billington, 1966).

However, he noted that trophoblastic giant cells caused loosening and disaggregation of liver cells which he attributed to the presence of a large 'dose' of giant cells in the organ. Al-Janabi (1988) concluded that the hamster trophoblast is invasive, but not particularly destructive. However, he could not determine the mechanism of invasion by the trophoblast cells but suggested that internal haemorrhage due to erosion of major blood vessels was the most likely cause of death in chicks examined histologically post-mortem.

(iii) Rat

Barber (1989) transplanted 8.5 day rat ectoplacental cones into the chick embryonic coelom, and found 19 of the 23 grafts demonstrating trophoblastic giant cell proliferation, migration of tongues of cells away from the graft site and invasion of blood vessels. He reported active migration of giant cells, both individually and as tongues of cells, into adjacent host tissues. He also noted that the invasiveness of rat trophoblast appeared to be dependent on the density of tissue faced, for instance, penetration of compact parenchymal structures appeared more difficult than the loose mesenchyme. He concluded that rat trophoblast when faced with chick tissues is invasive, by a mechanism that did not seem to involve phagocytosis of host cells, histolytic or cytolytic damage to host tissues.

7. **THE MECHANISM OF TROPHOBLAST INVASION**

The mechanism of trophoblast invasion in utero and in ectopic sites of various species involves physical and chemical factors (see Billington, 1971 for review).

Of the physical factors, the ability of trophoblast cells to invade host tissues by phagocytosis found limited support in the work of Fawcett (1950), but much more in those of Kirby (1960, 1962, 1963a & b, 1965) and of Billington (1965, 1966). Kirby (1965) reported that " trophoblast is primarily a tissue of destruction which unleashes its full invasive proclivities by phagocytosis, with the cytoplasmic processes of trophoblast

characteristically extending and engulfing host cells". However Kirby's observations (1965) could not be corroborated by Porter (1967), who failed to find any unequivocal evidence of phagocytosis, as only visceral yolk sac cells and none of the trophoblast cells within the transplant concentrated the injected trypan blue.

Similarly, De Souza (1986), Al-Janabi (1988) and Barber (1989) in their respective studies of the behaviour of mouse, hamster and rat trophoblast transplanted into the chick's intraembryonic coelom, found evidence of phagocytosis only of red blood cells, and that to a limited extent, and all agreed with Porter (1967) that phagocytosis could not have been the principal means by which trophoblast cells invaded tissues.

Glass et al. (1979) have also shown that mouse trophoblast cell is non-phagocytic when cultured with liver cells and with neoplastic cells.

Phagocytosis of healthy maternal uterine epithelial cells has been reported to occur to a certain extent in some species (in rat, guinea pig, rabbit, ferret, armadillo and bat, Enders and Schlafke, 1969; and in man, Boyd and Hamilton, 1970). On the other hand, other authors have only been able to observe the ability of trophoblast cells to phagocytose degenerating or dead maternal uterine epithelial cells (in mouse, Smith and Wilson, 1974; El-Shershaby and Hinchliffe, 1975).

Eaves (1973), in his studies on the mechanism of

invasion by malignant tumours, concluded that invasion could be explained solely on the basis of mechanical pressure exerted by a growing population of malignant cells. This view has been strongly refuted in some quarters, without excluding its role in some aspects of invasion (see Mareel, 1980 for review). Fawcett (1950) had earlier noted the effect of pressure exerted by the growing blastocyst transplanted under the kidney capsule, the expanding mass of fetal tissue was not only exerting pressure against the kidney cortex, but that trophoblastic giant cells were also invading and destroying it. A similar observation to those of Fawcett (1950) was made by Michie (1984) using the same species and a similar site, which strongly suggests that pressure does aid invasion of tissue by trophoblast cells. How this is achieved is not known at the moment but may not be totally unconnected with the view that the compression exerted by the growth of malignant cells contributes to degeneration of invaded tissues, expressed by Ozzello and Sanpitak (1970).

Separation and migration of individual cells or groups of cells away from the primary trophoblastic growth have been reported (in hamster, Billington, 1966; Pijnenborg et al., 1974; in mice, Tickle et al., 1978; and in rats, Barber, 1989). The current view on separation of malignant cells which facilitates invasion is that there first occurs reduced adhesiveness of tumour cells, leading to loss of cell-to-cell junctions (see Mareel, 1980, for review). Once a trophoblastic or malignant cell breaks away, active

individual cell migration follows (Strauli and Weiss, 1977; Tickle et al., 1978).

That trophoblast and tumour cells demonstrate active individual cell migration has found support in the findings of locomotory apparatus such as microfilament bundles and microtubular complexes within their cytoplasm (Willingham et al., 1977; De Mey et al., 1978 and Carpenter, 1982). Direct evidence for locomotion of malignant cells inside solid tissues has also been provided by Haemmerli and Strauli (1978) who demonstrated cinematographically the locomotion of invading leukemia cells in the rat mesentery.

A question that has been asked by many is what initiates the locomotion of a cell that is normally stationary? The concept of contact inhibition, defined as cessation of movement of a cell in the same direction after it has collided with another cell, originally proposed by Abercrombie and Heaysman (1954), is thought to offer some answer. Glass et al. (1979) demonstrated this concept using time-lapse cinematography. They showed that mouse liver cells in vitro retracted from trophoblast cells on making contact, with the trophoblast cells thereafter spreading into the areas vacated by the liver cells.

Enders and Schlafke (1969) have suggested a possible 'stapling' function for the desmosome-like structures found between trophoblast and uterine epithelial cells of some species (mouse, Potts, 1968; hamster, Young et al., 1968; rabbit, ferret, armadillo and bat, Enders and Schlafke,

1969) which might serve as some sort of anchorage from which cytoplasmic processes could flow to penetrate and dislodge the uterine epithelium in the early phases of invasion.

Active vascular dissemination of trophoblast has been reported to effect extensive structural alterations in the walls of the 'invaded' arteries of various species (in hamster, Orsini, 1954, 1968; Billington, 1966; Carpenter, 1982; in man, Brosens et al., 1972; Robertson et al., 1975; in man, monkey and baboon, Ramsey et al., 1975). These alterations, they postulate, are vital to the physiological adaptation of the arteries to the markedly increased blood flow demands of the developing fetoplacental unit.

Brosens et al. (1972) in their work on human placenta bed biopsy materials noted that trophoblast invasion and the associated structural alterations of the spiral arteries failed to extend beyond the decidua basalis in pregnancies complicated by pre-eclampsia. The observations made the authors suggest that the absence of the trophoblast-induced 'physiological changes' in the myometrial segments of the spiral arteries (as a result of unaltered elastic and smooth muscle tissue in the walls of these arteries) might be an important aetiological factor in the development of the symptoms of these disorders of pregnancy. Although the theory appears plausible, one might not be too cautious by not accepting it, in its entirety, if one remembers the statement of Ramsey et al.

variation in the amount of endometrial arterial wall replacement in man, monkey and baboon, with pre-eclamptic and eclamptic conditions by Brosens et al. (1972), noted that such clinical conditions were not known to occur in monkey or baboon.

The chemical factors implicated in trophoblastic giant cell invasion have been ascribed cytolytic and proteolytic functions by various authorities (Bryce, 1937; Hertig and Rock, 1945; Blandau, 1949; Kirby 1960, 1962; Billington, 1966).

It has been postulated that proteolytic enzymes cause the breakdown of tissues adjacent to trophoblast and tumour cells and thus create space into which these cells are able to move soon afterwards.

One of the early experiments to test the hypothesis of the probable release of proteolytic enzymes by the trophoblast was that of Blandau (1949). He cultured 6-8 day guinea pig blastocysts and 5-6 day rat blastocysts on various protein substrates such as gelatin, purified human fibrinogen and dried homologous plasma. He found that only guinea pig ova showed proteolytic activity, demonstrated by the localised dissolution of the protein substrates films. The 50 or more rat ova cultured under similar conditions as the guinea pig blastocysts, did not cause any dissolution of the protein films. The observation that implanted glass beads (artificial ova) failed to erode the uterine epithelium in the guinea pig, taken together with the

ability of cultured guinea pig ova to cause proteolysis in vitro, led him to conclude that the implanting guinea pig blastocysts secrete proteolytic enzymes.

A unique observation made by Denker (1974) on inversely orientated rabbit blastocyst further buttressed the hypothesis that trophoblast elaborates proteolytic enzymes. He noted that in the rabbit, as in the mouse, rat and hamster at nidation, it is the abembryonic pole of the blastocyst which first makes contact with the antimesometrial part of the endometrium, where shedding of the zona pelludica also commences. He observed that in the case of the reversed-orientation implanting blastocysts, when the embryonic pole first came into contact with the endometrium, the lysis or breakdown of the zona pelludica still occurred at the abembryonic pole despite the absence of antimesometrial endometrium, whilst no lysis was seen in the embryonic hemisphere despite its contact with the antimesometrial endometrium. He also observed blastocyst protease activity to be maximal in the abembryonic hemisphere of the rabbit blastocyst irrespective of orientation which he then considered to be a function of the trophoblast.

Possible localized proteinase secretion by trophoblast or membrane proteinases on the surface of trophoblast has also been implicated in the digestion of radio-labelled extracellular matrix by mouse blastocyst observed by Glass et al. (1983).

Fisher et al. (1985) cultured first-trimester human

placenta villi on radio-labelled extracellular matrices which contained basement membrane specific macromolecules, including type IV collagen, laminin and proteoglycan. Cytotrophoblastic cells grew from the villi cultures in both matrices which were observed to cause a marked focal dissolution of the underlying matrix, presumably due to the effect of a variety of proteolytic and glycosidic enzymes, possibly released by the cells.

That trophoblast causes tissue cytolysis was strongly supported by Kirby (1963a) who said of the effect of mouse trophoblast transplanted to the testis, "it appeared as if a cytolytic agent was diffusing ahead of the advancing border of the trophoblast causing the lysis of the host tissue". A similar cytolytic effect by hamster trophoblast transplanted to the testis was reported by Billington (1966).

Carpenter (1982) reporting on the arterial invasion of the pregnant hamster uterine vessels noted that the trophoblastic giant cells sequentially remove and replace the arterial endothelium and destroy nearly all the smooth muscle cells by an intense cytolytic activity.

By contrast, Porter (1967) did not find any effect attributable to a cytolytic agent in his studies of transplanted mouse trophoblast. His observations were further supported by the studies of Enders and Schlafke (1969) who remarked that cytolysis could not be found in association with trophoblast invasion in normal

implantation of the rat, rabbit, guinea pig, armadillo, bat and ferret. Neither did Glass et al. (1979) find any evidence of cytolysis in their in vitro culture of mouse blastocysts on various mouse cells.

Plasminogen activator (PA), whose production was demonstrated in mouse blastocyst (Strickland et al., 1976), converts plasminogen into plasmin, a trypsin-like enzyme, which causes degradation of fibrin (Nagy et al., 1977). P.A. is thought, in conjunction with other similar enzymes, to contribute to invasion (Liotta, 1986).

A 'stepwise' progression has been postulated for the invasion of host tissue by cancer cells (Liotta, 1986). The first step is tumour cell attachment via cell surface receptors which specifically bind to components of the matrix such as laminin (for the basement membrane) and fibronectin (for the stroma). The second step involves secretion of hydrolytic enzymes by the anchored tumour cells which are able to cause local matrix degradation. The third step is locomotion of the tumour cell into the region of the matrix modified by proteolysis. Continued invasion of the matrix is thought to take place by cyclic repetition of the three steps.

Loke et al. (1989) have demonstrated by immunohistologic methods that human first trimester trophoblast binds to laminin in vitro. Laminin, a basement membrane glycoprotein, was also seen surrounding individual cells both in vivo and in vitro by the same authors. Their observation offered supportive evidence that trophoblast-

laminin interaction possibly mediate the initial attachment of the trophoblast cells to host structures, as had been presented for tumour cells by Liotta (1986).

Tickle et al. (1978), found mouse trophoblast cells aligned along the basal lamina of the ectoderm of the chick wing bud, and suggested that the basal lamina might provide the necessary migratory cues in that situation, a view adopted by Barber (1989) in his observations on the invading rat trophoblast.

8. SUMMARY OF THE REVIEW OF LITERATURE ON THE 'INVASIVENESS' OF TROPHOBLAST

The following points emerge from the review of literature.

Blastocysts and ectoplacental cone grafts of mice, rat, rabbit, guinea pig and hamster yield luxuriant growths of trophoblast cells, which transform into giant cells in ectopic sites as they do in utero.

In animals with haemochorial placentae (e.g. mice, rats, hamsters) trophoblast is undoubtedly invasive, to varying degrees, in normal pregnancy. While the traditional view has been that one important role of the decidual reaction is to restrain and contain this invasive activity, the evidence that decidual degeneration is not dependent upon the activity of trophoblast, but is genetically programmed, suggests that the role of the decidua is, rather, to facilitate invasion.

The evidence on the invasive activity of ectopically transplanted trophoblast, of mice, rats and hamsters, is conflicting. Some authors (notably Kirby, 1960, 1963a & b and 1965) claim to have shown that it is highly invasive and destructive; others (notably Fawcett et al., 1947) regard trophoblast as a much more passive tissue, with limited powers of invasion.

The mechanism of trophoblastic invasion remains to be fully elucidated, as the claims that trophoblast invades by phagocytic, histolytic and cytolytic means have been questioned.

9. **RATIONALE OF STUDY**

In the present study, the behaviour of hamster trophoblast transplanted to the coelom of the chick embryo has been chosen for the following reasons:

1. hamster trophoblast was chosen because previous workers have shown it to have, in vivo, a greater migratory activity than that of other species. This migration has been described as highly selective towards arterial channels (Orsini, 1954), while invasion of veins and capillaries appears to be limited (Billington, 1966).

2. Chick intraembryonic coelom was chosen for two main reasons:

a. because the graft can be placed in position without any damage to the host during the grafting procedure, a unique advantage of this site.

b. In the chick embryo, the graft comes into contact with mesothelium, then with loose mesenchyme and then with various parenchymal organs. The first two of these might be regarded as offering little, if any, mechanical resistance to invasion, while the third group of tissues provides a useful test-bed of invasive activity and of the phagocytic, histolytic and cytolytic activities which have been claimed to underlie trophoblastic invasiveness.

MATERIALS AND METHODS

1. PREPARATION OF THE CHICK HOST FOR GRAFTING: INCUBATION AND WINDOWING OF EGGS

Ross Brown hens' eggs (Ross Poultry, Aberdeenshire) were incubated in a forced draught incubator (37-38^{o}C) for approximately 72 hours prior to "windowing". The eggs were usually "windowed" the night preceding grafting to allow developing embryos to recover from the disturbance, before they received a graft.

At "windowing", each egg was transilluminated with a Western Cellascope to locate the positions of the developing embryo and of the air sac, which were marked. A Casali Milano dental drill was then used to cut through the shell along the marked rectangular area, 2 cm x 1 cm, overlying the position of the embryo and at a pin point area over the air sac. In each case, the shell membrane was left intact. The egg was then wiped clean of shell dust with a swab soaked in Hibitane in 90% alcohol.

The drilled-out rectangular area of shell was lifted off, to expose the shell membrane and a pin-hole opening was made in the shell membrane over the air sac. Air was extracted from the air sac by a rubber teat while the shell membrane over the embryo was pierced with a needle. The negative pressure thus created within the shell caused the developing embryo and its membranes to "drop" away from the shell membrane, which was now removed. The dropped embryo at this stage usually lay on its left side, covered by the transparent avascular chorion. The window was re-sealed

with Sellotape, and the egg placed in a Western Curfew static incubator at 38-40^{o}C for a further 12 hours, prior to transplantation the following day, thereby making the total incubation period about 3.5d prior to grafting.

2. **PREPARATION OF THE GRAFTS**

a) Timed mating of hamsters

The golden hamsters used were obtained from a closed colony maintained in the Department. Breeding animals were kept in a light-reversed room, which was artificially lit from 19.00 - 10.00 hours, and in darkness from 10.00 - 19.00 hours.

Those females which showed the characteristic lordosis of oestrus in the presence of male hamsters were then put in the same cage with them, one female to three males, between 15.00 and 17.00 hours until mating was observed. At least four females were mated in preparation for each experiment.

b) Dissection of hamster egg cylinders at 7 d p.c.

The mated females were killed by cervical dislocation at noon on the seventh day after the observed mating. All procedures were carried out under antiseptic or aseptic conditions. The abdominal skin was shaved and swabbed with Hibitane in 90% alcohol, sterile instruments and glassware were used and all dissections were carried out under a transparent cover to exclude aerial contamination. The entire bicornuate uterus was excised, and stretched out on a sheet of cork, to which it was pinned in lightly

stretched condition. Under a Wild stereobinocular dissecting microscope, fitted with dust-excluding hood, each decidual swelling was exposed in situ. The serosa and myometrium were cut with fine scissors along the antimesometrial border and peeled away, exposing the pear-shaped decidual swellings.

Each decidual swelling was then opened from its anti-mesometrial side with fine curved scissors and the egg cylinder teased out using a fine needle point. The egg cylinders were then transferred to a concave slide, containing modified TCM 199 medium, in a petri dish and examined under the dissecting microscope. From these, various types of grafts were prepared for transplantation to the chick coelom:

1. Complete egg cylinder;
2. ectoplacental cone, cut off with cataract knife;
3. egg cylinder without the ectoplacental cone.

3. PREPARATION OF CONTROLS

Several types of controls were used.

a) Normal (in utero) implantation sites

Two intact decidual swellings, one at 7d, the other at 7.5d, after mating, were fixed in Bouin's fluid, processed to wax, cut in serial sections and stained with H & E. These controls enabled comparison of the behaviour of ectoplacental cone (EPC) tissue in contact with decidual tissue in utero, with that in the ectopic environment of the chick embryonic coelom. The 7d specimen showed the

appearance in situ of the EPC corresponding to that at the time of the removal of the egg cylinder for transplantation; the 7.5d specimen showed its behaviour in situ, for comparison in general with that of 12 hours explants.

b) Explants of hamster decidual tissue

Pieces of decidua were cut with fine curved scissors from the anti-mesometrial side of the decidual swelling of a 7 day pregnant uterus, transferred to a concave slide containing TCM 199 and cut into irregular cubes of about 1½ - 2mm side, in preparation for transplantation.

These controls were used for two different reasons:

1. because, when the egg cylinder was removed from the decidual swelling in preparation for its intracoelomic grafting, some decidual cells were likely to be taken with it. It was obviously important to know if effects ascribed to the explanted trophoblast owed anything to the inadvertent presence of decidual cells.
2. to obtain information about the behaviour and fate of decidual tissue in an ectopic site. Normal implantation involves interaction between trophoblast and decidua. Knowledge of the behaviour of explanted decidua might help our understanding of the respective contributions of trophoblast and decidua to this interaction.

c) Embryonic chick tissues

Fragments of chick limb bud (5.5d) were prepared for

transplantation to the chick coelom to determine what effect, if any, grafts of "neutral" chick tissue would have on the host chick.

d) Normal control chick embryos

Five normal (unoperated) chick embryos taken at 84, 92, 96, 100 and 108 hours of incubation were serially sectioned. These times were chosen to provide information about the normal undisturbed appearances of the embryo at the time chosen for placing the graft (84h) and at the various time intervals (8, 12, 16 and 24h) after grafting of experimental chicks.

4. **GRAFTING TO THE CHICK INTRAEMBRYONIC COELOM**

Grafting of experimental and control tissues to the chick intraembryonic coelom was carried out on the 'windowed' chick embryos at Stages 19-21 (Hamburger and Hamilton, 1951) when the embryonic and extraembryonic coeloms were still in free communication, using the method of Dossel (1954), with only slight modifications.

A small hole was made with a cataract knife in the avascular chorion overlying the umbilical ring. The graft was picked on the knife point and placed on the chorion near the incision. An L-shaped glass rod with a smoothly rounded tip was used gently to push the graft through the incision in the chorion and then through the umbilical ring, into the rostral part of the intraembryonic coelom. (Fig. 3). The whole procedure required no incision of the chick tissues, and great care was taken not to damage the

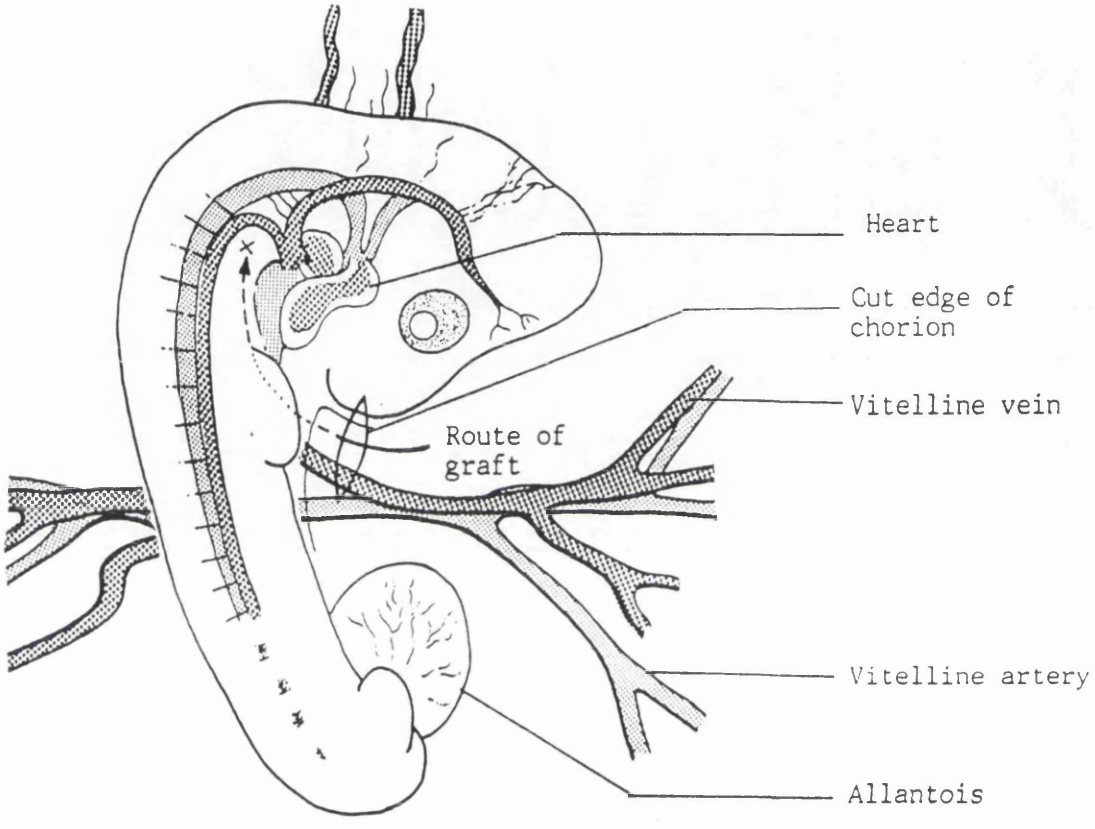

Fig. 3: Shows the route and position of intracoelomic grafts in a 3.5 day-chick embryo.

(After Barber, M.D., 1989, with acknowledgement)

embryo. The egg window was then resealed with a piece of Sellotape and the egg returned to the static incubator.

5. RECOVERY OF GRAFTS

When it became clear after the first experiment that the host chicks often died overnight, because of bleeding into the coelom, it was decided to monitor the eggs at regular hourly intervals, often right through the night, and to terminate each experiment when significant bleeding was observed on inspection through the egg window. A few chicks died in the one hour interval between inspections, but none had been dead for more than one hour when fixed for histology and tissue preservation was therefore always good. Some experiments were terminated at a predetermined time - 8, 12, 16 and 24 hours after grafting. Of these, only those chicks which were seen on inspection through the egg windows to be alive were fixed for study.

At termination, the shell was gently cracked and its contents emptied into a shallow dish. The embryo was dissected free of its membranes and vessels, and fixed in freshly prepared Bouin's solution for 24 hours, after which it was transferred to 70% ethanol. From each grafted chick a block of tissue was cut with a sharp razor blade. The aim was to include all the upper abdominal/lower thoracic region i.e. with the graft and the surrounding host tissues in an undisturbed relationship.

6. HISTOLOGICAL PROCEDURES

The histological blocks were processed to wax (see Appendix A) and cut serially in transverse sections at 5μm using a Spencer microtome. Every 10th section was mounted in an interrupted series and stained, to locate the position of the graft. Once the graft had been located, the remaining sections were mounted serially, from at least five sections rostral to the commencement of the graft until another five sections caudal to its termination, and stained with haematoxylin and eosin (H & E) for histological study.

7. SUMMARY OF MATERIAL PREPARED AND STUDIED

EXPERIMENTAL

Grafts of ectoplacental cone (EPC) or of complete egg cylinder (EC) to the chick intraembryonic coelom

All grafts were made to chicks of 3.5d incubation.

Incubation age of chick at termination (i.e. age at grafting + duration of graft)	No. of chicks
3.5d + 8h	15
3.5d + 12h	10
3.5d + 16h	9
3.5d + 24h	3
Total	37

CONTROLS

a. Normal implantation sites in utero

1 at 7d p.c.

1 at 7.5d p.c.

b. Grafts of 7d hamster decidua to chick coelom

Incubation age of chick at termination (i.e. age at grafting + duration of graft)	No. of chicks
3.5d + 8h	5
3.5d + 16h	2
3.5d + 24h	1
Total	8

c. Grafts of embryonic chick limb buds (5.5d) to chick coelom

Incubation age of chick at termination	No. of chicks
3.5d + 48h	1
3.5d + 72h	1
Total	2

d. Normal unoperated chick embryos

Incubation age of chick at termination	No. of chicks
3.5d	1
3.5d + 8h	1
3.5d + 12h	1
3.5d + 16h	1
3.5d + 24h	1
Total	5

e. Grafts of parts of egg cylinder excluding E.P.C.

Incubation age of chick at termination	No. of chicks
3.5d + 24h	2

RESULTS

1. PREAMBLE

The main aim of the experiments was to assess the capacity of the ectoplacental cone and its derivatives to "invade" the tissues of the chick embryo, when transplanted ectopically to the intraembryonic coelom, using a method which, in itself, caused no damage to the host tissues.

Subsidiary aims included:

1. Elucidation of the mechanism of invasion by the trophoblast, attempting to answer the following questions:

a. Does trophoblast invade by causing histolysis of host tissue? By histolysis is meant loosening or disaggregation of tissue components, particularly of epithelial tissues.

b. Does trophoblast invade by producing cytolysis of host cells with which it comes into close proximity or contact? By cytolysis is meant degenerative change leading to death of individual cells.

c. Does invasive activity of trophoblast depend wholly or in part on a capacity to phagocytose host cells?

d. Does invasion depend upon active movement of trophoblastic cells, either the local extension of "tongues" of cells into host tissues or the more distant migration of individual trophoblastic giant cells?

e. Do tissues other than trophoblast behave in an invasive fashion when transplanted to this ectopic site? In particular, the behaviour of the visceral layer of the yolk sac was studied, when it came close to the lining

of the chick coelom. Grafts of intact egg cylinders provided a particularly valuable opportunity to compare the behaviour of the ectoplacental cone with that of the yolk sac endoderm. Other control grafts included hamster decidua and chick limb buds.

2. Elucidation of the response, if any, of the host tissues to contact with trophoblast.
   a. An old view, long discarded but now returning to favour, maintained that trophoblastic invasion was largely a passive affair, encouraged and facilitated by spontaneous degeneration of the decidua. The interaction of trophoblast with the "neutral" tissues of the chick seemed a useful test of this hypothesis.
   b. Do various tissues of the chick exert a specific attraction on hamster trophoblast? In particular, do arteries and veins "attract" trophoblast?

Before giving a detailed account of individual experiments, it is necessary to sketch in the general background to the results.

A. **The graft site**

Grafts were placed in the right supraumbilical coelom. This includes the upper end of the future right peritoneal cavity and its direct rostral continuation, the right pericardio-peritoneal canal.

The position of the graft varied slightly from case to case, depending upon a number of factors: the size of the

host embryo at the time of grafting, the size of the graft, chance variation in the placement of the graft, and, in a very few cases, some caudal displacement of the graft postoperatively. In general, however, the position of the graft in ovo, as assessed from serial transverse sections at the end of the experiment was remarkably constant. The graft site is illustrated in Fig. 4, which shows the intact mesothelium of the upper right coelom and the neighbouring structures of the chick prior to placement of the graft. To assist further in the orientation of all subsequent illustrations, Fig. 5 gives a general low power view of an egg cylinder graft in situ. It lies within, and follows the contours of the right supraumbilical coelom. In this particular section the graft lies close to the coelomic mesothelium but makes no contact with it. The coelomic cavity is bounded:

(i) <u>dorsally</u> by the right half of the dorsal body wall, containing dorsal aorta, right posterior cardinal vein and, between and ventral to these, the right mesonephros and its duct. Dorsal to the dorsal aorta may be seen part of the notochord, sympathetic ganglia, and, on each side, a dorsal intersegmental vein, opening ventrally into its respective posterior cardinal vein.

(ii) <u>laterally</u>, by the right body wall (with the fore-limb bud at this level). Ventrally, where the body wall meets the septum transversum, is the right umbilical (allantoic) vein.

(iii) ventro-medially is the developing liver, largely occupying the septum transversum. This surface shows only a layer of mesothelium and a thin plate of hepatocytes separating the graft from a large venous channel, the ductus venosus. Ventral to the liver are the pericardial cavity and the heart.

(iv) medially lie the dorsal mesentery, containing part of the foregut (oesophagus, stomach or duodenum depending upon the craniocaudal level of the section), the bursa omentalis, and bulging from its left face, the developing spleen.

Table 1 shows the shortest distances between coelomic mesothelium and various key structures, measured from Fig. 5, as a guide to the distances which would have to be traversed by invading trophoblast to reach these structures:

Table 1: Distances between coelomic mesothelium and some host structures.

Structure	Distance from mesothelium in μm (approximate)
Ventral wall of aorta	30
Dorsal wall of aorta	300
R. sympathetic chain	350
Ventral border of notochord	400
Ectoderm, right body wall	120
Ductus venosus	10
Right umbilical vein	10
Bursa omentalis	60
Developing spleen	200
Pericardial cavity	80

These figures indicate the risk of severe bleeding in the host chick in the event of invasion of its tissues by

trophoblast.

B. **Nature of the graft**

At the time of transplantation, three types of graft were prepared:

1. the entire egg cylinder (E.C.)
2. the amputated ectoplacental cone (E.P.C.)
3. the egg cylinder minus the E.P.C.

In each case, the nature of the graft was checked in the serial sections at the end of the experiment: it usually agreed with that recorded in operative notes, but sometimes varied. It occasionally included some contaminating decidual cells, and the "pure" ectoplacental cone graft occasionally included some contaminating extraembryonic ectoderm, and/or some endodermal tissue (visceral yolk sac and/or embryonic endoderm).

Some of the variations in the final positioning of the graft of intact egg cylinders were particularly instructive. The ovoid graft was usually found with its long axis lying longitudinally in the coelom, but the ectoplacental cone sometimes lay at the rostral end, sometimes at the caudal end of the graft. This (unavoidable) variation obviously affected the precise relationship of the ectoplacental cone to organs of the chick host.

C. **Duration of the graft**

Grafts were studied, in situ, in serial sections of

the host embryo, at four time-intervals after grafting: 8 hours, 12 hours, 16 hours and 24 hours.

There was some variation in the exact duration of the graft: some experiments were terminated slightly earlier (or, sometimes, later) than these times, depending upon the occurrence of bleeding in the embryo, produced by vascular erosion. If bleeding occurred, termination of the experiment was not usually delayed for more than about 1 hour. In order to secure specimens at the later stages of grafting, some experiments were continued after bleeding had begun, provided it was minimal and the chick remained alive.

There was also some variation in the behaviour and fate of the graft and in the status of the host embryo within each of these four groups, but it was possible to construct a sequential account of the behaviour of the trophoblast and the response of the host.

We can now consider, in detail, results at each time interval after grafting.

2. 8-HOUR GROUP

In all, there were 15 grafted chicks in this group. A detailed summary of the findings in each individual chick is given in Appendix B. The following account attempts to construct a coherent general picture of the behaviour of the graft and the response of the host at this stage.

A. **Nature of graft**: all 15 grafts included the ectoplacental cone (EPC); in 12 it was part of an

apparently intact egg cylinder, in 3 it was a "pure graft" of EPC. The egg cylinder grafts were ovoid in shape, and generally lay with their long axis longitudinally, with the EPC either rostrally or caudally situated, the actual position being a matter of chance at the time the graft was placed. This chance variation in the position of EPC had important consequences, as we shall see.

B. **The graft site**

All 15 grafts were located in the right supraumbilical coelom and all had gained attachment to the host, by "erosion" of the coelomic mesothelium. The site of attachment was most frequently to the dorsal coelomic wall, overlying the dorsal aorta, in 11/15 embryos. In 4 of these attachment was solely to the dorsal wall. In the other 7, there was an additional area of attachment: to the dorsal mesogastrium (3), to the right body wall (5), to the dorsal surface of the liver (3). In 4/15 embryos the graft was attached to sites other than the dorsal wall: to the right body wall alone (3), or to the right body wall and dorsal surface of liver (1). **In each case, the position of attachment was clearly associated with the position of EPC and of giant cells derived from it.**

C. **Invasive behaviour of grafts**

The first host tissue encountered by graft EPC was of course, invariably coelomic mesothelium, and this layer had been breached, to varying degrees, in all experiments.

The earliest stages of invasion were seen particularly

clearly in embryos, numbers 31, 33, 34, 35, 37 and 38.

No.31 This specimen provided the first example of early invasion. The graft was an egg cylinder, with the EPC directed rostrally. The section shows three types of trophoblast: a central core of untransformed cells, small, round and compactly arranged; 'typical' giant cells, with large nuclei and dense cytoplasm, round the perimeter, and ventro-laterally a small cluster of 'vacuolated' giant cells, with vacuolated cytoplasm and shrunken angular nuclei. A breach in the mesothelium of the dorsal coelomic bay was observed where typical giant cells in the dorsolateral aspect of the graft came in contact with it (Figs. 6a and 6b).

It is noteworthy that the mesothelial cells that bounded the defect, as well as the underlying mesenchymal cells, appeared normal. Also, where giant cells came in close proximity to the coelomic mesothelium over the dorsomedial coelomic bay, right body wall and the dorsal surface of the liver, damage "at a distance" was not observed. In addition, Fig. 6a provides a low power view of the graft in its usual place, the upper right coelom, at the earliest stage of invasion of host structures after making contact with the coelomic mesothelium. It is representative of the position and behaviour of most grafts in this group.

No.33: The graft was an egg cylinder. The EPC was directed caudally and laterally and came into contact only with the right body wall. The mesothelium showed a very localized

defect, occupied by trophoblastic giant cells (Fig. 7). Host mesothelial cells at the boundaries of the defect, and mesenchymal cells deep to it, were histologically normal. The gap had clearly been produced by the giant cells but there was no certainty about the mechanism (see later).

No.34: In this, the third example of early invasion, the graft again was an egg cylinder, with the EPC facing laterally, in contact with mesothelium of the right body wall. The mesothelium had been breached by a mass of giant cells, which presented a smooth external surface bulging slightly into the underlying mesenchyme (Fig. 8). Again, the mesothelial cells surrounding the defect, and the mesenchymal cells deep to it, appear healthy despite their immediate proximity to giant cell cytoplasm.

No. 35: In this specimen, showing early invasion, the graft was an egg cylinder with the EPC situated rostrally, and attached to the dorsal wall of the coelom, immediately ventral to the dorsal aorta. There was a very localized defect in the mesothelium, produced by a single giant cell whose cytoplasm extended into it. (Fig. 9). Two features are noteworthy. The nuclei of the mesothelial cells which flanked the defect appeared normal, and the invading giant cell cytoplasm abutted directly upon the mesonephric duct, whose cells again appeared normal.

No.37: The graft was an EPC which included polar trophoblastic cells and trophoblastic giant cells. The mesothelium showed a localized defect where it was

penetrated by a tongue-like process of giant cell cytoplasm. Again, the mesothelium bordering the defect appeared normal histologically.

No.38: This provided the sixth example of early invasion. The graft was an EPC consisting largely of giant cells. It was attached to the dorsal coelomic wall, whose mesothelium again showed a localized defect, filled by a "tongue" of giant cell cytoplasm. (Fig. 10a). The mesothelium bordering the defect showed no sign of histolysis or cytolysis. Indeed the mesothelium to the immediate right of the giant cell cytoplasm was thickened, with massing of several nuclei, all of which appeared normal. The normal, unthickened, mesothelium of the left dorsal coelomic bay is shown in Fig. 10b, for comparison.

Later stages of invasion

1. Invasion of dorsal aorta

Once the mesothelium had been breached, the next structure most commonly eroded was the dorsal aorta. In 8 of the 15 embryos at this stage, the ventral wall of the aorta, which was still purely endothelial, had been destroyed by contact with giant cells of the EPC. In some cases this had resulted in bleeding into the coelom, occasionally leading to the death of the host chick. (Fig. 11). In other cases, although the aorta had been opened, the defect had been at least partially plugged by the mass of giant cells, so that there was only limited bleeding into the coelom. (Fig. 12).

2. Invasion of veins:

The frequency of erosion of the aortic wall prompted careful search for damage to other blood vessels. Three large veins lay relatively close to the graft site: the posterior cardinal vein (occasionally common cardinal), the ductus venosus, and veins in the right body wall. In 11 of the 15 embryos at this stage, none of these veins had been eroded. In the other 4, contact with giant cells had resulted in opening of the posterior cardinal vein in 3 and of a body wall vein in 1. Figure 13 shows a solitary migrant trophoblastic giant cell which lay in contact with the outer surface of the endothelium of the right posterior cardinal vein; the endothelium appears undamaged, maintaining its integrity as an epithelial sheet and without signs of cytolytic damage to individual cells.

3. **Invasion of other tissues**

Distinction was drawn between "local" and "distant" invasion.

(i) Local invasion was defined as extension into structures lying in the immediate vicinity of the main mass of the graft. When the invading cells maintained continuity with the parent graft they were described as tongues of cells. Study of the serial sections was essential to establish whether or not this continuity existed. Several examples of "tongues" have already been illustrated (Figs. 7, 8, 9, 10a) and further examples are shown in Figs. 14 and 19. In Fig. 14, the ventral part of the right wall of the aorta has been eroded, and replaced, by what appears to be a

syncitial mass of giant cells. This shows no sign of recently phagocytosed host cells, nor of endocytosis of the chick red cells with which it lies in intimate contact. Dorsally, the giant cell tongue comes into direct contact with the right mesonephric duct whose epithelum appears histologically normal.

When individual giant cells had separated from the main graft to invade immediately adjacent chick tissues, this is classified in the synopsis of results as "local invasion, individual giant cells". This was recorded in only 4 embryos at this stage (Nos. 14, 19, 30 and 54) in each of which only occasional giant cells were found migrating dorsally, in contact with the outer surface of the aortic endothelium.

(ii) distant spread. This was defined as spread of individual giant cells beyond the immediate environs of the graft. Typical examples of this are shown in Figs. 15 and 16, in which single giant cells lie in contact respectively with the dorsal wall of the aorta and with the notochordal sheath. One embryo (No.37) showed a few isolated giant cells lying in contact with the deep surface of the epidermis of right body wall. Fig. 17 shows a distinctly atypical example of distant spread. A single giant cell is lodged within a mesodermal somite, in what can only be a local enlargement of the myocoele. How it reached this unusual position is not clear: although it has produced a local enlargement of the myocoele it has not disrupted the

compact epithelial arrangement of the dermomyotome (laterally) and the sclerotome (medially). At an early stage in its development the myocoele communicates ventrally with the peritoneal coelom and it is possible that the giant cell found its way into the myocoele by this route and became sequestered there when the connection closed.

It is noteworthy that, of the small number of distant migrant giant cells, all were found in contact with a surface: that of the aorta, notochord, epidermis, and myocoele. (See Discussion).

It is also noteworthy that, of the 15 embryos in the 8-hour group, 7 showed no evidence of spread of individual giant cells, local or distant, from the main graft site.

D. **Possible mechanisms of invasion**

Some of these have already been mentioned incidentally, but the following account brings together results of all the observations made.

i. **Invasion requires intimate contact with trophoblast**

Chance variation of the orientation of grafts of the egg cylinder brought EPC into contact with several different areas of the coelomic lining. Invasive behaviour was shown only by EPC giant cells, and only by those which had made actual contact with the mesothelium. Of the 15 grafts in the 8-hour group, all showed erosion of the mesothelium where it came into contact with EPC, and 13 showed further local extension, into the subjacent

mesenchyme, of "tongues" of trophoblast cells. This is positive evidence of invasive capacity, but equally important is negative evidence i.e. those cases in which close proximity between graft and host tissues, without actual contact had not resulted in host tissue dissolution.

Figure 18 shows EPC giant cells lying very close to lung bud and body wall without causing any apparent damage to their mesothelial covering, or to the underlying cells. Figures 9 and 14 both show giant cell cytoplasm immediately adjacent to the mesonephric duct, which in each case appears normal.

The lack of histolytic or cytolytic damage to mesothelium immediately adjacent to a site of early penetration by trophoblast has already been illustrated (see Figs. 8 and 10a).

No examples were found of invasive activity by visceral yolk sac endoderm, although it often lay close to the coelomic mesothelium, although never in actual contact with it. Fig. 5 and Fig. 19 both show examples of visceral yolk sac following the contours of the coelomic surface, but separated from it by distances of about 10-20μm minimum. By contrast (Fig. 19) where a small cluster of giant cells have made contact with the mesothelium, there has resulted a localised defect.

ii. **Does invasion involve phagocytosis by trophoblast?**

The best evidence on this question was provided by study of the sites of early invasion of the mesothelium, which are illustrated at high magnification in Figs. 7, 8

and 10a. In each of these cases giant cell cytoplasm had apparently only recently penetrated the mesothelium and was beginning to spread into the underlying mesenchyme. In none of them, however, was there convincing evidence of recently phagocytosed host cells.

iii. **What is the mechanism of spread of isolated giant cells found at a distance from the main graft?**

Although "distant spread" was observed in 4 of the 15 embryos in this group, careful study gave no clear evidence of the mechanisms of spread. This is perhaps not surprising in view of the nature of the tissue traversed. Once the mesothelium had been breached, giant cells travelled through the gelatinous matrix of primitive embryonic mesenchyme; where they encountered an epithelium (e.g. the basal surface of the endothelium of blood vessels or of the epidermis), they came to lie at the epithelial-mesenchymal interface. They left no "track" of their route through the mesenchyme. There was no clear halo of dissolved matrix around them. They did not contain phagocytosed debris.

What did appear certain was that they had actively migrated through host tissue, travelling distances of up to about 400μm in the 8 hour period. Despite careful search of the serial sections no evidence was found at this stage of vascular dissemination of trophoblast. Although several examples were seen of penetration of veins (e.g. posterior cardinal and body wall veins) by giant cells, these were

not found inside blood vessels remote from the graft site.

Summary of findings in the 8-hour group

1. Of the 15 grafts, all were attached to host tissue, through "erosion" of the mesothelium by trophoblastic giant cells.
2. Local spread by the extension of "tongues" of giant cell cytoplasm was seen in 13 of the 15 embryos.
3. Local spread occurred most commonly into the dorsal coelomic wall and resulted in erosion of the aortic wall in 8 of the 15. Erosion of the right body wall was seen in 8 cases, and of the dorsal surface of the liver in 3.
4. Distant spread of individual giant cells was found in 4 host embryos. Of the few migrant cells found, almost all lay at an epithelial-mesenchymal surface.
5. Erosion of the aortic wall resulted not surprisingly in bleeding. This was not, however, always immediately fatal to the host, since the invading mass of giant cells seemed to form a temporarily effective plug in the defect.
6. The mechanism(s) involved in invasion were not clear, but no convincing evidence was found of histolysis, cytolysis or phagocytosis of host tissue.

Synopsis of Results - 8 hour group

No.	Graft	Attachment	Erosion of Mesothelium	Invasion	Vascular Erosion	Phago-cytosis
14	E.P.C.	dorsal	+	L:tongues + indiv.G.C.	Aorta RPCV	-
19	E.C.	dorsal; mesent. liver	+	L:tongues + indiv.G.C.	Aorta	-
23	E.C.	dorsal	+	L:tongues	Aorta	+ (of red cells only)
30	E.C.	R.B.W. liver	+	L:indiv.G.C.	R.C.C.V. R.P.C.V.	-
31	E.C.	R.B.W.	+	L:tongue (minimal)	-	-
33	E.C.	R.B.W.	+	L:tongues (minimal)	-	-
34	E.C.	R.B.W.	+	L:tongues (minimal)	-	+ -
35	E.C.	dorsal	+	-	-	-
37	E.P.C.	dorsal mesent. R.B.W.	+	L:tongue D:indiv:G.C. (Sub-epidermal)	Aorta R.P.C.V	-
38	E.P.C.	dorsal	+	L:tongue	-	-
40	E.C.	dorsal R.B.W.	+	L:tongue D:indiv.G.C. periaortic	-	+ (of red cells only)
45	E.C.	dorsal liver	+	L:tongues	Aorta R.C.C.V D.V.	+ (of red cells only)
48	E.C.	dorsal R.B.W.	+	L:tongues	Aorta	-
53	E.C.	dorsal mesent.	+	L:tongues D:indiv.G.C. periaortic & to L.P.C.V.	Aorta	
54	E.C.	dorsal R.B.W.	+	L:tongues D:indiv.G.C. periaortic	Aorta	+ (of red cells only)
Total =15	E.C=12 E.P.C= 3	dorsal = 11 R.B.W. = 8 Liver = 3	15	L:tongues=13 D:indiv.GC=4	Aorta=8 Veins=4	Phagocytosis (of red cells only=4)

Abbreviations: R.(L) P.C.V = right (left) posterior cardinal vein; D.V. = ductus venosus; RCCV = R.common cardinal vein; L = local; D = distant; Indiv.G.C. = Individual Giant cell; EPC = ecto-placental cone; E.C.= egg cylinder; R.B.W. = right body wall; mesent. = mesentery; dorsal = dorsal body wall.

3. 12-HOUR GROUP

In all there were 10 grafted chicks in this group. A detailed summary of the findings in each individual chick is given in Appendix C.

A. Nature of the graft

All 10 grafts included the EPC; in 5 it was part of an apparently intact egg cylinder, in the remaining 5 it was a pure graft of EPC. The orientation of the ovoid egg cylinder was longitudinal, and, as in the 8-hour group, the site of attachment coincided with the position of the EPC.

B. The graft site

All 10 grafts were situated in the right supraumbilical coelom; all had gained attachment to the host by erosion of the coelomic mesothelium. In 9 embryos, this attachment was to the dorsal wall of the coelom; to this alone in 2 cases, and associated in 7 with one or more other attachments: to the right body wall, the dorsal mesentery and the dorsal surface of the liver. In 1 experiment, (No.22), the graft (of an intact egg cylinder) was attached to the right body wall and to the liver and not to the dorsal wall of the coelom; the attachment matched the unusual situation of the EPC.

C. Invasive behaviour of grafts

A common feature in this group was further gradual progression from the initial stages of invasion already illustrated in the 8-hour group following breach of

coelomic mesothelium by trophoblastic giant cells.

Invasion of blood vessels

Macroscopic signs of internal bleeding were seen in 8 of the 10 embryos. In some, this prompted immediate termination of the experiment; in others, where the chick was not obviously dying, the experiment was continued for up to 1 hour, or, in one exceptional case, for 2 hr 45 min.

The source(s) of the bleeding became apparent on examination of the serial sections, on which the following accounts are based.

(i) **Invasion of dorsal aorta**

In all but one embryo (No.22), the EPC giant cells had eroded the ventral wall of the aorta; the reason why death was not always immediate was that the trophoblastic mass had, partially at least, sealed the defect in the aorta. The surface of the trophoblast which now constituted the aortic wall was characteristically concave dorsally, having been ballooned ventrally by the pressure of the blood (Figs. 20 and 21). In many embryos, surprisingly little blood had escaped into the coelom; indeed in one, (No.52), the sealing of the defect seemed to have been virtually complete, since very little bleeding into the coelom was found in the sections.

(ii) **Invasion of veins**

Invasion of one or more of the large veins in the vicinity of the graft was seen more frequently than in the 8-hour group. Seven of the 10 grafts had eroded

endothelium of the cardinal veins or ductus venosus. Fig. 21 shows erosion of the dorsal wall of the liver by giant cells, opening the ductus venosus.

(iii) **Invasion of other tissues**

A new feature in the 12-hour group was the frequency of spread of individual giant cells dorsally around the aorta. This was seen in 5 of the 10 experiments. Typical examples are illustrated in Figs. 22 and 23. Most of the giant cells had migrated along the basal surface of the aortic endothelium, in many cases without apparently damaging it, although some were seen penetrating the endothelium.

Invasive behaviour was always associated with trophoblastic giant cells and never with non-trophoblastic components of the graft. This contrasting behaviour is shown particularly clearly in Fig. 24. A small tongue of giant cells had produced localized erosion of the right body wall, while the visceral yolk sac endoderm, which in places lay virtually in contact with mesothelium on the dorsal surface of the liver, had produced no damage.

(iv) **Distant spread**

Five of the 10 embryos in this group each showed several examples of individual giant cells which had spread into host tissues further than the immediate environs of the graft. As in the 8-hour group, distant spread frequently brought giant cells into contact with an epithelial-mesenchymal interface. In 2 embryos (Nos. 10

and 52) a small number of individual cells were seen at the deep surface of the epidermis: this was usually associated with a localized blister-like separation of epidermis from mesenchyme (Fig. 24). Both these embryos also showed giant cells immediately adjacent to the notochordal sheath. Embryo 10 provided a second example of the unusual feature already seen in the 8-hour group, namely of a single giant cell lodged within the remnant of a myocoele, in intimate contact with dermatomal and myotomal cells. (Fig. 25).

Another unusual example of distant spread is shown in Fig. 26, in which a solitary giant cell lies adjacent to the ventral root of a spinal nerve, on the left side of the embryo, i.e. the side remote from the graft site.

Each of these examples of distant spread seemed to have involved migration through the mesenchyme: no evidence was found to suggest that these giant cells had been transported in blood vessels.

In one embryo (No.18) the EPC had breached the dorsal wall of the liver, and opened up the ductus venosus. Small numbers of single giant cells were seen apparently attached to the endothelial lining of the ductus and of associated smaller hepatic vessels. It was not possible to be certain how these cells had reached their new position. There are two possibilities: the first, that they had seeded from the main mass of giant cells into the venous blood circulating through the liver and had attached themselves to the endothelium; the second, that they had actively migrated along the endothelial surface. The random

distribution of isolated giant cells around the cross sectional profile of the ductus venosus supports the first possibility rather than the second. The fact that such spread would be retrograde i.e. against the general direction of blood flow, need not weaken this view. In early embryonic life, blood flow in the vascular system is not uniformly unidirectional: to- and -fro flow does occur, particularly in the very large venous reservoir in the liver, and especially when the circulation has been weakened through loss of blood, when major vessels have been eroded. The distinction between distant spread by active migration and by passive vascular transport is an important one which will be referred to again both later in the Results and in the Discussion.

It is worth emphasizing that although periaortic spread was seen in about half of the embryos at 12 hours (i.e. spread within a radius of about 500μm of the site of penetration of the mesothelium by the main mass of EPC), more distant spread, although dramatic in Experiments 10 and 52, was unusual.

(v) **Possible mechanisms of invasion**

The 12-hour group provided evidence of invasive behaviour which confirmed and extended that seen in the 8-hour group.

Embryo No.50 showed early invasion of the right side of the dorsal mesogastrium. Giant cells of the ectoplacental cone had eroded the ventral wall of the

aorta, had just made contact with the mesogastrium and were close to, but not in contact with the dorsal surface of the liver. Where the EPC made contact with the mesogastrium, there was a distinct, localized thickening of the mesothelium (Fig. 27). An adjacent section (Figs. 28 and 29) shows two cytoplasmic processes of a giant cell apparently beginning to penetrate this thickened area of mesothelium; it is noteworthy that although there is no general dissolution of epithelial continuity, no normal mesothelial nuclei are seen immediately adjacent to the giant cell cytoplasm. By contrast, these three Figs. (27, 28 and 29) also show, very clearly, the absence of effect of a sheet of giant cells on hepatic mesothelium, from which it was separated by some 25μm.

More distant spread of giant cells in the 12-hour group brought them in contact with a variety of chick tissues. Signs of damage were unusual: local separation of epidermis from mesenchyme seemed to result from damage to the bonds which hold them together rather than to the epidermal cells, which retained their epithelial arrangement (Fig. 24). This retention of epithelial cohesion was also seen in the dermatome and myotome flanking a migrant giant cell (Fig. 25). Where giant cells came into contact with notochord, spinal nerve and with mesenchyme, these tissues usually showed neither histolysis nor cytolysis. There were a few exceptions to this generalisation. In embryo No.49, the EPC was attached to the dorsal surface of the liver, and a tongue of giant

cells had penetrated the covering mesenchyme and through (or between) thin plates of hepatocytes. (Fig. 30). Where the giant cell cytoplasm lies in immediate contact with the edge of an hepatic plate, the hepatocytes are obviously damaged, with pyknotic nuclei and loosening of cellular contact. Similar appearances are seen in another sectional profile of an hepatic plate which, in this section, does not come directly into contact with giant cells. However another section, some 10μm further caudally, shows that similar contact with giant cells is present (Fig. 31) and the conclusion must be that giant cells <u>can</u> produce cytolytic damage. It is puzzling why such evidence of cytolytic damage is so sparse. The size of the giant cell mass may be a factor: most of the examples of no cytolytic damage were of tissues in contact with a single isolated giant cell, rather than a substantial tongue, as in Figs. 30 and 31.

(vi) **<u>Does invasion involve phagocytosis by trophoblast?</u>**

Despite careful search, no convincing evidence was found of phagocytosis of host cells by giant cells which were either <u>in the act of</u> invasion or which were remote from the main graft and, therefore <u>had</u> invaded. Fig. 29 shows a giant cell in the first category: although it has begun to invade the coelomic mesothelium, its cytoplasm shows no sign of recently phagocytosed host cells. Figs. 25 and 26 show giant cells in the second category. Each had migrated for distances of 500-600μm; neither contained

cellular debris, and neither was in the act of phagocytosis.

Occasionally, however, giant cells were seen to contain phagocytosed host cells e.g. the tongue of cells invading the liver (Figs. 30 and 31). Four embryos showed evidence of phagocytosis of avian erythrocytes by giant cells. Again, these were not on a large scale in each of the embryos concerned, as only a few sections in each case presented with phagocytosed nucleated erythrocytes (as opposed to hamster's, which were non-nucleated).

Summary of findings in 12 hour group

1. All 10 grafts (5 intact egg cylinders and 5 pure ectoplacental cones) had gained attachment to the host by erosion of the coelomic mesothelium. In each case, the site of attachment coincided with the position of EPC.
2. Local spread, either by the extension of tongues of giant cells or by periaortic spread of single giant cells, or both, was seen in all 10 experiments.
3. In 9 of the grafted embryos, local spread occurred into the dorsal coelomic wall and resulted in erosion of the aortic wall. In 5 embryos, local spread into the liver and the right body wall was observed and in 4, erosion of the dorsal mesogastrium was seen.
4. Distant spread, of small numbers of individual giant cells was found in 5 embryos , i.e. in relation to the notochord, epidermis, dermatomyotome and the ventral

root of a spinal nerve.

5. Erosion of the aortic wall (in 9 embryos) and of the ductus venosus (in 1 embryo) resulted in internal bleeding which was not instantly fatal, since the invading mass of giant cells formed a temporarily effective plug in the vessel. In one embryo, the sealing of the aortic wall was apparently complete, since no bleeding was noted (Embryo No.52).
6. The mechanism(s) involved in trophoblast invasion, as in the 8 hour group, were still not very clear. However, it became more apparent that (i) contact had to be made before trophoblast had any effect on chick tissues and (ii) that invasion did not seem to depend upon cytolytic, histolytic or phagocytic activity.

Synopsis of Results - 12 hour group

No.	Graft	Attachment	Erosion of Mesothelium	Invasion	Vascular Erosion	Phago-cytosis
10	E.P.C.	dorsal R.B.W.	+	L:tongue periaortic D:indiv.G.C.	Aorta (plugged) L.P.C.V.	+ (of red cells only)
18	E.C.	dorsal liver	+	L:tongues D:Indiv.G.C. in liver	Aorta (Early invasion) D.V.	+ (of red cells only)
20	E.P.C.	dorsal mesogastrium	+	L:single cells extension periaortic	Aorta P.C.V.	-
22	E.C.	R.B.W. liver	+	L:tongues only	Ductus venosus	+ (of host cell at invasion site - minimal)
24	E.P.C.	dorsal R.B.W. liver	+	L:tongues & periaortic spread of single cells.	Aorta P.C.V. D.V.	-
29	E.C.	dorsal R.B.W.	+	L:tongues G.C. D:indiv.G.C.	Aorta	+ (of red cells only)
49	E.P.C.	dorsal liver	+	L:tongues, single cells, periaortic	Aorta Ductus venosus	(of host cell at invasion site minimal)
50	E.C.	dorsal R.mesogastrium	+	Local:tongues	Aorta	+ (of red cells only
52	E.C.	dorsal R.B.W. d.mesogastrium	+	Local Distant	Aorta (sealed)	-
55	E.P.C.	dorsal d.mesogastrium liver	+	L:tongues & periaortic spread of single G.C.	Aorta	+ (of host cell at invasion site minimal)
Total =10	5 E.P.C=5 5 E.C. =5	dorsal - 9 liver - 5 RBW - 5 mesogastrium - 4	+	Local and peri-aortic = 5 distant = 4	Aorta = 9 R(L)PCV=3 D.V. = 4	Phagocytosis = 7 (of red cells only = 4; of host cells at invasion site = 3)

Abbreviations: R(L) P.C.V = right (left) posterior cardinal vein; D.V. = ductus venosus; L = local; D - distant; G.C. = Giant cell; E.P.C. = Ectoplacental cone; E.C. = Egg cylinder; R.B.W. = right body wall; dorsal = dorsal body wall.

4. **16-HOUR GROUP**

There were 9 grafts in this group. A detailed summary for each experiment is given in Appendix D.

A. Nature of graft

Of the 9 grafts, 7 were complete egg cylinders, the other 2 consisted only of EPC.

B. The graft site

Eight grafts lay in the usual position, in the right supraumbilical coelom. The 9th, No.28 lay further caudally at the level of the anterior intestinal portal, and this had altered the pattern of invasion by bringing the EPC in contact with a different group of host organs. Each of the grafts had gained attachment by eroding coelomic mesothelium. The most frequent site of attachment was the dorsal surface of the right lobe of the liver (7/9), in combination with two or three of: right body wall (6), dorsal coelomic wall (6) lung bud (4), and mesentery (2). Of the 2 not attached to the liver, 1 was attached to the dorsal coelomic wall and the lung bud, the other solely to the midgut mesentery.

C. Invasive behaviour of grafts

Although, in general, the extent of local invasion of host tissues had progressed beyond that seen in the 12-hour group, there was significant variation within this group, as in earlier groups. Examples of the two extremes of invasive behaviour are given first, before a more general

appraisal.

(a) More advanced invasion

No.5. The graft, an EPC, had been placed in the upper right coelom. It had become fragmented, probably at the time of transplantation, so that separate parts of it were attached to a variety of host sites. A minor fragment was attached to the right lung bud; a larger piece had eroded the right body wall (opening large tributaries of the posterior cardinal vein) and the dorsal surface of the liver (opening the ductus venosus). The two veins and coelom formed a common space within which lay the graft, partly attached, partly free. However, the aorta was intact (Fig. 32).

In the 1 in 10 series, some 25 individual giant cells were found, which had spread to a variety of sites. Spread appeared to have occurred by two routes:

(i) by direct migration around the outer surface of the aorta, and, further afield, to a position beneath the epidermis (Fig. 33), (4 cells) and in the right limb bud (4 cells). The cells had left no trace of their migratory route through the mesenchyme.

(ii) by vascular dissemination:

Examples were seen of individual giant cells which were adherent to the luminal surface of the endothelium lining a variety of vessels: liver sinusoids; in the right limb bud (1 cell); tributaries of the right posterior

cardinal vein (Fig. 32) (several cells); and the left pulmonary venous plexus (Figs. 34a and b). In this last, a single giant cell lay largely within the vessel, but a cytoplasmic process protruded through the endothelium. It is obviously not possible to be certain whether this cell was in process of entering or leaving the vessel, but vascular dissemination is favoured.

A curious finding was of four separate examples of individual giant cells lying closely adjacent to ganglia of the sympathetic chains (on both right and left sides, Fig. 35; on the right, Fig. 36 and on the left, Figs. 37a and b). These cells could have reached their position in one of two ways: by direct migration around the outside of the aorta or by retrograde spread in tributaries of the posterior cardinal vein, which form rich plexuses around each ganglion. None of the giant cells was seen, unequivocally, to lie <u>within</u> a blood vessel. On the other hand, several pieces of circumstantial evidence support the idea of dissemination by a vascular route: each of the cells lay at some distance from the aorta, whereas most cells migrating around the aorta tended to follow its outer surface closely; three of the four giant cells were found on the <u>medial</u> side of the ganglion, instead of on the lateral side which might have been expected if they had encountered the ganglion during periaortic spread; the appearance (Fig. 36) of a giant cell adherent to the lining of a tributary of the right posterior cardinal vein near to the position of a giant cell lodged near to the sympathetic

ganglion, at least suggests vascular dissemination; and, finally, careful search of the sections failed to reveal any giant cells in a comparable position, dorso-lateral to the aorta, at levels between ganglia. (See Discussion for further consideration).

Whatever the route of dissemination, it was noteworthy that of the various tissues with which giant cells came into close contact - primitive mesenchyme, epidermis, sympathetic ganglion cells - none showed signs of histolysis or cytolysis. As in some of the 12-hour group, however, it was noteworthy that where a giant cell had come to lie beneath the epidermis, this was raised like a blister.

No.26. In this experiment, internal bleeding was seen two hours before termination, at 16 hours after grafting. The graft was an EPC, which lay in the upper right coelom, attached to dorsal coelomic wall, the right body wall and the dorsal surface of the liver. The aorta, right posterior cardinal vein and ductus venosus had all been eroded and there was bleeding into the coelom. The omental bursa was distended with blood (Fig. 38).

As in No.5, spread of individual giant cells appeared to have occurred by two routes:

i) by direct migration, through mesenchyme, around the outer surface of the aorta (Fig. 38), and to a position beneath the epidermis of the right body wall.

ii) by vascular dissemination:

There were numerous examples of giant cells within liver sinusoids, attached to or penetrating the endothelial covering of plates of hepatocytes (Fig. 39).

Again, as in No.5, there were several examples (5 in all, in the 1/10 series), of single giant cells immediately adjacent to sympathetic ganglia (Figs. 40a, 40b and 41). (See Discussion for further consideration).

Again, it was noteworthy that the various cells in close contact with giant cells - epidermis, hepatocytes, sympathetic ganglion cells - all appeared normal. This absence of cytolytic and histolytic effect is shown particularly clearly in Fig. 41, in which a giant cell lies in contact with undamaged aortic endothelium and sympathoblasts.

(b) Less advanced invasion

Two specimens in the group showed minimal invasive activity - Nos. 43 and 44.

No.43: This embryo was alive and apparently healthy, with no sign of bleeding, when the experiment was terminated, at 16 hours.

In serial sections, the graft was found to be an egg cylinder. The EPC was situated rostrally, and had established very early attachment to the dorsal coelomic wall. Figure 42 shows several interesting features:

i. the EPC includes three types of trophoblast cell: untransformed trophoblast cells, small and closely packed;

'typical' giant cells, with very large nuclei and abundant, rather dense cytoplasm and large cells with clear, vacuolated cytoplasm and shrunken, angular nuclei.

ii. Early invasion of mesothelium. A typical giant cell appears to have just penetrated the mesothelium, which is intact on each side of the point of attachment. A nearby section at higher power (Fig. 43) shows the maximal extent of penetration of a tongue of 'typical' giant cells, extending dorsally towards the aortic endothelium, which was just breached, but not sufficiently to allow any bleeding. The leading giant cell contained a few phagocytosed nuclear remnants. On either side of this tongue was a cluster of vacuolated giant cells; even where these made direct contact with mesothelium, this appeared normal. A few migrant vacuolated giant cells were also seen in the dorsal mesentery and within a mesonephric glomerulus. (Fig. 44). There was no periaortic spread and despite careful search, no examples were found of giant cells which had migrated further from the main graft. In all respects, therefore, this embryo conforms more closely to those of the "early invasion" group at 8 hours, and does not show the further progression of migration which one might have expected at 16 hours.

No.44. In this experiment, the graft was an egg cylinder, and bleeding was observed in the embryo in situ about 1 hour before termination.

The EPC was found to be facing ventrally and a conical

mass of typical giant cells had eroded the dorsal wall of the liver (Fig. 45a). There was a very narrow zone of damaged hepatocytes at the interface with the EPC, one of the few examples found of such damage. Invasion had opened up liver sinusoids and allowed retrograde spread of numerous vacuolated giant cells into the sinusoids; most were attached to hepatic plates, whose cells appeared normal. No evidence was found of more distant migration of giant cells.

This specimen was remarkable not only for the fact that the graft showed early attachment, although it had been in place for 16 hours. It also showed what is assumed to be the result of erosion by primary giant cells. The right lung bud showed an arc of erosion, apparently where it came in contact with the visceral layer of the yolk sac. (Fig. 45a). Careful inspection at high power, however, showed that contact was in fact with the parietal layer of the yolk sac and Reichert's membrane (Fig. 45b). It has to be an assumption that primary giant cells were responsible, since none were found at the interface, nor migrant from it (see Discussion).

The behaviour of the remaining 5 specimens at 16 hours fell, in general, between that of the most and least invasive pairs just described. Some showed unusual features, which merit further description.

No.47. The graft was an intact egg cylinder in which the embryo had progressed to the neural fold stage (Fig. 46).

It lay much further caudally than usual, at the anterior intestinal portal. The visceral yolk sac endoderm projected dorsally into the intraembryonic coelom, lying close to, and in one place in actual contact with, the coelomic mesothelium, which was clearly intact at this level.

The EPC lay further caudally in the serial sections, and had made contact with the mid-gut mesentery (Fig. 47). A mass of giant cells, some "typical" and some vacuolated, had eroded the mesentery and the right wall of the vitelline artery, close to its origin from the aorta. At another level, the vitelline/portal vein had also been eroded (Fig. 48). Bleeding from both artery and vein had been limited because the mass of trophoblast cells had partially occluded the defects.

From the main mass of the EPC, a discontinuous column of vacuolated giant cells had invaded the dorsal mesentery and extended rostrally, following the outer surface of the portal vein. A very few giant cells had spread dorsally round the outside of the aorta and a single vacuolated giant cell was found outside the dorsal wall of the right posterior cardinal vein. All these appeared to be examples of the tendency of giant cells to migrate along the surfaces of epithelia (most probably along the basement membranes (See Discussion)). A few vacuolated giant cells, however, showed an apparently different pattern of behaviour. They were found in the mesenchyme of the right

body wall, close to, but usually quite clearly, outside, the right umbilical vein. There was no clue as to how they had reached this position, since no break was found in the mesothelium lining the right body wall. One possibility is that they had spread from the right vitelline vein (into which giant cells were found projecting) into the right umbilical vein, through the vitello-umbilical anastomosis, and had subsequently migrated outwards from the umbilical vein.

Two specimens showed extensive local erosion of host tissues, but no evidence of distant migration of giant cells.

No.41: Bleeding was observed about 1 hour before termination. The graft was an egg cylinder, in the usual position, and the sections showed extensive erosion of the right body wall, the right lung bud and the liver. This had resulted in opening up of the right posterior cardinal vein and the ductus venosus, which formed with one another and with the coelom a continuous blood-filled space. The aorta had not been eroded, since giant cells had not made contact with it.

No.42: This embryo was killed at 16 hours, at the first sign of bleeding. The graft was an EC which had eroded the ventral wall of the dorsal aorta, but the defect was largely plugged by the invading trophoblast. The liver had also been eroded, and the ductus venosus opened, as had a large vein in the right body wall. Despite the extensive

local damage, no giant cells had migrated far from the graft. Some vacuolated cells had migrated into the dorsal mesentery; two had made intimate contact with the thickened mesothelium, marking the site of the developing spleen (Fig. 49). The mesothelial cells appeared undamaged.

No.46: This graft was an egg cylinder, but the serial sections showed it to be atypical, since it included only a small part of the EPC. The rest had presumably been amputated in preparing the graft. Only a few giant cells were present, but sufficient to have eroded the aorta (in which the defect was plugged) the right body wall and the liver. No distant migrants were found, but this may not be significant, in view of the small mass of EPC which was grafted.

We can now consider the invasion of particular organs and tissues:

1. **Invasion of blood vessels**

a. Dorsal aorta

The aorta had been invaded in 5 of the 9 embryos, a smaller proportion than that seen at 12 hours (9/10). No particular significance is attached to this difference, which was clearly related to chance variation in the position of the active invasive agent, the EPC.

b. Veins

The frequency of invasion of major veins (7 of the 9

showed invasion of ductus venosus and/or cardinal veins; one showed invasion of portal vein) was higher than that seen at 12 hours (5/10). Again, however, the more frequent erosion of the ductus venosus appeared to result from the chance variation in position of the EPC, bringing it more frequently in contact with the liver. The more frequent involvement of the posterior cardinal vein (or, more precisely, of one or more of its large tributaries in the right body wall) may have been the result of deeper invasion of the body wall.

Invasion of these veins had interesting consequences for further dissemination of trophoblastic giant cells.

(i) Four of the 7 embryos with invasion of ductus venosus each showed the presence of many giant cells within the sinusoids of the liver, almost invariably adherent to the endothelial covering of plates of hepatocytes. The surface of the liver plate was often indented by the giant cell, and it was difficult to be certain whether or not the endothelium was intact. What was clear, however, was that the hepatocytes in contact with a single migrant giant cell appeared normal histologically. It is noteworthy that all the migrant giant cells within liver sinusoids were of the large, clear vacuolated variety. Where "typical" giant cells came in contact with liver (e.g. at the site of initial attachment in No.44, Fig. 45a) a narrow arc of hepatocytes at the interzone included damaged cells.

(ii) The numerous examples of single (vacuolated) giant cells in close contact with sympathetic ganglia, in embryos

5 and 26, have already been referred to. As indicated, it seems likely that they had been disseminated by a retrograde venous route.

2. **Invasion of other tissues**

It had been expected that the 16-hour group would show more examples of distant migration of individual giant cells than seen at 12 hours. This, however, was not uniformly so. Even in those embryos which showed the most extensive invasion (Nos. 5 and 26), the total numbers of distant migrants were small. The remaining embryos of the group each showed local migration of a few isolated giant cells, but few, if any, which had migrated further.

3. **Possible mechanisms of invasion**

Study of this group confirmed the findings at 8 and 12 hours: that tissues with which "typical" giant cells came in direct contact showed erosion, but it did not contribute much further information on the mechanism.

Distant migration had brought individual giant cells into contact with a wide variety of tissues, including hepatocytes, sympathetic ganglion cells, the mesothelial precursor of the spleen and the epidermis. None showed histolytic or cytolytic damage, although there were some examples of separation of epidermis from the underlying mesenchyme, to form a localised blister.

As at earlier stages, migrant giant cells left no trace in the mesenchyme of the route which they had followed.

Does invasion involve phagocytosis by trophoblast?

A convincing answer - positive or negative - to this question can only be provided under a rather special set of circumstances, in which trophoblast is just beginning to "erode" an epithelial tissue, or where its cytoplasm is in intimate contact with mesenchymal cells. When these criteria were satisfied, there was no clear evidence of phagocytic activity.

Summary of findings in 16-hour group

1. All nine grafts (2 EPC and 7 EC) were attached to host tissue by "erosion" of coelomic mesothelium. The site of attachment coincided with the position of the EPC. In one embryo, attachment had, in addition, apparently been effected by primary giant cells.
2. Local spread of "tongues" of trophoblast cells occurred in all 9 experiments. Because of chance variation in the orientation of the EPC, the liver was more frequently invaded, and the dorsal coelomic wall (and aorta) less frequently invaded, than at 12 hours.
3. Retrograde vascular dissemination (in liver sinusoids and via the posterior cardinal vein) was seen in 4 of the 9 embryos.
4. Two embryos (Nos. 5 and 26) each showed several examples of individual giant cells lying close to ganglia of the sympathetic chains. Their route of travel is uncertain.
5. Distant spread, by migration through mesenchyme, was seen in only 4 of the 9 embryos.

6. Distant migrants were mostly of the large pale vacuolated type: host cells with which they came in contact seemed undamaged, although the epidermis was often elevated as a localized blister.
7. Local erosion was effected by "typical" giant cells; the mechanism was not clear, but seemed not to include histolysis, cytolysis or phagocytosis.

Synopsis of Results - 16 hour group

No.	Graft	Attachment	Erosion of Mesothelium	Invasion	Vascular Erosion	Phago-cytosis
5	E.P.C.	R.B.W. R.Lung bud Liver	+	L:tongues D:individ.GC: Direct migrat-ion and via veins	R.C.C.V. D.V.	-
26	E.P.C.	Dorsal R.B.W. Liver	+	L:tongues D:individ.GC: Direct migrat-ation and via liver sinusoids	Aorta R.P.C.V. D.V.	+ (red cells only)
28	Egg Cylinder	Dorsal R.B.W. Mesentery Liver	+	L:tongues D:Individ.G.C: Direct migrat-ion and via liver sinusoids	Aorta D.V.	+ (degenerated red cells only)
41	Egg Cylinder	Dorsal R.Lung bud Liver Mesent.	+	L:tongues	R.P.C.V. D.V.	+ (red cells only)
42	Egg Cylinder	Dorsal R.B.W. Liver	+	L:tongues	Aorta R.P.C.V. D.V.	-
43	Egg Cylinder	Dorsal Lung bud Mesogastrium	+	L:tongues	Aorta (minimal)	-
44	Egg Cylinder	R.B.W. Liver Lung bud	+	L:tongues D:indiv.G.C. into liver	D.V.	-
46	Egg Cylinder (Small EPC)	Dorsal R.B.W. Liver	+	L:tongues	Aorta D.V.	+ (red cell only)
47	Egg Cylinder	Mesentery	+	L:tongues D:few indiv. G.C. around aorta and in R.B.W.	Vitelline artery Portal vein	-
Total= 9	E.P.C.=2 E.C.=7	Dorsal: 6 Liver: 7 Lung: 4 R.B.W.: 6 Mesent: 4	9	Local: 9 D:direct:4 veins: 3	Aorta: 5 Vit.art:1 D.V. :6 C.V. :4 Portal vein:1	Red cells only: 4

R(L) PCV = right (left) post. cardinal vein; D.V. = ductus venosus; L = local; D = distant; G.C. = giant cell; E.P.C. = ectoplacental cone; R.B.W. = right body wall; mesent. = mesentery; dorsal = dorsal body wall.

5. **24-HOUR GROUP**

Only 3 embryos in this group survived for this length of time. It had been intended to examine similar numbers at each time interval. All embryos were inspected in situ in the egg, at intervals after grafting. When signs of internal bleeding were seen, the experiment was terminated usually within an hour or so. This increased the numbers in the earlier groups, but obviously reduced those available at 24 hours. This is made clear by the following Table, which shows the number of embryos in which bleeding had/had not begun before termination.

Table 2: Number of embryos bleeding/not bleeding at termination.

Group	No. bleeding	No. not bleeding
8 hour	9 (60%)	6
12 hour	10 (91%)	1
16 hour	9 (100%)	0
24 hour	3 (100%)	0

In view of the early onset of bleeding in the other groups, it is perhaps surprising that any embryos survived for 24 hours. Study of the 3 survivors showed that 2 of them were atypical in that the graft had not been successfully transplanted in the usual place, or it was unusually small.

The results from this group do not, therefore, fit into the general pattern established for the earlier stages. They are included, however, because they help to

expand and illuminate the earlier findings. The findings for each embryo are presented separately.

No.3

This was the only one of the three in which the graft, an EPC, had been placed in the usual position. Some bleeding was first observed about 2 hours before the experiment was terminated at 24 hours. Figure 50 shows a typical section through the graft: it was attached to dorsal coelomic wall, the right lung bud, the right body wall, and the dorsum of the liver. Each of these sites had been eroded by the graft. The right ventro-lateral wall of the aorta was missing, but bleeding was limited because the defect was effectively sealed by the trophoblast.

Ventrally the graft bulged directly into a large vascular space within the liver, essentially an enlarged ductus venosus. The right lung bud had been extensively eroded, the right body wall much less so. The right posterior cardinal vein had not been breached, but a few small veins in the right body wall had been invaded.

Despite extensive local erosion of host tissues by the main graft, only 52 individual migrant giant cells were found in serial sections totalling 900μm in thickness. Most had spread into mesenchyme close to the main graft: into the dorsal mesentery (meso-oesophagus, -gastrium and -duodenum); around the ventral and lateral surfaces of the aorta and of the two posterior cardinal veins. Exceptionally, one was seen intercalated in the endothelium of the dorsal wall of the aorta (Fig. 51), but it was

impossible to tell whether it was invading inwards, towards the lumen, or outwards.

Further afield, a surprisingly small number of giant cells had seeded from the main graft to become attached to hepatocyte cords (Fig. 52). As usual, the adjacent hepatocytes appeared normal. The small number of giant cells was surprising because a large area of the main graft faced directly into the ductus venosus, but its cells had largely retained their epithelial character, so that few had been shed.

Further afield again, three examples were found of giant cells close to sympathetic ganglia, all on the right side. Figure 53 shows a single giant cell intercalated at one pole into the endothelium of a dorsal intersegmental artery, while its other pole is intimately related to the ventro-medial pole of a sympathetic ganglion. A tributary of the right posterior cardinal vein is close by. As in the case of previous examples, (at 16 hrs), this one provided no clear evidence of how the giant cell had reached this position, whether by a vascular route - arterial or venous - or by direct migration through the mesenchyme. The second example is illustrated in Fig. 54, which shows a single giant cell virtually embedded in a sympathetic ganglion further down the chain. The third example (Fig. 55) is of a giant cell which clearly lay <u>within</u> a tributary of the right posterior cardinal vein, and closely adjacent to a few rather scattered sympathetic ganglion cells. It is noteworthy that none of these three

examples shows evidence of histolytic or cytolytic damage to the nerve cells, despite their very close proximity to giant cells.

Two single giant cells were found in the heart wall, one in that of the sinus venosus (Fig. 56) and the other in that of the right atrium (Fig. 57). It seems likely that these had reached the heart in venous blood and "seeded out" into the heart wall.

Two individual giant cells seemed to have spread within the coelom: one had apparently "seeded out" in the wall of the pericardium (Figs. 58a and b). The other was attached to the mesothelium of the left face of the dorsal mesogastrium (Figs. 59a and b) where it was adherent to the apical ends of 2 or 3 mesothelial cells, which appeared undamaged.

Finally, one giant cell was found at the epidermal-dermal interface, at the root of the left limb bud (Fig. 60). It was not within a blood vessel, nor was there one nearby, so it must be assumed to have migrated through mesenchyme. On this occasion, there was no separation between epidermis and mesenchyme, to form a blister.

Taken together, the results in this experiment indicate erosion of tissues with which the main graft made contact; local invasion by small numbers of migrant giant cells; somewhat more distant spread of a few giant cells, some by "seeding" from the venous blood and, finally, only a single example of a giant cell which had migrated for the maximum horizontal distance, to the epidermis on the side

of the trunk away from the graft site.

No.25

The graft was an EPC, and bleeding was noticed about 1 hour before termination. The graft had an unusual shape and position. It formed an elongated flattened sheet of giant cells, the lower end of which lay in contact with the right face of the upper end of the mid-gut mesentery, which had been eroded, together with part of the right wall of the vitelline artery, and of the vitelline/portal veins. Bleeding had been limited because giant cells plugged the defect (Fig. 61). It is noteworthy that those giant cells which occupied the defect, and therefore were responsible for having made it, showed no evidence of recent phagocytosis, while those further away (to the reader's left) were packed with phagosomes.

Traced rostrally through the series of sections, the sheet of giant cells was adherent to the inner surface of the right body wall, whose mesothelial lining it had replaced. The sheet formed the right boundary of a large vascular space, bounded, ventrally by a thin sheet of hepatocytes, and to the left by the eroded right face of the mesoduodenum (Fig. 62). This vascular space represents a distorted ductus venosus, greatly enlarged by erosion of the liver by giant cells. The ventral end of the sheet of giant cells had invaded the right umbilical vein (Fig. 63).

Single giant cells had seeded extensively from the main sheet into the enlarged ductus venosus: they were

seen in large numbers adherent to the hepatocyte sheet which bounded the space ventrally, and within small veins in the dorsal mesoduodenum (Fig. 64).

Systematic search of the serial sections showed several individual migrant giant cells, in positions similar to those in earlier experiments. Eight were found closely adjacent to sympathetic ganglia, 6 of them on the right (the same side as the graft), 2 on the left. A further 4 were found in a comparable position, but associated with an intersegmental artery (or vein) rather than with the ganglion itself. Of the total of 12 giant cells in this position, 4 were probably within a blood vessel, the others were almost certainly not. Selected examples from this group of cells are shown in Figs. 65, 66, 67, 68a,b and 69.

The second largest group of individual migrant giant cells, 7 in all, had spread dorsally and rostrally on the outer surface of the aorta. One (unusually) was found adherent to the inner surface of the aorta (Fig. 70).

A total of 5 individual cells were found inside vessels in the limb bud (4 on the right, 1 on the left) and in a body wall vessel near the root of the right limb bud. For those on the right side it seems likely that they reached a vessel in the body wall, and then those in the limb bud, by retrograde venous dissemination from the right umbilical vein. For those in the left limb bud, the route taken is less certain, but again retrograde venous spread from the vitelline vein, to the left umbilical vein, to

body wall vein, to limb bud vein is considered possible.

The possibility of anterograde dissemination of giant cells through the chick's circulation was considered in this, and previous examples, and evidence for and against was looked for:

(i) No giant cells were seen within the lumen of the heart (although 2 were found in the wall of the sinus venosus and the right atrium in Experiment 3), in aortic arch arteries or in other arteries, such as those supplying the mesonephros;

(ii) most giant cells found in, or near, veins were present on the right side of the body, the side of the graft. Had dissemination been by an arterial route, they would have been expected to be more equally distributed to right and left;

(iii) The limb vessels in which giant cells were seen were large vessels at the root of the limb. To judge by the size of giant cells relative to these vessels, it seemed unlikely that they had traversed smaller, more peripheral vessels first.

The question of the route followed by giant cells is considered important because of its bearing on their "invasiveness". Passive transport in the blood cannot be equated to active invasive movement through the mesenchyme. In this embryo only 7 of about 23 giant cells found at a distance from the graft were regarded as truly "migrant", and these were all found around the aorta, close to its

endothelial lining.

No.15

In this experiment, the graft was only a small fragment of EPC, and it did not lie within the abdominal coelom. The pericardial wall was missing in the sections, whether through agenesis or due to damage when the embryo was removed at termination, is not known. The graft therefore appeared to be attached to the ventral free edge of the right body wall, immediately adjacent to the right umbilical vein (Fig. 71). Giant cells had invaded the umbilical vein (Fig. 72) whence they had spread into (a) right body wall vein (in the same figure), (b) liver sinusoids (Figs. 73a,b and 74), and (c) veins at the root of the right limb bud (Figs. 75a and b). Giant cells were also found further afield: in the left umbilical vein (Fig. 76a and b) and near a tributary of the right posterior cardinal vein and a spinal nerve (Fig. 77). All these examples of apparent "migration" of giant cells could reasonably be accounted for by spread via veins. Distant spread by active migration was not seen, though a few giant cells had migrated for about 125μm along the deep surface of body wall ectoderm (Fig. 78).

In view of the histological findings in this embryo, it is not surprising that it was healthy, and showed little evidence of bleeding at the time of termination. Its healthy survival for 24 hours is attributable to the small size of the graft and the fact that there was only localized erosion of a single vessel (the right umbilical

vein) and this defect was sealed, in part, by the EPC.

Two features are particularly noteworthy:

1. The absence of <u>active</u> migratory spread of giant cells (as opposed to <u>passive</u> dissemination via blood vessels) and
2. Once again, the absence of signs of damage by giant cells of the various tissues with which they came in contact, notably the myocardium, vascular endothelium hepatocytes, and sympathoblasts.

CONTROLS

1. **<u>THE CONCEPTUS IN UTERO</u>**

a. **7-day**

Figures 79a and b give a general view of the 7-day conceptus. It lies within an ovoid implantation cavity which measures about 0.9 mm greatest width x 0.4 mm along the mesometrial-antimesometrial axis, and is situated eccentrically in the decidual swelling, separated from, and peripheral to, the remains of the uterine cavity. The conceptus itself is roughly circular in cross-sectional profile, and measures about 0.4 mm in greatest diameter. It consists of two epithelial layers, an inner of ectoderm and an outer of visceral endoderm, with some interposed mesoderm at the anti-mesometrial pole. At the mesometrial pole, the ectoplacental cone is forming from the extraembryonic ectoderm. It is a rather mushroom-shaped mass (Fig. 80) consisting mostly of small, compactly

arranged basophilic cells; some of those more peripherally placed are larger, with pale vacuolated cytoplasm and an occasional secondary giant cell is seen.

At the margin of the EPC, the visceral yolk sac endoderm becomes continuous with the parietal endoderm. This is a discontinuous layer of rounded cells, resting on Reichert's membrane.

The implantation cavity is lined by a discontinuous layer of primary giant cells. These lie close to, or in contact with, a thin layer of mostly necrotic decidual cells (Fig. 81). Outside this layer, the decidua consists of densely packed large cells, with pale vacuolated cytoplasm indicating the former presence of glycogen, dissolved in tissue preparation. These are referred to subsequently as "glycogen-rich" cells. The decidua is being interpenetrated by many large, irregular sinusoidal vessels, some of which have been opened by primary giant cells, allowing maternal blood to enter the implantation cavity. (Fig. 82).

The ectoplacental cone, also bathed by maternal blood, faces towards the remnant of the uterine lumen, but is separated from it by a thin sheet of maternal tissue containing few surviving cells (Fig. 80). The uterine cavity contains red blood cells, polymorphonuclear leucocytes, and cellular debris. It is noteworthy that little remains of the uterine epithelium, even on the <u>mesometrial</u> side of the uterine cavity, even though this lies some 0.4 mm from the ectoplacental cone.

Careful search was made for invasion of maternal tissue by giant cells, primary and secondary. There was some evidence of very limited local invasion of the decidua by primary giant cells, but none was found further than a short distance (< 50μm) from the limits of the implantation cavity. A sample of 20 sections of the 1:10 series (representing a total thickness of 1 mm) was searched systematically for the presence of trophoblastic giant cells within maternal blood vessels. Only three were found. One lay within a thin-walled vessel (probably a vein) at the endometrial-myometrial junction. (Fig. 83). The other 2 were situated in arterioles, in the mesometrial half of the decidual swelling (Fig. 84).

This study of the 7d conceptus in situ was carried out for several reasons:

i. to define the nature of the material transplanted to the chick coelom. By comparing the appearance of the conceptus in utero, with what was seen in serial sections of the graft in the chick, it became clear that in most cases, an "intact egg cylinder" included everything seen in the conceptus in utero with the exception, in all but a very few cases, of the parietal endoderm and Reichert's membrane, and primary giant cells.

Inevitably the graft occasionally included parts of one or more of: parietal endoderm; primary giant cells; maternal red cells and leucocytes; necrotic decidual cells. It was also evident that when giant cells recovered in the

chick were found to contain phagocytosed red cells and cellular debris, these could well have been of maternal origin: (Fig. 85) shows two primary cells, lying within the implantation cavity, their cytoplasm packed with phagocytosed red blood cells.

(ii) To determine the extent of local invasion, and destruction of endometrial tissue associated with, and presumptively attributable to, primary and secondary giant cells of the conceptus. Two concentric zones surrounded the conceptus: the inner, about 0.1 - 0.15 mm in diameter, lay between the parietal endoderm and Reichert's membrane on the one hand, and the ring of primary giant cells in contact with decidua on the other. This zone contained maternal blood. Its thickness gives some measure of the amount of decidua lost between the time of initial attachment of the conceptus (about 5d) and the time when the implanted conceptus was examined at 7d. The outer zone consisted of the ring of recently necrotic decidua in contact with giant cells. This outer zone (Fig. 79b) measured about 300 μm in thickness. Its cells had pyknotic nuclei, and their cytoplasm was ragged and strongly eosinophilic (Fig. 81). The giant cells in contact with it contained phagocytosed red blood cells, but only very occasional (nucleated) decidual cells. Externally, this recently necrotic zone gave way quite sharply to what appeared to be healthy, glycogen-rich decidual cells.

(iii) To define the nature of the grafts of decidual tissue transplanted as controls.

These grafts were prepared from decidual swellings at 7d p.c., and consisted of small masses, cut by cataract knives, usually from the anti-mesometrial side of the swelling, although subsequent histological study of the grafts, in situ in the chick, suggested that, occasionally, some mesometrial decidua had been inadvertently included. The three possible varieties of decidua are illustrated in Figs. 82, 86 and 87.

Figure 82 shows large, pale, vacuolated 'glycogen-rich' decidual cells.

Figure 86, from the edge of the antimesometrial side of the swelling, shows decidual cells at an earlier stage in their development; their enlargement is still incomplete, and their cytoplasm dense, with few, if any vacuoles. Some of the most peripheral cells are spindle-shaped, (non-decidualized) stromal cells.

Figure 87, from the mesometrial decidua, shows an admixture of the first two of these three cell types, with an additional noteworthy feature, namely that the venous sinusoids contain large numbers of polymorphonuclear leucocytes.

b. **7.5d**

Figure 88 gives a general view of the conceptus at 7.5d p.c. It lies in the implantation cavity, which is situated towards the anti-mesometrial side of the centre of the decidual swelling. The limits of the implantation cavity are defined by a discontinuous line of giant cells

arranged roughly in a circle (in sectional profile) of about 0.9 mm in diameter, with the embryo itself sectioned transversely. The three germ layers of the embryo are well-established and the neural folds are forming. When the embryonic endoderm is traced to the edges of the embryo it becomes continuous, on each side, with the visceral layer of yolk sac endoderm, a tall columnar epithelium, which in turn becomes continuous with the parietal layer of yolk sac endoderm, a discontinuous layer of small rounded cells lying on Reichert's membrane. Four cavities are present within the conceptus: the cavity of the inverted yolk sac, between visceral and parietal endoderm; the amniotic cavity; the exocoelom and the ectoplacental cavity, lined by extraembryonic ectoderm.

The ectoplacental cone lies on the mesometrial side of the conceptus (Fig. 89). It consists of an epithelial plate, forming the roof of the ectoplacental cavity and containing many layers of compactly arranged small basophilic cells and, extending mesometrially from this, widely spaced, irregular anastomosing cords of larger cells. As these cords are traced towards the mesometrium, their cells are seen to contain large pale vacuoles. A few are of giant cell dimensions. The interstices between the trophoblastic cords are filled with maternal blood and some of the trophoblast cells have phagocytosed red blood cells.

Proceeding further mesometrially, the remains of the uterine cavity is seen. It contains maternal blood, with

large numbers of polymorphonuclear leucocytes. A few remnants of the original uterine epithelium may be seen on the mesometrial side of the cavity.

The ectoplacental cone occupies the mesometrial sector of the perimeter of the conceptus. The rest is made of primary giant cells derived from the mural trophoblast (Fig. 90). Most of these lie flattened tangentially to the decidua. Detailed search was made of the 1:10 serial sections for evidence of invasion of the decidua by giant cells.

(i) **Local invasion**

In a sample of 29 sections of the 1 in 10 series (a thickness of about 1.5 mm) only a few examples were found of giant cells which had penetrated the zone of partially necrotic decidua (about 200μm in thickness) surrounding the implantation cavity (e.g. Figs. 91a and b) and each of them seemed to lie inside an endometrial sinusoid, which opened freely into the implantation cavity.

(ii) **Distant invasion**

By contrast, in the same 29 sections, no fewer than 21 giant cells were found within the lumen of decidual arteries/arterioles. Selected examples of these are shown in Figures 92 and 93. All lay in arterioles in the mesometrial side of the swelling; all were large, basophilic, 'typical' giant cells; none were found outside arteries.

Enlargement of the implantation cavity

The extent of growth (in diameter) of the conceptus,

and of the implantation cavity in which it lies, between 7 and 7.5d is illustrated by comparison of Figs. 79b and 94, each at the same magnification. On each photograph the following measurements were made: (i) maximum diameter of conceptus: this was taken as the maximum diameter of the yolk sac, measured through the sectional profile of the parietal layer of endoderm and Reichert's membrane.
(ii) maximum diameter of implantation cavity: measured between the inner limits of the zone of necrotic decidual cells, in contact with primary giant cells.

The results are shown in Table 3 below.

Table 3: Diameters of 7 and 7.5 day hamster conceptuses and implantation cavities.

Age of Conceptus	Maximum Diameter of Conceptus	Maximum Diameter of Implantation Cavity
7d	0.38 mm	0.9 mm
7.5d	1.1 mm	1.25 mm

As outlined in the Introduction, enlargement of the implantation cavity occurs by centrifugal spread of the zone of necrosis in the decidua, which advances at the expense of the zone of healthy vacuolated, glycogen-rich decidual cells lying peripherally to it. This process is made clear by comparison of Figs. 82 and 95, which show the two zones at 7d and 7.5d respectively. The enlargement of the implantation cavity during this period of 0.5d is achieved by the necrosis of a shell of decidua about 0.17

mm in thickness (i.e. half the difference between 1.25 mm and 0.9 mm). The extent to which decidual necrosis is caused by contact with giant cells, or by an intrinsic process of degeneration, will be examined in the Discussion. At present it should simply be noted that, **if we assume that degeneration results from the activity of trophoblastic giant cells, we might reasonably expect a corresponding amount of tissue necrosis in the tissues of the chick embryo carrying a graft of EPC.**

Close attention was paid to the appearance of the antimesometrial decidua for a second reason, namely to allow comparison of the changes which it undergoes, in situ, between 7 and 7.5d, with those which occur when it is transplanted ectopically to the chick's coelom. Comparison of Figs. 86 (7d) and 96 (7.5d) makes it clear that the antimesometrial decidua <u>at the extreme periphery of the decidual swelling</u> undergoes little, if any, change between 7 and 7.5d in situ: each shows closely packed, non-vacuolated decidual cells. At first glance, comparison of Figs. 82 (7d) and 95 (7.5d), might suggest a similar lack of change in the antimesometrial decidua <u>adjacent to the implantation cavity</u>, until it is recalled that this part of the decidua is succumbing progressively to a centrifugal process of cell necrosis. In the calculation given above it was suggested that a shell of decidua about 0.17 mm in thickness would have undergone necrosis to provide for the enlargement of the implantation cavity between 7 and 7.5d.

This process of necrosis which the decidua undergoes in situ will obviously have to be kept in mind in interpreting the changes which it undergoes when transplanted, the subject of the next section of Results.

2. Grafts of hamster decidua to chick coelom

Grafts taken from decidual swellings at 7d p.c. were placed in the coelom of 3.5d chick embryos in the same manner as used for grafts of EPC and recovered at 8 and 16 hours after grafting.

A. 8 hour grafts

Serial sections of 5 specimens were studied: DC1, 2, 3, 4 and 5.

In each case the graft occupied most, or all, of the upper right coelom and made contact with its four walls: right body wall, dorsal wall, dorsal mesogastrium and liver.

The appearance of the graft itself varied. In DC1, DC4 and DC5, most of its cells were necrotic. In DC2 there was a large central area of healthy cells with a narrow peripheral rim of necrotic cells, while in DC3, there was a small central core of healthy cells and a thick peripheral layer of necrotic ones.

DC4 Figure 98a shows a general view of the graft, which occupied all the available space in the coelom, apart from the extreme right dorso-lateral coelomic bay. Its right margin bulged convexly into the right body wall, and here

most of the coelomic mesothelium was absent. The dorsal, ventral and left walls of the coelom were also encroached upon. Coelomic mesothelium could not be seen on the dorsal surface of the liver. The status of the mesothelium of the dorso-medial coelomic bay was difficult to assess; the normal well-defined sheet of low cuboidal epithelium was replaced by a thick ill-defined zone of dead cells, intermingled with apparently healthy cells of epithelial appearance, which did not, however, in any way resemble decidual cells. (Fig. 98b).

Most of the graft cells were necrotic. The cytoplasm was strongly eosinophilic, and many of the nuclei were either pyknotic or fragmented; the few which were still intact and appeared normal, showed only very weak basophilia. Groups of polymorphonuclear leucocytes were scattered through the graft.

Despite the encroachment of the graft on the walls of the coelom, there was no evidence of the movement of decidual cells away from the graft, nor any invasion of blood vessels. Bleeding was therefore absent.

DC3 The graft occupied all available space in the upper right coelom, apart from a dorso-lateral remnant of the coelomic bay (Fig. 99a). At the centre of the graft was a sharply circumscribed ovoid mass of healthy decidual cells, with normal nuclei and cytoplasm filled with large clear vacuoles. These were clearly "glycogen-rich" cells such as those seen in the more central part of the antimesometrial

decidua (see e.g. Figs. 82 and 95). Around this healthy core was a band of necrotic cells, similar to those already described for DC4, although nuclei in early stages of pyknosis or fragmentation were more commonly seen here. As in DC4, the graft bulged into the surrounding chick tissues. There was no trace of mesothelium on the dorsal surface of the liver (Fig. 99b).

Elsewhere, although a defined (epithelial) mesothelial profile was absent, the boundary between graft and host tissue was marked by a band of more condensed (most probably mesenchymal) cells, giving the appearance of an epithelioid layer. There was no evidence of spread of decidual cells beyond the limits of the graft, no vascular invasion and no bleeding.

DC2 The major (central) part of the graft had survived. As Figures 100a and b show, it consisted mainly of decidual cells similar to those seen at the periphery of the decidual swelling. (Compare with Figs. 86 and 96). Their cytoplasm was abundant and stained with both haematoxylin and eosin. A minority of cells contained large clear vacuoles and corresponded to "glycogen-rich" decidual cells (Figs. 100b and 101). Around this healthy central core was a thin rim of necrotic decidual cells. Where these abutted upon chick tissues, the mesothelium was either present, as over the lateral and dorsal body walls, or a rather disorganized discontinuous layer (as over the dorsal mesogastrium) (Fig. 100b), or absent (as over the liver)

(Fig. 101).

There was no spread of cells away from the main graft, no vascular invasion and no bleeding.

DC1 This graft occupied the usual position (Fig. 102a), but virtually all its cells were dead, with pyknotic or karyorhectic nuclei, although an occasional surviving nucleus was seen. The cytoplasm was very palely eosinophilic and ill-defined, but some, at least, of the cells seemed to have belonged to the "glycogen-rich" category. (Fig. 102b). The coelomic mesothelium facing the graft was either absent or represented by a discontinuous "epithelioid" layer of mesenchymal cells, as in the other specimens in this group. (Fig. 102b).

DC5 The graft lay in the usual place, the upper right coelom. Careful search of the 1/10 series showed no surviving decidual cells, but there were large collections of polymorphonuclear leucocytes and much brick red 'fibrinoid' material, such as that seen in mesometrial decidua, immediately adjacent to the implantation cavity. An unusual feature was the presence in the graft of two or three typical trophoblastic giant cells, embedded within the graft and therefore not in contact with chick tissue (Fig. 103a). The state of the mesothelium was of interest. On the lateral body wall some remained, but most had been eroded. It was absent over the dorsal surface of the liver (Fig. 103a). On the dorsal and medial walls of the coelom it appeared more like an irregular layer of condensed

mesenchyme rather than an organized epithelial layer (Fig. 103b).

No migration or vascular invasion by graft cells was seen and there was no bleeding.

B. **16 hour grafts**

There were two grafts (DC7 and DC8) in this group.

DC7 The graft lay in the usual place, but its narrow rostral end was clearly embedded within the mesenchyme of the lateral body wall, without any investing mesothelium (Fig. 104). At the rostral end none of the grafted cells was healthy; further caudally, there was a central core of apparently healthy cells, whose nuclei, although weakly stained, were not pyknotic or fragmented. Their cytoplasm was pale and vacuolated and the cells were reminiscent of the "glycogen-rich" cells of central decidua. (Fig. 105). The coelomic mesothelium showed the familiar pattern of disruption and, as usual, there was no spread of cells away from the graft, no vascular invasion and no bleeding.

DC8 This presented a most remarkable appearance. The rostral 2/3rd or so of the graft showed cells in an early stage of necrosis. The large nuclei showed early karyorrhexis. The cytoplasm was densely eosinophilic and non-vacuolated, similar to that of decidual cells seen at the periphery of a decidual swelling (Fig. 106). This impression was confirmed by appearances at the caudal end

of the graft, which showed a sharply circumscribed compact ball of mainly healthy decidual cells, similar to those at the periphery of the in situ antimesometrial decidua, (compare Figs. 107 and 96).

Where the necrotic part of the graft met coelomic mesothelium this was either absent (as over the right face of the dorsal mesogastrium) or was somewhat disorganised and discontinuous (as that lining the right body wall) or appeared to show pressure atrophy. This was seen in the dorsal coelomic wall (See Fig. 106) where the cells were smaller and paler than those immediately adjacent which do not abut upon the graft. Where the ball of apparently healthy decidual cells contacted the dorsal coelomic mesothelium, this appeared to be thickened (see Fig. 107).

Again, there was no migration of decidual cells away from the graft, no vascular invasion and no bleeding.

3. **Grafts of egg cylinders, EPC amputated**

There were two grafts (HM12 and HM16) of egg cylinders from which the EPC had been amputated, and each shows some interesting features:

HM12 The abembryonic pole of the conceptus faced dorsally, (Fig.108). It consisted of a compact mass of extraembryonic ectoderm (from which the EPC had presumably been amputated), covered by a thin layer of extraembryonic endoderm. Some giant cell transformation was seen at the dorsolateral pole of the ectoderm, but the giant cells did not come into intimate contact with the coelomic

mesothelium, which was undamaged.

HM16 This specimen showed a somewhat similar situation. The abembryonic pole faced dorsally and a small focus of trophoblastic giant cells was present at its dorsolateral angle (Fig. 109). However, the giant cells were covered by a layer of extraembryonic endoderm, which prevented contact with the coelomic mesothelium, which was undamaged.

4. **Grafts of 5.5d chick limb buds**

Two grafts of 5.5d limb buds LGC1 and LGC2 were examined, after 48h and 72h respectively in the coelom.

LGC1 This graft (Fig. 110) consisted of a core of embryonic mesenchyme, (in which scleroblastemal condensation was just beginning) covered by a layer of ectoderm. It made only very limited contact with the mesothelium: in this particular section there were two very narrow pedicles of tissue linking graft and host. At these points, the mesothelium and ectoderm appeared to be continuous with one another. The graft had apparently been nourished by diffusion of coelomic fluid, since there was no vascular connection with the host. The coelomic mesothelium was not damaged by the presence of the graft. The contact between graft and host was so limited that there was scarcely an opportunity for migration of graft cells - epidermal or mesenchymal - into the host.

LGC2 A similar graft, but resident for 24 hours longer in the coelom, had grown substantially and showed more advanced development of the precartilaginous skeleton. There were two areas of contact between graft and host; at one of these, the coelomic mesothelium and the graft epidermis were absent, allowing graft and host mesenchymes to come into contact (Fig. 111X); at the other (Fig. 111Y), part of the contact was between the two mesenchymes, part between graft epidermis and host mesenchyme. At none of these three areas of contact was there any evidence of invasion of the host tissue by graft cells: the boundaries between graft and host were clearly defined, and there was no vascular invasion and no bleeding.

It is worth emphasizing that there was no sign of cell necrosis in either graft, despite the large size of the grafts and their apparent dependence on coelomic fluid for nutrition and gaseous exchange. Moreover, not only did the grafts survive, but the older one showed continued skeletal development. The possible implications of the better survival of grafts of limb bud than of grafts of decidua will be considered in the Discussion.

DISCUSSION

This study of the behaviour of the egg cylinder of the 7d hamster, when transplanted to the chick's intraembryonic coelom, was designed to assess the invasive capacity of the ectoplacental cone when brought into contact with the soft, compliant tissues of the embryo.

The hamster was chosen because

i. its ectoplacental cone yields a luxuriant growth of trophoblastic giant cells (Billington, 1966).

ii. in pregnancy, its giant cells demonstrate a remarkable migratory activity including migration into uterine arteries (Orsini, 1954; Pijnenborg et al., 1974).

The intraembryonic coelom was chosen as the graft site because it is possible to insert grafts of the size of the egg cylinder (and even larger) without causing operative damage, a great advantage over other sites previously used. This site had been used previously in a series of studies in the Department, which have provided information on the behaviour of trophoblast of mouse (De Souza, 1986), rat and pig (Barber, 1989) and, in some preliminary experiments, of hamster (Al-Janabi, 1988). This approach, using a standard experimental procedure, allows comparison of the "invasive" and "destructive" properties of trophoblast from four different species, three with haemochorial placentae, one with an epitheliochorial placenta.

In the present study, the use of control tissues was limited, partly to allow more detailed study of hamster trophoblast, partly because Al-Janabi (1988) had already made an extensive study of a wide range of control grafts.

The aims of the investigation were set out in the Preamble to the Results and this Discussion will attempt to answer the questions raised there.

I. THE INVASIVE CAPACITY OF HAMSTER TROPHPOBLAST

The first tissue with which the graft came into contact was the coelomic mesothelium. The results provide answers to several questions:

1. Do any of the graft components cause damage to the mesothelium before they make direct contact with it?

a) Ectoplacental cone

Although attachment of the graft had occurred, by 8 hours after grafting a few examples were found of trophoblastic giant cells lying close to, but not directly in contact with, coelomic mesothelium. (see e.g. Fig. 18). There was no evidence, at least at the highest power of the light microscope, of damage to the mesothelium, such as loosening of the epithelial layer (histolysis) or damage to individual cells (pyknosis, cytolysis).

b) Other graft components

i) Visceral endoderm

Many examples were seen in which a layer of visceral endoderm lay close to the coelomic mesothelium (e.g. Figs. 5, 19, 24) or even in direct contact with it (Fig. 46), again without any sign of damage to mesothelium.

2. **Which components of the graft become attached to, and penetrate the mesothelium?**

Most of the earliest stages in attachment were seen at 8 hours, when already 15/15 of the grafts were attached. In each case the site of attachment coincided with the position of the ectoplacental giant cells, indicating that these were the active agents in the process.

By contrast, (with one very interesting exception), attachment of other components of the egg cylinder (principally the visceral endoderm) to the mesothelium was never seen. The exception (see Fig. 45a) was the quite extensive erosion of the right lung bud where it lay (apparently) in contact with a layer of visceral endoderm. On closer inspection, however, it turned out that the parietal endoderm and Reichert's membrane were in fact the contacting tissues and it seems a reasonable presumption that attachment and erosion had been effected by primary giant cells formerly adherent to the external surface of Reichert's membrane.

3. **Was there a preferred site for attachment?**

When the graft was correctly placed at operation, it lay in the upper right portion of the coelom; this was achieved in all the experiments in the 8, 12 and 16 hour groups, in total 34. In 26 of these, attachment was to the dorsal wall of the coelom, either alone (4/34) or in combination with one or more of dorsal mesentery (10/34), liver (13/34), right body wall (14/34) and lung bud (3/34). A total of 8/34 grafts were not attached to the dorsal

coelomic wall, but to one or more of: right body wall, liver or mesentery.

Although a substantial majority of all grafts were attached to the dorsal coelomic wall (26/34), only 4/34 were attached to the dorsal wall alone. The position(s) of attachment always corresponded to the position(s) of contact with the ectoplacental cone, which was usually the most rostral part of an egg cylinder graft, when this was "nudged" into position with the glass rod at the time of grafting. The most likely interpretation is that when the graft was introduced, the EPC followed "the outer side of the curve" i.e. the dorsal wall of the coelom, and that the apparent small preference for attachment to the dorsal wall was a matter of chance in placement of the graft.

4. **How is penetration of the mesothelium effected?**

The best evidence is provided by the 6 examples of early penetration seen in the 8-hour group. In each of these, penetration was very localized, and had been effected by "typical" giant cells (i.e. those with densely stained cytoplasm) rather than by the "vacuolated" variety. Other features common to all of these examples of early penetration were: absence of signs of histolysis or cytolysis of the mesothelium on either side of the breach (indeed some showed thickening of the bordering mesothelium), and absence of unequivocal signs of phagocytosis of host cells.

5. **After penetration of the mesothelium, how does invasion of chick tissues progress?**

Further invasion always involved giant cells and occurred by one or more of three methods:

a. local invasion

b. vascular dissemination

c. intracoelomic spread.

5a. Local invasion by tongues of cells or by individual cells

Local invasion occurred from the site(s) of first attachment and therefore, most frequently into the dorsal coelomic wall. In 8/15 of the 8-hour group, this had led to destruction of the ventral wall of the aorta. The resultant bleeding was restrained by the mass of trophoblast, which presented a concave face towards the opened aorta. The trophoblastic giant cells had been stretched out into a thin sheet by the pressure of blood; the sheet was attached on either side to the margins of the defect in the aortic endothelium. This very characteristic appearance was also seen in most of the embryos of the 12 hour group (see, especially, Fig. 20), and in about half of the 16-hour group. In all, invasion of the ventral wall of the aorta was seen in 26/37 embryos (70%). This should be compared with invasion of one, or more, of the large veins which lie adjacent to the graft site: posterior (or common) cardinal, umbilical veins and the ductus venosus (see Figs. 4 and 5). Venous invasion was seen in 21/37 embryos (57%). These figures might suggest some preference for arterial

invasion, and to support Billington's (1966) suggestion that the preference of migrant hamster trophoblast for arteries rather than veins (both in utero and in ectopic sites) might indicate attraction by the higher oxygen tension in arterial blood. This suggestion is not, however, supported by the present findings. Firstly, the 'preference' is not nearly so marked as that claimed by Billington (1966) and, secondly, if higher oxygen tension were the attraction, invasion in the embryo would in fact be more frequently seen into the umbilical (allantoic) vein, which carries oxygenated blood from the chorio-allantoic membrane, than into the abdominal aorta, which carries partially deoxygenated blood. The small 'preference' for aortic rather than venous invasion seems much more likely to be due to the (chance) greater proximity of the aorta to the EPC, corresponding to the more frequent attachment of the graft to the dorsal wall of the coelom, already noted.

Erosion of the walls of the aorta and major venous channels would have led rapidly to death in more embryos had it not been for the "plugging" of the defect - even if only partially and temporarily - by the trophoblast. "Plugging" of arterial and/or venous channels was seen in 31/37 embryos (84%).

Temporarily, at least, a sheet of flattened trophoblastic giant cells, functioned as an integral part of the vessel wall, reducing bleeding into the coelom.

This implies not only good adhesion between adjacent trophoblast cells themselves which behaved as a cohesive reticular sheet (see Fig. 18), but also between trophoblast and host endothelium. Many of the experiments were terminated 1-2 hours after the onset of bleeding was first noticed. The fact that only 3 embryos survived for 24 hours, taken together with the incidence of bleeding at the earliest stages (See Table 2, 24 hour group p.109), indicates that most chicks, if left undisturbed, would have died from bleeding within the 24 hour period.

Local invasion (i.e. of tissues immediately adjacent to the main part of EPC, was effected either by a cluster of giant cells (so-called tongue) which often appeared to be syncitial, or by individual giant cells. Tongues of cells were best seen in examples of early invasion (see e.g. Figs. 10a, 14, 19 and 43), and they were made up of "typical" giant cells. Tongues did not extend far, and careful study of the serial sections did not suggest the penetration of the host tissues by elongated tapering columns of cells. Indeed at the earliest stages, an invading tongue presented a smooth convex face to the chick tissues (Figs. 7, 8 and 10a).

Local spread of individual giant cells

This was not a prominent feature in the 8-hour group being seen in only three embryos. It was more frequent in the 12-hour group, most commonly, (5/10 embryos), in a dorsal direction, following closely the outer surface of the aorta (despite the fact that the ventral wall of the

aorta had often been completely replaced by trophoblast, which could easily have spread on the internal surface). Very few giant cells were seen adherent to the internal surface, and even these seemed to have penetrated locally from a previously external position.

More distant spread of individual giant cells

At 8 hours, only 8/15 embryos showed more distant spread of individual giant cells. Of the very small number of migrant cells seen, all lay at an epithelial-mesenchymal boundary, in contact with the outer surface of the aorta (or of a large vein), of the notochord or of the deep surface of the ectoderm.

At 12 hours, distant spread was still seen in only 4/10 embryos, and at 16 hours, only 4/9 embryos; in each group the pattern of distribution was similar to that seen at 8 hours. At each stage, the "distant migrants" seemed to belong to the "vacuolated" variety of giant cell: this feature was particularly clear in the 16-hour group.

At each stage, the "distant migrants" appeared to have travelled through the mesenchyme (rather than in blood vessels) and as individual cells: study of the serial sections did not suggest that they represented the remaining tip of a tapering cord of cells, continuous with the main graft. The implication is that this migration involved inherent cell motility (see later).

5b. Intravascular spread of giant cells

Despite frequent erosion of the aorta, and the

occasional penetration of one or more of the large veins, no evidence was found at 8 hours of vascular dissemination of giant cells, none being found inside blood vessels remote from the graft site.

At 12 hours, in a single embryo (No.18), the graft of EPC had eroded the dorsal wall of the liver, opening the ductus venosus, and small numbers of individual giant cells were seen attached to the endothelial covering of hepatic plates. They were all of the vacuolated variety.

At 16 hours, evidence of intravascular dissemination was more frequently seen. Four embryos in which the graft had eroded the dorsal wall of the liver all showed many individual giant cells - again of the vacuolated variety - adherent to endothelium of hepatic vessels. In addition, at this stage, there were examples of giant cells within veins further from the graft: in the right posterior cardinal vein, the left pulmonary plexus and the right limb bud. In most cases, the giant cells were adherent to the inner surface of the endothelium, and it seems likely that they had travelled within the particular blood vessel: whether actively by migration along the endothelium or carried passively (anterogradely or retrogradely) by the blood, it is not possible to be certain.

The curious finding, at 16 hours and at 24 hours of fifteen separate examples of giant cells lying adjacent to ganglia of the sympathetic chains from 2 grafts has already been commented upon in the Results. They are included here as examples of intravascular dissemination because, on

balance, the evidence seems to support that view. The sympathetic ganglia are richly supplied with vessels which drain by intersegmental veins into the cardinal system. Of the total of 20 giant cells which were found in relation to ganglia at 16 and 24 hours, 5 lay within blood vessels, and 1 was intercalated at one pole into the wall of an intersegmental artery. In 2 embryos, other giant cells were also seen within the associated intersegmental vein. The remaining evidence is circumstantial.

The distribution of the giant cells suggests vascular dissemination, unless it is postulated that sympathetic ganglia exert a tropic attraction on giant cells migrating through the peri-aortic mesenchyme: no giant cells were seen in a corresponding position, dorsolateral to the aorta, at interganglionic levels. Moreover had the giant cells migrated to the ganglia by the peri-aortic route, they would have been expected to be found on the lateral side of the ganglia, rather than the medial side, which was usually the case.

The 24-hour group showed abundant examples of dissemination of giant cells by veins. This greater frequency seems to have been related to chance proximity of the EPC to large veins rather than to any "attraction" exerted by veins, or to a progression of the invasive process. In embryo No.25, it will be recalled, the graft lay more caudally than usual, at the anterior intestinal portal, and extensive sheets of giant cells had eroded the

mesentery, right body wall and dorsal surface of the liver, opening up the vitelline/portal veins, the ductus venosus and the right umbilical vein. From these points of penetration of the venous system, giant cells had spread widely: into the liver, the right body wall and both limb buds. The evidence that this spread was passive and in some cases anterograde, in others retrograde, has already been presented in the Results (p.115). The distinction between active migration and passive dissemination is an important one: in this embryo, some 23 giant cells were found remote from the graft site, and of these only 7 were not found within veins and could therefore be safely regarded as true "migrants".

The findings in Embryo No.15 point to a similar conclusion: invasion of the right umbilical vein had led to spread into the liver (and left umbilical vein) and into veins in the right limb bud. Again, few truly "migrant" giant cells were found, and these had spread along the deep surface of body wall ectoderm.

The results from these two embryos are of particular significance. Their survival for 24 hours was almost certainly attributable to the anomalous placing of the graft, which avoided early erosion of the aorta and premature termination of the experiment. The significance of the findings is that, even given 24 hours' survival of the host, active invasion by migrant giant cells was very limited, and that which did occur was confined to fairly local spread around the aortic endothelium and beneath body

wall ectoderm. This limited invasion can hardly be attributed to "resistance" offered by the soft gelatinous mesenchyme of the host embryo, but seems more likely to reflect a limited invasive capacity of the giant cells, at least during the 24 hour period of the experiment.

5C. **Intra-coelomic spread of giant cells**

Since the grafts had been placed within the coelom, transcoelomic spread of individual giant cells might have been expected to occur frequently. In fact, only a single embryo (No.3, 24 hour group) showed clear evidence of this occurrence: 1 cell attached to the inner surface of the left side of the pericardium and 1 cell attached to the dorsal mesogastrium. It may be, of course, that more giant cells had been seeded into the coelom, but failed to gain attachment to the mesothelium and were therefore lost in the coelomic fluid at termination of the experiment.

As already indicated in the Results, the 2 examples of individual giant cells within what could only represent a re-expanded former myocoele, posed the problem of how they arrived there. It is just possible that they entered by the communication which exists between abdominal coelom and myocoele, but this has normally closed at a much earlier stage than that used for grafting.

We can now summarise the progress of invasion of host tissues by trophoblastic giant cells:

1. Much of the spread was local, into tissues immediately adjacent to the graft.

2. The commonest route of further spread by tongues of trophoblast, was through the ventral wall of the aorta. Invasion of large veins was less frequent.
3. Further spread, by individual giant cells, was most commonly around the outer surface of the aorta, and less commonly, of one of the large veins.

The frequency of <u>perivascular</u> migration suggests that the endothelial basement membrane provides a migratory cue for giant cells (see later).

4. <u>True</u> migration (i.e. by active movement through the mesenchyme) further afield was surprisingly limited, both as to the number of cells involved and the distances travelled. Combining the data from all 37 embryos at 4 stages, four embryos had showed, at most, "a few" giant cells, which had come to lie at the ectodermal-mesenchymal interface; two showed single giant cells in contact with the notochordal sheath; one showed a single giant cell in contact with a spinal nerve on the left side (i.e. away from the site of the graft).

The distances travelled by truly 'migrant' individual giant cells were surprisingly small: from the dorsal coelomic wall to the dorsal wall of the aorta was about 300μm, and to the lateral side of the notochord about 400μm.

Although a few giant cells (in 4/37 embryos) had migrated to the deep surface of the ectoderm, in only one embryo did the migration appear to have taken place <u>from the graft site</u> along the ectodermal-mesenchymal interface;

in the others, migration had apparently occurred directly through the mesenchyme and had been arrested when the giant cells contacted the ectoderm (or its basement membrane). Giant cells seemed similarly to have been halted in their invasion by contact with the notochordal sheath, a particularly well developed basement membrane.

5. Intravascular dissemination accounted for most of the distant spread of individual giant cells, apparently occurring almost exclusively by veins, both retrogradely and anterogradely.

Despite the frequency with which the aorta was breached, only 2 embryos each showed a single giant cell attached to the inner surface of the aorta.

II. THE MECHANISM OF INVASION

A. of the coelomic mesothelium

Most of the evidence here is negative.

i. No evidence was found of histolytic or cytolytic damage to mesothelium by giant cells (or indeed by any of the other tissues of the egg cylinder) before contact was established, even where there was very close proximity between the two.

ii. Where a tongue of trophoblast had recently penetrated the mesothelium, the gap showed no cellular remnants, margins were made up of apparently healthy cells, and there was no clear-cut evidence that penetration had involved phagocytosis.

B. of the ventral wall of the aorta

Erosion of the thin layer of mesenchyme (about 30μm) which separates the dorsal coelomic mesothelium from the ventral wall of the aorta, and the aortic endothelium itself, occurred rapidly (8/15 embryos at 8 hours). To judge from the earliest stages in this process it was effected by broad tongues of compactly arranged "typical" giant cells (see e.g. Figs. 10a, 14 and 43) which presented a flat or dorsally convex profile on the invasion front. As indicated frequently in the Results, no evidence was found of histolysis, cytolysis or phagocytosis of the mesenchyme.

Once the aortic wall had been breached, the invasion front became markedly concave dorsally, presumably reshaped by the aortic blood pressure, which may also have contributed to aortic rupture. Certainly the speed and frequency of initial penetration of the aorta by tongues of invading giant cells contrasts with the apparent resistance of other arterial and venous endothelia to penetration by small numbers of migrant giant cells.

C. of epithelial structures

i. mesonephric duct. This lay along the route of dorsal invasion which overtook the aorta, and several examples were seen of close proximity to or even in contact with giant cells without any histological signs of damage to the ductal epithelium (see e.g. Figs. 9, 12 and 14, at 8 hours; Fig. 20 at 12 hours). However at 16 hours, one example was

seen of erosion of the ventral wall of the right mesonephric duct, although the remainder of its epithelial wall appeared normal (Fig. 38).

ii. Liver: The close proximity of the dorsal wall of the liver to the graft site is emphasized by Figs. 4 and 5: a plate of hepatocytes is covered only by the coelomic mesothelium and a very thin layer of mesenchyme. Because of the position of the EPC, the liver was spared early invasion: at 8 hours, giant cells had contacted liver in only 3/15 embryos and in only 1 of these was liver eroded and the ductus venosus opened. Examples of early erosion (see e.g. Fig. 21, 12-hour group) gave little clue as to the mechanism of erosion: there was simply a gap in the plate of hepatocytes, and those bordering the gap appeared normal. Some embryos however, did show evidence of damage: Fig. 31 (12-hour group) shows a substantial tongue of giant cells invading the dorsal surface of the liver and in contact with a cluster of hepatocytes, which are clearly damaged. Another example is shown in Fig. 45a (16-hour group) in which the ectoplacental cone fits into a reciprocal concavity in the dorsum of the liver and faces a narrow crescentic zone of damaged hepatocytes. A third example (Fig. 50, 24-hour group) showed complete erosion of the dorsal wall of the liver by an extensive sheet of "typical" giant cells, which bulged convexly into a much enlarged ductus venosus. These examples make it clear that **when a substantial sheet or tongue of giant cells makes direct contact with hepatic epithelium, this is "eroded";**

unfortunately they do not make clear the mechanism of erosion, although this does not seem to involve phagocytosis and if a lytic agent is concerned, this has only a very local range of action.

In contrast to the destructive effect of sheets or tongues of "typical" giant cells on hepatocytes, single (usually 'vacuolated') giant cells, which had seeded into the liver and become attached to hepatic plates, produced no histological damage, at least in the limited time available (see e.g. Fig. 39, 16-hour; Figs. 52 and 73b, 24-hour). The difference in response must be due to one (or both) of two factors: a "dosage" factor, single cells producing too little of the putative lytic factor, or a difference in the capacity for destructive effect of "vacuolated" as compared with "typical" giant cells.

iii. Vascular endothelium

Wherever substantial collections of giant cells (particularly of the "typical" variety) came in contact with either arterial or venous endothelium, erosion occurred. In contrast, many examples were seen of individual (and, usually, "vacuolated") giant cells in contact with the outer surface of blood vessels, without apparently causing any damage (see e.g. Figs. 13, 15 and 41). Examples of individual giant cells adherent to or intercalated within, the internal surface of veins (particularly within the liver) were also numerous (see e.g. Figs. 34b, 36, 55, 67 and 76a). Surprisingly few

giant cells were found adherent to, or intercalated within, arterial endothelium: almost all those found are illustrated in Figs. 51, 53 and 70: perhaps the most striking feature of intravascular giant cells was the fact that they were adherent to the endothelium.

iv. Surface ectoderm

The presence of giant cells at the ectodermal-mesenchymal boundary has already been referred to. It was commonly associated with blister-like separation of the ectoderm from the mesenchyme, presumably due to damage to the basement membrane, an important contributor to ectodermal adhesion. The ectoderm itself appeared (histologically) normal: it was never penetrated by a giant cell, nor did it show loosening of contact between its cells (histolysis) or of pyknosis (cytolysis).

v. Other tissues

Migration or vascular dissemination brought giant cells into contact with a variety of other tissues.

a. Mesenchyme: no clues were found as to how giant cells penetrated the mesenchyme. Mesenchymal cells in their immediate vicinity invariably appeared normal histologically; there was no sign of phagocytosis; the intercellular matrix showed no evidence of dissolution. These findings differ from those of Tickle et al. (1978), considered later.

b. Sympathetic ganglion cells

The frequent presence of individual giant cells close to, or even embedded in, sympathetic ganglia is shown in

the Results. Here it only needs to be emphasized that this intimate contact was never seen to produce histological signs of damage to the ganglion as a whole or its constituent cells.

c. Notochord

Only 2 or 3 examples were seen of individual giant cells which had come into contact with the notochord. Each was apparently adherent to the notochordal sheath, without penetrating it.

d. Mesodermal somites

How giant cells came to lie within a somite in what appeared to be a remnant of the myocoele, has already been discussed. Whatever the route of entry, it resulted in very intimate contact between an individual giant cell and epithelial cells of the dermato-myotome: once again, these appeared undamaged.

e. Neural tube and spinal nerves

No examples were found, in serial sections of any of the 37 embryos, of giant cells in contact with the neural tube.

Two or three examples were found of giant cells close to spinal nerves; yet again no damage was evident.

We can now summarize evidence bearing on the mechanism of invasion by trophoblast and the resistance offered by various tissues to invasion.

Tongues of trophoblast travelled only short distances; they readily penetrated coelomic mesothelium, mesenchyme

and vascular endothelium; other epithelia, such as the mesonephric duct and plates of hepatocytes seemed more resistant.

Individual giant cells migrated through the mesenchyme but seemed to be arrested when they came in contact with the outer surface of endothelia and of the notochord. When they were disseminated within blood vessels, they most frequently became adherent to the endothelium. They gave no indication, in contact with a wide variety of tissues, that they were phagocytic, histolytic or cytolytic.

Inherent giant cell motility

A point in favour of the inherent giant cell motility contributing to trophoblastic invasion is the demonstration of locomotory apparatus like microtubules and microfilaments within the cytoplasm of hamster trophoblastic giant cells by Carpenter (1982), a finding suggestive of locomotory ability by trophoblastic giant cells.

III. BEHAVIOUR OF CONTROL TISSUES TRANSPLANTED TO THE COELOM

1. Grafts of decidua

Small pieces of 7d decidua were transplanted to the coelom and examined at either 8h or 16h later.

Interpretation of the results is difficult, partly because histological examination suggested that there was variation in the type of decidual tissue which had been transplanted. One graft (DC5) appeared to be of mesometrial decidua, since it contained large numbers of

polymorphonuclear leucocytes and fibrinoid material, as did mesometrial decidua in the control conceptuses in situ. Of the other grafts, two (DC4 and DC1) were completely necrotic and it was not possible to identify their site of origin. Two (DC2 and 8) each contained a healthy central core of what resembled peripheral mesometrial decidua, while two contained glycogen-rich decidua cells.

All however showed two features in common: i. more or less extensive necrosis of decidual cells, either involving the whole graft or a peripheral layer; ii. more or less complete loss of the coelomic mesothelium with which they came in contact but absence of invasion of chick tissues beyond the level of the mesothelium. These features are now examined in turn:

i. Necrosis of decidual grafts

From a very wide experience in the Department of a variety of tissues, graft necrosis was an unexpected finding. The commonest cause of necrosis of grafts is nutritional, when they are too large to be supported by diffusion of oxygen and nutrients. However, this type of necrosis is invariably central in character, overtaking those cells furthest from the source of supply. None of the 7 decidual grafts showed only central necrosis: when surviving healthy cells were seen they were centrally situated, with a surrounding rim of necrotic cells.

A possible cause of decidual cell death to be considered is that it represented the process of programmed cell death which is known to overtake deciduomata in

pseudo-pregnant animals, as described for the hamster by Turnbull and Kent (1963). They saw maximal decidual response at 7-8d after sterile mating and decidual necrosis began spontaneously near the end of the 8th day and was completed by the end of the 9th day after mating. Necrosis in the grafts seems to have occurred more rapidly than would be expected from this time-scale, although grafts removed from a position close to the conceptus would probably have undergone early necrosis, as they would if left in utero. Resolution of the cause of graft necrosis obviously requires further study, but the possibility that it represents programmed cell death is an interesting one.

ii. Loss of coelomic mesothelium

This was seen to greater or lesser extent in relation to all the decidual grafts. There are several possible explanations:

a. that mere contact with a substantial, rather firm graft, was sufficient to cause abrasion of the mesothelium;
b. that dead decidual cells release materials toxic to chick tissue;
c. that decidual cells actively eroded the mesothelium before they died.

2. Limb bud grafts

The only other control material prepared for this study was very limited, being provided by two grafts of chick limb bud, left in place for 48h and 72h. These,

however showed some interesting contrasts. Despite their large size, the limb bud grafts showed no evidence of necrosis; indeed they continued actively to grow and develop. Contact with the host was limited in each case. Where it occurred there was minimal loss of mesothelium.

This limited control material may be usefully supplemented by reference to previous work in the Department, in which similar procedures were used.

Al-Janabi (1988) studied the behaviour and fate of a wide variety of embryonic chick tissues and organs transplanted to the coelom including neural tube, mesonephros, optic cup and lens, nasal capsule, limb bud, allantois, heart, body wall, liver and otic vesicle. Most of the chick hosts were killed and studied at 7 days. Most of the grafts showed excellent survival. Where they made contact with the host (usually the liver) the mesothelium was missing, but in no case as extensively as seen with decidual grafts in the present study. With two exceptions none of the grafts showed invasion of host tissues by graft cells: the 2 exceptions were the rather special cases of nerve fibres growing out from spinal cord and optic cup grafts.

Barber (1989) used grafts of chick mesonephros, liver, heart and chorioallantoic membrane as controls for his study of rat trophoblasts, with results very similar to those of Al-Janabi (1988).

Most of his grafts were healthy when retrieved at intervals of up to 5 days; they had established limited

attachment to one or more of the organs bounding the coelom; although at these sites the mesothelium was missing, there was no further invasion of chick tissues, nor any bleeding.

This very extensive control material strengthens the feeling that the behaviour of decidual grafts in the present study was anomalous, both in the extensive necrosis of graft cells and in the virtually complete erosion of the coelomic mesothelium. This "erosion" raises the possibility that it had been brought about actively by decidual cells, before they died. This possibility finds some support in the demonstration that, in normal implantation in the rat, the basal lamina of the uterine luminal epithelium is penetrated not by trophoblast, but by processes from underlying decidual cells (Parr et al., 1986; Schlafke et al., 1985).

3. Observations on the egg cylinder in situ

The extent to which spread of decidual necrosis in advance of 'invasive' spread of (primary) trophoblastic giant cells was due to the latter's effect or to programmed cell death in the decidual cells remains to be determined. It would seem that a cooperative interaction of trophoblast and decidual cells is responsible for trophoblast invasion.

IV. PRESENT FINDINGS RELATED TO PREVIOUS STUDIES

As indicated in the Introduction, any discussion of the invasiveness of trophoblast must consider not only the possibility of large species variations in the properties

of the trophoblast itself, but also variations in the nature of the material invaded, whether the natural site of the uterus or the wide range of ectopic sites used experimentally.

The present findings are compared first with those obtained by others, using different varieties of trophoblast but a similar site, namely the tissues of the 3.5d chick embryo.

Barber (1989) compared the behaviour of grafts of rat ectoplacental cone with that of pig allanto-chorion. While the behaviour of **rat trophoblast** was, in general, similar to that of hamster trophoblast, reported in the present study, there were important quantitative differences:

1. Longer survival of chick hosts

Of 24 chicks grafted, 12 were still alive when sacrificed after 3 days. This is in marked contrast with the present study, in which it seems likely that all 37 recipient chicks would have died in the first 24h from bleeding from major blood vessels, had the experiments not been terminated earlier.

2. Different pattern of invasion

Unlike hamster trophoblast, rat trophoblast sent out very long tapering tongues of cells, which tended to follow epithelial-mesenchymal boundaries e.g. around the outer surface of aorta and major veins, beneath coelomic mesothelium. These tongues of trophoblast were much less destructive of endothelium than were comparable structures

in the hamster. This explained why there was little or no bleeding with rat grafts.

Distant migration of individual cells of rat trophoblast was virtually absent. Groups of cells remote from the main graft seemed to represent the sequestered tips of former elongated tongues.

These differences apart, there were many resemblances in the behaviour of rat and hamster trophoblast:

. both failed to produce histolytic or cytolytic damage "at a distance" i.e. when not in immediate contact with e.g. coelomic mesothelium.

. both produced only limited damage (and then not consistently) when in immediate contact with epithelial parenchyma, such as that of liver, mesonephros or lung bud.

. neither showed much evidence of migration within blood vessels such as that seen in normal pregnancy in rats, mice, hamsters and humans (Pijnenborg et al., 1981).

. neither showed consistent phagocytosis of host tissue.

These findings for hamster and rat may now be compared with those of De Souza (1986) who studied **mouse** trophoblast, explanted to the same site, mouse trophoblast behaved very similarly to that of the rat. Of a group of 32 embryos carrying a single EPC graft, 13 (40%) were healthy when the experiment was terminated at 3 days. Although, as in the rat, tongues of trophoblast were seen in very close contact with the abluminal surface of the aorta and major abdominal veins, the endothelium was frequently intact and, where it was not, the defect had

been effectively plugged by trophoblast, so that bleeding was minimal, as in chicks carrying trophoblast grafts, so accounting for the lower host mortality. Tongues of trophoblast and trophoblast cells single or in small clusters, penetrated tissues adjacent to the graft site, but again, as in both hamster and rat, they left no evidence of the mechanism of invasion and appeared not to have damaged the various tissues with which they came into contact: hepatocytes, mesonephric tubules, bile duct and gall bladder epithelium, general mesenchyme, adrenal gland. As in the rat, distant migration of individual giant cells was not a feature.

The results from this trio of trophoblasts, all from species showing haemochorial placentation, may now be compared with those obtained with intracoelomic grafts of the allanto-chorion of the pig, which has an epitheliochorial placenta. Barber (1989) showed that the grafts, which were considerably larger than a single rat EPC, formed multiple areas of attachment to the coelomic epithelium, but the chorionic epithelium was either greatly thinned or absent in at least some areas of attachment. There occurred substantial growth of the allantoic mesenchyme and "lobes" of the membrane were seen "invading" into adjacent tissues, notably the liver. This "invasion" however seemed to represent more the result of growth expansion of the mesenchyme rather than the more active penetration of host tissues demonstrated by trophoblast

from rat (and mouse and hamster).

We must now refer to the work of Tickle et al. (1978) who studied the behaviour of mouse trophoblast (EPC), and of a wide variety of other tissues, normal and malignant, when transplanted to the embryonic chick limb bud. They chose this site because it provided a 3-dimensional substrate of primitive mesenchyme on which to test the invasiveness of various types of cells. The site was not unlike that used in the present study, but differed in an important respect: in making a pocket in which to place the graft, "between one half and one third of the depth of the wing mesenchyme was removed, together with the overlying ectoderm". Moreover, the graft was sometimes kept in place by a platinum wire pin, 25 μm thick. This amount of operative trauma may complicate the interpretation of experiments designed to assess the "invasive" and "destructive" properties of trophoblast.

Despite this disadvantage, however, many of Tickle et al's (1978) findings anticipate those of the present study: in particular, trophoblast cells were seen extending along the basement membrane of the surface ectoderm, and lying closely apposed to blood vascular endothelium, or even intercalated into the vessel wall. Their conclusions on the mechanism of invasion were, however rather different, claiming that destruction of host tissue "may be important in the invasiveness of trophoblast". As evidence for this they cited: the space which often developed around trophoblast cells under the ectoderm, and places where

endothelial cells have been "destroyed and usurped"; crater-like cavities lined by trophoblast. (Their Fig. 3 showed a cavity some 50μm in depth lying along the developing skeletal cartilage, which was undamaged apart from absence of a perichondrium); denudation of ectoderm in 2 experiments in which a piece of trophoblast had been pinned to the outside of the limb bud.

These findings might suggest that mouse trophoblast is more destructive of mesenchyme than is hamster trophoblast. Certainly, separation ofsurface ectoderm from mesenchyme was seen in the present study but it seemed to be due to damage to the basement membrane rather than to either of the separated tissues. No cavities lined by trophoblast were seen in the present study. The apparently greater destruction by mouse trophoblast may owe at least something to the trauma of the operative technique. This belief is reinforced by the fact that when mouse trophoblast (as used by Tickle et al (1978)) is placed into the chick coelom, it is less destructive than hamster trophoblast (De Souza, 1986). Another minor but interesting difference in their findings was that what they called "vacuolated" 'frothy' giant cells did not migrate: in the present study most of the truly migrant giant cells were of the vacuolated variety.

We can now relate the present findings to a selection of the more significant papers on the behaviour of trophoblast in ectopic sites and in utero. Opinion on the

'destructiveness' and 'invasiveness' of trophoblast covers a wide spectrum, of which the work of Kirby represents one extreme. In his review paper (1965) he emphasized the destructive character of trophoblast, mediated through phagocytosis in a variety of sites, as indicated in the following direct quotations:

"Trophoblast destroys the renal tissue by phagocytosis. Usually small clumps of renal cells are engulfed by the trophoblastic cytoplasm, but not infrequently entire Bowman's capsules are ingested".

"Trophoblast destroyed the spleen by phagocytosis".

"Trophoblast destroyed (brain tissue) by phagocytosis".

"Trophoblast invaded (liver) by phagocytosis".

"In uteri not protected by a decidua the full invasive proclivities of trophoblast are unleashed and that portion of the uterus is virtually destroyed".

For grafts placed in the testis, however, Kirby (1963a) reported a different mechanism of invasion: lysis of host tissues by a "cytolytic agent" diffusing ahead of the trophoblast and Billington (1966) endorsed this view: "cytolytic action of the trophoblast causes erosion of the seminiferous tubules and interstitial tissue". But he added, "it seems that cellular debris is ultimately ingested by phagocytosis".

The findings of Fawcett (1950) on the other hand, suggest that trophoblast is a much more benign tissue. Giant cells were only "occasionally" found among the renal

tubules. Phagocytosis was not conspicuous and "the parenchyma adjacent to active growths of trophoblastic giant cells showed no clear evidence of damage attributable to a cytolytic agent".

It is not easy to reconcile these widely divergent views, each based on the use of mouse trophoblast. However, the results of the present study and those of de Souza (1986) and Barber (1989) using a similar method, all conform much more closely to those of Fawcett (1950) than they do to those of Kirby (1963a and b).

There is also a growing body of evidence from electron microscopic studies of implantation in utero, which questions the concept of trophoblast as a highly destructive and highly invasive tissue.

Enders and Schlafke (1967), studying the activity of primary giant cells during early stages of implantation in the rat, concluded that "the histolytic and invasive properties of the early trophoblast appear to be very feeble in the first stages of normal implantation", although phagocytosis of already sloughed uterine epithelial cells by trophoblast was seen.

In a subsequent paper, Enders and Schlafke (1969) extended their study of the cytology of early implantation to rabbit, guinea pig, armadillo bat and ferret, in addition to rat. Again for the rat, and also for the bat, they found sloughing of uterine lining epithelium "some distance from trophoblast cells" and again concluded that

"trophoblast is not a very active agent in penetration in the rat" (and bat). In the remaining species, although the trophoblast appeared to play an active role in penetrating the lining epithelium, by extending processes between its cells, there was "little evidence of destruction of epithelial cells in any of these early implantation stages". In the armadillo, later stages of penetration provided "good examples of highly invasive trophoblast" with destruction of uterine glands. In all of the species studied, trophoblast showed the capacity to phagocytose cells and other debris, but the authors emphasized that "phagocytosis bears no direct relationship to invasiveness of trophoblast". They further noted the resistance offered by basement membranes, both of the uterine epithelium and of maternal endothelium, to penetration by trophoblast.

Some of these conclusions were reinforced by El-Shershaby and Hinchliffe (1975), who studied the interaction of mural trophoblast and the antimesometrial epithelium in the mouse: "Evidence... favours the hypothesis that the process of deterioration of the anti-mesometrial epithelium is mediated by self-digestion rather than by trophoblast attack or secretion" and "Trophoblast phagocytosis of the whole uterine epithelium begins ... <u>after</u> the epithelial cells have undergone considerable deterioration".

Enders and his colleagues (Schlafke, Welsh and Enders, 1985) returned to the problem of penetration of the basement membrane of uterine epithelium. They were

themselves surprised to find that "the initial breaching of the basal lamina is an activity of the decidual cells and that contact of basal lamina with trophoblast is not necessary to permit this penetration". They were clearly worried by the apparent demotion of trophoblast as an invasive tissue and reassured themselves that "evidence of trophoblast invasiveness derives from later stages of implantation" e.g. secretion of plasminogen activator by trophoblast does not become maximal until 7-9d, while the basement membrane is penetrated at 6d.

A further paper (Welsh and Enders, 1985) again questioned the destructive and invasive capacity of rat trophoblast, this time in relation to the antimesometrial decidua. They concluded that "like the breakdown of the uterine luminal epithelium early in pregnancy, decidual cell degeneration is primarily autolytic". "It appears that trophoblast is not cytolytic in nature. In fact other than surrounding effete decidual cells and decidual cell debris, the trophoblast may not be very active at all in the degeneration of the antimesometrial decidua".

Welsh and Enders (1987) then tackled the problem of penetration of maternal endothelium by trophoblast in the establishment of the maternal circulation in the rat's yolk sac placenta. They found electron microscopic evidence of two of the mechanisms (i) initial penetration of maternal endothelium by <u>decidual</u> cell processes, subsequently substituted by trophoblast; (ii) a "more direct approach to

maternal blood by trophoblast" although "the fate of endothelial cells after they come in contact with trophoblast is not clear" (they found no evidence of apoptosis of endothelial cells). As a further possibility, they suggested that if mitosis in vascular endothelium failed to keep pace with the growth of the implantation chamber, the endothelium might become discontinuous without extensive cell death.

V. INTRAARTERIAL MIGRATION OF TROPHOBLAST

One of the aims of this study was to re-examine the remarkable phenomenon of retrograde migration of hamster trophoblast into maternal uterine arteries (Orsini, 1954; Billington, 1966; Carpenter, 1982) a phenomenon also seen in humans (Hamilton and Boyd, 1966), rats and mice (Pijnenborg et al, 1981).

In hamsters this vascular invasion occurs in two waves, the first associated with development of the yolk sac placenta, into the circumferential arteries, the second, in association with chorioallantoic placental development, into the spiral arteries. Intravascular invasion in hamsters is followed by destructive changes in the vessel wall, trophoblast effectively replacing the native constituents, and, possibly freeing the altered arteries from vasomotor control, and providing increased maternal blood flow through the placenta (see Pijnenborg et al., 1981).

As it turned out, the present study throws little new light on this phenomenon. The main features in the

relationship between trophoblast and blood vessels of the chick embryo (represented at this stage by little more than endothelium) can be summarised as follows:

1. Endothelium is eroded by tongues of trophoblast, without assistance of other cell types (such as decidual cells, in utero).
2. The aorta was somewhat more frequently eroded (by 13%) than were the veins, but the difference was probably a matter of chance in positioning of the EPC.
3. While spread round the outer surface of the aorta (and to a lesser extent of the veins) was a preferred route, spread along the internal surface of the aorta was rarely seen.
4. There was evidence (already discussed) of spread of single trophoblastic giant cells, or of small clusters, into venous channels, both with the direction of blood flow and against it.
5. Adhesion of giant cells to endothelium was very commonly seen, both to the internal surface of veins and venules, and to the edge of defects produced in the aorta (but very seldom to the internal surface of the aorta).

The failure to duplicate in the chick embryo, the intravascular migration seen in utero, may simply be due to short survival of chicks carrying hamster EPC grafts. However, the findings were similar with grafts of rat (Barber, 1989) and mouse (De Souza, 1986) trophoblast,

despite survival of their hosts for up to 4 days after grafting. It is interesting to speculate that another factor may be involved. Pijnenborg et al (1974) found that structural changes occurred in the uterine arteries before trophoblast migration started, and postulated that the arterial changes "represent a conditioning of the vessel walls prior to trophoblast migration". They recalled that, in hamsters, the same vascular changes occur in deciduomas i.e. in the absence of trophoblast (Turnbull and Kent, 1963; Orsini, 1968). Once again it seems that trophoblast invasiveness may depend upon cooperative interaction with uterine tissues.

CORRELATIONS

This Discussion ends with an attempt to correlate the data provided by this study, on one major topic and two minor ones:

i. The behaviour, during a 24-hour period in the chick's intraembryonic coelom, of the two principal constituents of the 7-day hamster egg-cylinder, namely the ectoplacental cone and the endoderm of the visceral yolk sac;

ii. the behaviour of trophoblastic giant cells and the extent of degeneration of decidual tissue in the uterus between 7 and 7.5d of normal pregnancy in the hamster;

iii. the behaviour of "control" grafts of chick limb bud and of decidual tissue, explanted to the chick coelom.

1. The behaviour of trophoblast in the chick coelom compared with that in utero

Within 8 hours after explantation to the coelom, the egg cylinder had gained attachment to, and eroded, the coelomic mesothelium; by 12 hours, local extension of tongues of giant cells had eroded principally the aorta and, to a lesser extent, other adjacent chick tissues. Individual giant cells had migrated for short distances locally, mainly around the outer surface of the aorta. More distant spread of migrant giant cells was infrequent, both at this, and subsequent stages, but occurred more frequently by way of veins, particularly at 16 and 24 hours. Although erosion of the ventral aortic wall was dramatic and usually led to premature termination of the experiment, it required the destruction of only a thin layer of tissue (about 30 μm), and local destruction of other tissues by the main graft was surprisingly limited. Moreover, individual migrant giant cells which came into contact with a variety of chick tissues, appeared to have caused little, if any damage.

This picture of relatively benign behaviour of trophoblast in the ectopic site contrasts sharply with that seen in utero over a comparable period of time. In utero, during the enlargement of the implantation cavity between 7 and 7.5d, there had clearly occurred much more extensive degeneration of decidual tissue, than the destruction of tissue seen in the chick embryo. It is highly unlikely that this was because the soft, gelatinous tissues of the

chick offered more "resistance" to invasion and destruction by trophoblast. Much more likely is that the more extensive breakdown of decidual tissue is attributable to programmed cell death, for which the fate of experimental deciduomata provides strong evidence.

In utero, local invasion of decidua by individual giant cells was limited (during the 12-hour period) to a zone around the implantation chamber about 200 μm in depth, a distance similar to that travelled by the (few) individual migrants in the chick. By contrast, however, there occurred more distant spread of giant cells into arteries of the mesometrial decidua, a phenomenon which did not have its true counterpart in the chick. The absence of endoarterial spread of giant cells in the chick was surprising, particularly in view of the frequency of erosion of the aortic wall. Time available for spread may have been the limiting factor. Another possibility lies in the suggestion of Pijnenborg et al. (1981), that the structural changes which they observed in the walls of uterine arteries before trophoblast migration started "represent a conditioning of the vessel walls prior to trophoblast migration".

2. The behaviour of "control" grafts of chick limb bud and of hamster decidua explanted to chick coelom

As already described, there were striking differences in the behaviour and fate of these two types of "control" grafts. Limb bud grafts remained healthy, developed and

grew, and produced only limited erosion of the coelomic mesothelium. This behaviour was similar to that described by Al-Janabi (1988) for a large range of control tissues. Decidual grafts, by contrast, all showed more or less extensive necrosis, with surviving cells, when present, confined to the central part of the graft, unlike the central necrosis seen in grafts which are too large to be supported by diffusion of oxygen from the host tissues. This finding is consistent with the suggestion that decidual breakdown in utero is an example of programmed cell death, rather than attributable to the "destructive" action of trophoblast, but further work is required to test this interpretation of the fate of decidual grafts.

APPENDICES

APPENDIX A

Processing of chick embryos for wax histology

1. Immediately after termination of each experiment, the chick embryo was fixed in Bouin's solution (12-24 hours) and processed for histology using a Reichert-Jung Histokinette (2000) automatic tissue processor which operated as follows:

. Dehydration through ascending concentrations of ethanol (70% and 90%)	- 2 hours each
. Absolute ethanol in celloidin (3 changes)	- 2 hours each
. Amyl acetate (3 changes)	- 2 hours each
. Paramat extra wax (2 changes)	- 4 hours each

The tissues were then embedded in paramat extra wax blocks and left to harden.

II. Trimmed blocks, sectioned at 5μm were mounted on slides, stretched in an electrothermal water bath at 45^{o}C, air dried and thereafter dried overnight in a Hearson oven at 37^{o}C.

III. The mounted sections were then dewaxed and hydrated as follows:

. Dewax in xylene	- 10 minutes
. Hydrated through descending concentrations of ethanol absolute ethanol (2 changes)	- 30 seconds each
. 90% and 70% ethanol	- 1 minute each

Wash in water

IV. Tissue staining was performed as follows:

Stain in Harris' haematoxylin - 4 minutes

Wash in water

Differentiate in acid alcohol - 15 seconds

Wash in water

Dip in Scott's solution.

Wash in water.

Counterstain in Eosin - 1 minute

Differentiate in 70% ethanol - 15 seconds

V. The stained sections were then dehydrated as follows:

90% ethanol - 30 seconds

Absolute ethanol (3 changes) - 1 minute each

Xylene - 5 minutes

After which coverslips were applied using Histomount mounting medium.

APPENDIX B

8 HOUR GROUP

Experiment: MB HM 14

Duration of graft: 8 hours

State of Host Embryo: Died about 1 hour before termination.

Nature of graft: Ectoplacental cone with some visceral yolk sac endoderm.

Position of graft and size: Upper right coelom, 450μm x 500μm, * See footnote.

Attachment of graft: Dorsal coelomic wall.

Erosion of mesothelium: Present only at site of attachment.

Invasion: Local spread: by extension of tongues of cells and individual cell migration.

Distant spread: Absent

Invasion of mesenchyme: Present

Erosion of endothelial wall of: Aorta: Present

Posterior cardinal vein: Present

Ductus venosus: Not applicable (no contact with EPC).

Trophoblastic giant cells plugging hole in vessel wall: Incomplete

Internal bleeding : Present, into EPC and coelom.

Phagocytosis : Absent

Other comments: In spite of close proximity of giant cells to mesothelium covering lung bud, lower and right body wall, no sign of histolytic damage to it or underlying tissue.

*The dimensions given are in each case for <u>length</u>, and greatest <u>diameter</u> of the graft. The length was calculated by number of transverse sections in which graft was present x thickness of sections (5μm).

Experiment: MB HM 19

Duration of graft: 10 hours

State of Host Embryo: Died about 1 hour before termination.

Nature of graft: Egg cylinder

Position of graft and size: Upper right coelom, 700μm x 350μm.

Attachment of graft: Dorsal coelomic wall, overlying aorta; the dorsal mesentery and the dorsum of the liver.

Erosion of mesothelium: Present only at sites of attachment.

Invasion: Local spread: By extension of tongues of cells, around aorta and into dorsal mesentery.

Distant spread: Present, isolated giant cell seen in somite cavity (myocoele).

Invasion of mesenchyme: Present

Erosion of endothelial wall of: Aorta: Present

Cardinal vein: Not applicable (no contact with EPC).

Ductus venosus: Not applicable (no contact with EPC).

Trophoblastic giant cells plugging hole in vessel wall: Partial

Internal bleeding: Present; into giant cell mass.

Phagocytosis: Not observed.

Other comments: Although EPC cells have eroded aortic wall, no histolytic or cytolytic damage to closely adjacent mesonephric duct. No damage to those tissues closely adjacent to visceral yolk sac endoderm.

Experiment:	MB HM 23
Duration of graft:	6 hours
State of Host Embryo:	Termination 30 mins. after bleeding observed.
Nature of graft:	Egg cylinder
Position of graft and size:	Upper right coelom, 850μm x 400μm.
Attachment of graft:	To dorsal coelomic wall, overlying aorta.
Erosion of mesothelium:	Present
Invasion: Local spread:	By extension of tongues of cells present.
Distant spread:	Absent
Invasion of mesenchyme:	Present
Erosion of endothelial wall of: Aorta:	Present
Cardinal vein:	Not applicable (no contact with EPC).
Ductus venosus:	Not applicable (no contact with EPC).
Trophoblastic giant cells plugging hole in vessel wall:	Virtually complete.
Internal bleeding:	Present into giant cell mass.
Phagocytosis:	Only of red cells.

Other comments: Several examples of giant cells in close proximity to mesothelium of right lateral body wall and of lung bud with no evidence of histolytic or cytolytic damage.

Experiment:	MB HM 30
Duration of graft:	8 hours
State of Host Embryo:	Healthy at termination. No macroscopic bleeding.
Nature of graft:	Egg cylinder
Position of graft and size:	Upper right coelom, 500μm x 400 μm.
Attachment of graft:	To the right body wall and dorsum of the liver.
Erosion of mesothelium:	Present, over liver and right body wall.
Invasion: Local spread:	By individual giant cell migration between aorta and right posterior cardinal vein.
Distant spread:	Absent
Invasion of mesenchyme:	Present
Erosion of endothelial wall of: Aorta:	No: (no contact with EPC).
Common and posterior cardinal veins:	Present
Ductus venosus:	Not applicable (no contact with EPC).
Trophoblastic giant cells plugging hole in vessel wall:	Not applicable (no contact with EPC).
Internal bleeding:	Absent
Phagocytosis:	Not observed

Other comments: Several examples of giant cells in contact with mesenchymal cells, hepatocytes and the mesonephric duct epithelium with no evidence of histolytic or cytolytic damage.

Experiment:	MB HM31
Duration of graft:	8 hours
State of Host Embryo:	Healthy at termination. No macroscopic bleeding.
Nature of graft:	Egg cylinder, EPC directed rostrally.
Position of graft and size:	Upper right coelom, 950μm x 450μm.
Attachment of graft:	To right body wall.
Erosion of mesothelium:	Present at site of attachment.
Invasion: By local spread:	Absent
By distant spread:	Absent
Invasion of mesenchyme:	Absent
Erosion of endothelial wall of: Aorta):	Not applicable (as they were not in contact with EPC).
Cardinal vein):	
Ductus venosus):	
Trophoblastic giant cells plugging hole in vessel wall:	Not applicable.
Internal bleeding:	Not observed.
Phagocytosis:	Not observed.

Other comments: Although giant cells were in contact with the coelomic mesothelium and dorsal surface of the liver, neither histolytic nor cytolytic damage was observed.

Experiment:	MB HM 33
Duration of graft:	8 hours
State of Host Embryo:	Healthy at termination. No macroscopic bleeding.
Nature of graft:	Egg cylinder, EPC directed caudally.
Position of graft and size:	Upper right coelom, 600μm x 450μm.
Attachment of graft:	To right body wall, adjacent to EPC.
Erosion of surface mesothelium:	Present; early.
Invasion: Local spread:	Minimal into right body wall by extension of a tongue of trophoblastic giant cells.
Distant spread:	Absent
Invasion of mesenchyme:	Just beginning.
Erosion of endothelial wall of: Aorta, cardinal vein and ductus venosus:	Not applicable, as none of them lay adjacent to EPC.
Trophoblastic giant cells plugging hole in vessel wall:	Not applicable.
Internal bleeding:	Absent (no erosion).
Phagocytosis:	Giant cell in process of penetrating mesothelium showed no sign of having phagocytosed host cells.

Other Comments: 1) This specimen showed the earliest stage of invasion. No damage apparent to mesothelium immediately adjacent to site of initial erosion by EPC. 2) No damage to those tissues closely adjacent to visceral yolk sac endoderm. 3) Evidence of local thickening of mesothelium adjacent to giant cell.

Experiment: MB HM 34

Duration of graft: 8 hours

State of Host Embryo: Healthy at termination.
No macroscopic bleeding.

Nature of graft: Egg cylinder; EPC directed laterally, in contact with right body wall.

Position of graft and size: Upper right coelom, 950μm x 250μm.

Attachment of graft: The right body wall.

Erosion of surface mesothelium: Present; early.

Invasion: Local spread: By tongue of giant cells with smooth convex external surface.

Distant spread: Absent.

Invasion of mesenchyme: Absent.

Erosion of endothelial wall of: Aorta, cardinal vein and ductus venosus: Not applicable as none of them lay adjacent to EPC giant cells.

Trophoblastic giant cells plugging hole in vessel wall: Not applicable.

Internal bleeding: Absent.

Phagocytosis: Not observed.

Other comments: Another example of initial stages of invasion. Little evidence of histolytic or cytolytic damage to mesothelial cells adjacent to site of initial penetration by giant cell; evidence of thickening of mesothelium at edge of invasion site.

Experiment: MB HM 35

Duration of graft: 8 hours

State of Host Embryo: Healthy at termination. No macroscopic bleeding.

Nature of graft: Egg cylinder; EPC directed rostrally.

Position of graft and size: Upper right coelom, 650μm x 250μm.

Attachment of graft: To dorsal wall of coelom.

Erosion of mesothelium: Present; early.

Invasion: Local spread: Absent

Distant spread: Absent

Invasion of mesenchyme: Absent

Erosion of endothelial wall of: Aorta: Not applicable, as none of them lay
Cardinal vein: adjacent to ectoplacental cone
Ductus venosus: giant cells.

Trophoblastic giant cells plugging hole in vessel wall: Not applicable.

Internal bleeding: Absent

Phagocytosis: Not observed despite close contact with EPC.

Other comments: Another example of early penetration of coelomic mesothelium by EPC giant cells. Adjacent cells of mesothelium and of mesonephric duct, appear normal despite close proximity to giant cell.

Experiment: MB HM 37

Duration of graft: 8 hours

State of Host Embryo: Healthy at termination, macroscopic bleeding not observed.

Nature of graft: Ectoplacental cone, consisting of trophoblastic giant cells.

Position of graft and size: Upper right coelom, 400μm x 300 μm.

Attachment of graft: To dorsal wall of coelom, ventral to the aorta; the mesogastrium; right body wall.

Erosion of mesothelium: Present; early.

Invasion: Local spread: By extension of trophoblastic giant cells.

Distant spread: Present, individual giant cells in right body wall, subepidermally.

Invasion of mesenchyme: Present

Erosion of endothelial wall of Aorta: Present

Posterior cardinal vein: Present

Ductus venosus: Not applicable (not adjacent).

Trophoblastic giant cells plugging hole in vessel wall: Observed

Internal bleeding: Present

Phagocytosis: Absent

Other Comments: Localised penetration of coelomic mesothelium by a tongue of giant cells. Mesothelial cells adjacent to defect appear healthy.

Experiment:	MB HM 38
Duration of graft:	8 hours
State of Host Embryo:	Healthy at termination. No macroscopic bleeding observed.
Nature of graft:	Ectoplacental cone.
Position of graft and size:	Upper right coelom, 300μm x 250 μm.
Attachment of graft:	To dorsal wall of coelom.
Erosion of mesothelium:	Present; early.
Invasion: Local spread:	Minimal, by extension of tongue of trophoblast cells.
Distant spread:	Absent
Invasion of mesenchyme:	Present
Erosion of endothelial wall of: Aorta) Cardinal vein) Ductus venosus)	Not applicable as none of them lay in proximity to EPC.
Trophoblastic giant cells plugging hole in vessel wall:	Not applicable.
Internal bleeding:	Not applicable.
Phagocytosis:	Absent.

Other comments: Localized penetration of coelomic mesothelium by large tongue of giant cell cytoplasm. Adjacent mesothelium, normal and thickened. No phagocytic debris in giant cell cytoplasm.

Experiment:	MB HM 40
Duration of graft:	8 hours
State of Host Embryo:	Killed 1.5 hours after the onset of bleeding.
Nature of graft:	Egg cylinder
Position of graft and size:	Upper right coelom, 640μm x 350μm.
Attachment of graft:	To right body wall, dorsal wall of coelom.
Erosion of mesothelium:	Present
Invasion: By local spread:	By extension of tongue of cells and individual giant cells around aorta.
By distant spread:	Present
Invasion of mesenchyme:	Present
Erosion of endothelial wall of: Aorta):	Not applicable (not adjacent to EPC).
Cardinal vein):	
Ductus venosus):	
Trophoblastic giant cells plugging hole in vessel wall:	Partial
Internal bleeding:	Present, into EPC and coelom.
Phagocytosis:	of red cells only.

Other comments: In this specimen, giant cells were observed in contact with 1. mesenchymal cells of the dorsal body wall. 2. endothelium of the aorta and left posterior cardinal vein without causing histolytic or cytolytic damage.

Experiment:	MB HM 45
Duration of graft:	9 hours
State of Host Embryo:	Macroscopic bleeding observed for 1 hr 30 mins before termination.
Nature of graft:	Egg cylinder
Position of graft and size:	Upper right coelom, 450μm x 250μm.
Attachment of graft:	To dorsal wall of coelom and to dorsal surface of liver.
Erosion of mesothelium:	Present
Invasion: By local spread:	By extension of tongues of trophoblastic giant cells.
By distant spread:	Absent
Invasion of mesenchyme:	Present
Erosion of endothelial wall of: Aorta:	Present (severe).
Posterior cardinal vein:	Present
Ductus venosus:	Present
Trophoblastic giant cells plugging hole in vessel wall:	Present (partial).
Internal bleeding:	Present
Phagocytosis:	of red cells only.

Other comments: Several examples of giant cells in close proximity to mesothelium of dorsal coelomic wall and hepatocytes with no evidence of histolytic or cytolytic damage.

Experiment: MB HM 48

Duration of graft: 9 hours

State of Host Embryo: Died 40 minutes before termination.

Nature of graft: Egg cylinder

Position of graft and size: Upper right coelom, 720μm x 500μm.

Attachment of graft: To dorsal wall of coelom and right body wall.

Erosion of mesothelium: Present

Invasion: Local spread: By extension of tongues of cells into dorsal and right body wall.

Distant spread: Absent

Invasion of mesenchyme: Present

Erosion of endothelial wall of: Aorta: Present

Cardinal vein): Absent (no contact with EPC)
Ductus venosus):

Trophoblastic giant cells plugging hole in vessel wall: Partial

Internal bleeding: Present into coelom and EPC.

Phagocytosis: Absent

Other comments: Dorsal and right walls of coelom eroded by giant cells, but no apparent damage to right mesonephric duct despite close proximity to giant cell.

Experiment:	MB HM 53
Duration of graft:	10 hours
State of Host Embryo:	Terminated at first sign of bleeding into coelom.
Nature of graft:	Egg cylinder
Position of graft and size:	Upper right coelom, 420μm x 500μm.
Attachment of graft:	Dorsal wall of coelom and right surface of the dorsal mesogastrium.
Erosion of mesothelium:	Present
Invasion: Local spread:	Present, several examples of migration of individual giant cells: around aorta; to side of dorsal mesogastrium and (furthest) to left side of left posterior cardinal vein.
Distant spread:	Absent
Erosion of blood vessel wall of: Aorta:	Present
Ductus venosus and cardinal vein:	Not applicable (not in contact with EPC).
Trophoblastic giant cells plugging hole in vessel wall:	Present
Internal bleeding:	Present, into EPC.
Phagocytosis:	Absent

Other comments: This specimen showed giant cells in contact with mesenchymal cells of coelomic wall and dorsal mesogastrium; and with endothelium of the left posterior cardinal vein without causing histolytic or cytolytic damage.

Experiment:	MB HM 54
Duration of graft:	9 hours
State of Host Embryo:	Killed at first sign of bleeding into the coelom.
Nature of graft:	Egg cylinder
Position of graft and size:	Upper right coelom, 420μm x 600μm.
Attachment of graft:	To dorsal wall of coelom and right body wall.
Erosion of mesothelium:	Present
Invasion: Local spread:	By extension of tongues of trophoblast cells and individual giant cells peri-aortically.
Distant spread:	Absent
Invasion of mesenchyme:	Present
Erosion of endothelial wall of: Aorta:	Present
Cardinal vein):	Absent (Not in contact with EPC).
Ductus venosus):	"
Trophoblastic giant cells plugging hole in vessel wall:	Partial
Internal bleeding:	Present
Phagocytosis:	of red cells only observed in a few giant cells.

Other comments: 1) This specimen showed periaortic spread of giant cells. No damage apparent to aortic endothelium and mesenchymal cells by giant cells. 2) No damage to epimyocardial reticulum of the right atrium in close contact to visceral yolk sac endoderm.

APPENDIX C

12 HOUR GROUP

Experiment:	MB HM 10
Duration of graft:	12 hours
State of Host Embryo:	Bleeding observed for 2 hours 45 minutes before termination.
Nature of graft:	Ectoplacental cone
Position of graft and size:	Upper right coelom, 525μm x 350μm.
Attachment of graft:	Dorsal wall of coelom Right body wall
Erosion of mesothelium:	Present
Invasion: local spread:	By individual cell migration and extension of tongues of cells.
distant spread:	of individual giant cells
Invasion of mesenchyme:	Present
Erosion of endothelial wall of: Aorta:	Present
Posterior cardinal vein:	Present (N.B. <u>left</u> vein)
Ductus venosus:	Not applicable (no contact with EPC).
Trophoblastic giant cells plugging hole in vessel wall:	Present, partial.
Internal bleeding:	Present
Phagocytosis:	of avian red cells observed.

Other comments: This specimen showed local, periaortic, spread and distant spread of individual giant cells.

Experiment:	MB HM 18
Duration of graft:	12 hours
State of Host Embryo:	Sudden and severe bleeding prompted instant termination of experiment.
Nature of graft:	Egg cylinder
Position of graft and size:	Upper right coelom, 575μm x 600μm.
Attachment of graft:	To dorsal wall of coelom and dorsal surface of liver.
Erosion of mesothelium:	Present at both sites of attachment.
Invasion: local spread:	By extension of tongues of trophoblast cells.
distant spread:	Present, 2 to 3 individual giant cells within liver vessels.
Invasion of mesenchyme:	Present
Erosion of endothelial wall of: Aorta:	Present
Cardinal vein:	Absent (not in contact with EPC).
Ductus venosus:	Present
Trophoblastic giant cells plugging hole in vessel wall:	Present (N.B. aorta).
Internal bleeding:	Present

Phagocytosis: of avian red cells observed.

Experiment:	MB HM 20
Duration of graft:	11 hours
State of Host Embryo:	Killed instantly at the onset of bleeding.
Nature of graft:	Ectoplacental cone
Position of graft and size:	Upper right intraembryonic coelom, 430μm x 250μm.
Attachment of graft:	Dorsal wall of coelom and right surface of dorsal mesogastrium.
Erosion of mesothelium:	Present
Invasion: Local spread:	By extension of tongues of cells and periaortic spread of single giant cells.
distant spread:	Absent
Invasion of mesenchyme:	Present
Erosion of endothelial wall of: Aorta:	Present
Posterior cardinal vein:	Present
Ductus venosus:	Absent
Trophoblastic giant cells plugging hole in vessel wall:	Present
Internal bleeding:	Present
Phagocytosis:	Absent

Other comments: 1. Dorsal coelomic wall and right surface of dorsal mesogastrium eroded by giant cells, but no apparent damage to right mesonephric duct, despite close proximity to giant cells. 2. Hepatocytes in close contact with giant cells appeared undamaged.

Experiment:	MB HM 22
Duration of graft:	14 hours
State of Host Embryo:	Macroscopic bleeding observed for one hour before termination.
Nature of graft:	Egg cylinder, with EPC directed laterally and ventrally.
Position of graft and size:	Upper right coelom, 960μm x 400μm.
Attachment of graft:	Right body wall and dorsum of the liver.
Erosion of mesothelium:	Present
Invasion: local spread:	By extension of tongues of trophoblast cells.
distant spread:	Absent
Invasion of mesenchyme:	Present
Erosion of endothelial wall of: Aorta:	Not applicable (not in contact with EPC).
Cardinal vein:	Not applicable (not in contact with EPC).
Ductus venosus:	Present
Trophoblastic giant cells plugging hole in vessel wall:	Present (in ductus venosus).
Internal bleeding:	Present
Phagocytosis:	Observed in giant cells (in form of cellular debris within the cytoplasm) in intimate contact with hepatocytes.

Other comments: In this specimen, giant cells were observed in intimate contact with: 1. mesenchymal cells of the dorsal body wall and mesogastrium, 2. Sheets of hepatocytes, without causing histolytic damage.

Experiment:	MB HM 24
Duration of graft:	12 hours
State of Host Embryo:	Terminated immediately on observation of macroscopic bleeding.
Nature of graft:	Ectoplacental cone
Position of graft and size:	Upper right coelom, 780μm x 250μm.
Attachment of graft:	Dorsal wall of coelom, right body wall and dorsum of liver.
Erosion of mesothelium:	Present at each site of attachment.
Invasion: local spread:	By extension of tongues of trophoblast cells and by periaortic spread of single giant cells.
distant spread:	Absent
Invasion of mesenchyme:	Present
Erosion of endothelial wall of: Aorta:	Present and severe.
Cardinal vein:	Present
Ductus venosus:	Present
Trophoblastic giant cells plugging hole in vessel wall:	Absent
Internal bleeding:	Present
Phagocytosis:	Absent

Other comments: 1. Erosion of the ventral wall of the aorta by tongues of giant cells. 2. Intimate contact of giant cell with mesonephric duct and hepatocytes without apparent damage (histolytic or cytolytic) to these host tissues.

Experiment:	MB HM 29
Duration of graft:	11 hours
State of Host Embryo:	Found dead, about 1 hour after last inspection.
Nature of graft:	Egg cylinder
Position of graft and size:	Upper right coelom, 900μm x 500μm.
Attachment of graft:	Dorsal wall of coelom, and right body wall.
Erosion of mesothelium:	Present
Invasion: local spread:	By extension of tongues of cells.
distant spread:	Present (individual G.C.).
Invasion of mesenchyme:	Present
Erosion of endothelial wall of: Aorta:	Present
Cardinal vein:	Not applicable (no contact with EPC).
Ductus venosus:	Not applicable (no contact with EPC).
Trophoblastic giant cells plugging hole in vessel wall:	Present (partial)
Internal bleeding:	Present
Phagocytosis:	of avian red cells observed.

Other Comments: In this specimen, several examples were seen of giant cells in contact with coelomic mesothelium, mesenchymal cells of the right body wall, mesonephric duct, epithelium and mesothelium of the lung bud without causing any histolytic or cytolytic damage.

Experiment:	MB HM 49
Duration of graft:	12 hours
State of Host Embryo:	Healthy at termination; no macroscopic bleeding seen.
Nature of graft:	Ectoplacental cone
Position of graft and size:	Upper right coelom, 450μm x 300μm.
Attachment of graft:	To dorsal wall of coelom and dorsal surface of liver.
Erosion of mesothelium:	Present
Invasion: Local spread:	By extension of tongues of trophoblastic giant cells; periaortic spread of several isolated giant cells.
distant spread:	Absent
Invasion of mesenchyme:	Present
Erosion of endothelial wall of: Aorta:	Present
Cardinal vein:	Not applicable (no contact with EPC).
Ductus venosus:	Present
Trophoblastic giant cells plugging hole in vessel wall:	Present
Internal bleeding:	Present
Phagocytosis:	of nuclear material of host cells in contact with hepatic plates.

Other comments: Several examples of giant cells in contact with mesenchymal cells of the dorsal coelomic wall and hepatocytes of the dorsal surface of the liver, without apparent histolytic or cytolytic damage.

Experiment:	MB HM 50
Duration of graft:	12 hours
State of Host Embryo:	Terminated immediately on observation of macroscopic bleeding.
Nature of graft:	Egg cylinder
Position of graft and size:	Upper right coelom, 400μm x 250μm.
Attachment of graft:	To dorsal wall of coelom and right wall of dorsal mesogastrium.
Erosion of mesothelium:	Present
Invasion: local spread:	By extension of tongues of cells.
distant spread:	Absent
Invasion of mesenchyme:	Present
Erosion of endothelial wall of: Aorta:	Present
Cardinal vein:	Not applicable (not in contact with EPC).
Ductus venosus:	Not applicable (not in contact with EPC).
Trophoblastic giant cells plugging hole in vessel wall:	Present
Internal bleeding:	Present
Phagocytosis:	of avian red cells observed.

Experiment:	MB HM 52
Duration of graft:	12 hours
State of Host Embryo:	Healthy at termination.
Nature of graft:	Egg cylinder
Position of graft and size:	Upper right coelom, 500μm x 350μm.
Attachment of graft:	Dorsal wall of coelom R. body wall R. mesogastrium
Erosion of mesothelium:	Present at sites of attachment.
Invasion: local spread:	Present
distant spread:	of individual giant cells, close to left spinal nerve and to ectoderm-mesenchyme boundary.
Invasion of mesenchyme:	Present
Erosion of endothelial wall of: Aorta:	Present
Cardinal vein:	Not applicable (not in contact with EPC).
Ductus venosus:	Not applicable (not in contact with EPC).
Trophoblastic giant cells plugging hole in vessel wall:	Present (complete).
Internal bleeding:	Absent
Phagocytosis:	Absent

Experiment:	MB HM 55
Duration of graft:	11 hours
State of Host Embryo:	Terminated 15 minutes after macroscopic bleeding was observed.
Nature of graft:	Ectoplacental cone
Position of graft and size:	Upper right coelom, 565μm x 350μm.
Attachment of graft:	To dorsal wall of coelom, right surface of dorsal mesogastrium and dorsum of liver.
Erosion of mesothelium:	Present, at both sites of attachment.
Invasion: local spread:	By extension of tongues of trophoblastic giant cells and periaortic spread of isolated giant cells.
distant spread:	Present
Invasion of mesenchyme:	Present
Erosion of endothelial wall of: Aorta:	Present
Cardinal vein:	Not applicable (not in contact with EPC)
Ductus venosus:	Not applicable (not in contact with EPC)
Trophoblastic giant cells plugging hole in vessel wall:	Present
Internal bleeding:	Present
Phagocytosis:	By a giant cell in the area of the developing spleen observed. The giant cell contained cellular debris.

Other comments: Localized penetration of dorsal coelomic wall and right surface of dorsal mesogastrium by giant cells as well as distant spread of individual giant cell into the area of the developing spleen observed. No evidence of histolytic or cytolytic damage seen.

APPENDIX D

16 HOUR GROUP

Experiment:	MB HM 5
Duration of graft:	18 hours
State of Host Embryo:	Macroscopic bleeding observed for about an hour prior to termination.
Nature of graft:	Ectoplacental cone
Position of graft and size:	Upper right coelom 400μm x 200μm.
Attachment of graft:	To right body wall, right lung bud, and dorsal surface of liver.
Erosion of mesothelium:	Present
Invasion: local spread:	of tongues of trophoblast cells and individual giant cells.
distant spread:	several giant cells at sites remote from main graft: sub-epidermal, sympathetic ganglia, right limb bud, notochord, liver.
Invasion of mesenchyme:	Present
Erosion of endothelial wall of: Aorta:	Nil, despite limited periaortic spread of individual giant cells.
Cardinal vein:	Present
Ductus venosus:	Present
Trophoblastic giant cells plugging hole in vessel wall:	Absent
Internal bleeding:	Present
Phagocytosis:	Absent

Other Comments: (i) The EPC graft had eroded wall of posterior cardinal vein and ductus venosus which were confluent and opened widely into coelom.

(ii) evidence of intravascular spread of individual giant cells.

Experiment:	MB HM 26
Duration of graft:	16 hours
State of Host Embryo:	Macroscopic bleeding observed 2 hours prior to termination.
Nature of graft:	Ectoplacental cone.
Position of graft and size:	Upper right coelom, 540μm x 350μm.
Attachment of graft:	To dorsal coelomic wall, liver, and right body wall.
Erosion of mesothelium:	Present
Invasion: Local spread:	By extension of tongues of trophoblastic giant cells.
Distant spread:	Present: many solitary giant cells situated away from the main graft: periaortic;
Invasion of mesenchyme:	Present
Erosion of endothelial wall of: Aorta:	Present
Posterior cardinal vein:	Present
Ductus venosus:	Present

Trophoblastic giant cells plugging hole in vessel wall: Present; partial.

Internal bleeding: Present

Phagocytosis: Observed

Other Comments: Several examples of individual giant cells; within liver vessels, closely applied to hepatocytes; adjacent to sympathetic ganglia. No histolysis, no cytolysis.

Experiment:	MB HM 28
Duration of graft:	16 hours
State of Host Embryo:	Macroscopic bleeding observed for three hours prior to termination.
Nature of graft:	Egg cylinder
Position of graft and size:	Upper right coelom, extending to anterior intestinal portal region. 1.05 mm x 450μm.
Attachment of graft:	To dorsal wall of coelom; to right body wall, to dorsal surface of the liver, and to dorsal mesogastrium.
Erosion of mesothelium:	Present
Invasion: local spread:	of tongues of trophoblast cells.
distant spread:	of individual giant cells present.
Invasion of mesenchyme:	Present
Erosion of endothelial wall of: Aorta:	Present
Cardinal vein:	Not applicable (no contact with EPC).
Ductus venosus:	Present
Trophoblastic giant cells plugging hole in vessel wall:	Present
Internal bleeding:	Present
Phagocytosis:	of degenerated red cell observed.

Other Comments: Erosion of dorsal coelomic wall, of its underlying mesenchyme and of aortic endothelium led to bleeding into the graft and coelom, which was plugged (only in part) by the graft.

Experiment:	MB HM 41
Duration of graft:	15 hours
State of Host Embryo:	Macroscopic bleeding observed for about an hour before termination.
Nature of graft:	Egg cylinder
Position of graft and size:	Upper right coelom 350μm x 400μm.
Attachment of graft:	To dorsal wall of coelom, dorsal mesentery, right lung bud and the liver.
Erosion of mesothelium:	Present
Invasion: local spread:	of tongues of trophoblast.
distant spread:	Absent
Invasion of mesenchyme:	Present
Erosion of endothelial wall of: Aorta:	Not applicable (no contact with EPC).
Posterior cardinal vein:	Present
Ductus venosus:	Present
Trophoblastic giant cells plugging hole in vessel wall:	Present; partial.
Internal bleeding:	Present
Phagocytosis:	of red cells observed.

Other Comments: Wide communication between right posterior cardinal vein and ductus venosus, resulting in bleeding into coelom.

Experiment:	MB HM 42
Duration of graft:	16 hours
State of Host Embryo:	Killed instantly at onset of bleeding.
Nature of graft:	Egg cylinder.
Position of graft and size:	Upper right coelom, 350μm x 400μm.
Attachment of graft:	To dorsal wall of coelom. Right body wall. Liver.
Erosion of mesothelium:	Present

Invasion: By local spread: of tongues of trophoblast cells.

By distant spread: Absent

Invasion of mesenchyme: Present

Erosion of endothelial wall of: Aorta: Present

Posterior cardinal vein: Present

Ductus venosus: Present

Trophoblastic giant cells plugging hole in vessel wall: Present

Internal bleedine: Present

Phagocytosis: of cellular debris observed in an individual giant cell in contact with mesenchyme of the dorsal mesentery.

Other Comments: Erosion of the aorta, ductus venosus and a large vein in the right body wall which were plugged in part most probably prevented the host from bleeding to death. Erosion of parts of the right mesonephros was also observed.

Experiment:	MB HM 43
Duration of graft:	16 hours
State of Host Embryo:	Alive and healthy at termination. No macroscopic bleeding observed.
Nature of graft:	Egg cylinder; EPC rostrally situated.
Position of graft and size:	Upper right coelom, 585μm x 500μm.
Attachment of graft:	To dorsal wall of coelom and right face of mesogastrium and right lung bud.
Erosion of mesothelium:	Present
Invasion: local spread:	of small groups of vacuolated giant cells.
distant spread:	Absent: no periaortic spread.
Invasion of mesenchyme:	Present
Erosion of endothelial wall of: Aorta:	Present but very minimal.
Cardinal vein:	Not applicable (no contact with EPC).
Ductus venosus:	Not applicable (no contact with EPC).
Trophoblastic giant cells plugging hole in vessel wall:	Not applicable.
Internal bleeding:	Absent
Phagocytosis:	Absent

Other Comments: An example of early attachment and invasion by trophoblastic giant cell in contact with the dorsal coelomic wall. No histolysis or cytolysis observed.

Experiment:	MB HM 44
Duration of graft:	16 hours
State of Host Embryo:	Macroscopic bleeding observed for about an hour prior to termination.
Nature of graft:	Egg cylinder
Position of graft and size:	Upper right coelom, 650μm x 400μm.
Attachment of graft:	To right body wall, dorsal surface of the liver and right lung bud.
Erosion of mesothelium:	Present
Invasion: local spread:	of tongues of trophoblast cells and individual vacuolated giant cells found in the liver.
distant spread:	Absent
Invasion of mesenchyme:	Present
Erosion of endothelial wall of: Aorta:	Not applicable (no contact with EPC).
Cardinal vein:	Not applicable (no contact with EPC).
Ductus venosus:	Present
Trophoblastic giant cells plugging hole in vessel wall:	Observed
Internal bleeding:	Absent
Phagocytosis:	Absent

Other Comments: Unusual specimen, showing erosive activity of primary giant cells. No distant migration through tissues seen. Trophoblastic giant cells plugged hole in the ductus venosus, thus preventing the host from bleeding to death.

Experiment:	MB HM 46
Duration of graft:	15 hours, 30 minutes
State of Host Embryo:	Healthy, killed at onset of bleeding.
Nature of graft:	Egg cylinder with very small EPC.
Position of graft and size:	Upper right coelom, 400μm x 350μm.
Attachment of graft:	Right body wall, surface of liver, and dorsal wall of coelom.
Erosion of mesothelium:	Present
Invasion: local spread:	of tongues of trophoblastic giant cells.
distant spread:	Absent
Invasion of mesenchyme:	Present
Erosion of endothelial wall of: Aorta:	Present
Cardinal vein:	Not applicable (no contact with EPC).
Ductus venosus:	Present
Trophoblastic giant cells plugging hole in vessel wall:	Present
Internal bleeding:	Present
Phagocytosis:	of red cells observed.

Other Comments: Although EPC was rather small trophoblastic giant cells were observed spreading locally to subjacent structures like the mesentery and the dorsal coelomic wall. No histolysis or cytolysis seen.

Experiment:	MB HM 47
Duration of graft:	16 hours
State of Host Embryo:	Killed instantly at the onset of bleeding.
Nature of graft:	Egg cylinder
Position of graft and size:	At the level of the anterior intestinal portal; 700μm x 600μm.
Attachment of graft:	To dorsal mesentery.
Erosion of mesothelium:	Present
Invasion: local spread:	Present: perivascular.
distant spread:	Present: but very few.
Invasion of mesenchyme:	Present
Erosion of endothelial wall of: Vitelline artery:	Present
Cardinal vein:	Absent
Portal vein:	Present
Trophoblastic giant cells plugging hole in vessel wall:	Present; partial.
Internal bleeding:	Present
Phagocytosis:	Absent

Other Comments: The graft lay in anterior intestinal portal with EPC directed caudally. This position and orientation resulted in pattern of invasion different from usual.

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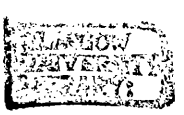

INVASIVE BEHAVIOUR OF HAMSTER TROPHOBLAST TRANSPLANTED TO CHICK INTRAEMBRYONIC COELOM

By

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VOLUME 2 - FIGURES

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Fig. 4: Shows the normal appearance of the graft's position in ovo, the upper right coelom. Note (i) the intact coelomic mesothelium which is largely squamous except for the dorsolateral aspect that appears to be cuboidal. (ii) neighbouring structures of the chick in an undisturbed state.

*This is representative of all unoperated controls.

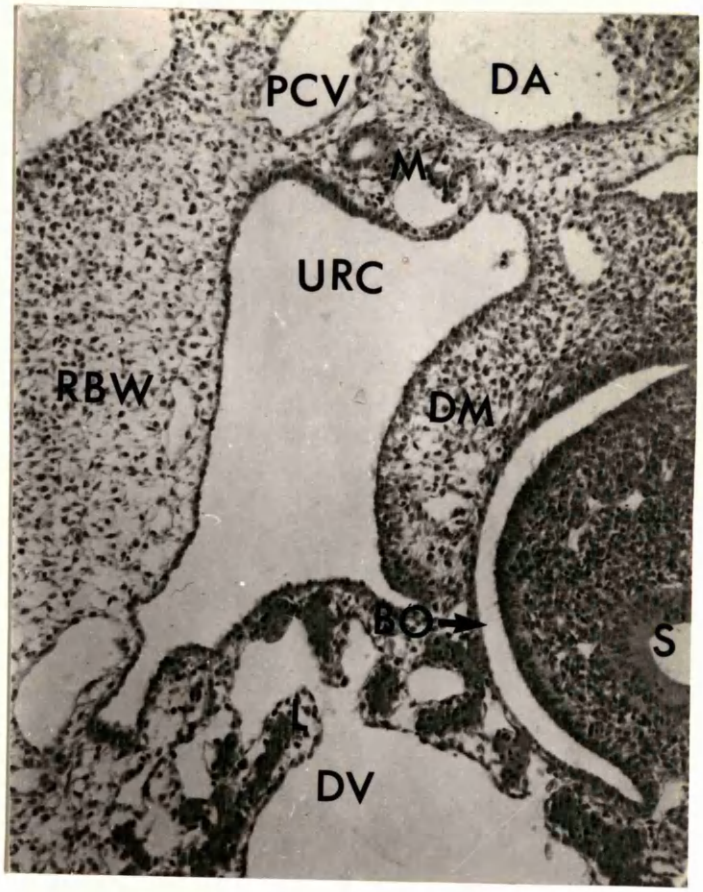
(R.B.W. - Right body wall; P.C.V. - Posterior cardinal vein; M - Mesonephros; D.A. - Dorsal aorta; DM - Dorsal mesogastrium; BO - Bursa omentalis; S - Stomach; L - Liver; DV - Ductus venosus; URC - Upper right coelom)

(U.C.2:1/10:8:4:8 - 8 hour group) x 250

Fig. 5: Shows an egg cylinder graft (now at neural fold stage) within right upper coelom and neighbouring structures of the chick .

(EC - Egg cylinder; M.D. - Mesonephric duct; U.V. - Umbilical vein; L.B. - Limb bud).

(Embryo No.12:1/10:8:2:8 - 8 hour group) x 80


PCV
DA
M
URC
RBW
DM
BO
S
DV

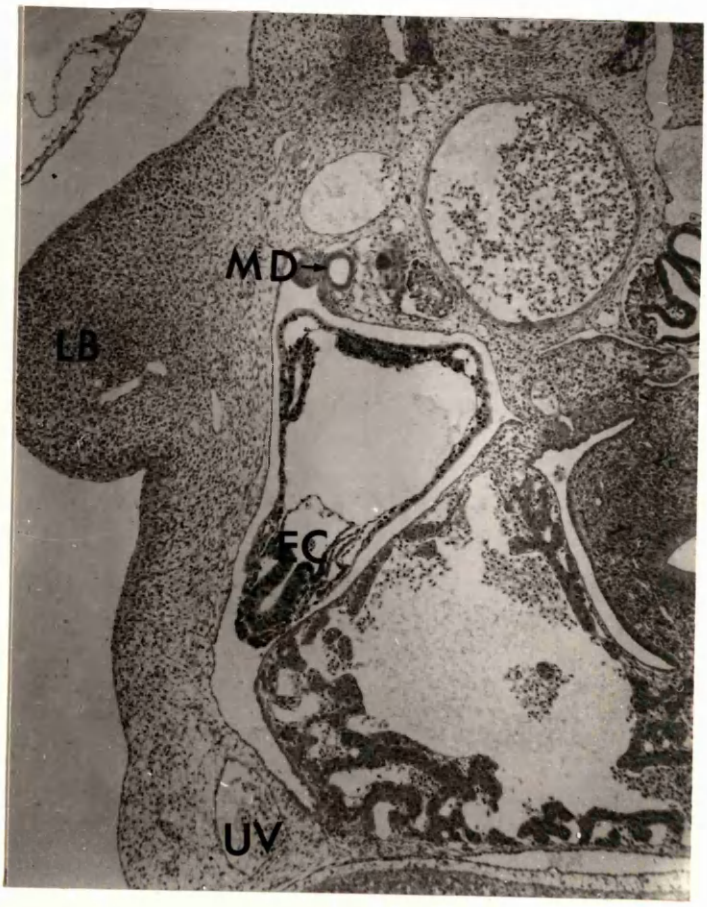

MD
LB
FC
UV

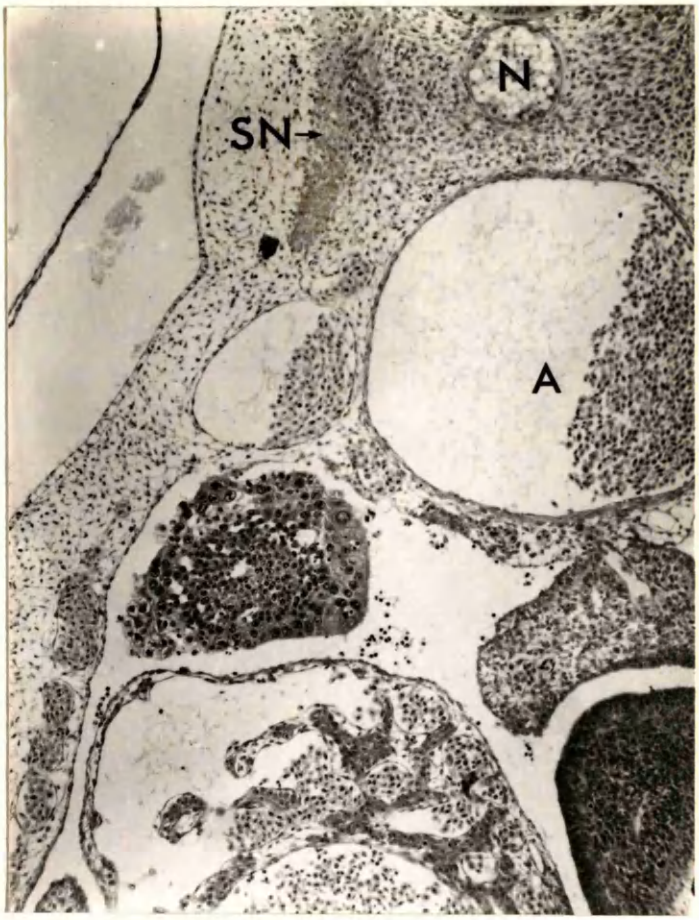
Fig. 6a: Shows EPC part of an egg cylinder graft in its usual position, the upper right coelom, which had caused a breach of the dorsolateral coelomic bay.

(N - Notochord; S.N. - spinal nerve)

(Embryo No. 31:1/10:1:4:8 - 8 hour group)
x 100

Fig. 6b: Shows trophoblastic giant cells (GC) which caused a breach in the dorsolateral coelomic mesothelium on making contact with it. Note that 1) the adjacent mesothelial cells and the underlying mesenchymal cells to the giant cells appear healthy; 2) although giant cells were in close proximity to the dorsomedial coelomic mesothelium, right body wall and the dorsal surface of the liver, damage "at a distance" was not observed and also 3) three types of trophoblast cells.

(Embryo No.31:1/10:1:4:8- 8 hour group)
x 250


N
SN
A

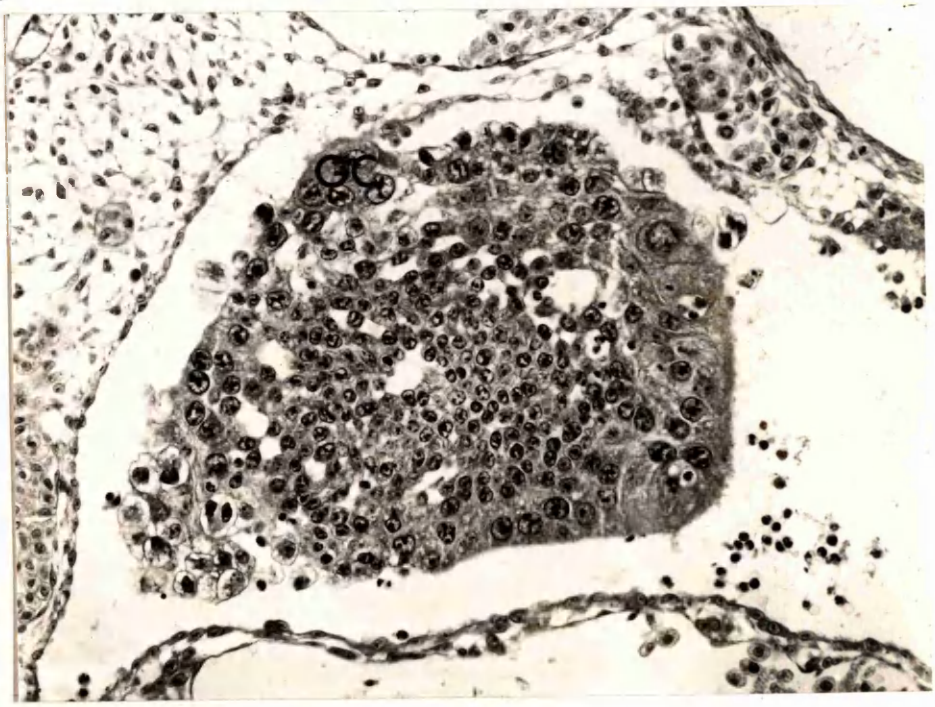
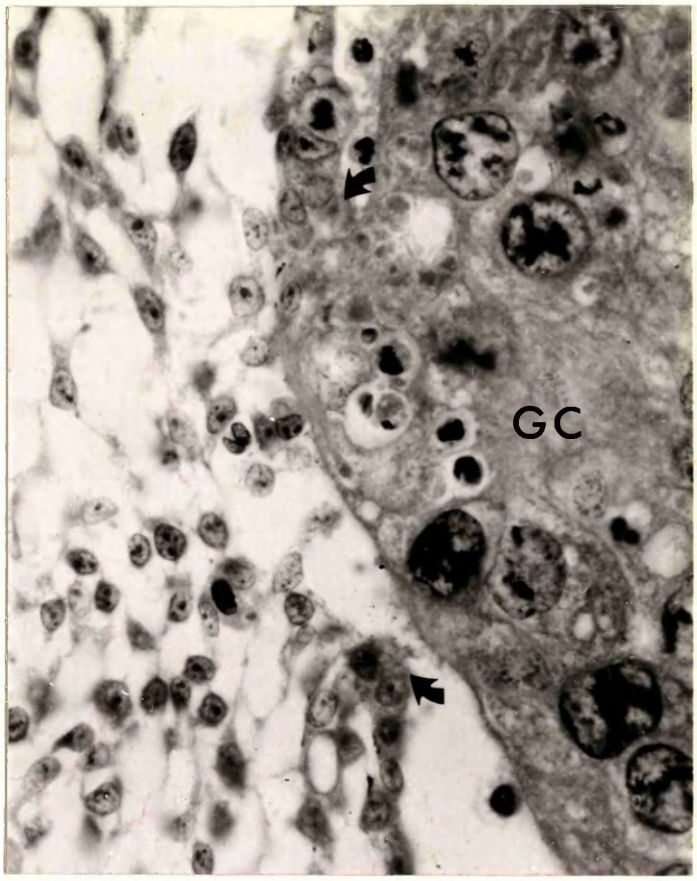

GC

Fig. 7: High power view of early invasion of right body wall by trophoblastic giant cells (GC). Notice thickening of coelomic mesothelium at point of contact with the graft (arrows).

(Embryo No.33:1/10:2:2:1 - 8 hour group)
x 400

Fig. 8: Early invasion of the right body wall by trophoblastic giant cells. Mesothelium at margins of defect and mesenchymal cells deep to it appear healthy (arrows).

(Embryo No.34:1/10:1:3:7 - 8 hour group)
x 400


GC

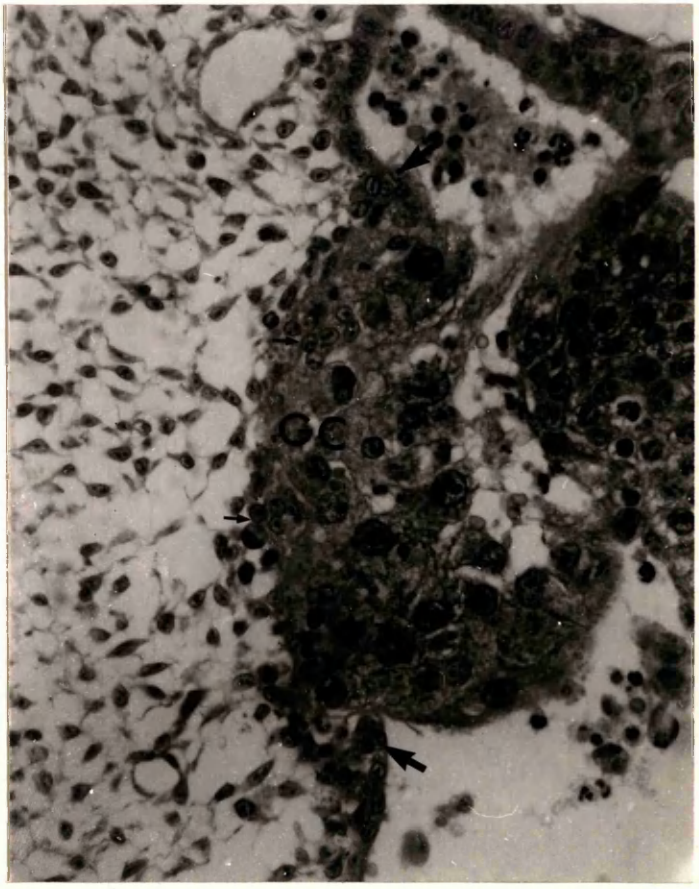


Fig. 9: High power view of very early penetration of coelomic mesothelium by the cytoplasmic process of a single trophoblastic giant cell which came into direct contact with epithelial cells of ventral wall of mesonephric duct, whose nuclei appear normal.

(Embryo No.35:1/10:1:2:1 - 8 hour group)
x 1000

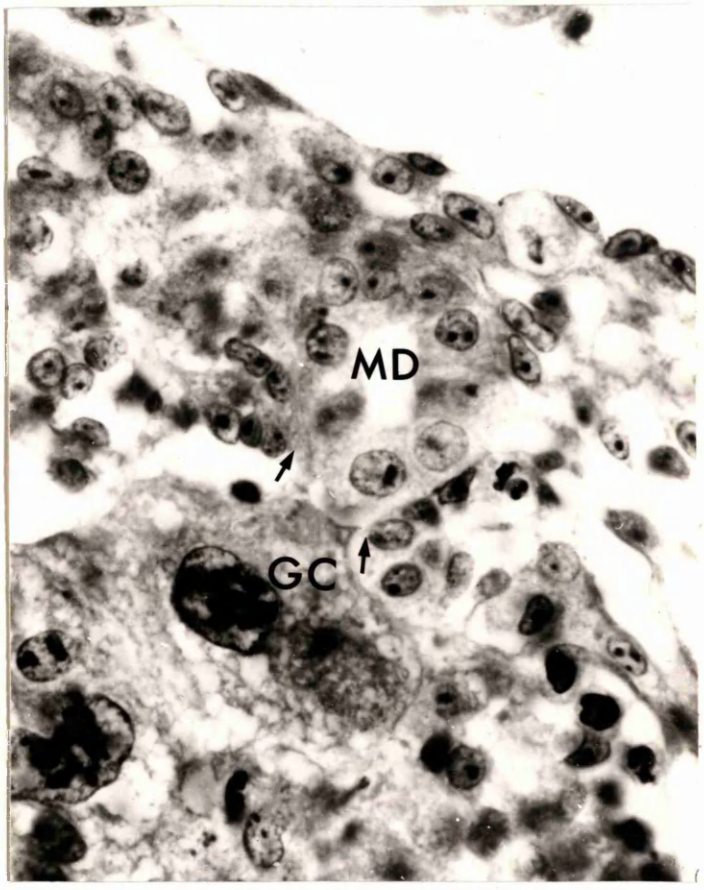

MD
GC

Fig. 10a: Shows early invasion of dorsal coelomic mesothelium by giant cells. Note:

(i) very localized erosion of coelomic mesothelium where giant cell cytoplasm came in contact with it.

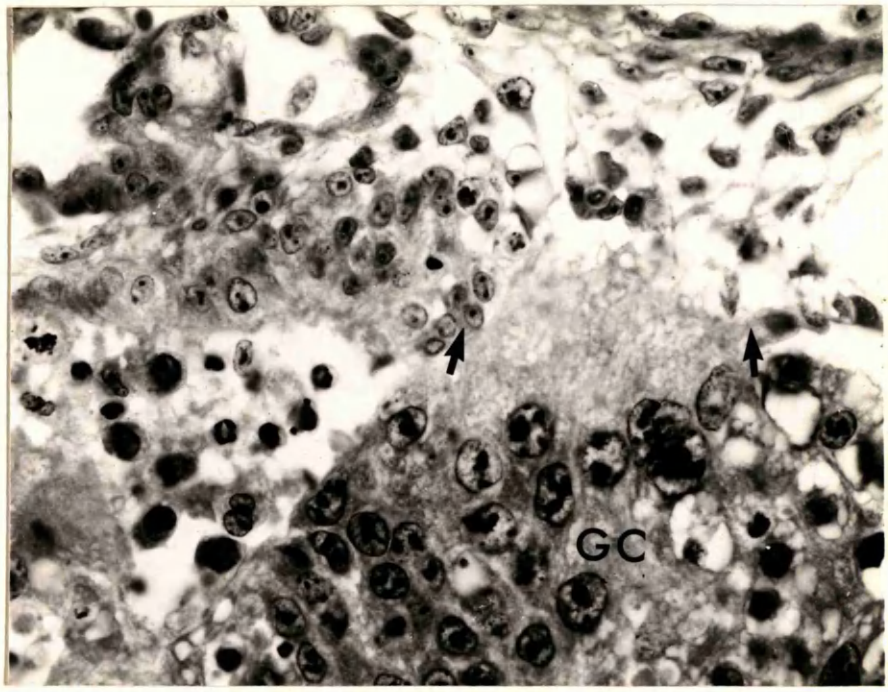
(ii) the invading giant cell cytoplasm appears devoid of cellular debris.

(iii) thickening of the coelomic mesothelium to the right side of the defect: compare with Fig.10b.

(Embryo No.38:1/10:1:4:5 - 8 hour group)
x 1000

Fig. 10b: High power view of dorsal wall of upper left coelom, to show the normal appearance of the dorsal coelomic mesothelium. This varies in height from squamous to low columnar, but is nowhere as thick as that adjacent to invading giant cell cytoplasm in right coelomic bay (same section as 10a).

(Embryo No.38:1/10:1:4:5 - 8 hour group)
x 1000


GC

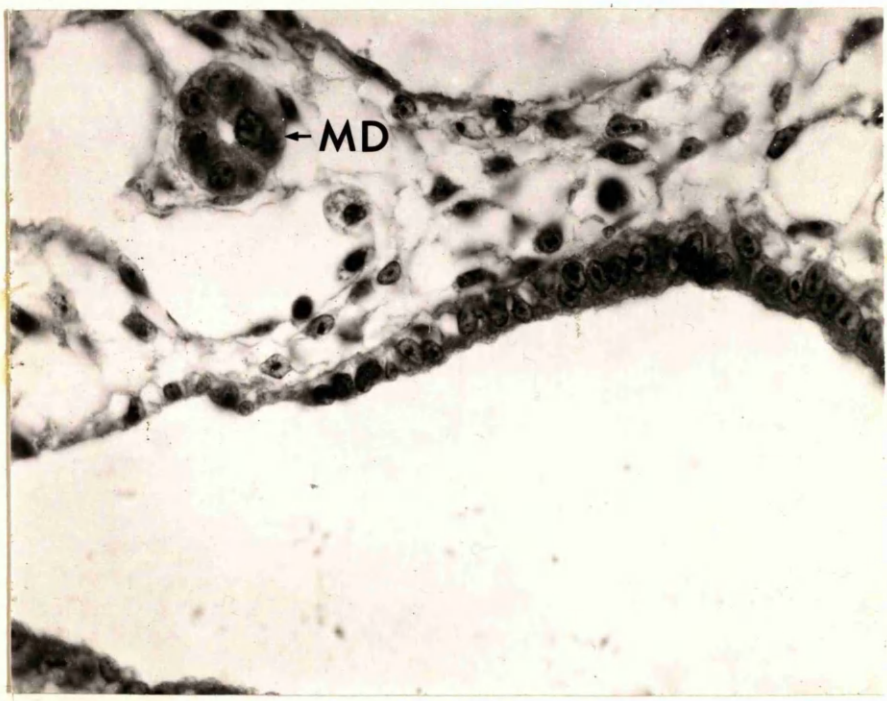
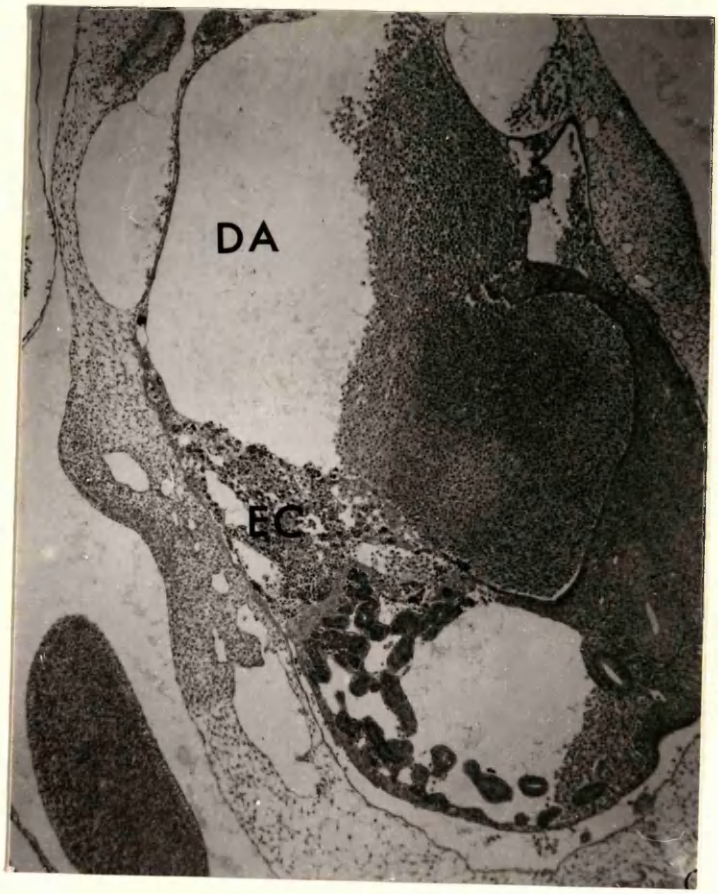

MD

Fig. 11: Low power view of graft (EC) in right coelom. The graft has eroded the ventral wall of the aorta and the right wall of bursa omentalis, which is filled with blood. Ventrally, the graft is attached to the right body wall and to the dorsal surface of the liver.

(Embryo No.54:1/10:2:4:9 - 8 hour group)
x 100

Fig. 12: Shows erosion of the dorsal body wall with destruction of ventral wall of aorta. Bleeding has apparently been partly contained by plugging of the defect in the aorta by a sheet of trophoblastic giant cells, which presents a concave surface to the escaping blood. Notice the apparently normal mesothelial cells adjacent to the right margin of the trophoblastic mass (arrowhead).

(Embryo No.23:1/10:1:2:5- 8 hour group)
x 250


DA
EC

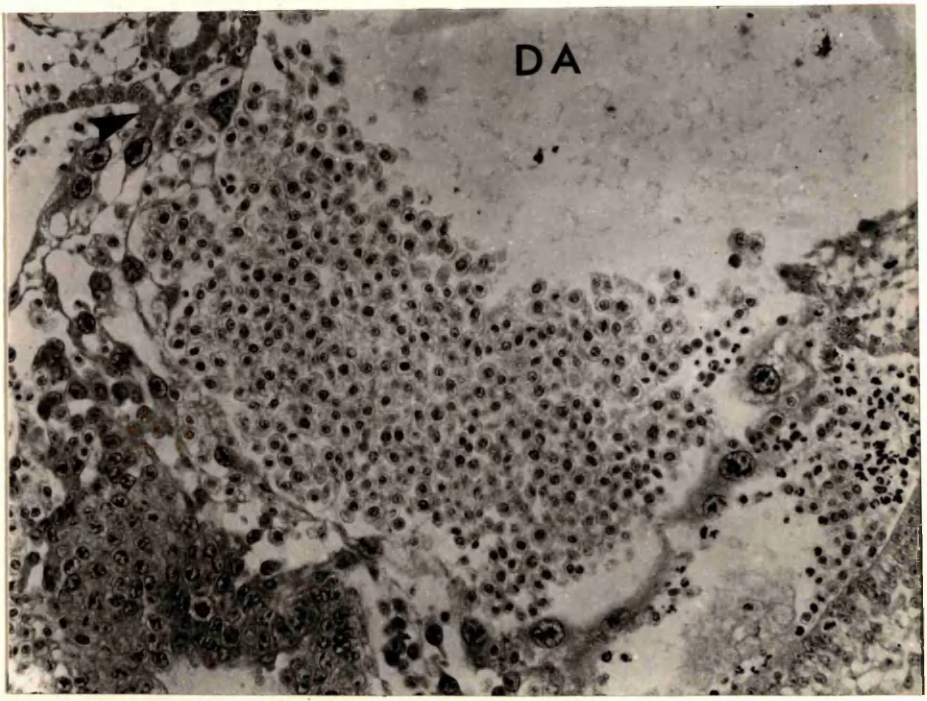
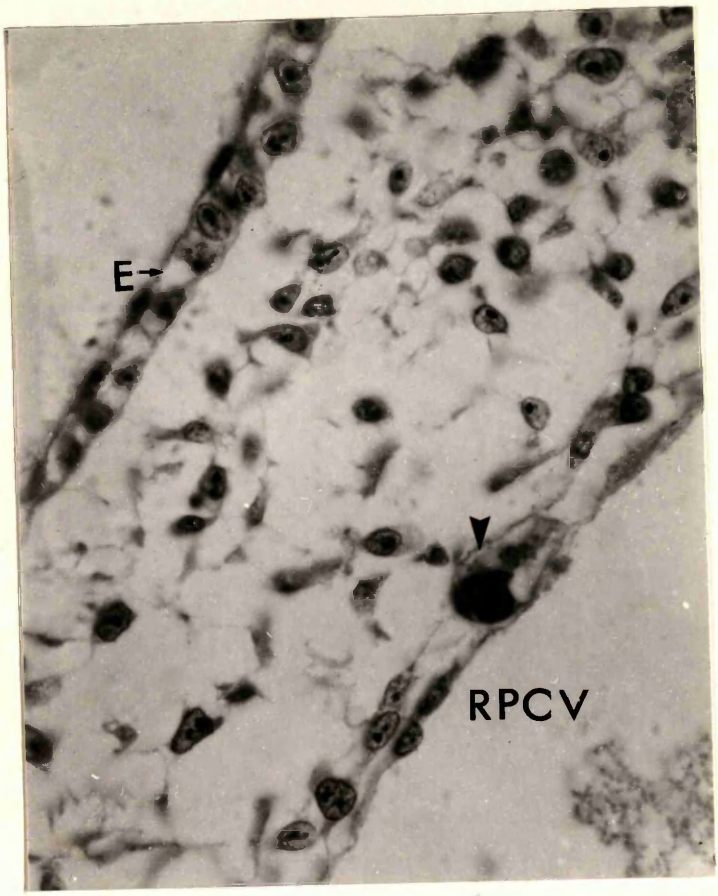

DA

Fig. 13: High power view of a migrant trophoblastic giant cell (arrowhead) which lay on the outer surface of the right posterior cardinal vein (RPCV), without causing any apparent damage to the endothelium (E - Epidermis).

(Embryo No.30:1/10:1:1:6 - 8 hour group)
x 800

Fig. 14: Shows a tongue of trophoblastic giant cells in upper right coelom. It has eroded and replaced the right wall of the aorta and comes into direct contact with the right mesonephric duct (arrow) which appears undamaged.

(Embryo No.19:1/10:2:1:4 - 8 hour group)
x 700


E
RPCV

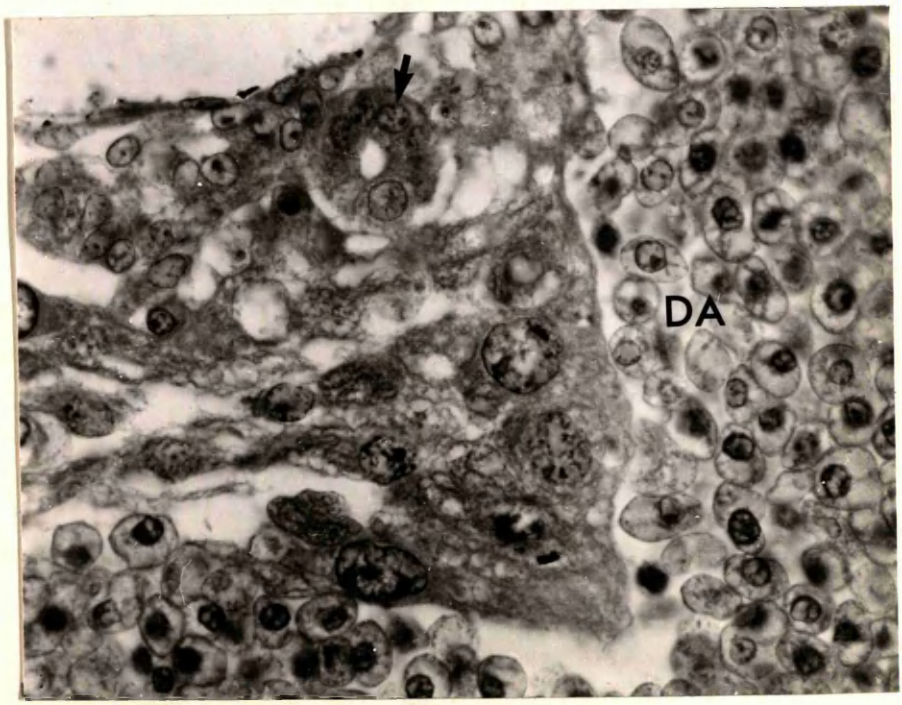

DA

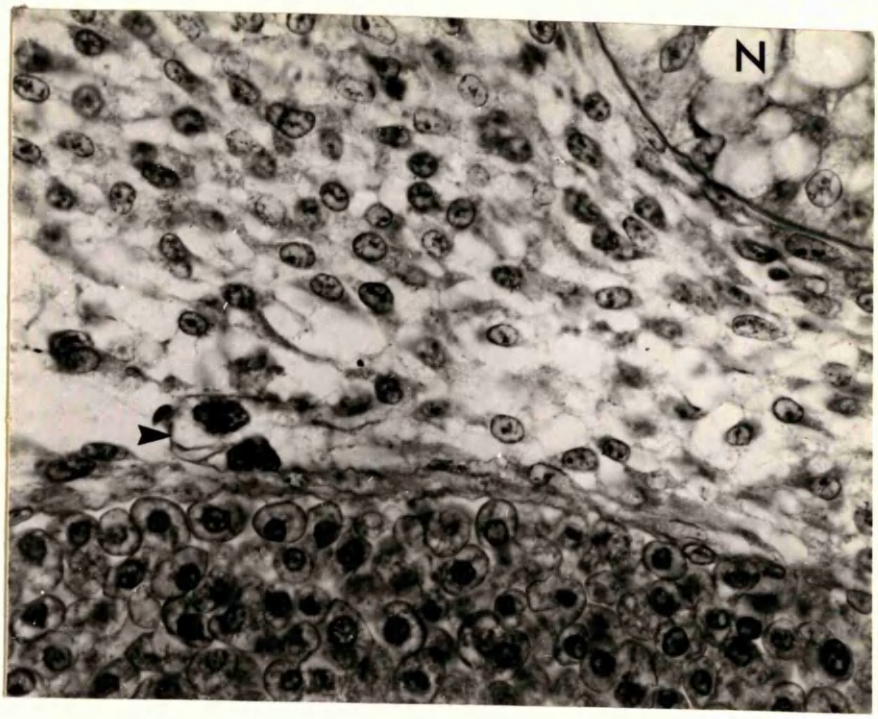
Fig. 15: Shows a migrant binucleated giant cell (arrowhead) which lay dorsal to the aorta (A). Notochord (N).

(Embryo No.19:1/10:2:1:1 - 8 hour group)
x 650

Fig. 16: A migrant trophoblastic giant cell (GC) in intimate contact with the notochordal sheath. Neither histolytic nor cytolytic damage to the surrounding mesenchymal cells was observed.

N - Notochord; NT - Neural tube.

(Embryo No.19:1/10:3:4:8 - 8 hour group)
x 400


N

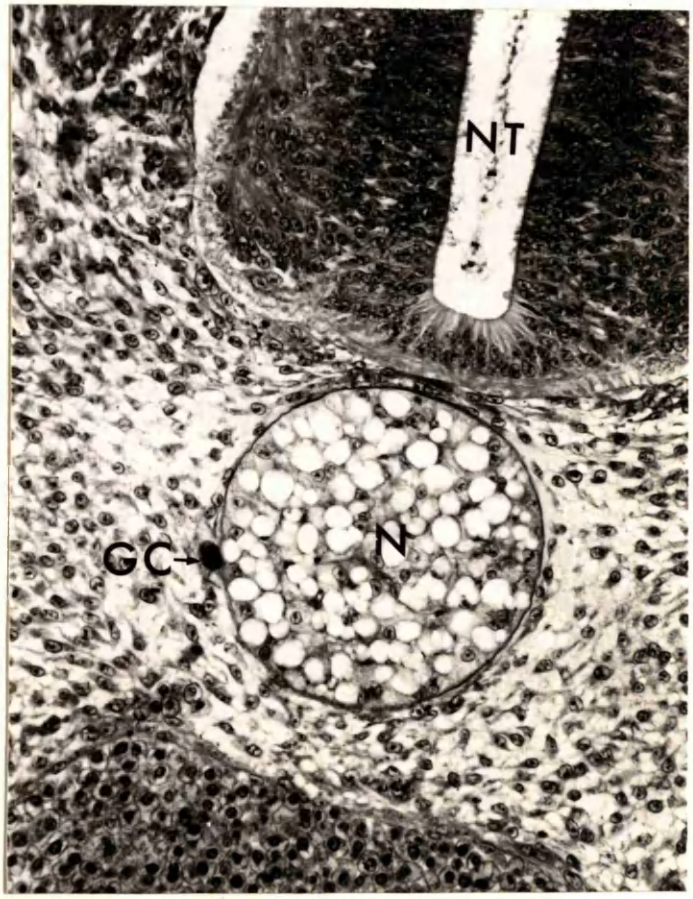
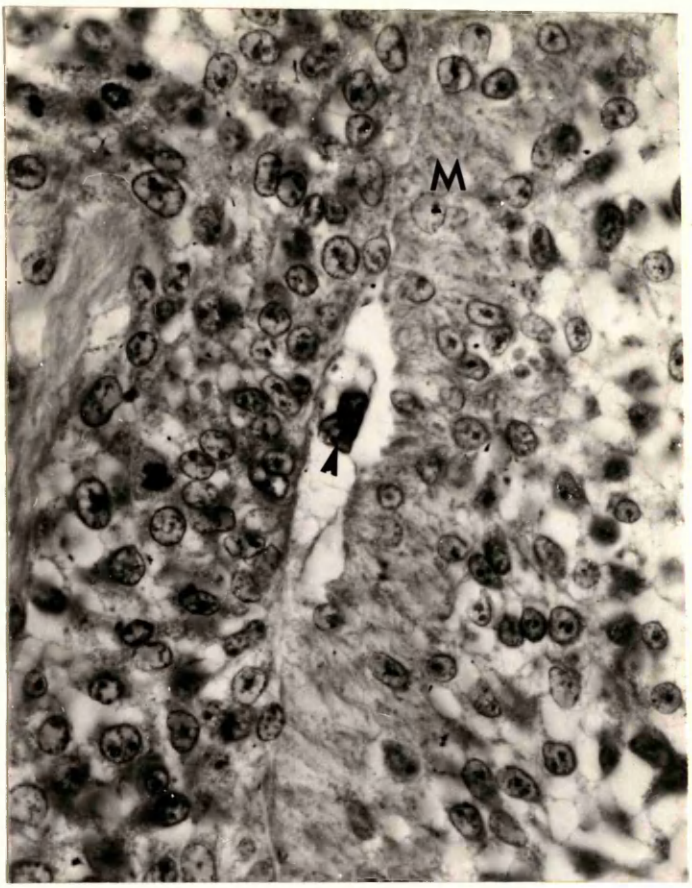

NT
N
GC

Fig. 17: Shows a high power view of a trophoblastic giant cell (arrowhead) in the right myocoele. Myotome (M).

(Embryo No.19:1/10:2:1:3 - 8 hour group)
x 800

Fig. 18: Shows a sheet of trophoblastic giant cells in close proximity to the mesothelium of the right body wall and of the right lung bud. Notice absence of damage at a distance.

(Embryo No.23:1/10:1:1:10 - 8 hour group)
x 250


M

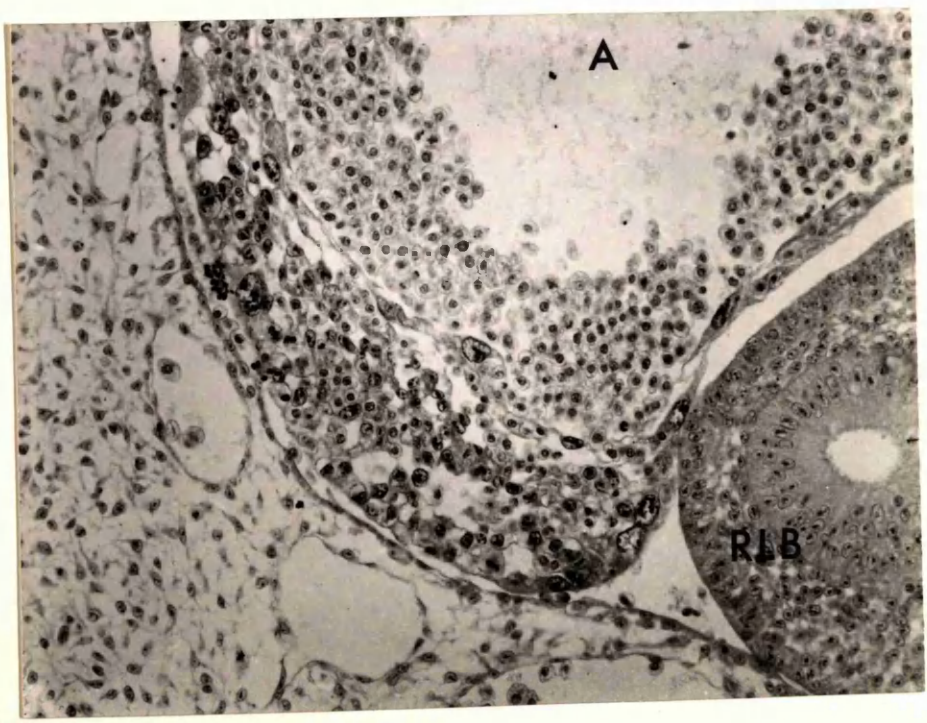

A
RIB

Fig. 19: Shows an egg cylinder graft in the upper right coelom with the EPC directed ventrally and laterally. A tongue of trophoblastic giant cell (GC) has produced a localised defect in the coelomic mesothelium and is penetrating the mesenchyme of the right body wall.

(Embryo No.31:1/10:2:3:8 - 8 hour group)
x 250

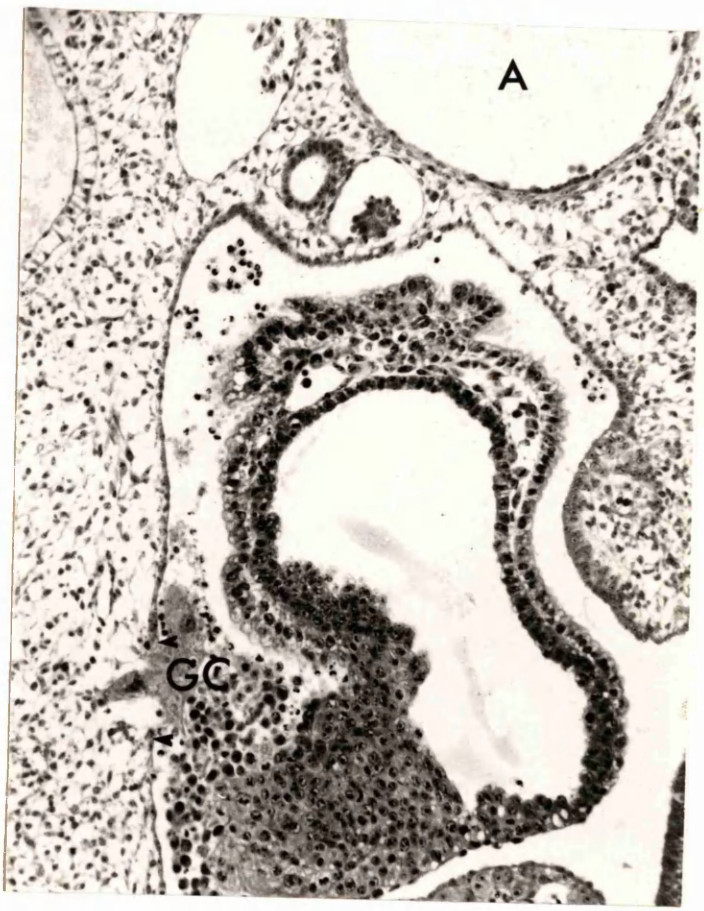

A
GC

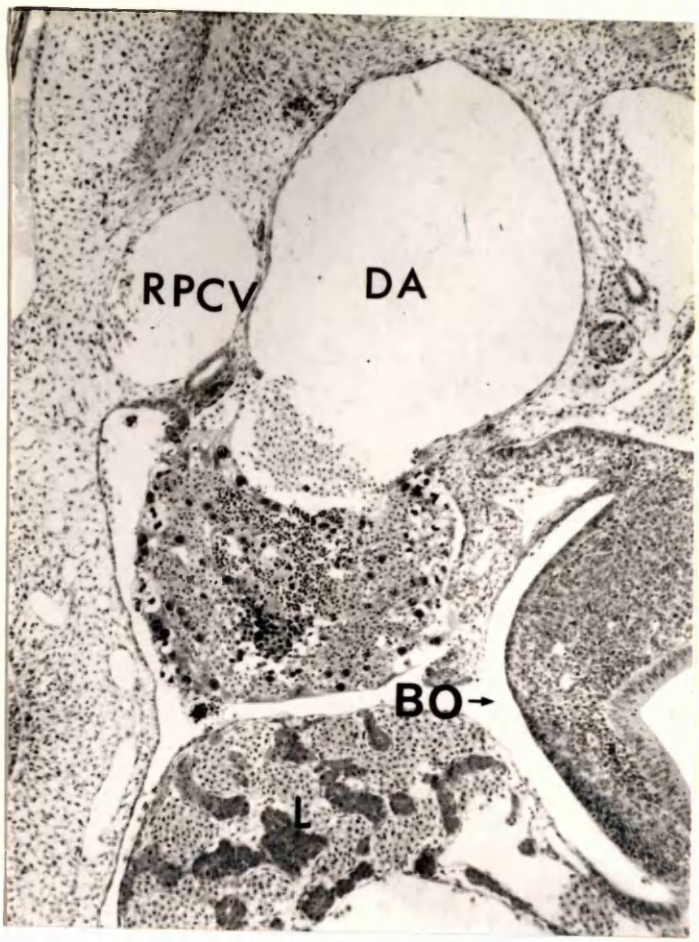
Fig. 20: Shows egg cylinder graft. It has eroded right ventral wall of aorta, but bleeding is limited by plugging of defect by trophoblast. It also has small area of contact with dorsal mesogastrium, which shows localized erosion. Elsewhere giant cells at periphery of graft lie within 20μm of right body wall and dorsum of liver without causing damage.

(Dorsal aorta - D.A., Liver - L, Right posterior cardinal vein - RPCV, Omental bursa - OB, Stomach - S)

(Embryo No.50:1/10:2:1:2 - 12 hour group)
x 100

Fig. 21: Shows an egg cylinder graft which had eroded the ventral aortic wall and 'plugged' it. Note also, erosion of the dorsum of the liver and of the ductus venosus. A few isolated trophoblast cells (arrows) have 'seeded' from the main graft, and come to lie in contact with the endothelial lining of the ductus venosus.

(Embryo No. 18:2:3:6 - 12 hour group)
x 100


RPCV
DA
BO
L

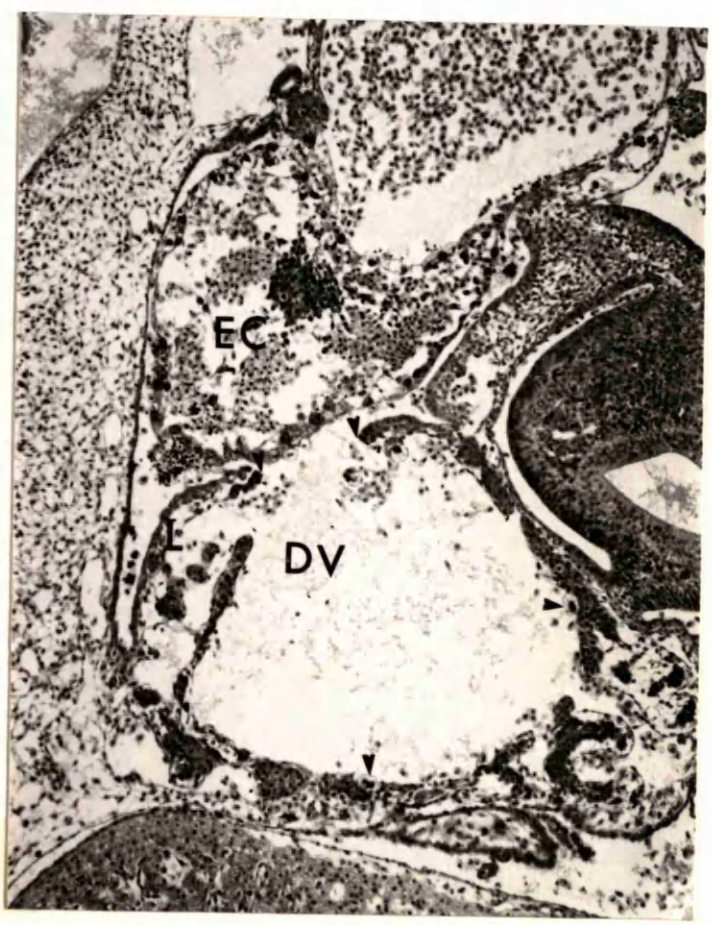
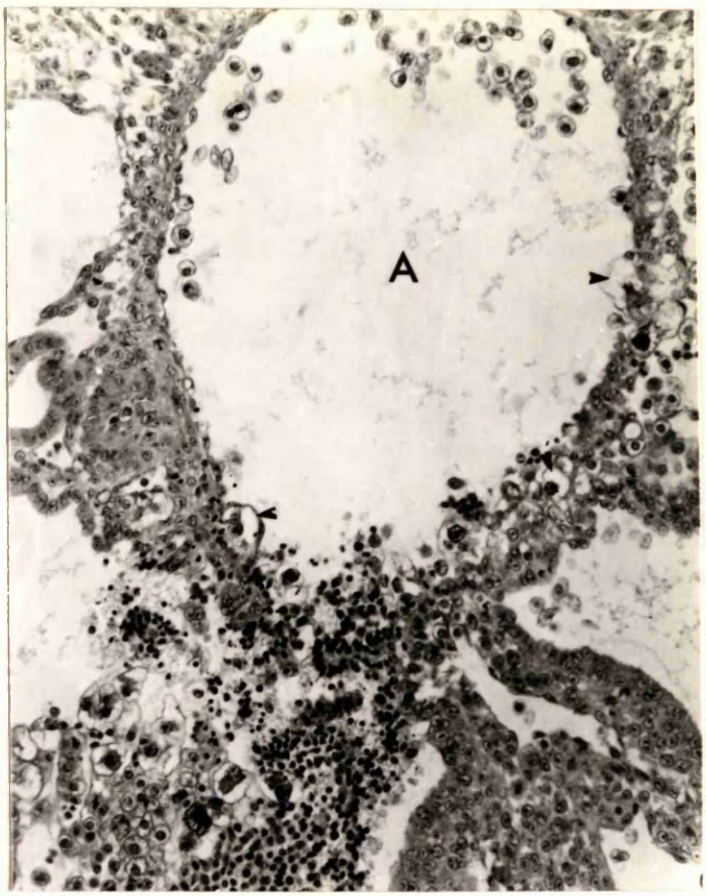

EC
DV

Fig. 22: Shows part of an ectoplacental cone graft which had eroded the ventral surface of the aorta. It also shows spread of vacuolated trophoblastic giant cells around aortic wall, particularly on left side (arrow), in places replacing the endothelium.

(Embryo No. 10:2:3:9 - 12 hour group) x 250

Fig. 23: Shows periaortic spread of several isolated trophoblastic giant cells (arrows), mostly along the outer surface of the endothelium, which appeared undamaged.

(Embryo No. 24:1/10:1:3:4 - 12 hour group) x 300


A

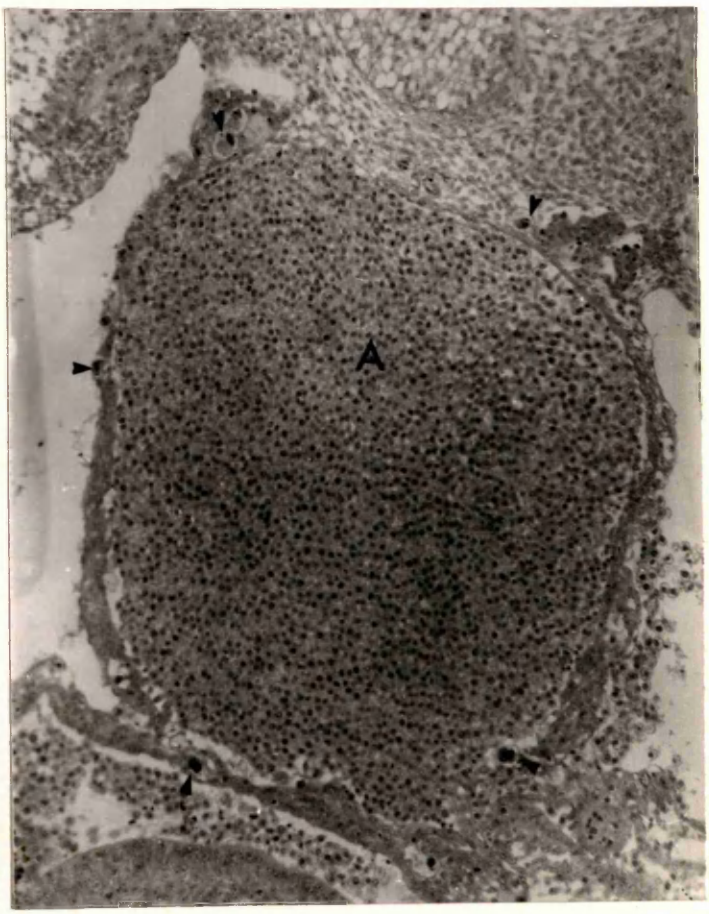

A

Fig. 24: Shows an egg cylinder graft.

Note: i. invasion of right body wall by giant cells

ii. lack of effect of visceral yolk sac endoderm on hepatic mesothelium, despite close proximity.

iii. migrant giant cell lying at deep surface of epidermis, which is separated, blister-like, from the mesenchyme (arrow).

(Right body wall - R.B.W., Epidermis - E)

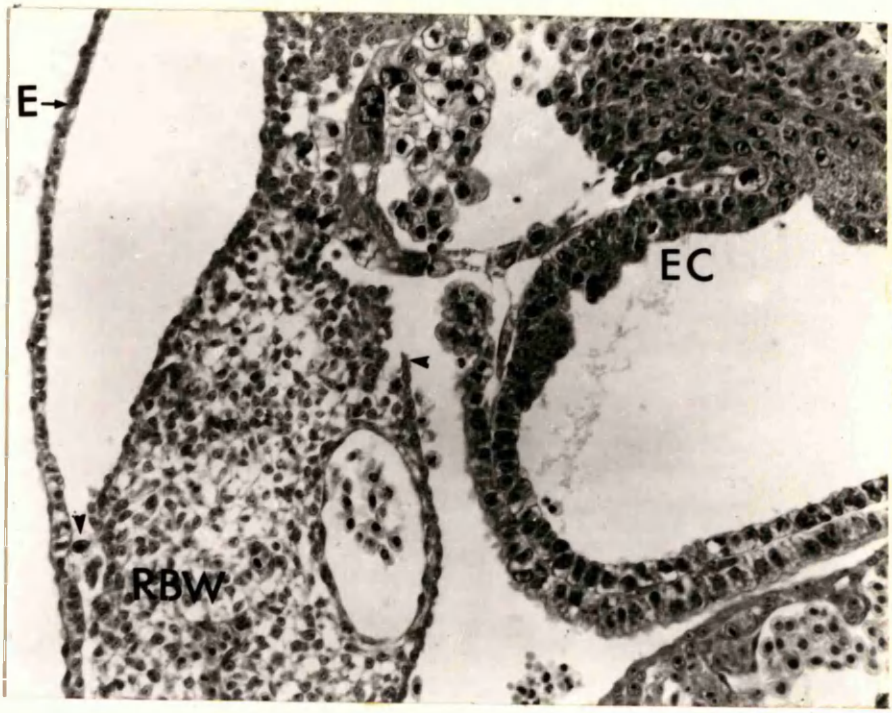
(Embryo No. 52:3:1:10 - 12 hour group) x 250

Fig. 25: Shows a binucleate trophoblastic giant cell (arrow) lying within what appears to be remains of the myocoele. Note:

i. absence of cytolytic or histolytic damage to closely adjacent cells of dermatomyotome.

ii. absence of phagocytosed material in giant cell.

(Embryo No. 10:1/10:1:1:5 - 12 hour group) x 1000


E
EC
RBW

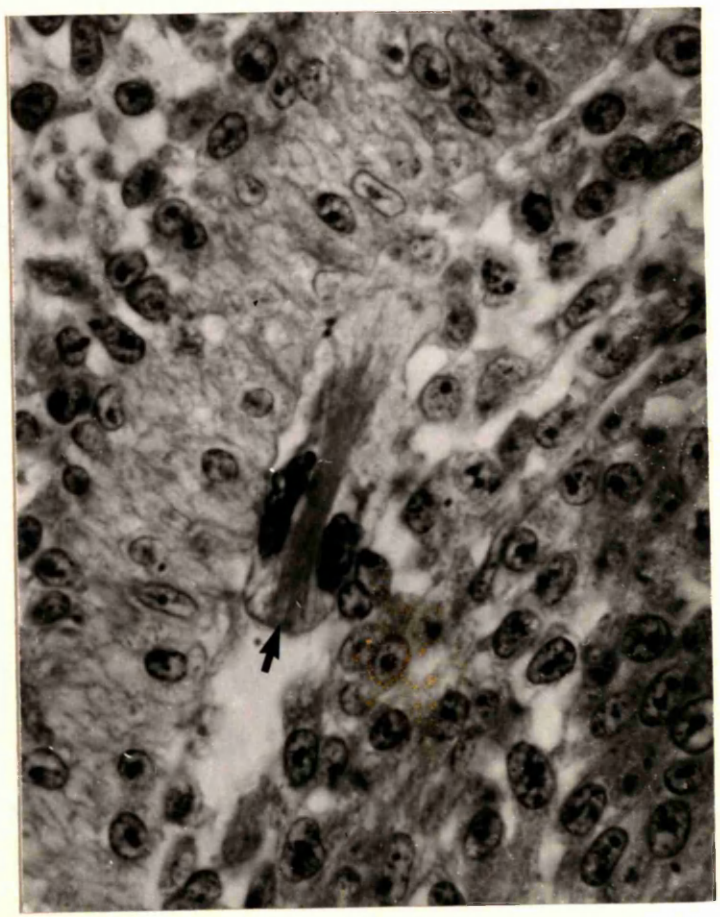


Fig. 26: Shows a trophoblastic giant cell in close proximity to the ventral root of a spinal nerve (arrow). Note:

i. that surrounding mesenchymal cells are normal.

ii. absence of phagocytosed material in giant cell.

(Spinal nerve - S.N., Notochord - N).

(Embryo No. 52:1/10:3:1:7 - 12 hour group) x 400

Fig. 27: Shows an ectoplacental cone graft which has eroded ventral wall of aorta (DA). Note:

i. plugging of aortic defect, limiting bleeding.

ii. giant cells in contact with mesothelium of right side of dorsal mesentery (D.M.) which shows local thickening.

iii. sheet of trophoblastic giant cells lies close to dorsal surface of liver (L); mesothelium and hepatic plates undamaged.

(Embryo No. 50:1/10:2:4:7 - 12 hour group) x 250


N
SM

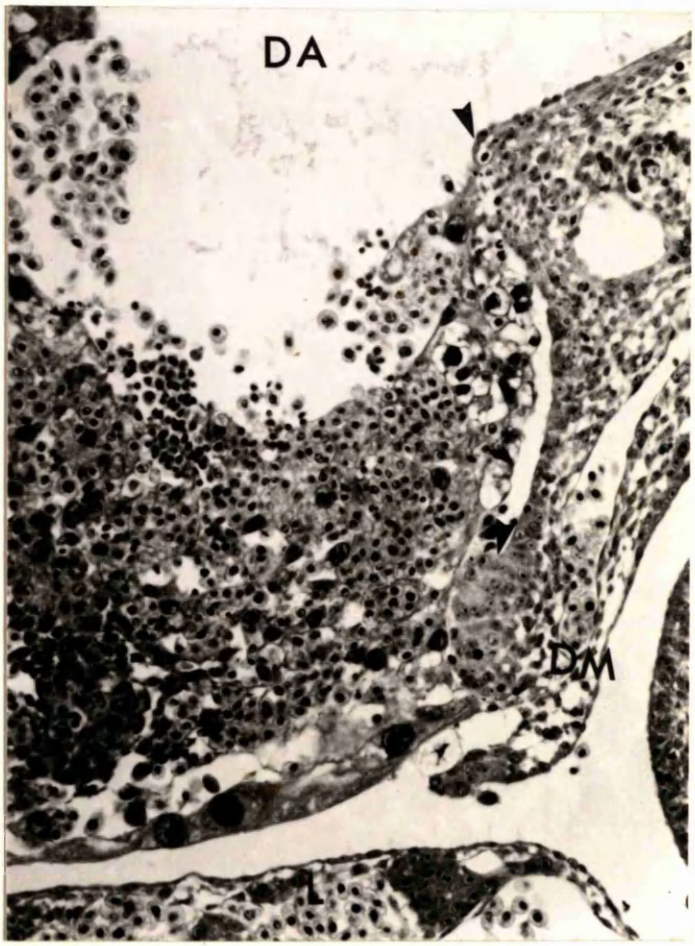

DA
DM

Fig. 28: Shows part of an ectoplacental cone graft in the right upper coelom. Note:

i. cytoplasmic process of a trophoblastic giant cell (arrowhead) in intimate contact with thickened coelomic mesothelium, which appeared to have been damaged.

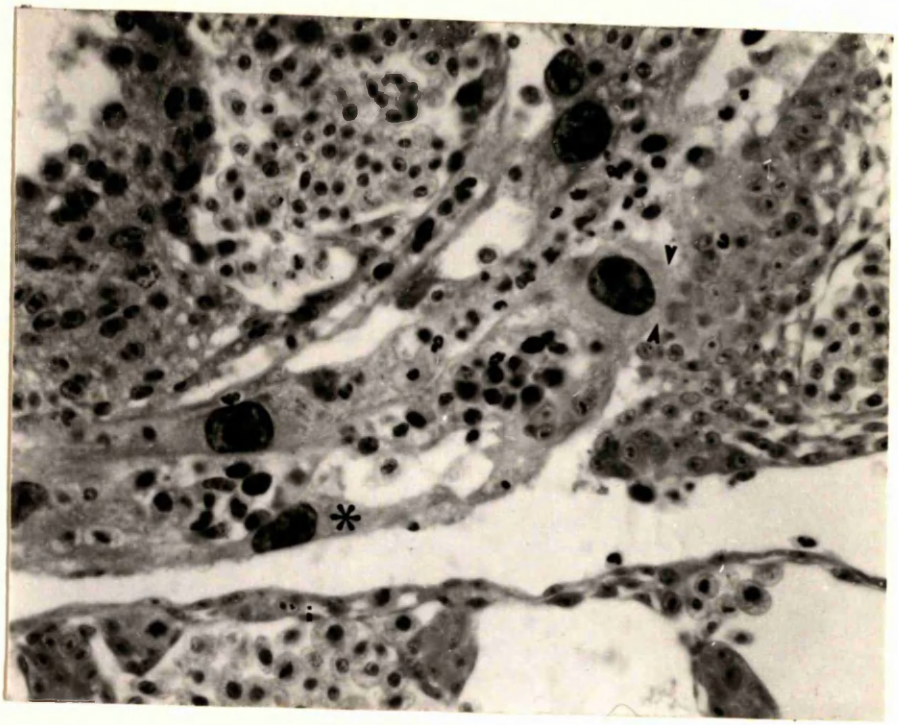
ii. trophoblastic giant cell (asterisk) close to the dorsum of the liver, which was undamaged.

(Embryo No.50:1/10:1:4:10 - 12 hour group)
x 400

Fig. 29: High power view of Fig. 28.

(Trophoblastic giant cell - G.C.)

(Embryo No.50:1/10:1:4:10 - 12 hour group)
x 600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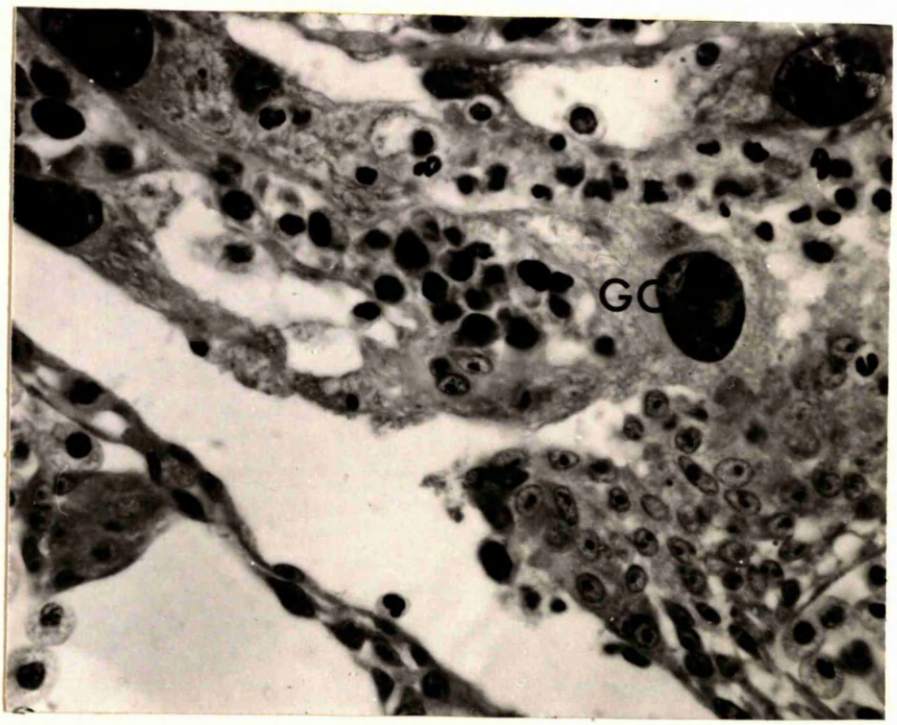
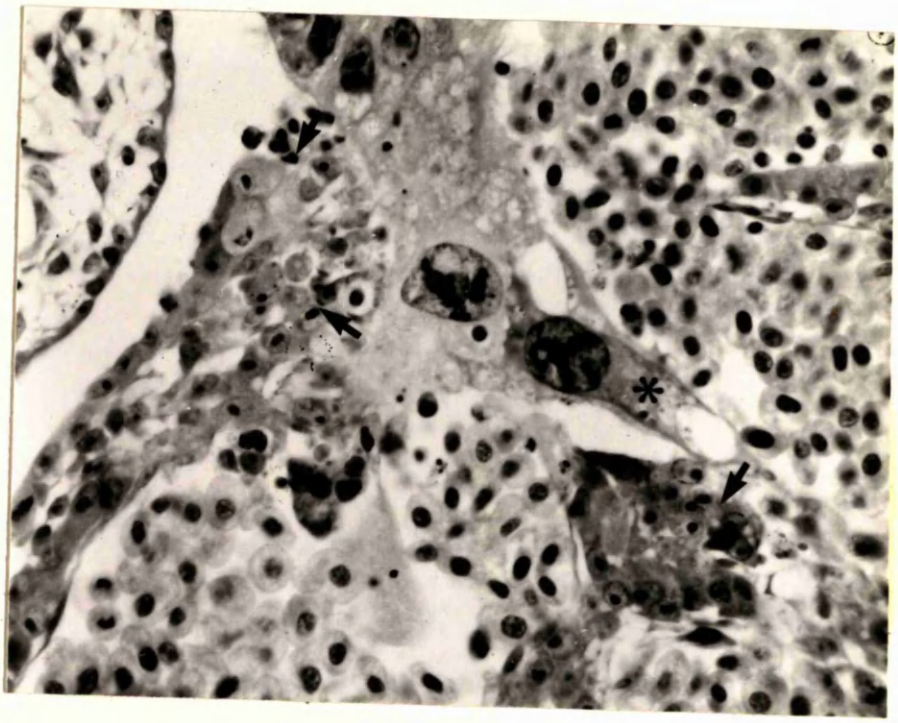

GC

Fig. 30: Shows a tongue of trophoblastic giant cells (asterisk) which has invaded the dorsum of the liver. Where it comes into contact with hepatic plates, the hepatocytes appear to be damaged (arrows). Note: evidence of phagocytic debris in giant cell.

(Embryo No.49:1/10:1:3:12 - 12 hour group) x 600

Fig. 31: Shows tongue of giant cells (arrow) which had eroded the dorsum of the liver (L) and the ductus venosus (DV). Where it comes in contact with an hepatic plate, the hepatocytes appear to be damaged.
Note: evidence of phagocytosis by giant cell.

(Embryo No.49:1/10:1:4:2 - 12 hour group) x 500



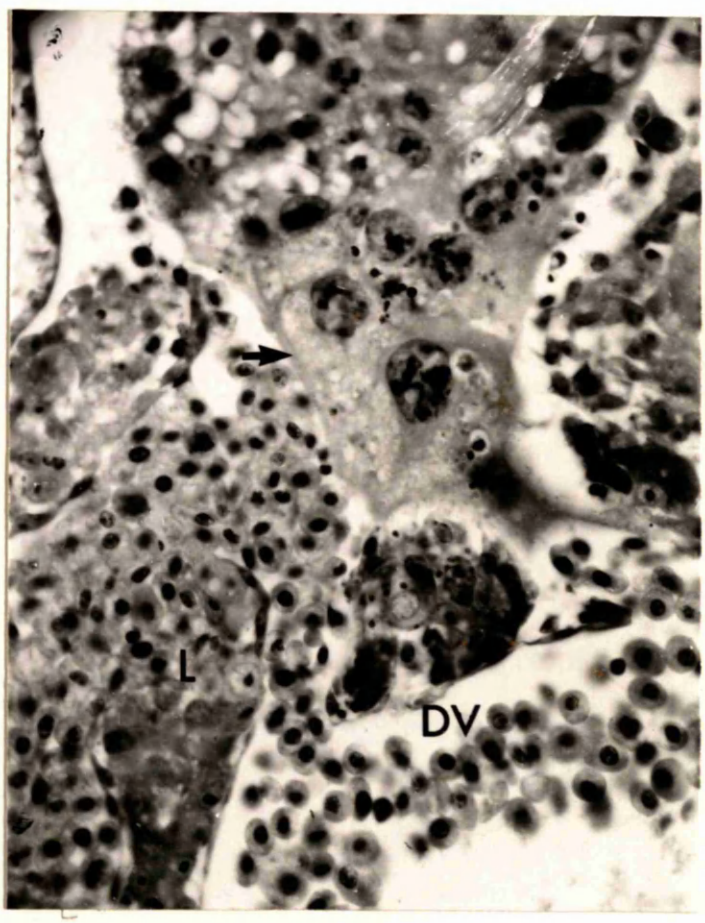

L
DV

Fig. 32: Shows a small fragment of an EPC graft (arrow) attached to the right lung bud (RLB). The graft had eroded the right body wall, and opened the right posterior cardinal vein (RPCV) and the ductus venosus, which are in continuity through the coelom.

Note: i. the aorta (A) is intact
ii. individual giant cells (arrows) within tributaries of posterior cardinal vein.

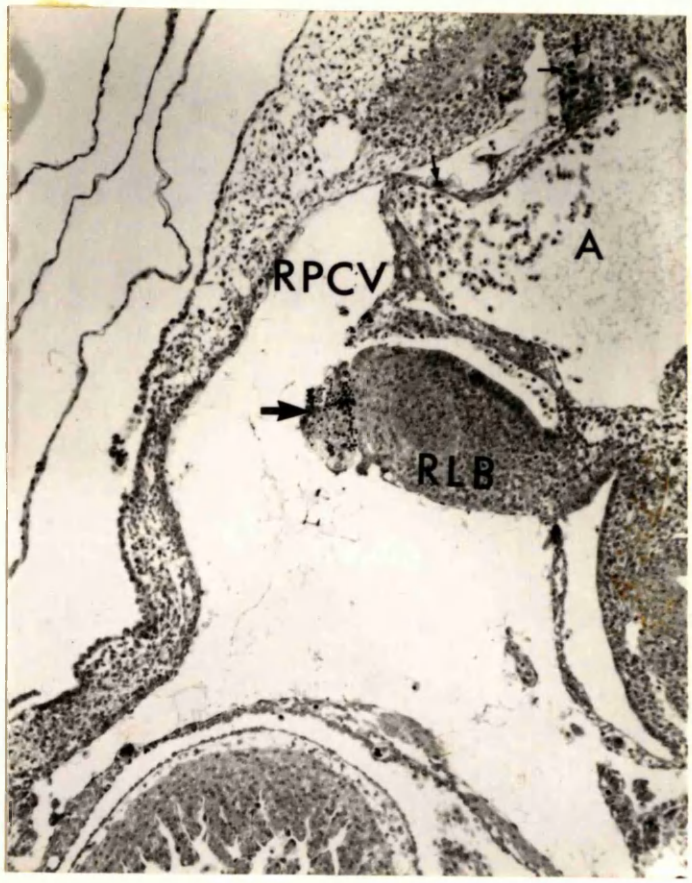
(Embryo No.5:1/10:1:3:4 - 16 hour group)

x 100

Fig. 33: High power view of a migrant trophoblastic giant cell (arrow) beneath the epidermis which, although it is elevated from the mesenchyme, shows no histolysis or cytolysis. Adjacent mesenchymal cells also appear normal.

(Embryo No.5:1/10:1:2:8 - 16 hour group)

x 630


A
RPCV
RLB

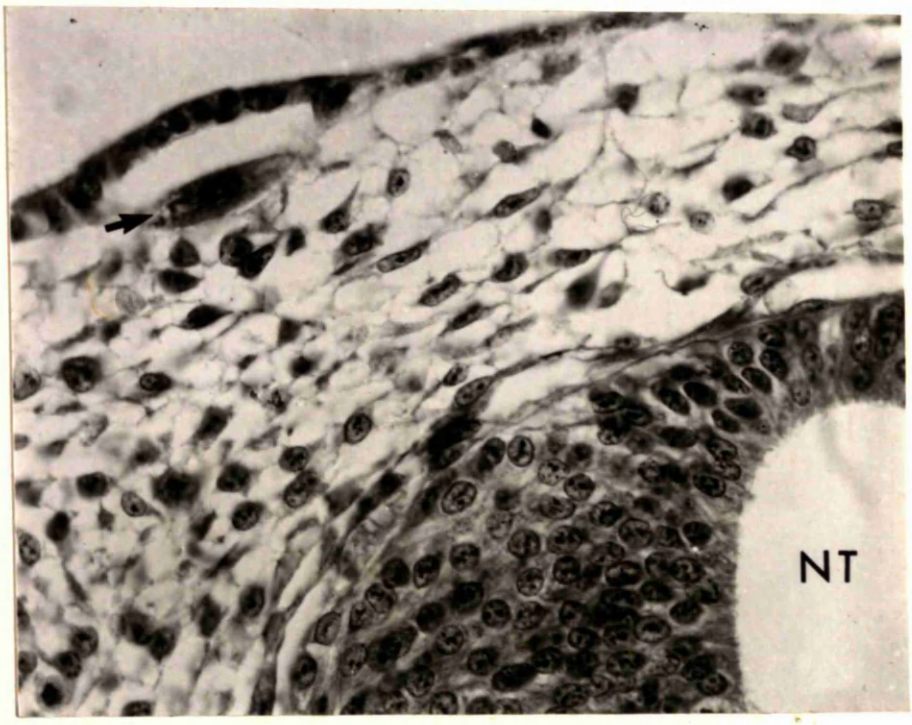

NT

Fig. 34a: Low power view of a trophoblastic giant cell (arrowhead) in the lumen of the left pulmonary venous plexus.

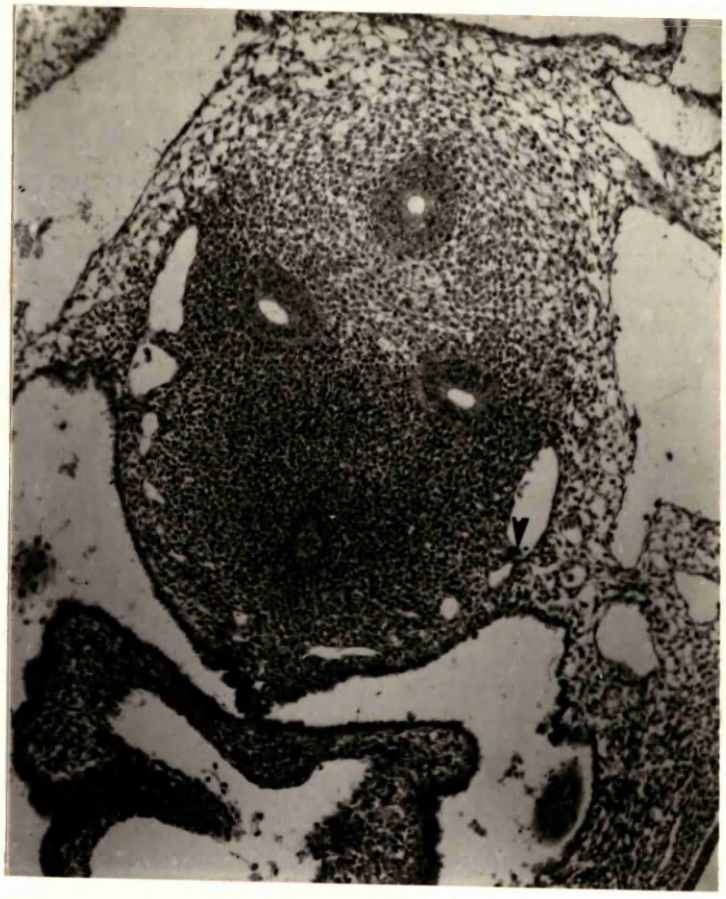
(Embryo No.5:1/10:1:2:1 - 16 hour group)

x 100

Fig. 34b: High power view of part of Fig. 34a showing intraluminal trophoblastic giant cell (GC) lodged in a defect in the venous endothelium.

(Embryo No.5:1/10:1:2:1 - 16 hour group)

x 1250



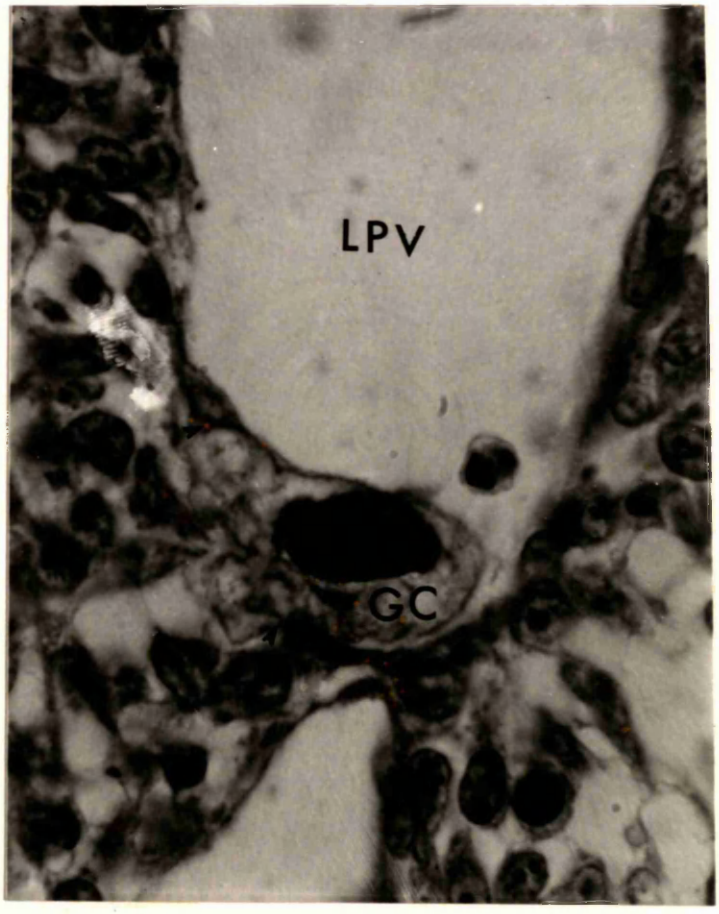

LPV
GC

Fig. 35: Shows trophoblastic giant cells (arrowheads) in close contact with ganglia of the right and left sympathetic chains. No histolytic or cytolytic damage to the sympathoblasts (S) was observed. (Notochord - N; Dorsal Aorta - DA).

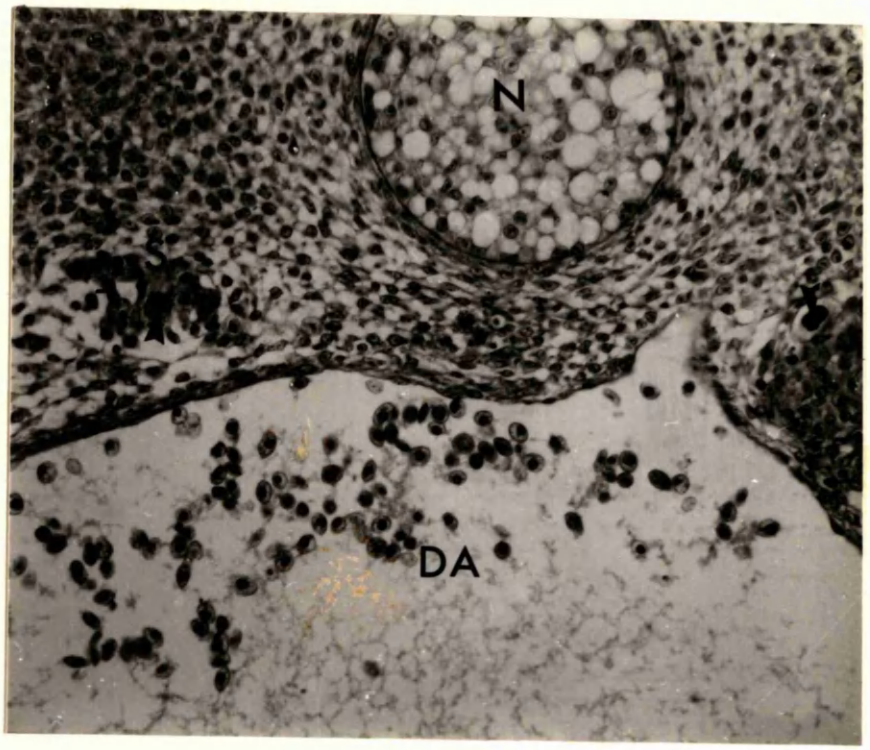
(Embryo No.5:1/10:1:2:9 - 16 hour group)

x 280

Fig. 36: Shows (a) a binucleated trophoblastic giant cell (arrowhead) closely related to a ganglion of the right sympathetic chain. (b) another giant cell (arrowhead) adherent to the endothelial lining of a tributary of the right posterior cardinal vein.
(Spinal nerve - SN; Aorta - A)

(Embryo No.5:1/10:1:3:4 - 16 hour group)

x 400


N
DA

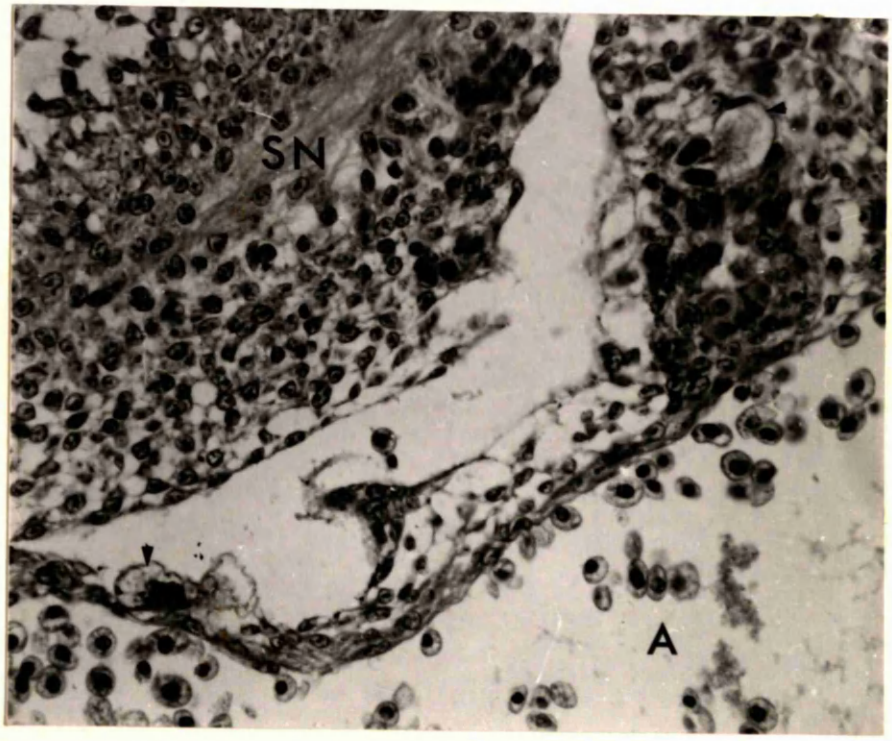

SN
A

Fig. 37a: Shows a trophoblastic giant cell in close proximity to a ganglion of the left sympathetic chain (Aorta - A).

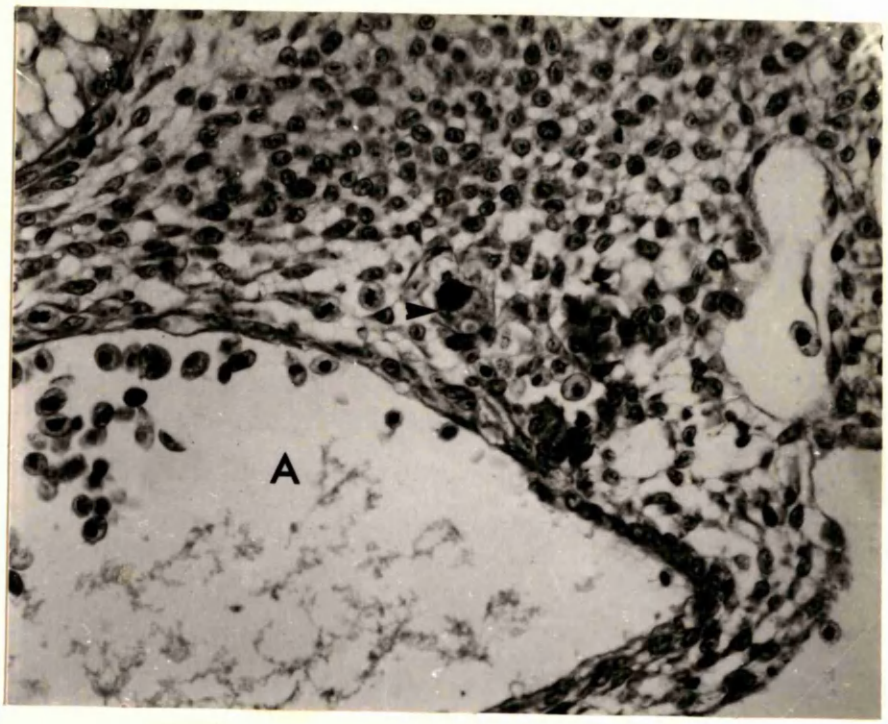
(Embryo No.5:1/10:1:4:9 - 16 hour group)

x 400

Fig. 37b: High power view of Fig. 37a. The giant cell contains a cytoplasmic vacuole. Neighbouring mesenchymal cells and sympathoblasts appear normal.

(Embryo No.5:1/10:1:4:9 - 16 hour group)

x 1000


A

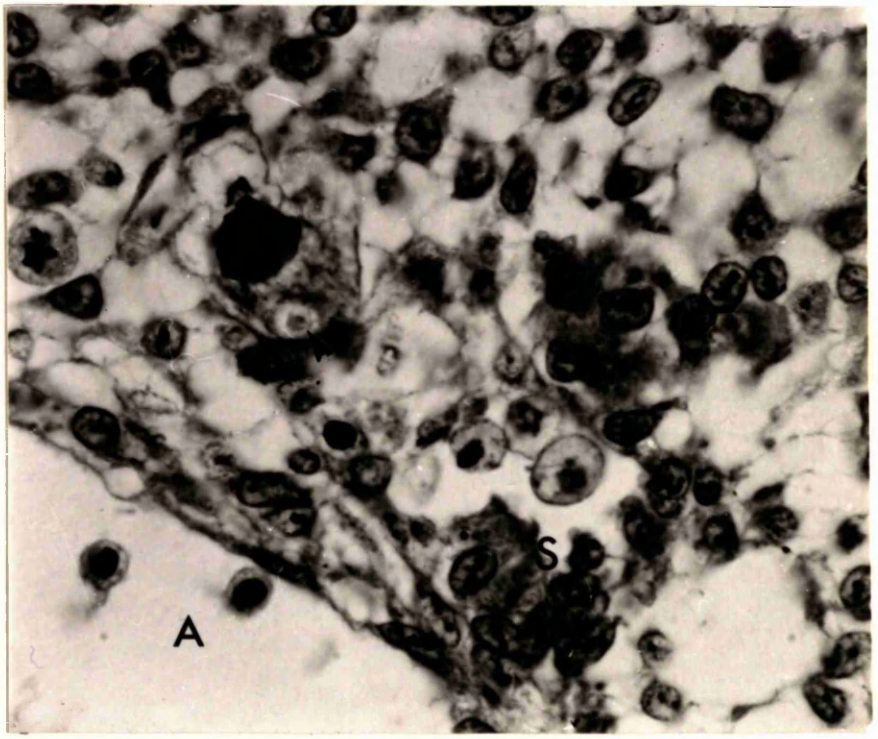

S
A

Fig. 38: Shows an EPC graft in the upper right coelom which had eroded the right mesonephric duct (arrowhead), the dorsal surface of the liver (L), the dorsal coelomic wall and ventral part of the dorsal aorta, with bleeding into the coelom; bursa omentalis (BO) is filled with blood.

Note: a migrant giant cell (arrowhead) is shown dorsal to the aorta.

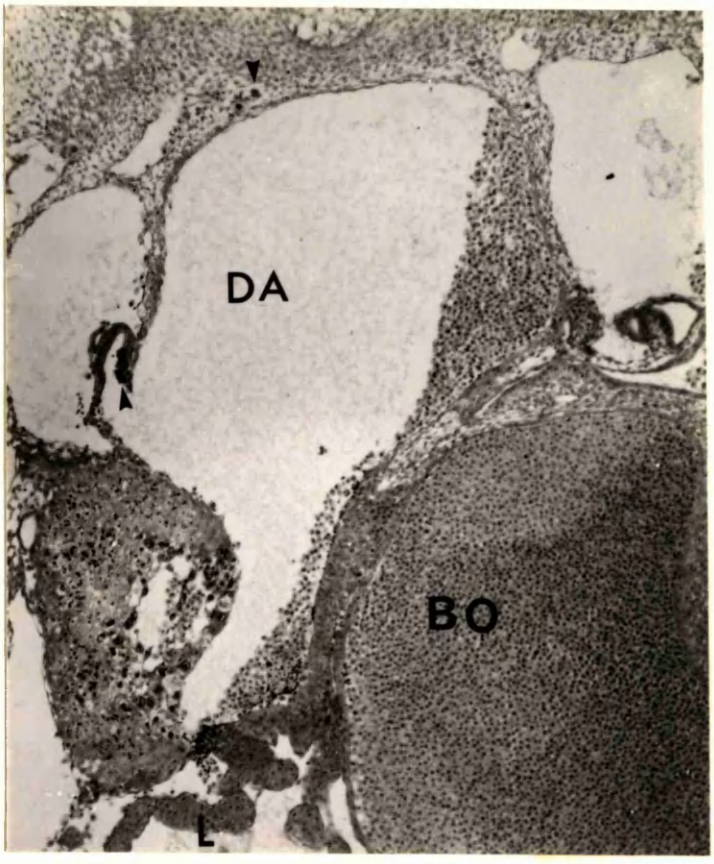
(Embryo No.26:1/10:1:3:8 - 16 hour group)

x 90

Fig. 39: High power view of individual giant cells which indent the surface of an hepatic plate. They have probably eroded the endothelium, but the hepatocytes appear healthy.

(Embryo No.16:1/10:1:3:9 - 16 hour group)

x 800


DA
BO
L

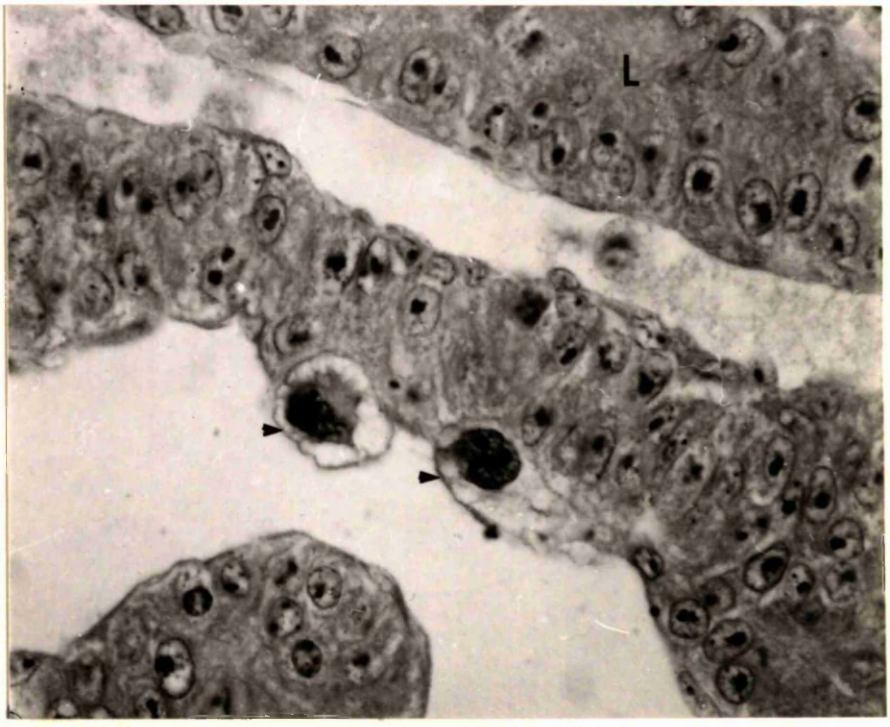

L

Fig. 40a: Low power view of an individual trophoblastic giant cell (arrowhead) adjacent to a ganglion of the left sympathetic chain.

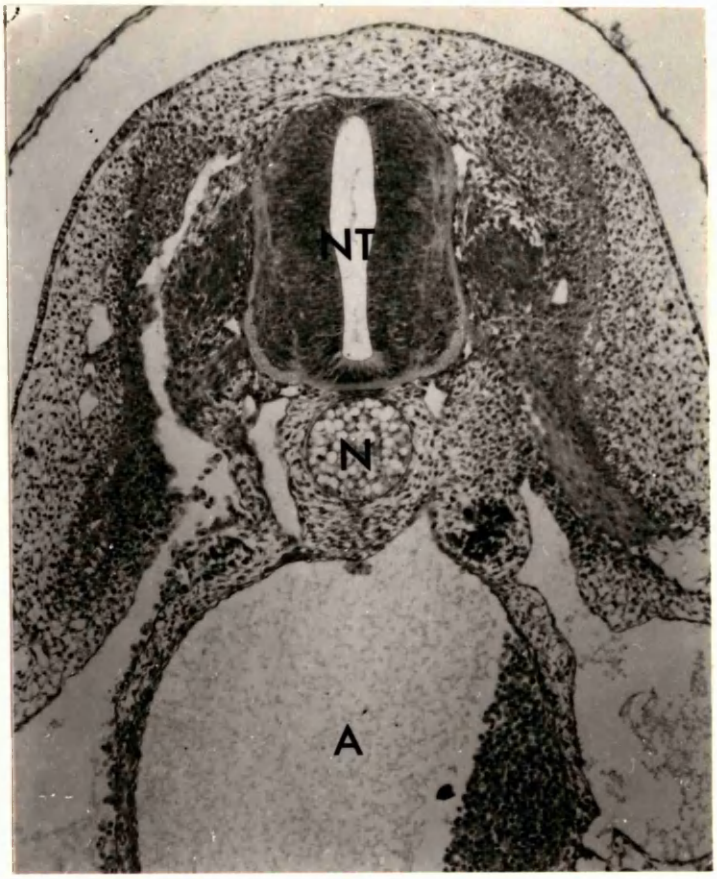
(Embryo No.26:1/10:1:3:3 - 16 hour group)

x 100

Fig. 40b: High power view of Fig. 40a, showing trophoblastic giant cell in close contact with apparently normal sympathoblasts.

(Embryo No.26:1/10:1:3:3 - 16 hour group)

x 800


NT
N
A

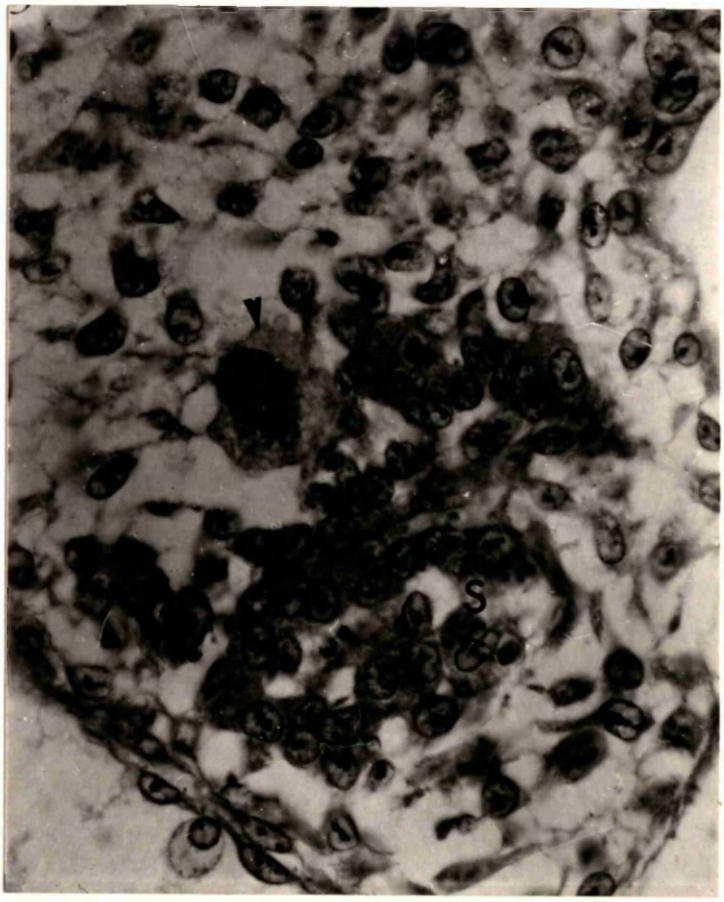

V
S

Fig. 41: High power view of another trophoblastic giant cell in intimate contact with sympathoblasts of a right sympathetic chain and aortic endothelium.

(Embryo No.26:1/10:1:4:1 - 16 hour group)

x 800

Fig. 42: Shows the EPC of an egg cylinder graft, with very early invasion of dorsal wall of coelom.

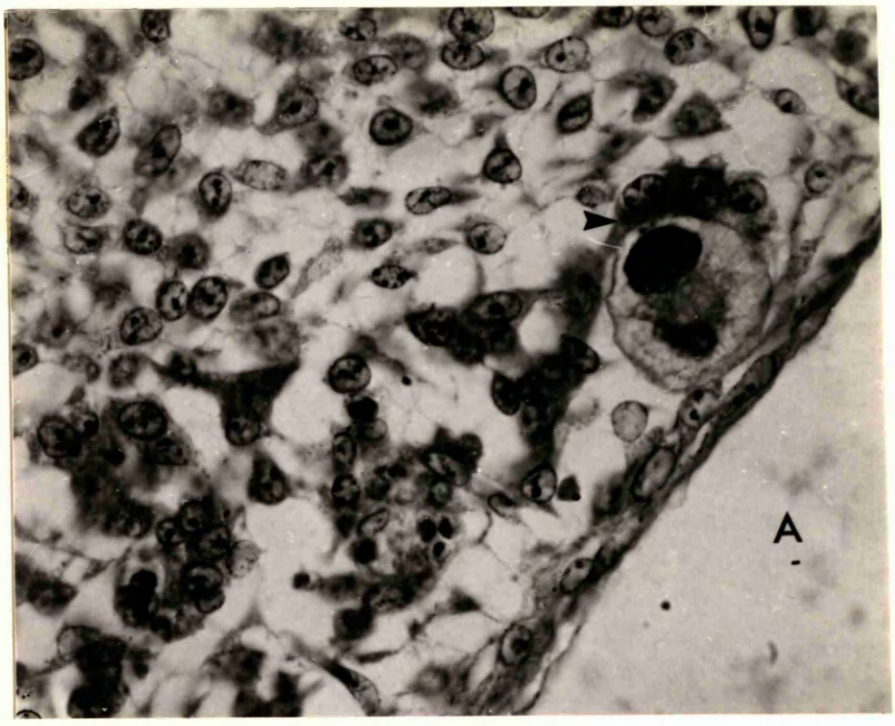
(a) small closely packed untransformed trophoblast cells.

(b) 'typical' giant cells with large nuclei and dense cytoplasm, and

(c) vacuolated giant cells with shrunken and angular nuclei, and clear vacuolated cytoplasm.

(Embryo No.43:1/10:2:2:4 - 16 hour group)

x 250


A

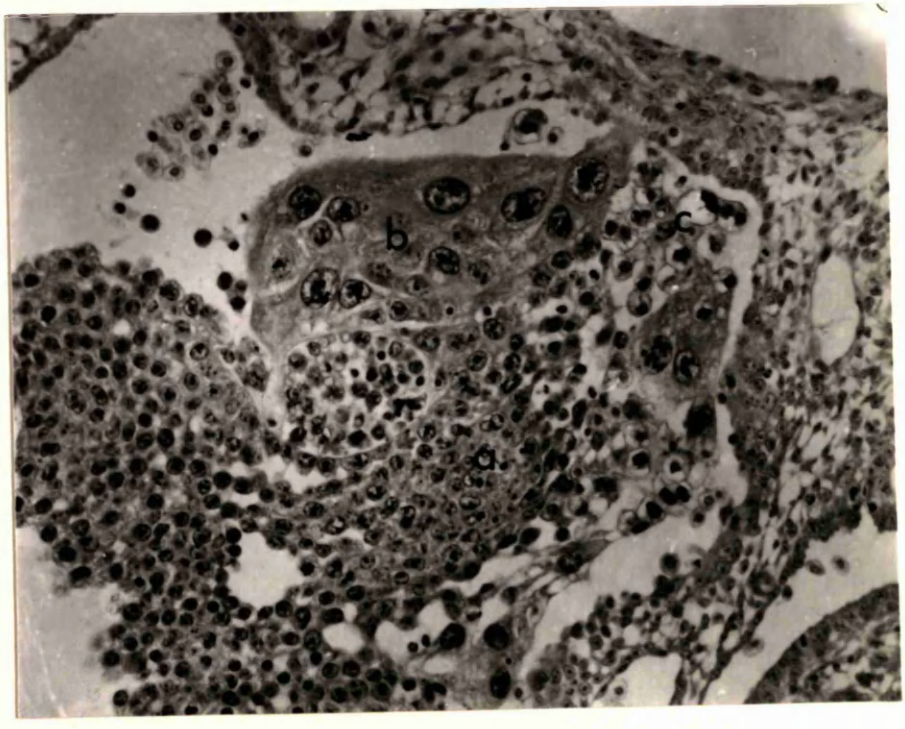

b
c
a

Fig. 43: High power view of a tongue of trophoblastic giant cells (arrowhead) extending towards the aorta, and making a small defect in the endothelium. Note:

(i) that the endothelium appears normal on either side of the defect.

(ii) phagocytosed cell debris contained in the leading giant cell cytoplasm.

(Embryo No.43:3:1:6 - 16 hour group)

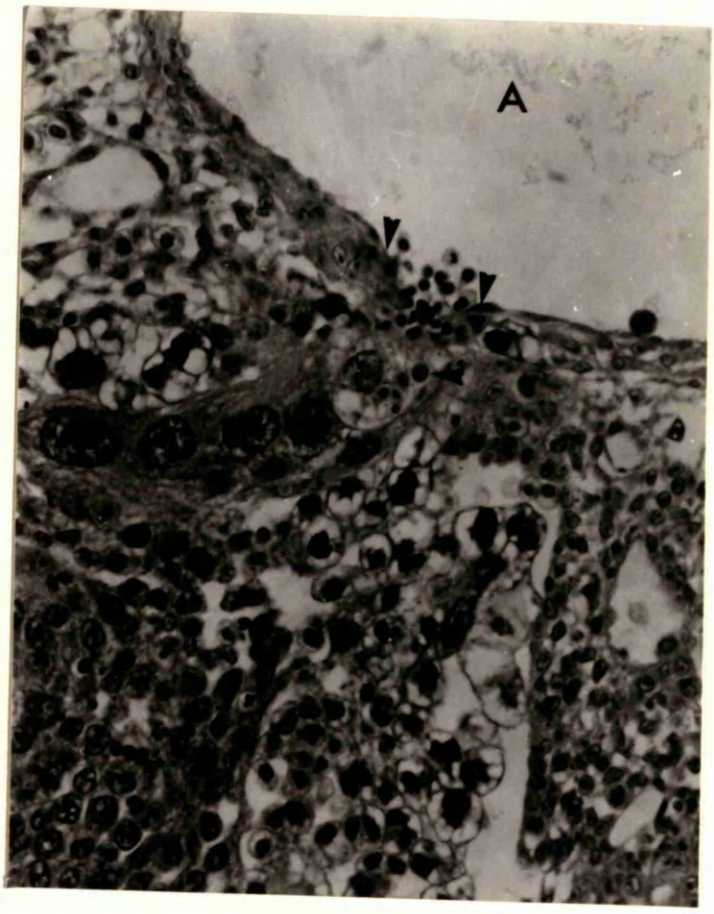
x 400

Fig. 44: Shows (i) a cluster of vacuolated giant cells in the coelom. The adjacent mesothelium (reader's right) appears normal, but that on reader's left has been penetrated.

(ii) small groups of vacuolated giant cells are seen in dorsal mesentery and adjacent to a mesonephric capsule.

(Embryo No.43:2:3:9 - 16 hour group)

x 250


A

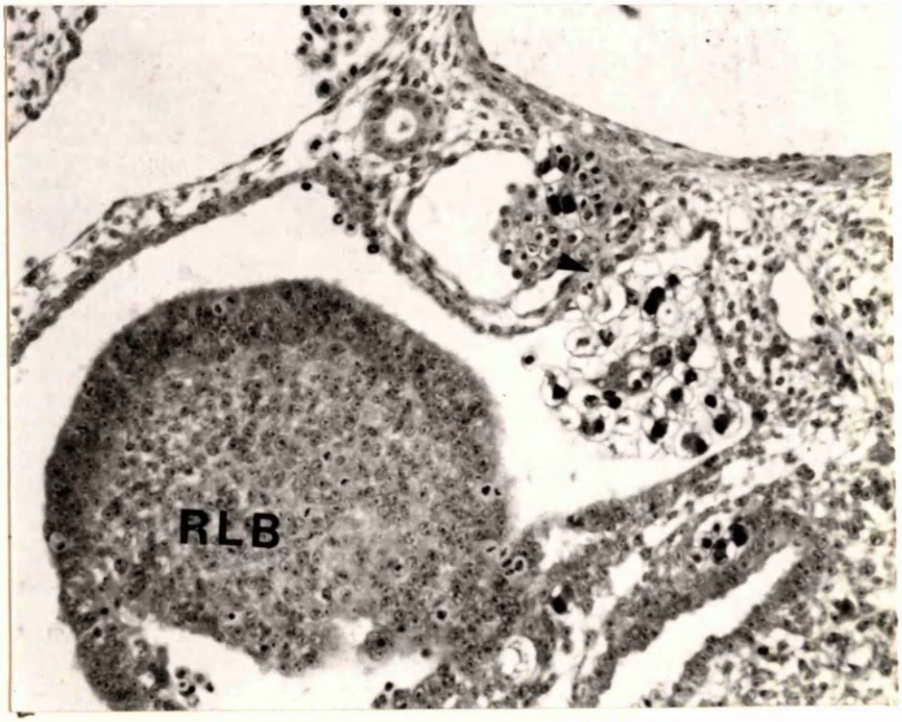

RLB

Fig. 45a: Shows part of an egg cylinder graft, adherent to right body wall, right lung bud and dorsum of liver. Notice a narrow zone of damaged hepatocytes at the interface with the EPC and erosion of the lung bud by what appears to be the visceral layer of the yolk sac endoderm.

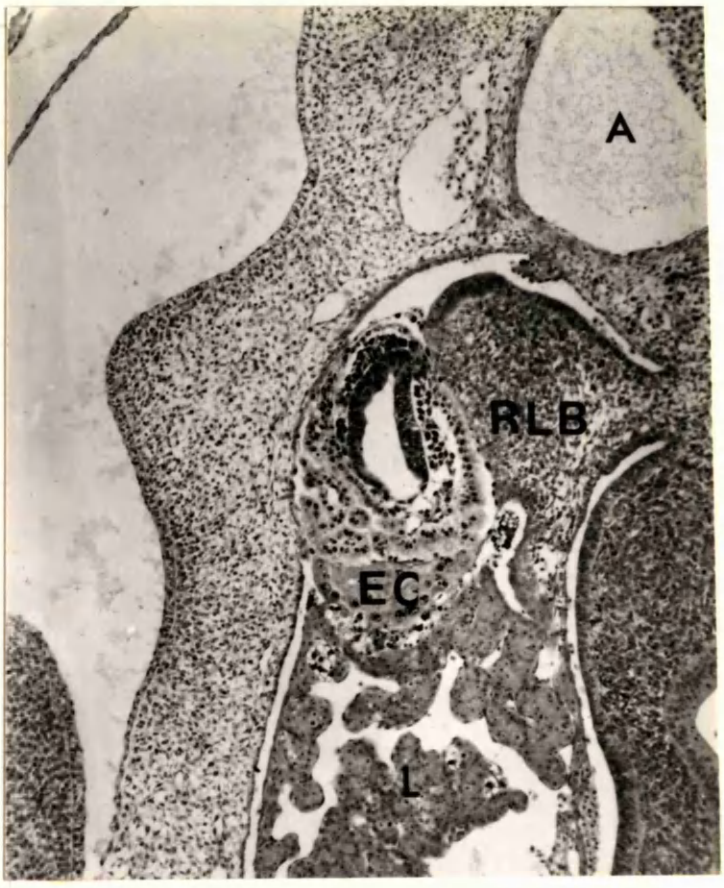
(Embryo No.44:3:4:8 - 16 hour group)

x 100

Fig. 45b: High power view of Fig. 45a showing that the eroded part of the right lung bud is in contact with the parietal layer of the yolk sac and Reichert's membrane.

(Embryo No.44:3:4:8 - 16 hour group)

x 400


A
RLB
EC
L

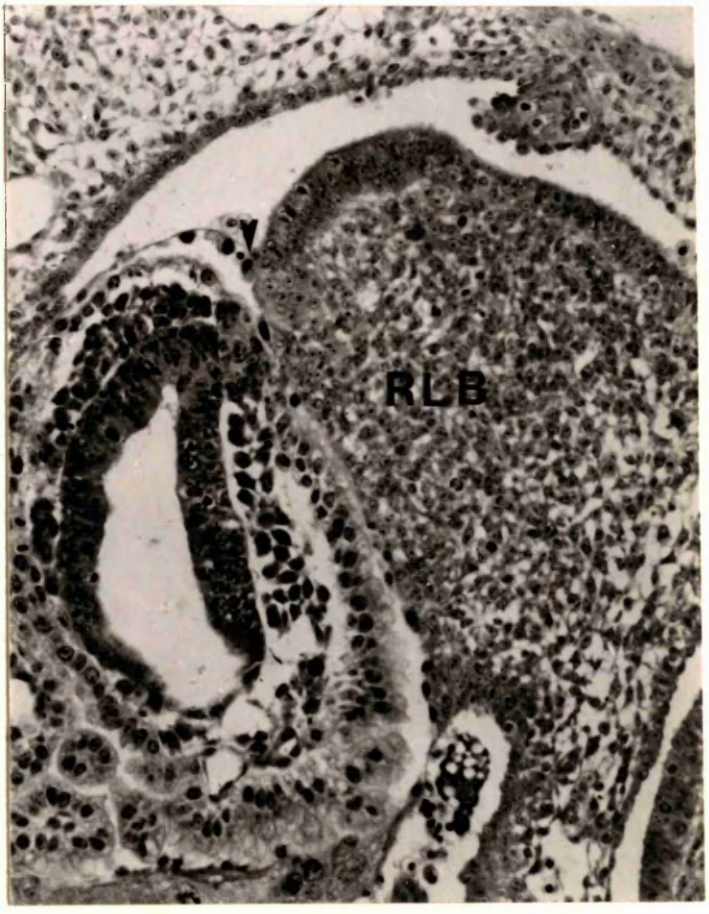

RLB

Fig. 46: Shows an egg cylinder graft which had progressed to the neural fold stage (arrowhead). It lies at the anterior intestinal portal. Notice contact by the visceral layer of the yolk sac with coelomic mesothelium which is undamaged.

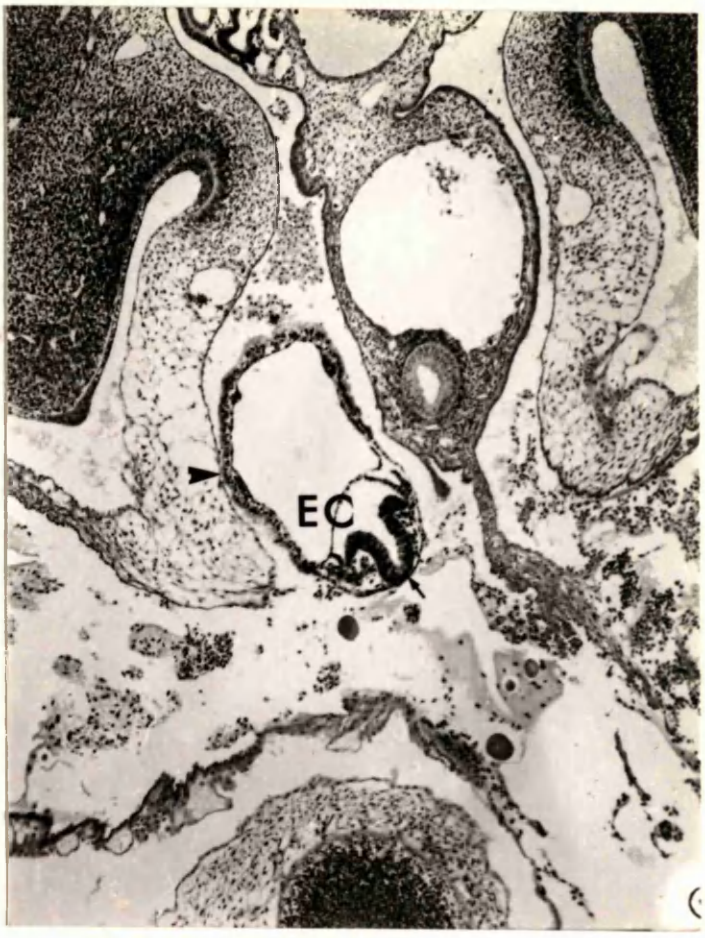
(Embryo No.47:1:4:12 - 16 hour group)

x 100

Fig. 47: Shows the EPC of an egg cylinder graft which had contacted the mid-gut mesentery and had eroded the right wall of the vitelline artery. Notice presence of both typical and vacuolated giant cells in the invading mass of EPC.

(Embryo No.47:2:5:9 - 16 hour group)

x 250


EC

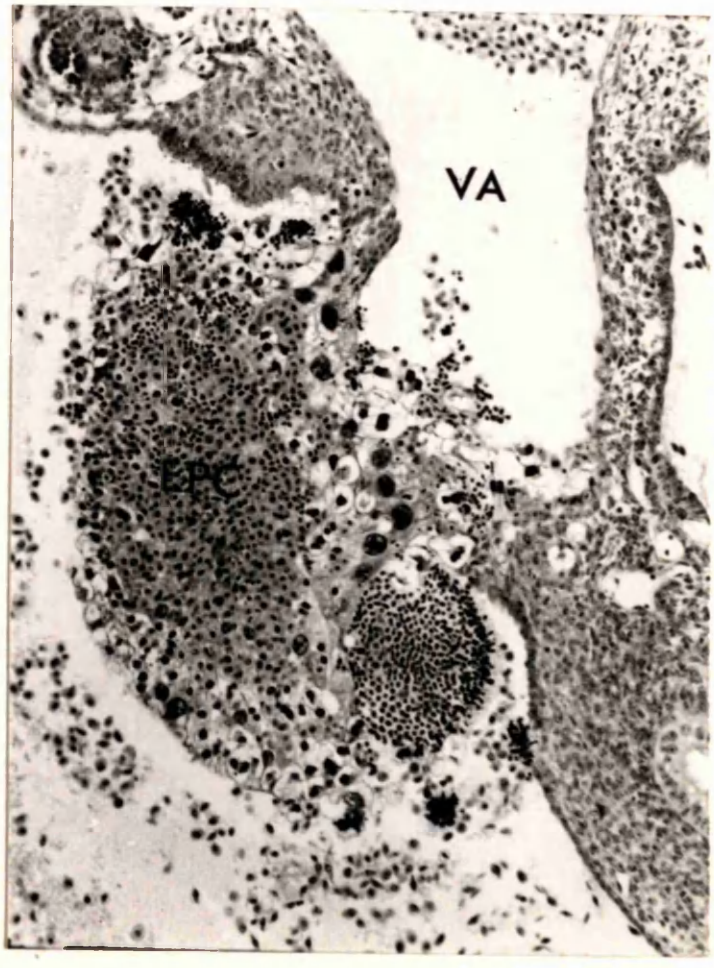

VA

Fig. 48: Another section through the same graft as in Fig. 47, showing localised erosion of the right wall of the portal vein which the graft had partially occluded.

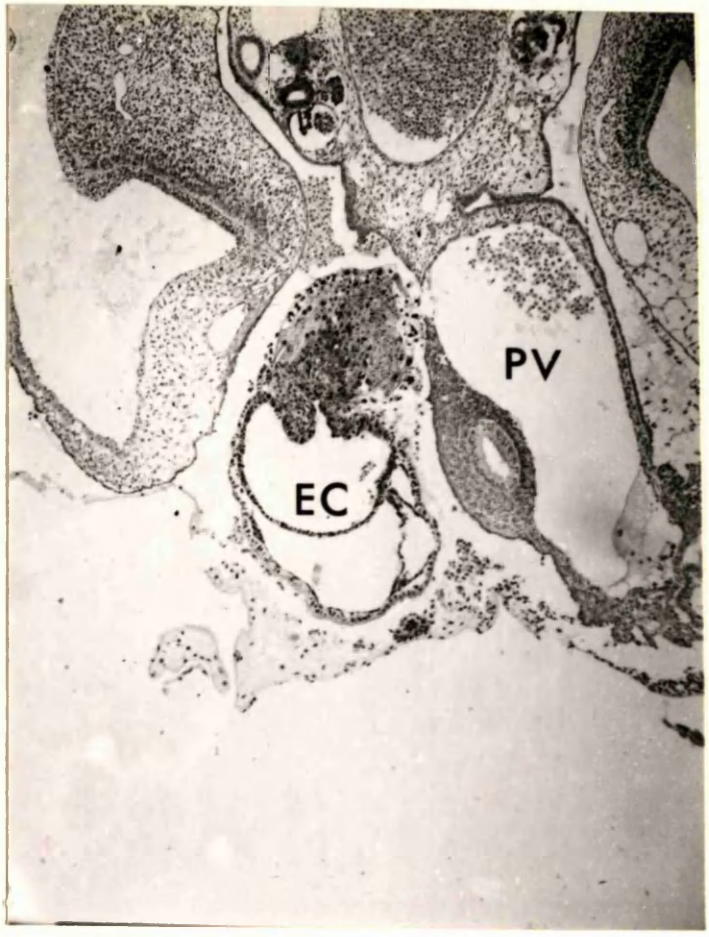
(Embryo No.47:2:2:9 - 16 hour group)

x 100

Fig. 49: Shows invasion of dorsal mesentery by several giant cells, all of vacuolated type (arrows). Two giant cells (arrows) have reached the deep surface of the thickened mesothelium which marks the beginning of development of the spleen; this appears undamaged.

(Embryo No.42:3:1:4 - 16 hour group)

x 250


PV
EC

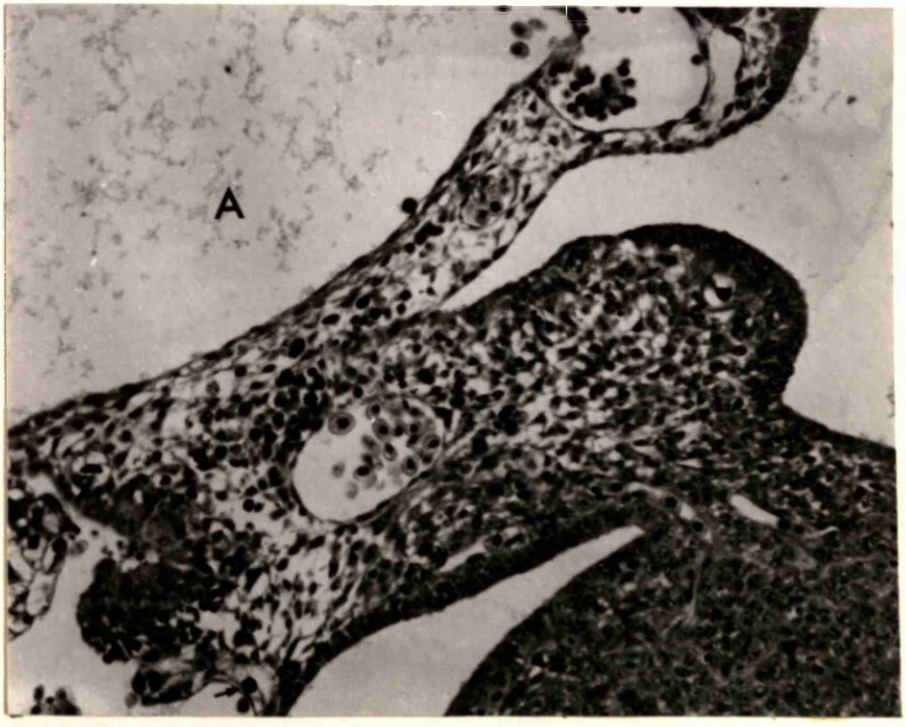
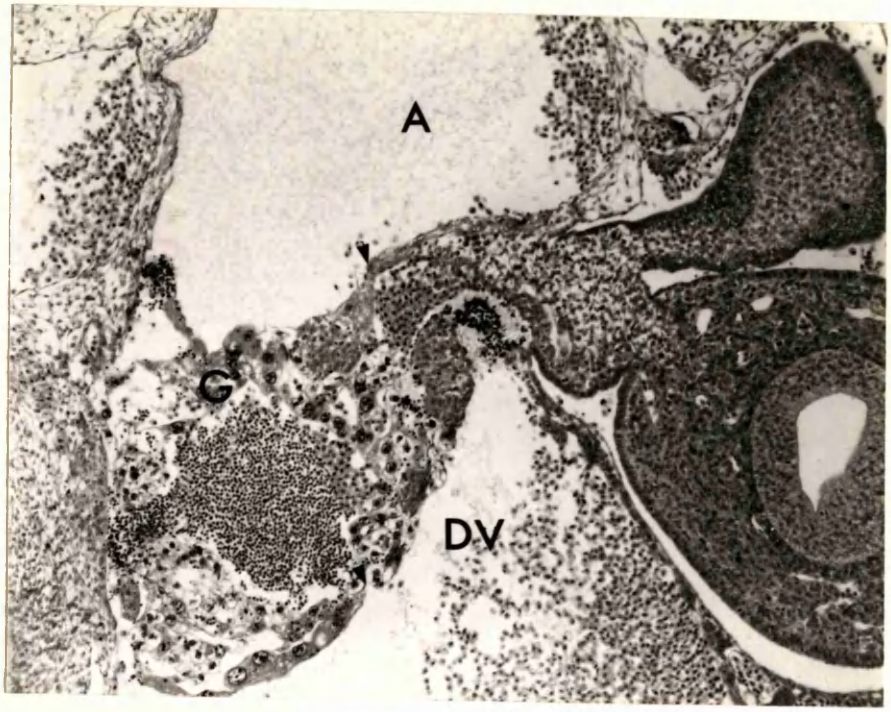

A

Fig. 50: Shows the graft (G) attached to dorsal coelomic wall, right body wall and the dorsal surface of the liver. Note erosion of the dorsal coelomic wall, ventral wall of the aorta, dorsal wall of ductus venosus (arrowheads).

(Embryo No.3:3:4:3 - 24 hour group) x 100

Fig. 51: Shows a migrant trophoblastic giant cell (arrowhead) intercalated in the endothelium of the dorsal wall of the aorta. Adjacent endothelial cells appear undamaged. No phagocytosed debris in giant cell. (Aorta - A; Notochord - N).

(Embryo No.3:3:3:9 - 24 hour group) x 500


A
G
DV

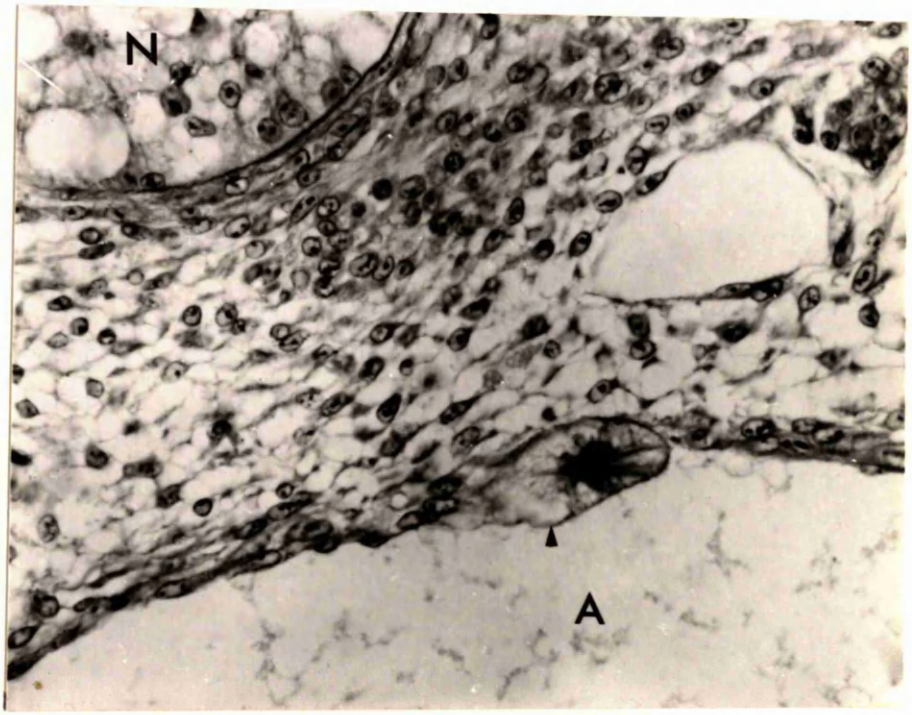
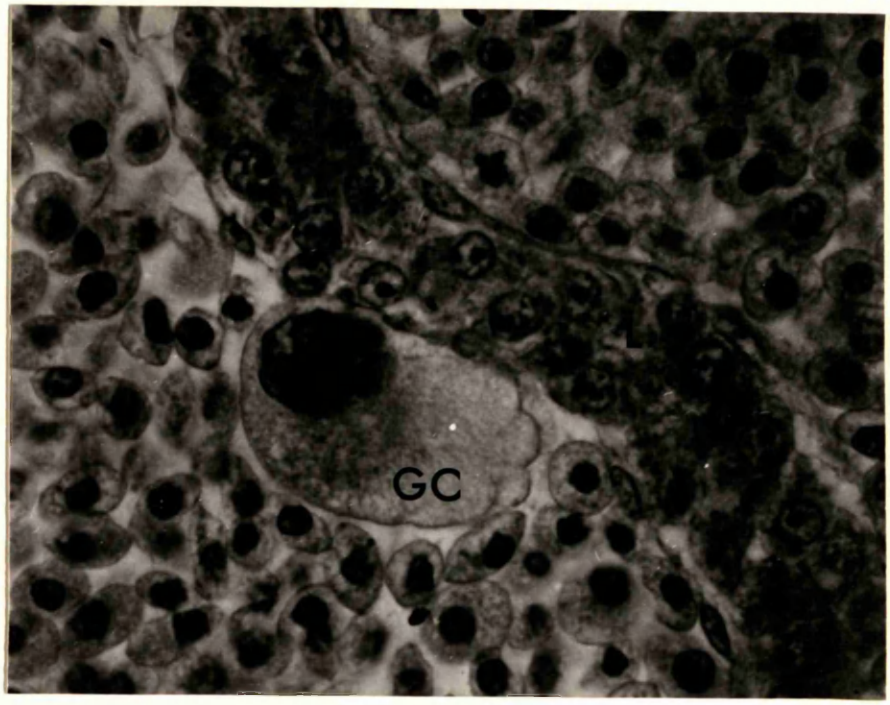

N
A

Fig. 52: Shows a trophoblastic giant cell (GC) attached to the surface of a plate of hepatocytes. Note (i) normal appearance of hepatocytes contacted by the giant cell. (ii) absence of evidence of phagocytosis by giant cell.

(Embryo No.3:4:1:8 - 24 hour group) x 100

Fig. 53: Shows a migrant giant cell (arrowhead) intercalated at its dorsal pole into the endothelium of a dorsal intersegmental artery, while its ventro-lateral pole is in close contact with the medial aspect of a right sympathetic ganglion.

(Embryo No.3:3:4:1 - 24 hour group) x 500


GC

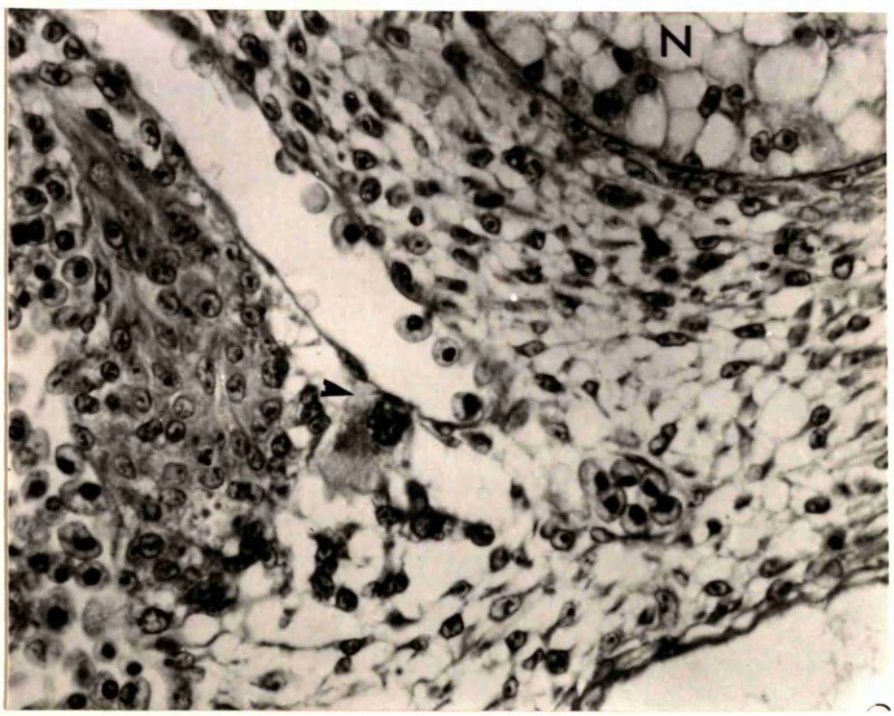
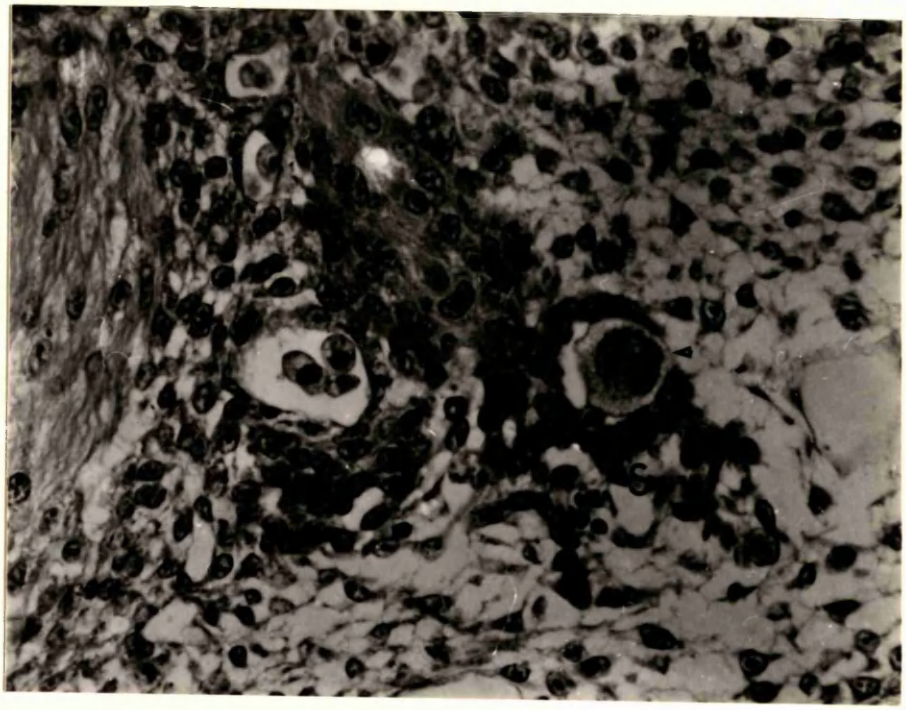

N

Fig. 54: Shows a migrant trophoblastic giant cell embedded in a sympathetic ganglion (arrowhead) on the right side. Note the surrounding healthy sympathetic ganglion cells.

(Embryo No.3:4:3:6 - 24 hour group) x 500

Fig. 55: Shows a trophoblastic giant cell (arrowhead) within a tributary of the right posterior cardinal vein, closely adjacent to a few rather scattered sympathetic ganglion cells. No evidence of phagocytic activity in giant cell.

(Embryo No.3:4:4:7 - 24 hour group) x 1000



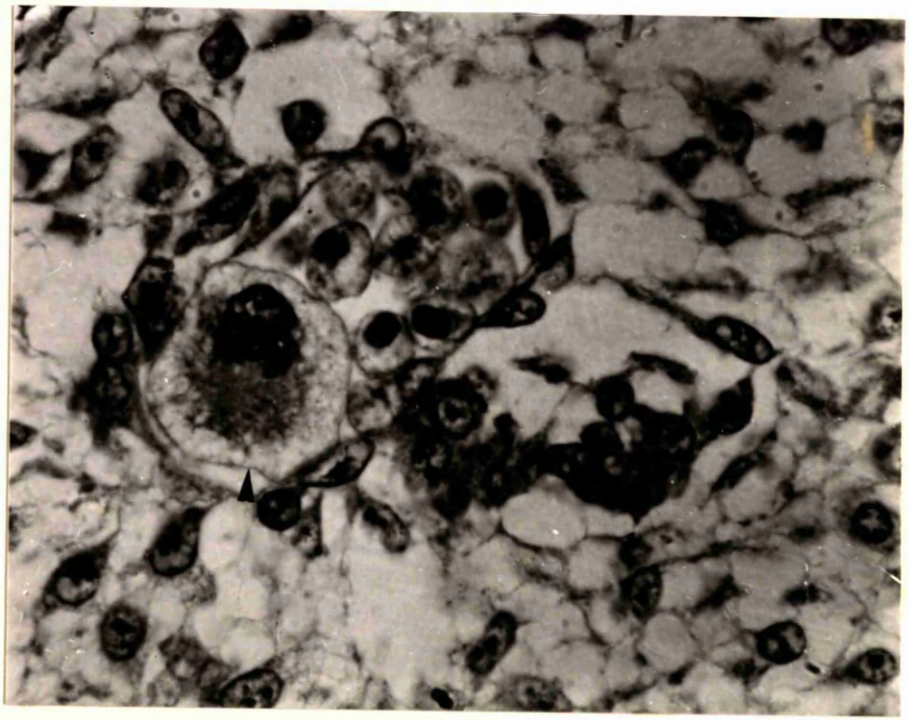
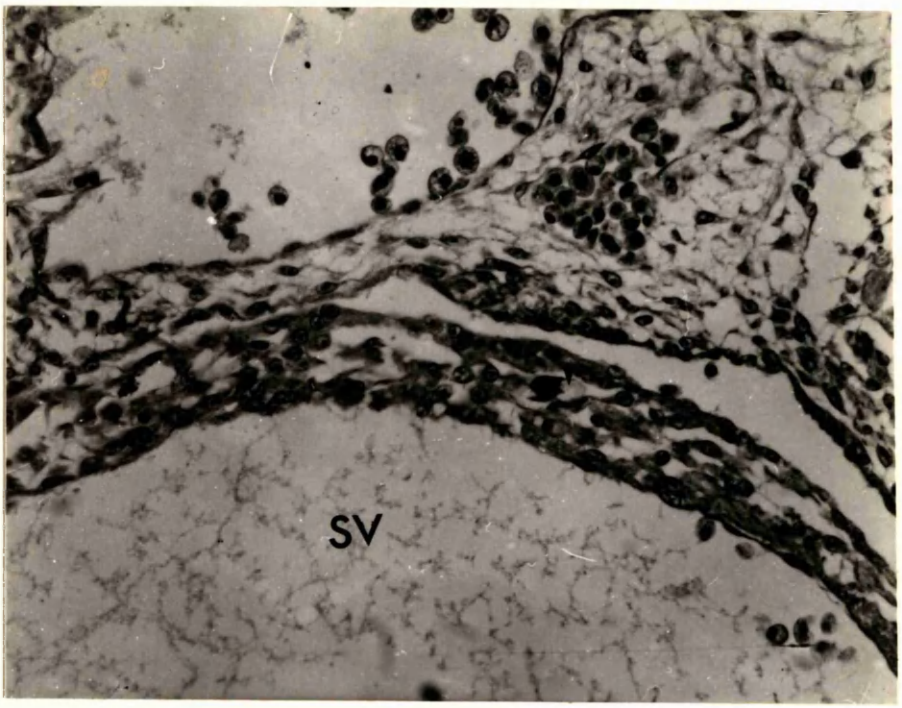


Fig. 56: Shows a single giant cell (arrowhead) in the wall of the sinus venosus (SV).

(Embryo No.3:1:2:1 - 24 hour group) x 310

Fig. 57: Shows a trophoblastic giant cell (arrowhead) in the wall of the right atrium (RA).

(Embryo No.3:2:1:2 - 24 hour group) x 125


SV

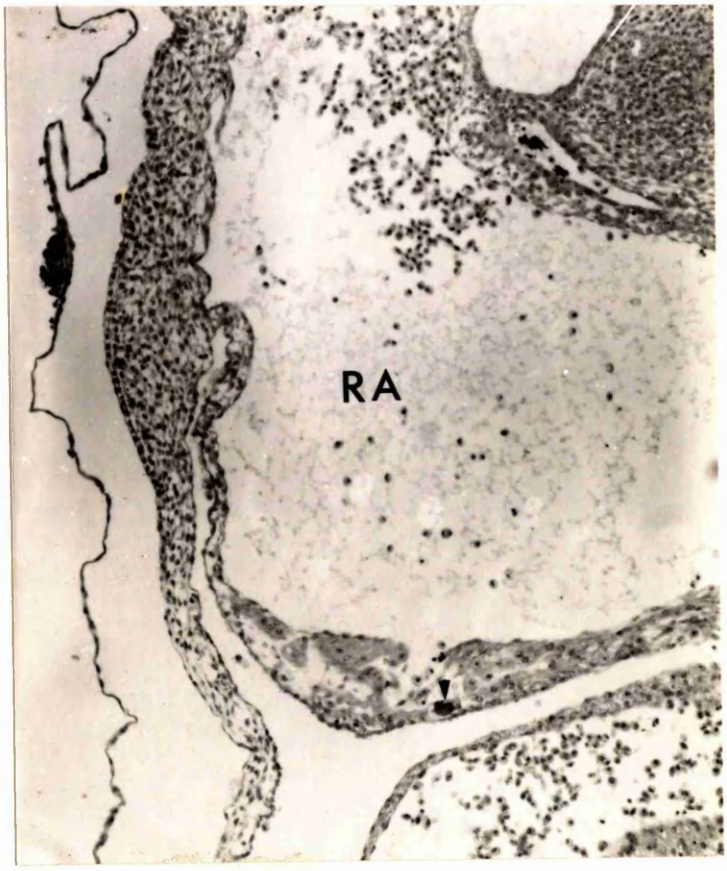
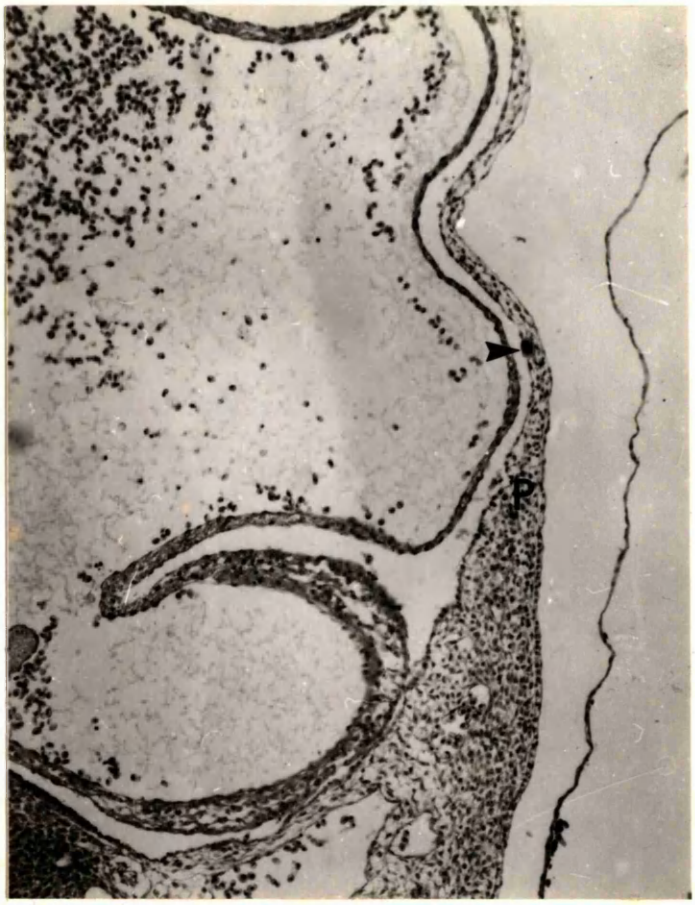

RA

Fig. 58a: Shows a migrant trophoblastic giant cell (arrowhead) in the pericardial wall (P).

(Embryo No.3:1:1:1 - 24 hour group) x 100

Fig. 58b: High power view of part of Fig. 58a showing a migrant trophoblastic giant cell (arrowhead) in the pericardial wall (P). Adjacent mesothelial and mesenchymal cells appear undamaged.

(Embryo No.3:1:1:1 - 24 hour group) x 1250


P

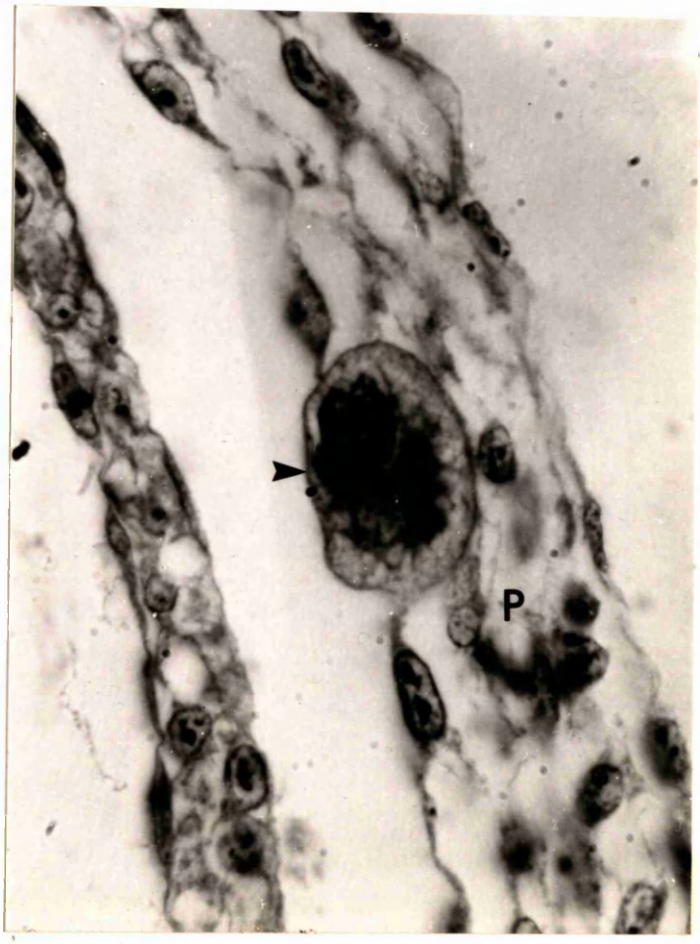

P

Fig.59a: Shows a 'seeded' trophoblastic giant cell (arrowhead) attached to the mesothelium of the dorsal mesogastrium.

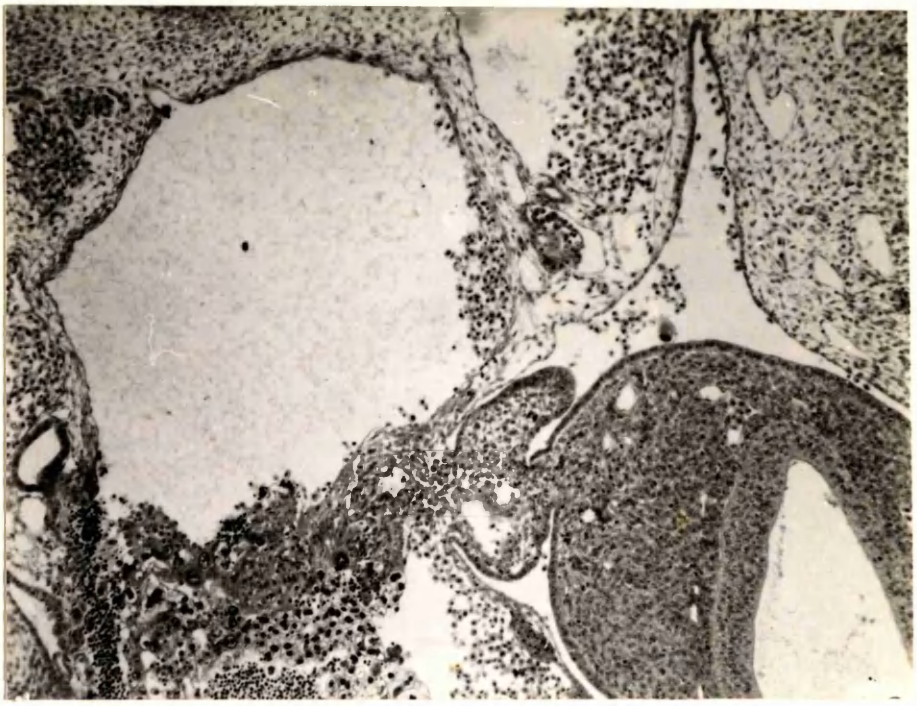
(Embryo No.3:4:2:4 - 24 hour group) x 100

Fig. 59b: High power view of part of Fig. 59a showing a 'seeded' trophoblastic giant cell (arrowhead) attached to the mesothelium of the dorsal mesogastrium.

Note: (i) no evidence of histolytic or cytolytic damage to the underlying mesothelium.

(ii) no sign of phagocytic activity by giant cell.

(Embryo No.3:4:2:4 - 24 hour group) x 1250



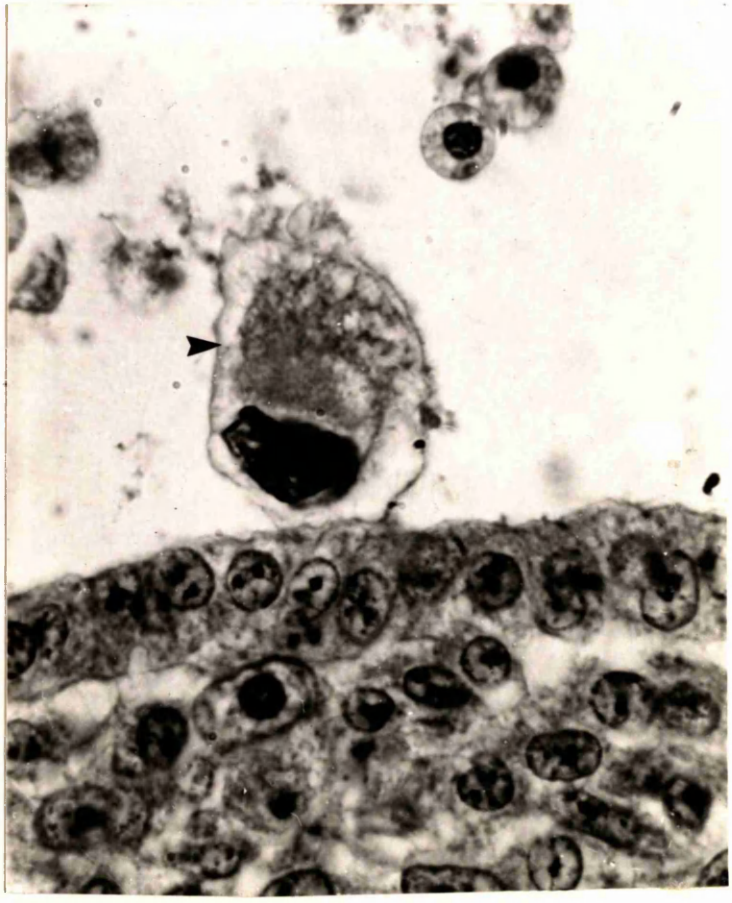


Fig. 60: Shows a binucleated trophoblastic giant cell (arrowhead) at an epidermal-dermal interface of the left limb bud.

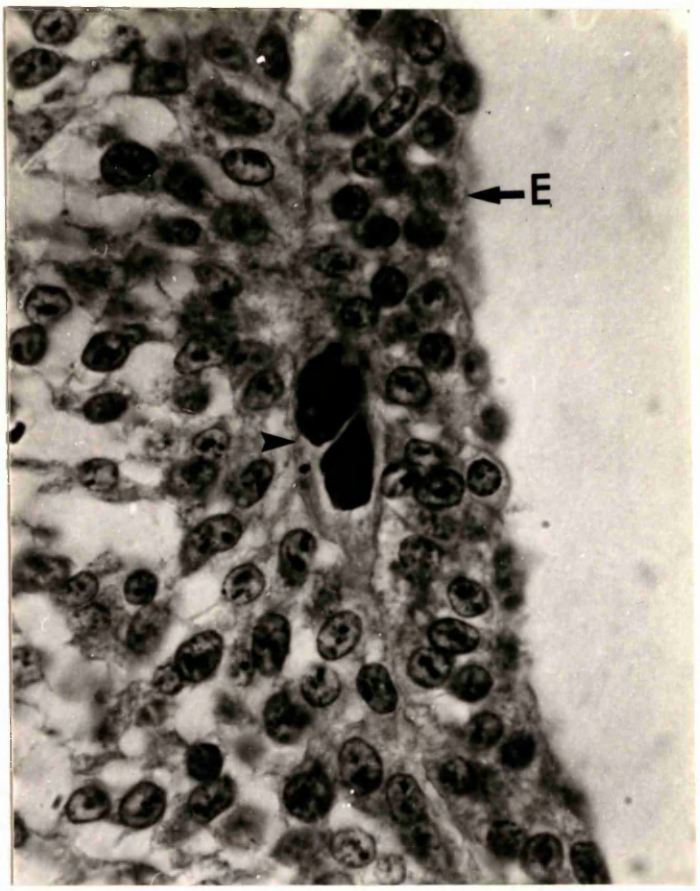
Note: (i) absence of damage to epithelium and to dermal mesenchyme.

(ii) no "blister" formation at site of giant cell.

(Embryo No.3:4:1:3 - 24 hour group) x 1000

Fig. 61: Shows erosion of the right wall of the vitelline artery (V.A.) by trophoblastic giant cells (arrowheads) which sealed the defect. Notice phagosomes are abundant in giant cells further away from the invasion site (to the reader's left) but absent from those at site of erosion.

(Embryo No.25:5:3:9 - 24 hour group) x 250


E

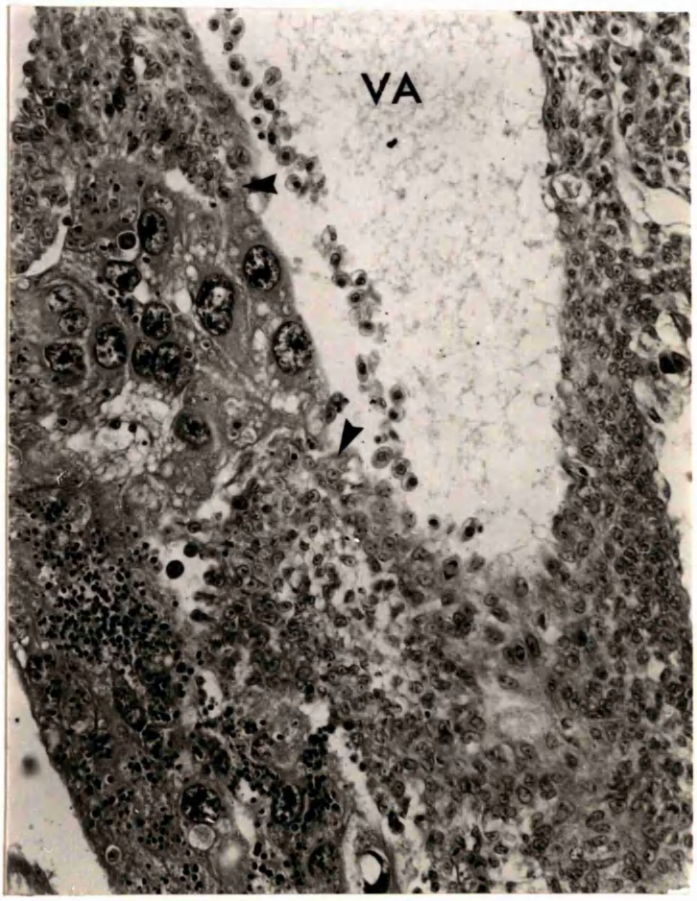
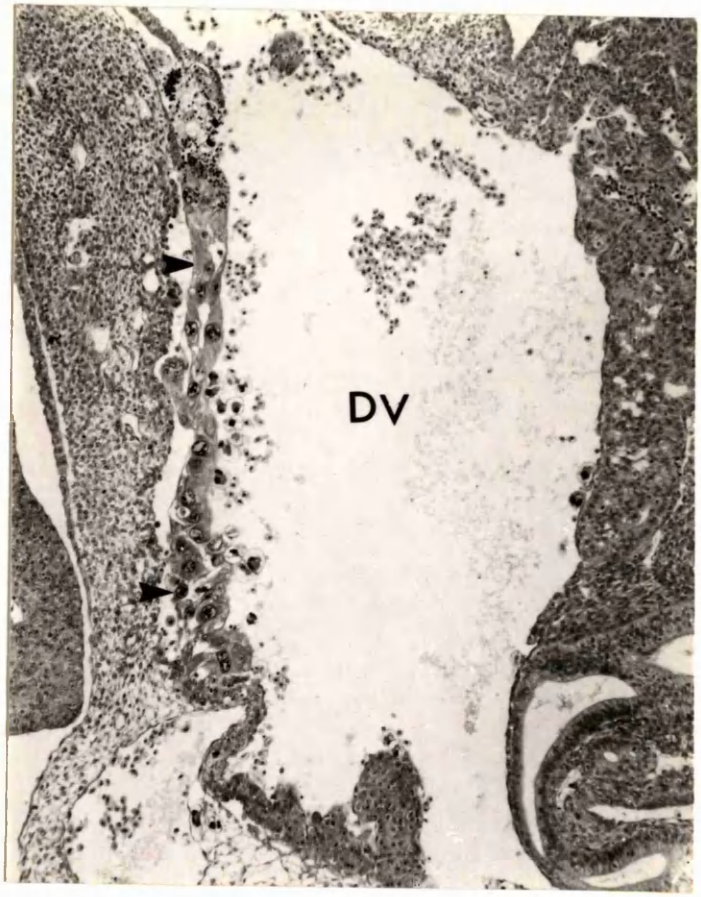

VA

Fig. 62: Shows an epithelial sheet of trophoblastic giant cells (arrowheads) forming the right boundary of the distorted ductus venosus (DV).

(Embryo No.25:2:3:5 - 24 hour group) x 100

Fig. 63: Shows the ventral end of a sheet of trophoblastic giant cells (arrowheads) which had eroded the medial wall of the right umbilical vein. Note: majority of giant cells are of vacuolated type.

(Embryo No.25:3:4:8 - 24 hour group) x 250


DV

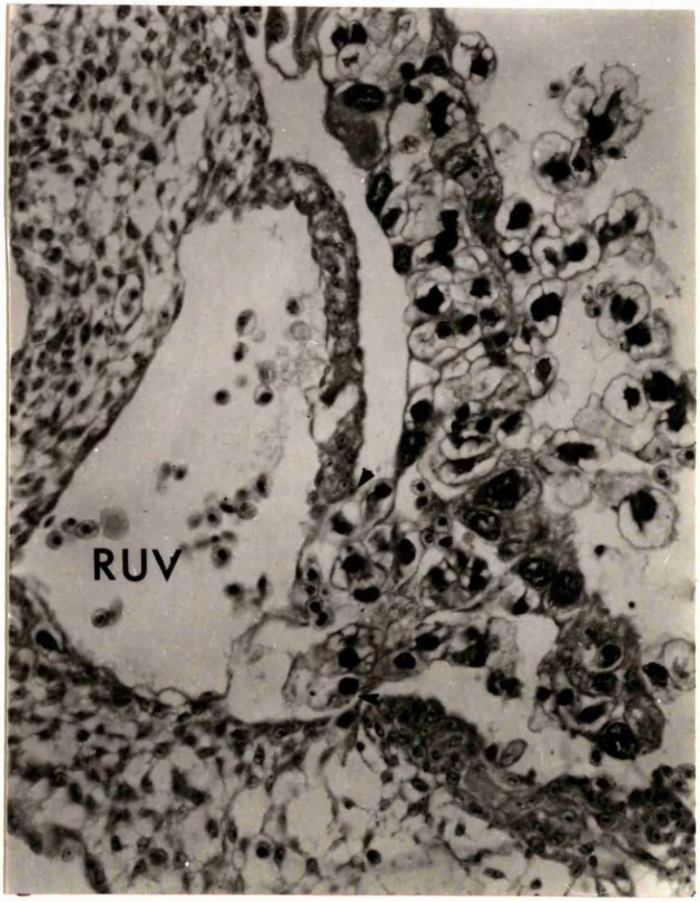
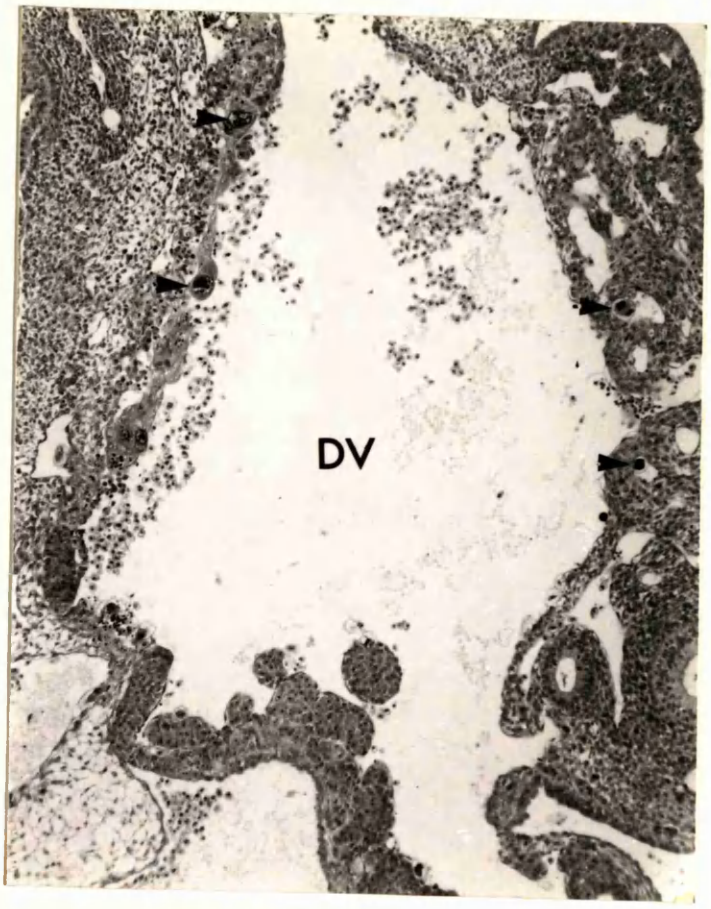

RUV

Fig. 64: Shows trophoblastic giant cells (arrowheads) which had "seeded" from the main sheet of graft on to the hepatocyte sheet which bounded the distorted ductus venosus (DV) and also within small veins in the dorsal mesoduodenum.

(Embryo No.25:2:2:6 - 24 hour group) x 100

Fig. 65: Shows a single trophoblastic giant cell (arrowhead) probably within a tributary of the right posterior cardinal vein, and closely adjacent to the sympathoblasts (S) of a right sympathetic ganglion.
(Notochord - N; Dorsal aorta - D.A.)

(Embryo No.25:1:1:11 - 24 hour group) x 400


DV

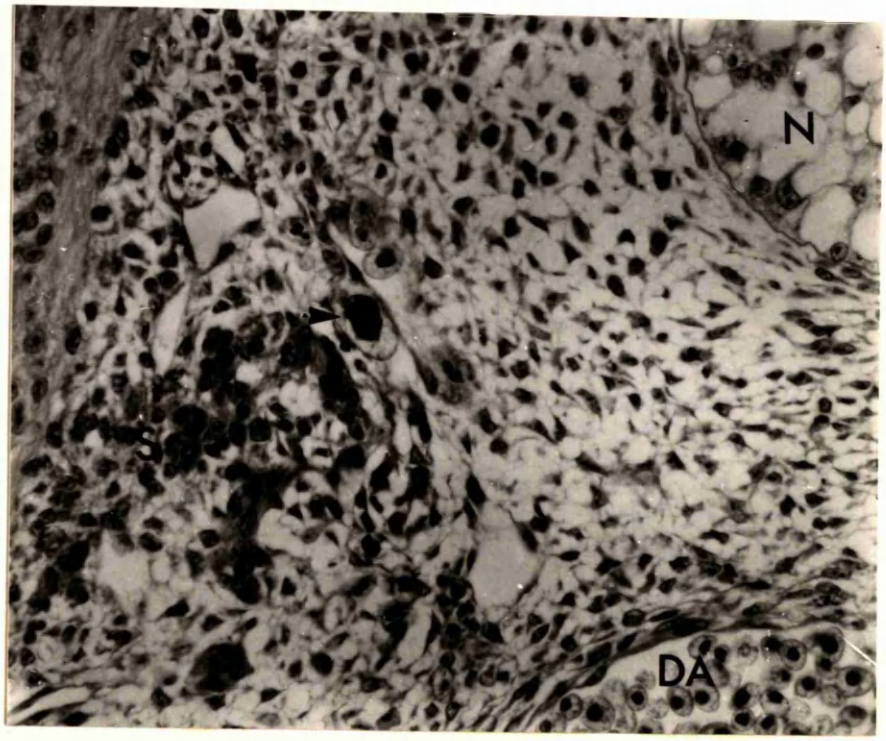
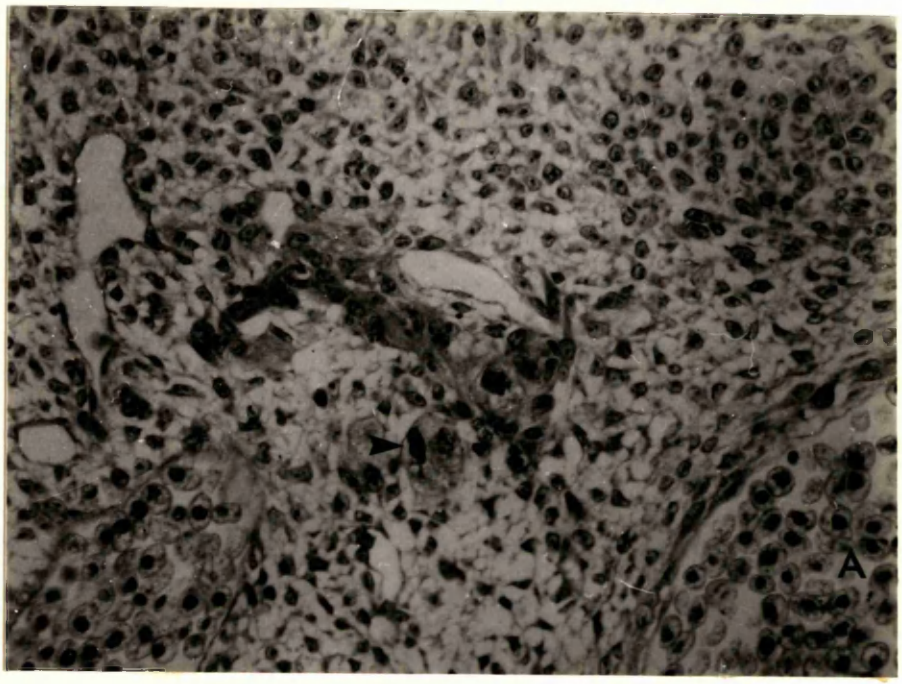

N
DA

Fig. 66: Shows two trophoblastic giant cells (arrowheads) in close contact with sympathoblasts of a right sympathetic ganglion. The more dorsal one appeared to be embedded within the ganglion.

(Embryo No.25:1:4:1 - 24 hour group) x 400

Fig. 67: Shows a binucleated giant cell (arrowhead) intercalated into the endothelium of the dorsal wall of an intersegmental artery of the left side.

(Embryo 24:1:4:8 - 24 hour group) x 400


A

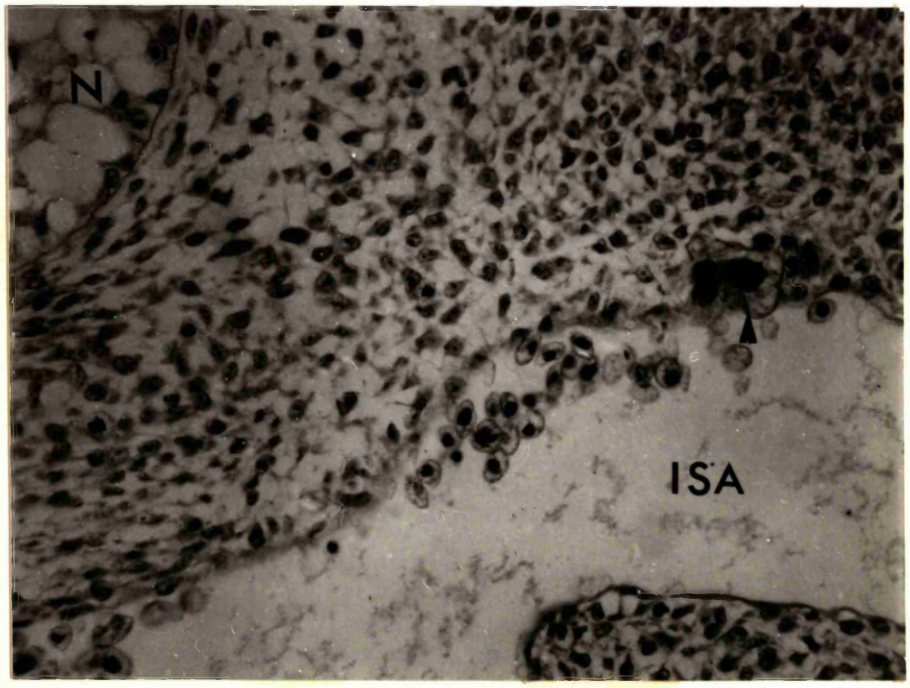
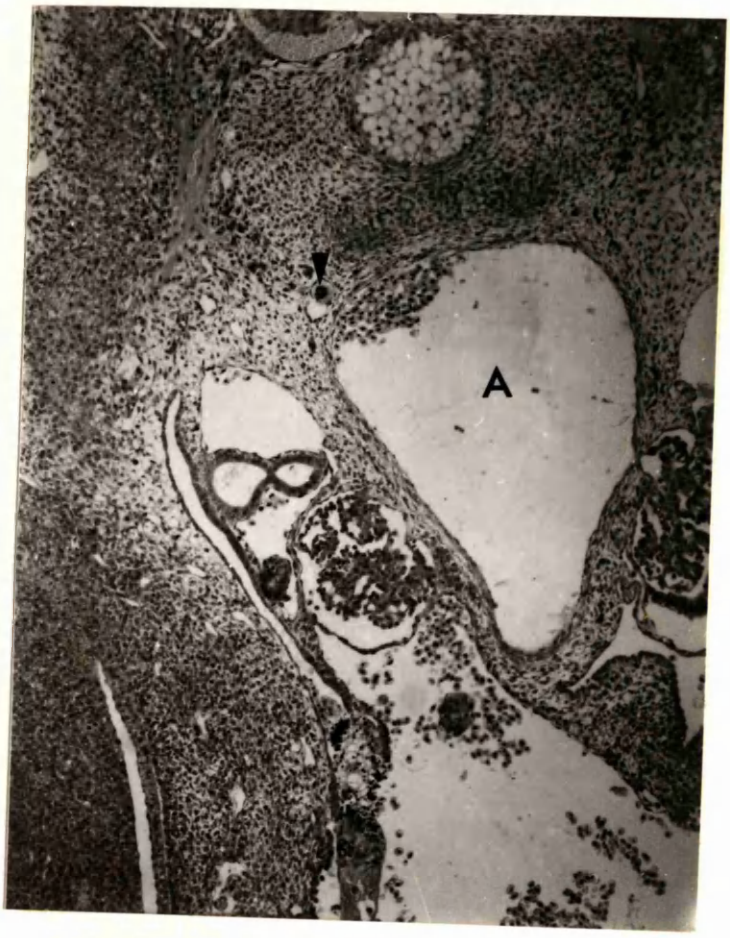

N
ISA

Fig. 68a: Shows a binucleated trophoblastic giant cell (arrowhead), probably within a small vein dorsolateral to the aorta, and closely related to a right sympathetic ganglion.

(Embryo No.25:2:4:5 - 24 hour group) x 100

Fig. 68b: A part of Fig. 68a at higher power showing a binucleated trophoblastic giant cell within a small vein, dorsal and to the right of the dorsal aorta (D.A.) and closely adjacent to the healthy looking sympathoblasts of a right sympathetic chain (arrowheads).

(Embryo No.25:2:4:5 - 24 hour group) x 1000


A

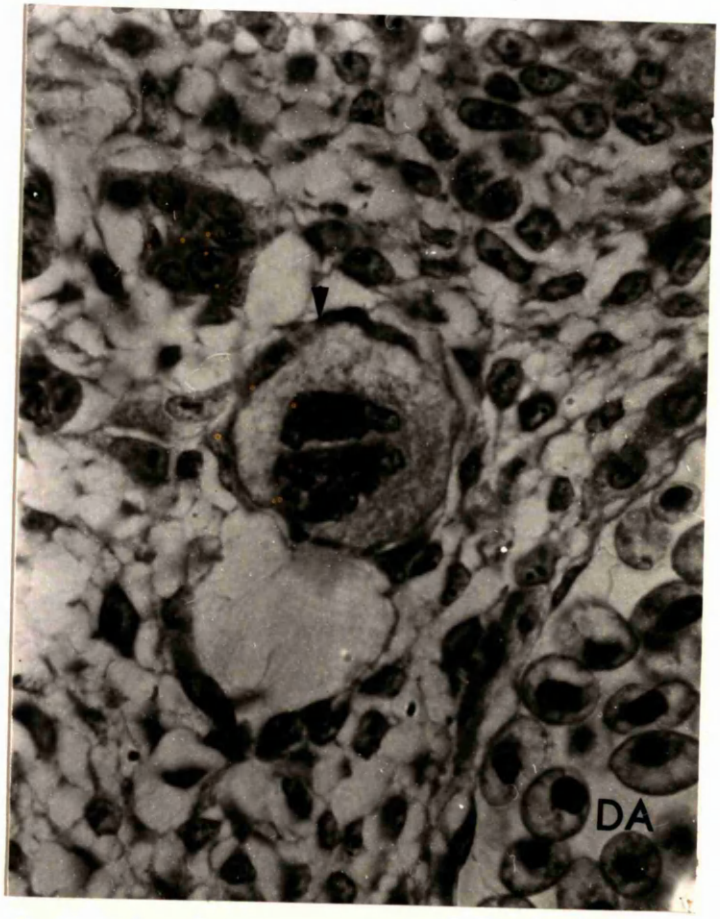
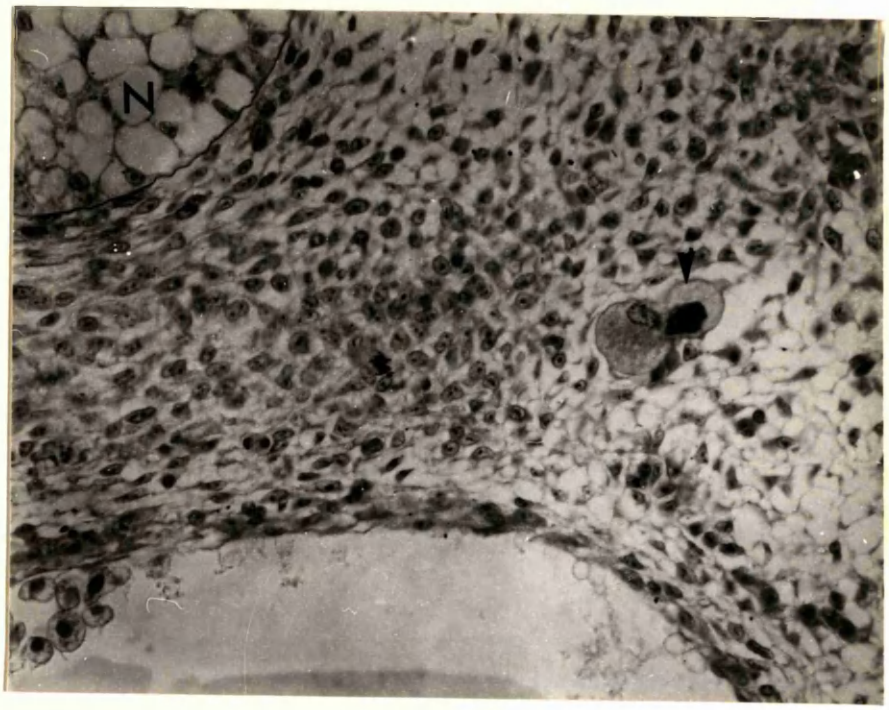

DA

Fig. 69: Shows a binucleated trophoblastic giant cell (arrowhead), possibly within a vein and closely related to the rather scattered sympathoblasts of a left sympathetic ganglion.

(Embryo No.25:3:4:8 - 24 hour group) x 400

Fig. 70: Shows an individual giant cell (arrowhead) adherent to the inner surface of the aortic endothelium. (Very few examples were seen of giant cells adherent to the internal surface). Note: absence of phagocytic activity by giant cell, despite close proximity of chick red cells.

(Embryo No.25:1:2:5 - 24 hour group) x 400


N

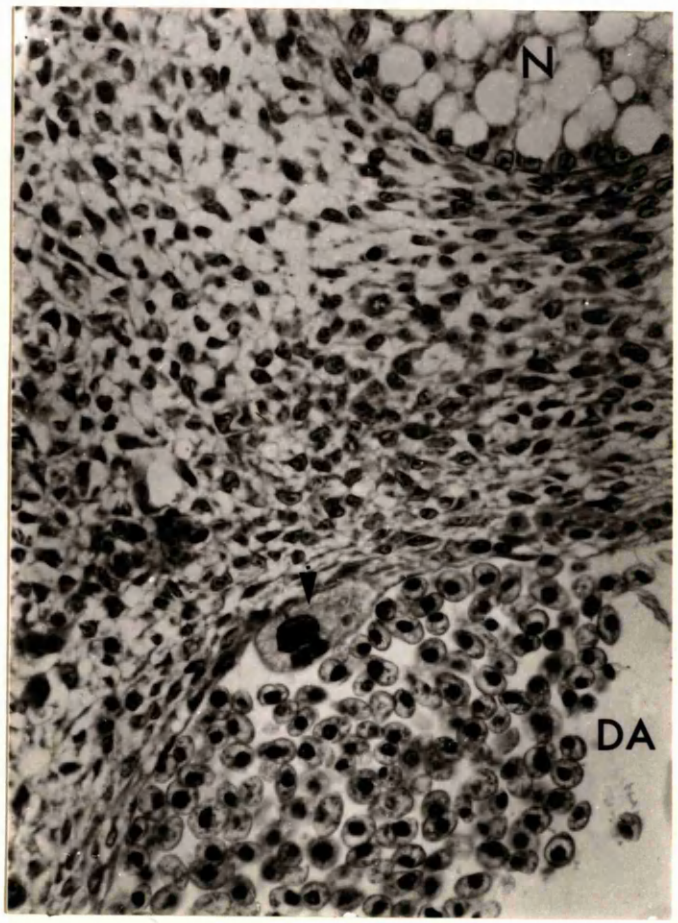
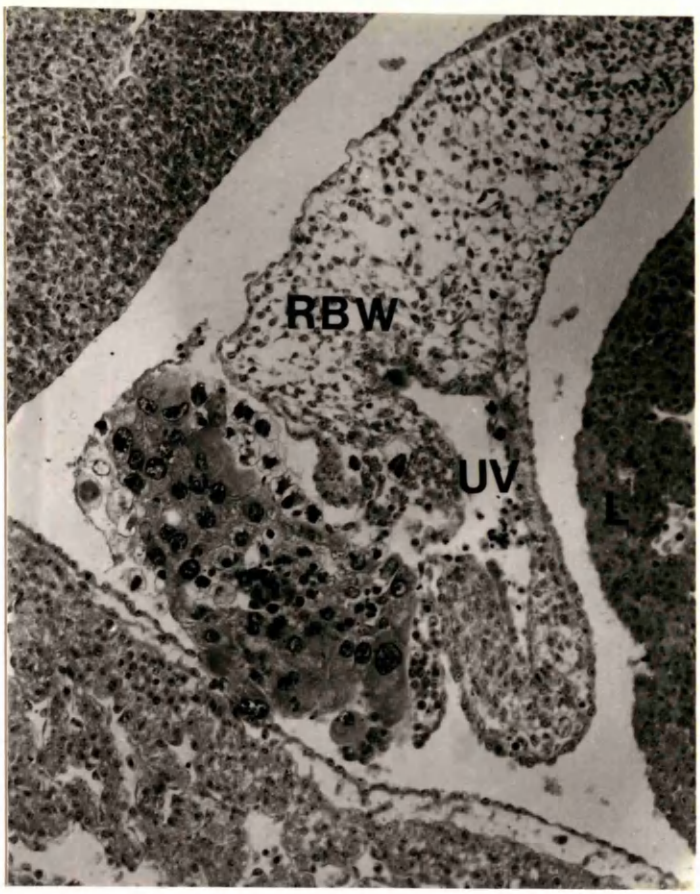

N
DA

Fig. 71: Shows an EPC graft (arrowhead) attached to the ventral edge of the right body wall (RBW), containing the umbilical vein (UV) which has also been eroded. (Liver - L).

(Embryo No.15:2:1:2 - 24 hour group) x 250

Fig. 72: Shows invasion of the right umbilical vein (RUV) by trophoblastic giant cells (arrowheads). Note: single giant cell (arrow) inside a small vein in the body wall. In serial sections this vein was traced into continuity with umbilical vein.

(Embryo No.15:2:2:6 - 24 hour group) x 250


RBW
UV
L

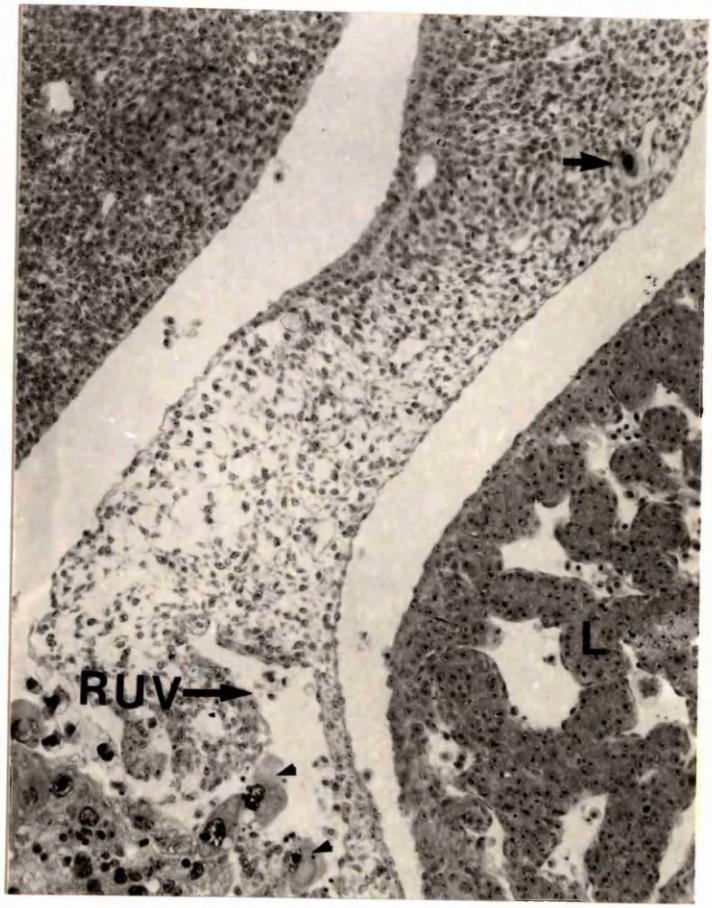
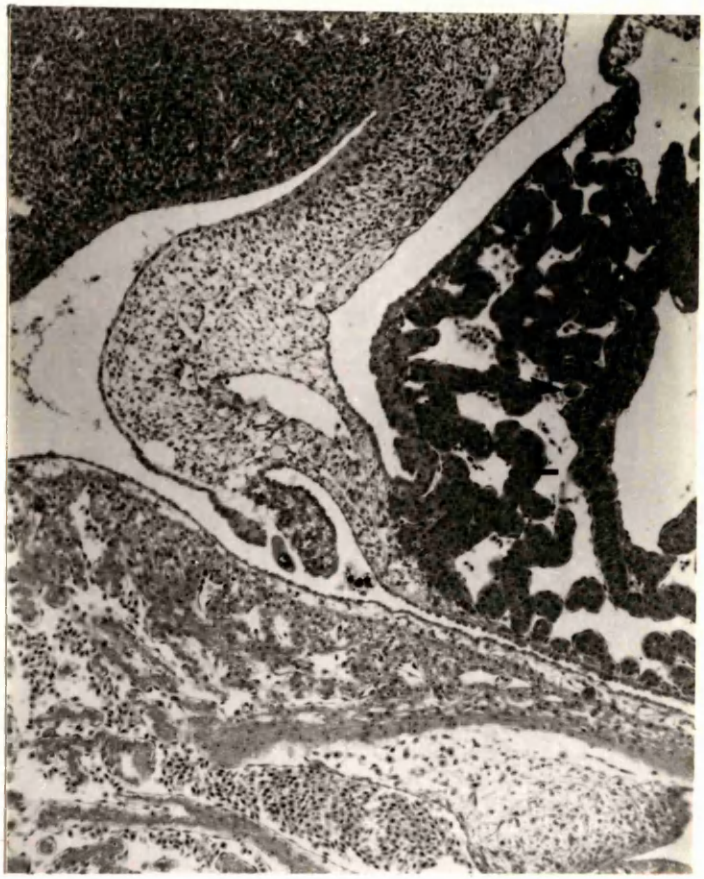

L
RUV

Fig. 73a: Shows a single trophoblastic giant cell (arrowhead) within a liver sinusoid.

(Embryo No.15:1:3:12 - 24 hour group) x 100.

Fig. 73b: High power view of part of Fig. 73a, showing trophoblastic giant cell (arrowhead) within a liver sinusoid. Note: Adjacent endothelial cells and hepatocytes appear undamaged: no histolysis or cytolysis.

(Embryo No.15:1:3:12 - 24 hour group) x 1250



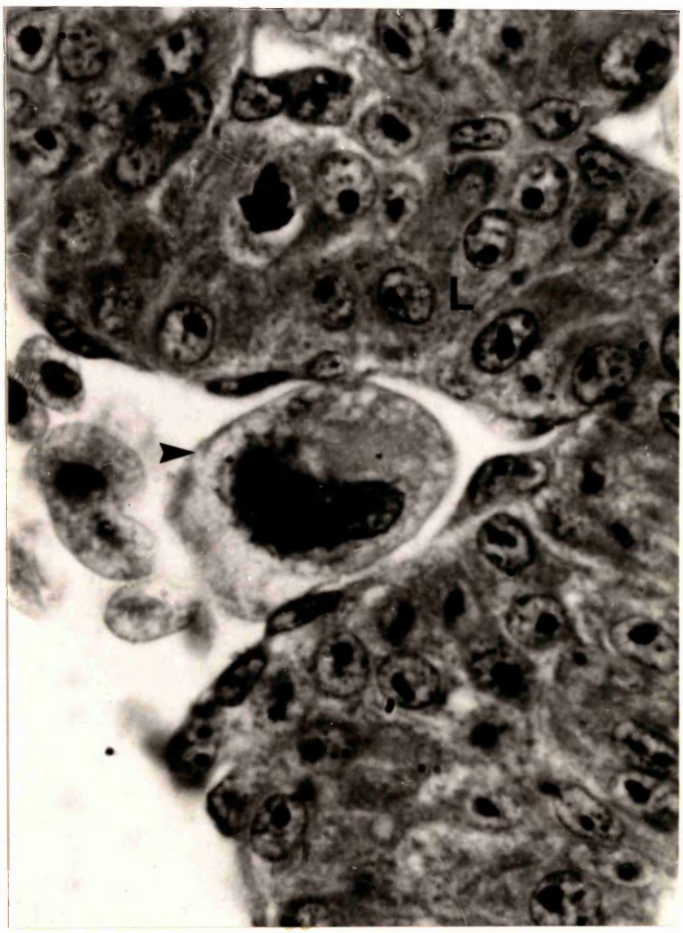

L

Fig. 74: Shows a single trophoblastic giant cell (arrowhead) within a liver sinusoid in contact with apparently healthy endothelium and hepatocytes. In serial sections, this sinusoid was traced into continuity with the right umbilical vein.

(Embryo No.15:1:5:6 - 24 hour group) x 250

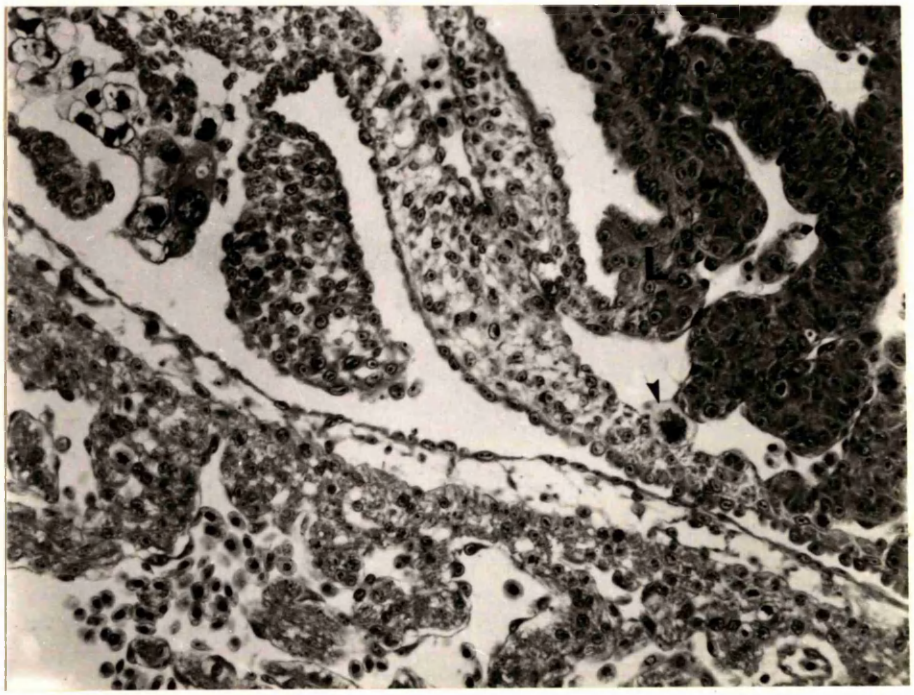
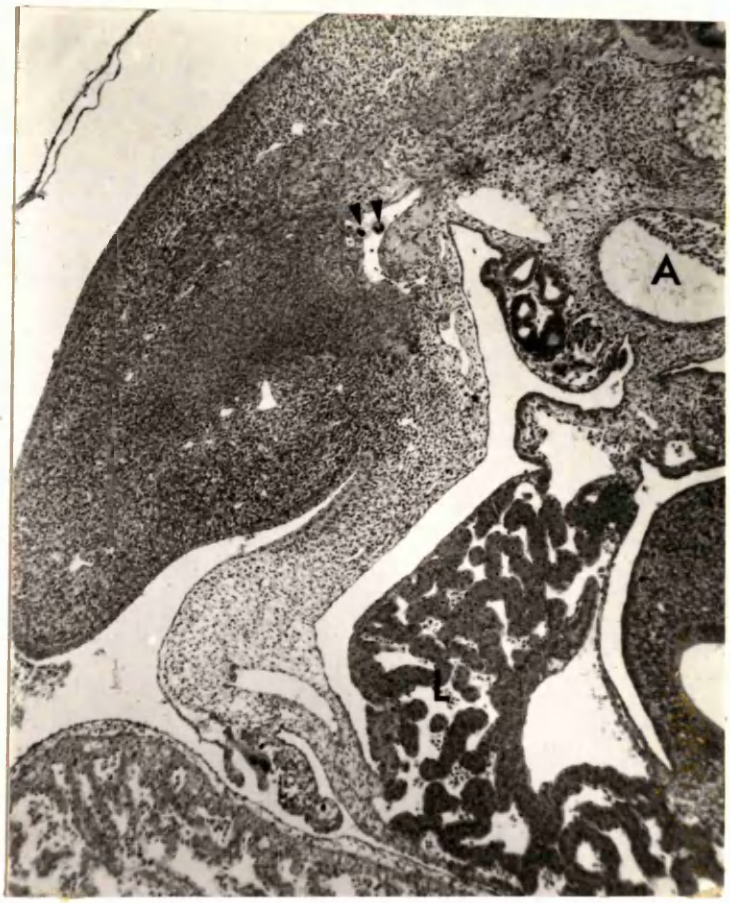

L

Fig. 75a: Shows two giant cells (arrowheads) within a vein at the root of the right limb bud. Note a binucleate trophoblastic giant cell at the site of the graft's attachment.

(Embryo No.15:1:4:10 - 24 hour group) x 100

Fig. 75b: High power view of part of Fig. 75a, showing trophoblastic giant cells (arrowheads) in the lumen of a vein at the root of the right limb bud. The mesenchymal cells adjacent to the more ventral of the two giant cells show no sign of histolytic disaggregation nor of cytolysis.

(Embryo No.15:1:4:10 - 24 hour group) x 400


A

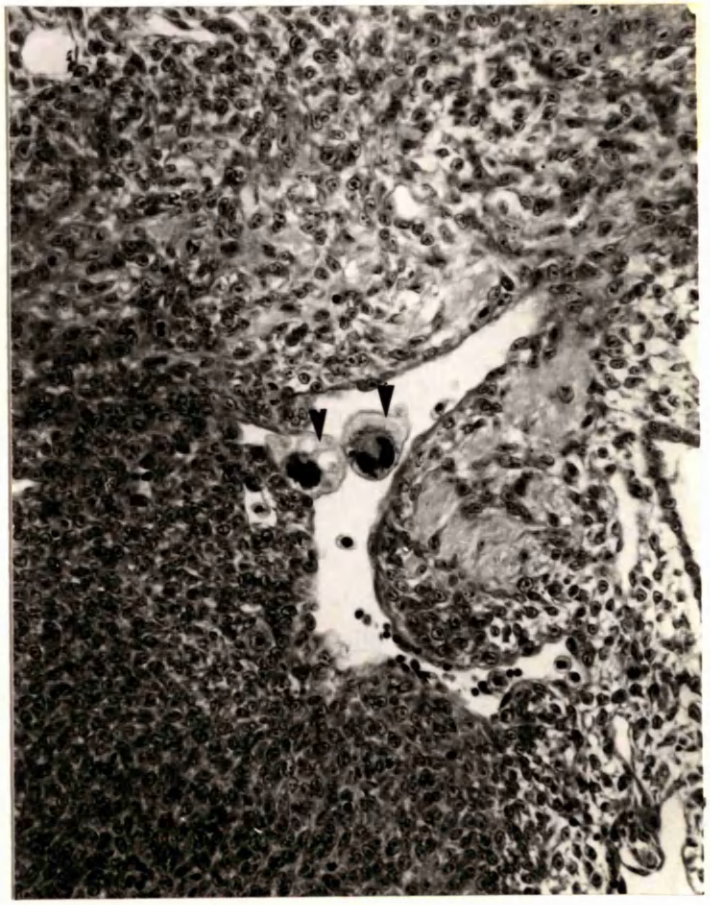
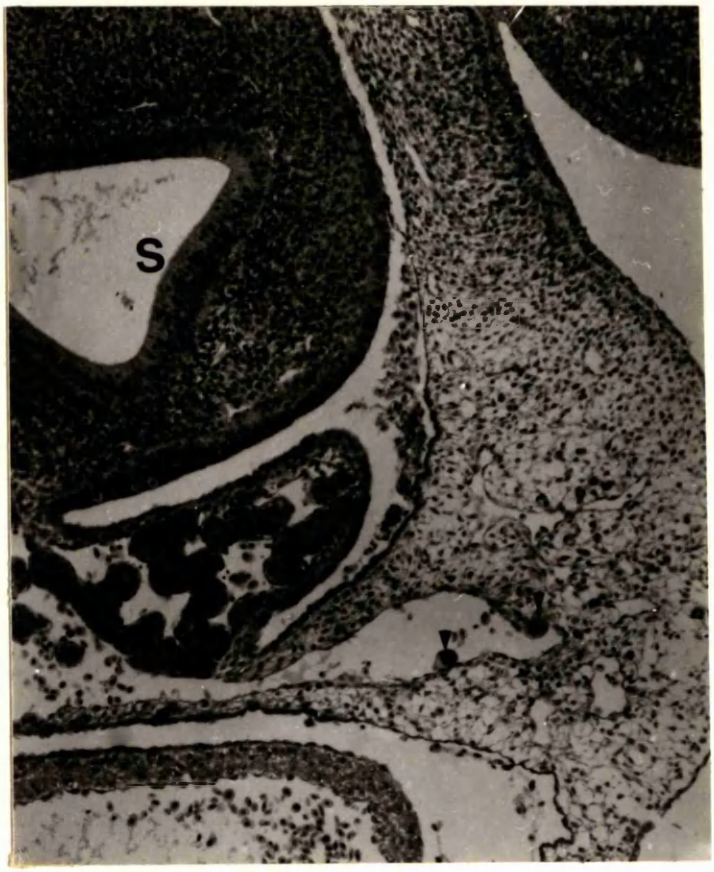


Fig. 76a: Shows two giant cells (arrowheads) within the left umbilical vein.

(Embryo No.15:1:5:7 - 24 hour group) x 125.

Fig. 76b: High power view of part of Fig. 76a, showing giant cells (arrowheads) in the left umbilical vein. The dorsally placed one had intercalated into the endothelial lining, while the ventrally placed one lay in contact with the luminal surface of the endothelium.

(Embryo No.15:1:5:7 - 24 hour group) x 500


S

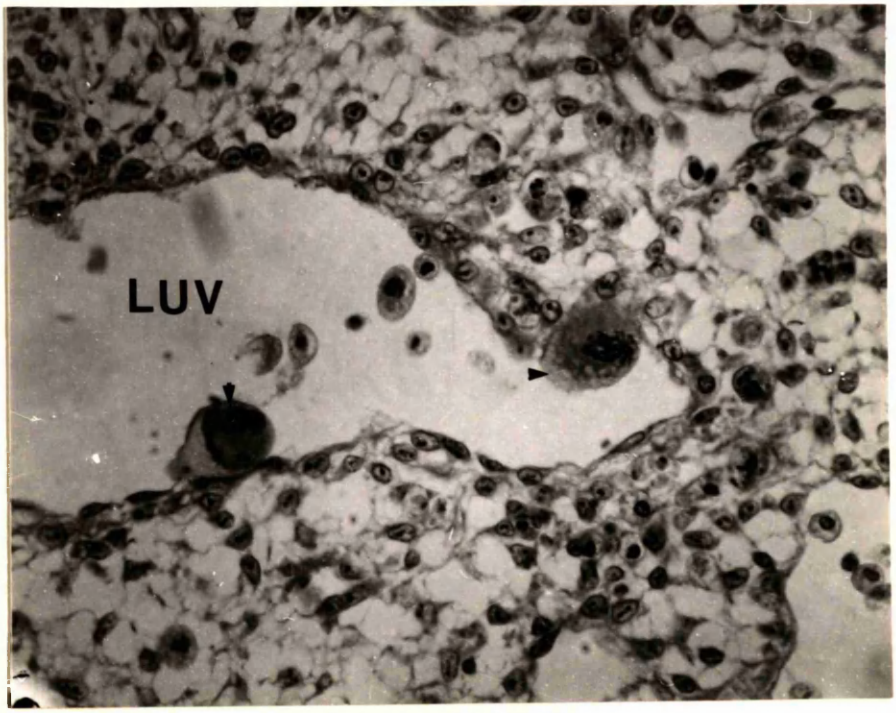
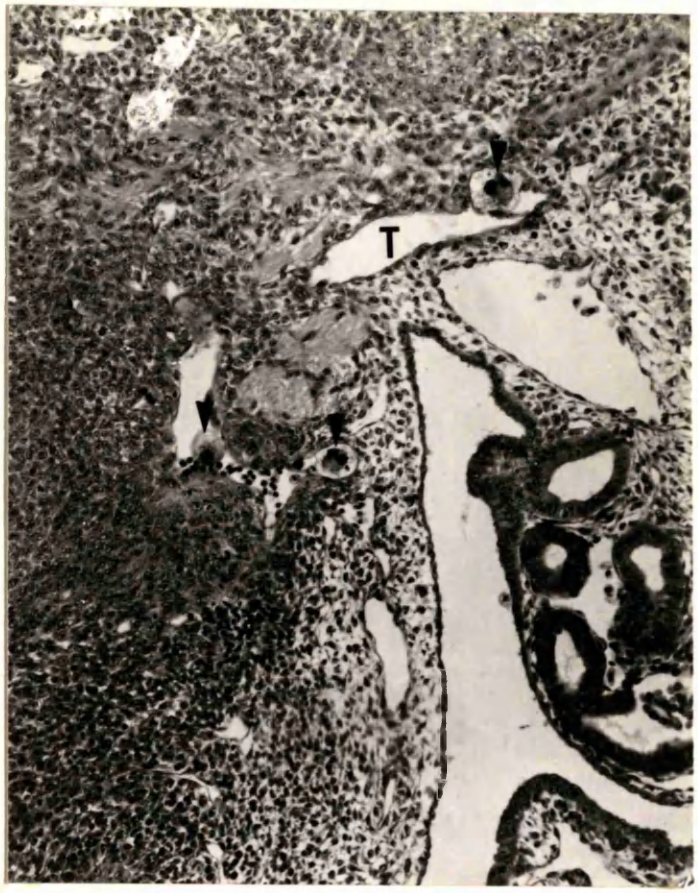

LUV

Fig. 77: Shows individual giant cells (arrowheads) (a) dorsal to a tributary of the right posterior cardinal vein (T), and (b) within a vein at the root of the right limb bud.

(Embryo No.15:2:4:2 - 24 hour group) x 250

Fig. 78: Shows (i) migrant trophoblastic giant cells (arrowheads) at the epidermal-dermal interface of the ventral part of the right body wall, close to the original site of attachment of the graft. Epidermal cells appear undamaged. (ii) single giant cell (arrow) within a small vein in body wall.

(Embryo No.15:1:3:1 - 24 hour group) x 400


T

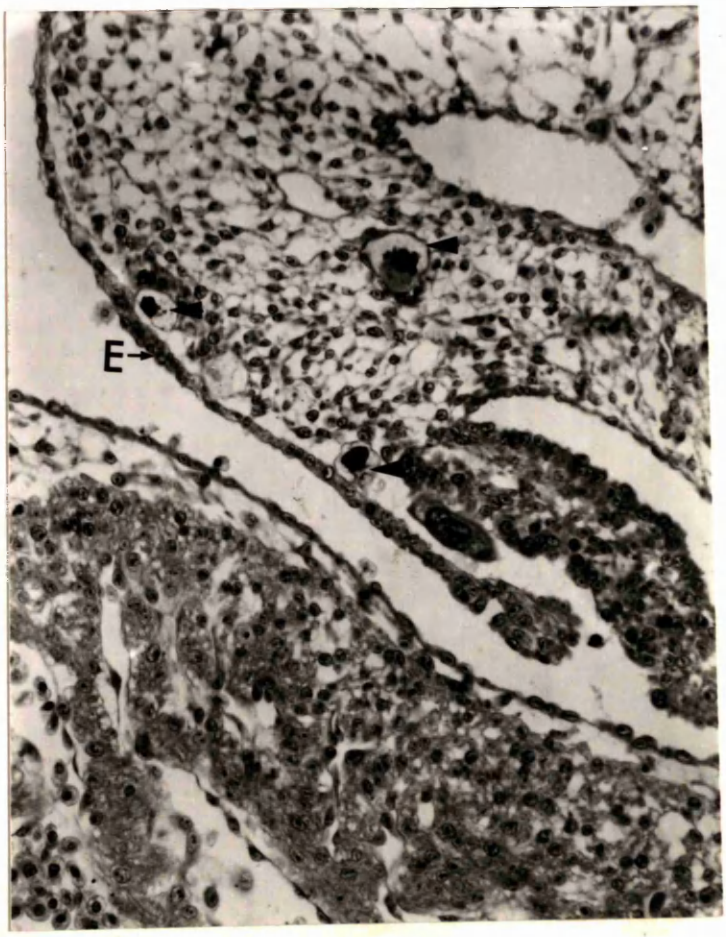
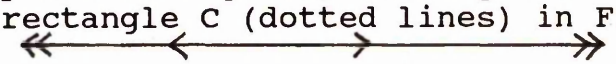

E

Fig.79a: General view of a 7-day conceptus in situ (arrowhead). It lies in an ovoid implantation cavity (IC). Note: antimesometrial decidua (AD); uterine cavity (UC); mesometrial decidua (MD); myometrium (M). Rectangle marked A is shown at higher power in Fig. 79b, that marked B in Fig. 86.

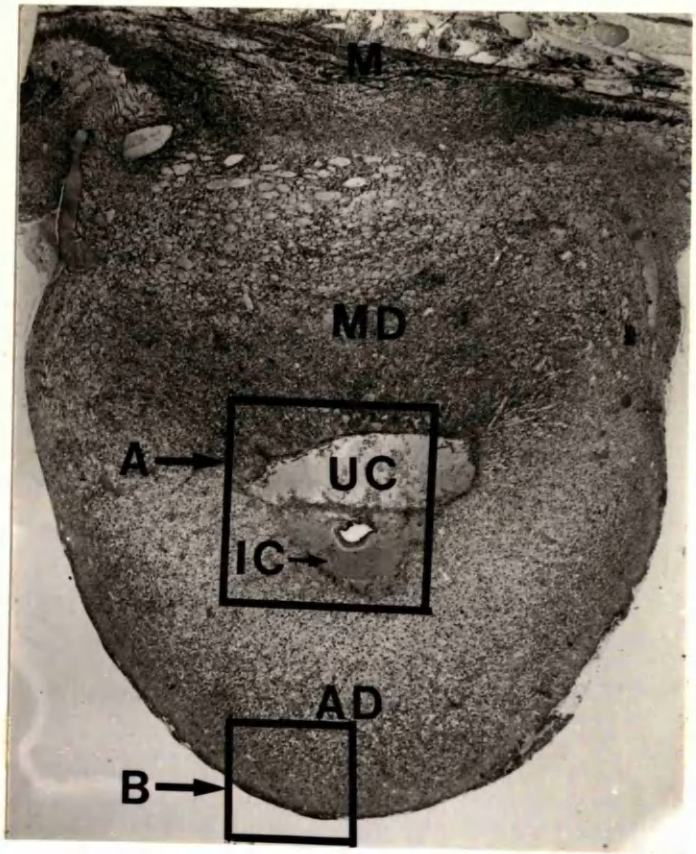
(7-day conceptus in situ: 1/10:1:3:6) x 20

Fig. 79b: Higher power view of rectangle marked A in Fig. 79a. The ectoplacental cone is just forming at the mesometrial pole. Implantation cavity (IC), uterine cavity (UC). Rectangle (A) is shown at higher power in Fig. 80, rectangle B in Fig. 81, rectangle C (dotted lines) in Fig. 82.



; Indicates maximum diameter of conceptus and of implantation cavity at 7 days; for comparison with corresponding measurements at 7.5 days, see Fig. 94, at the same magnification.

(7-day conceptus in situ: 1/10:1:3:6) x 80


M
MD
A
UC
IC
AD
B

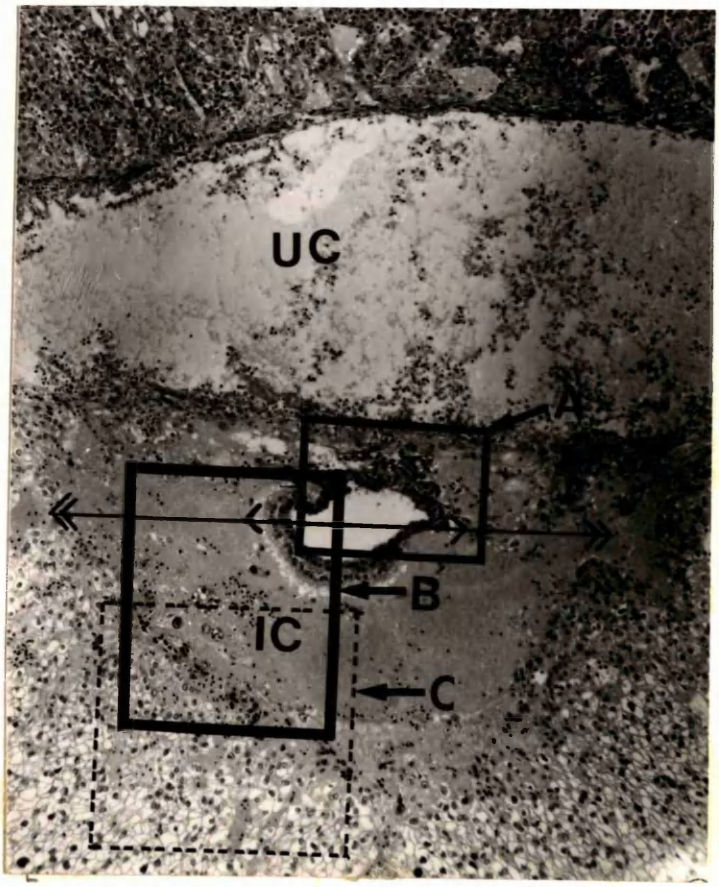
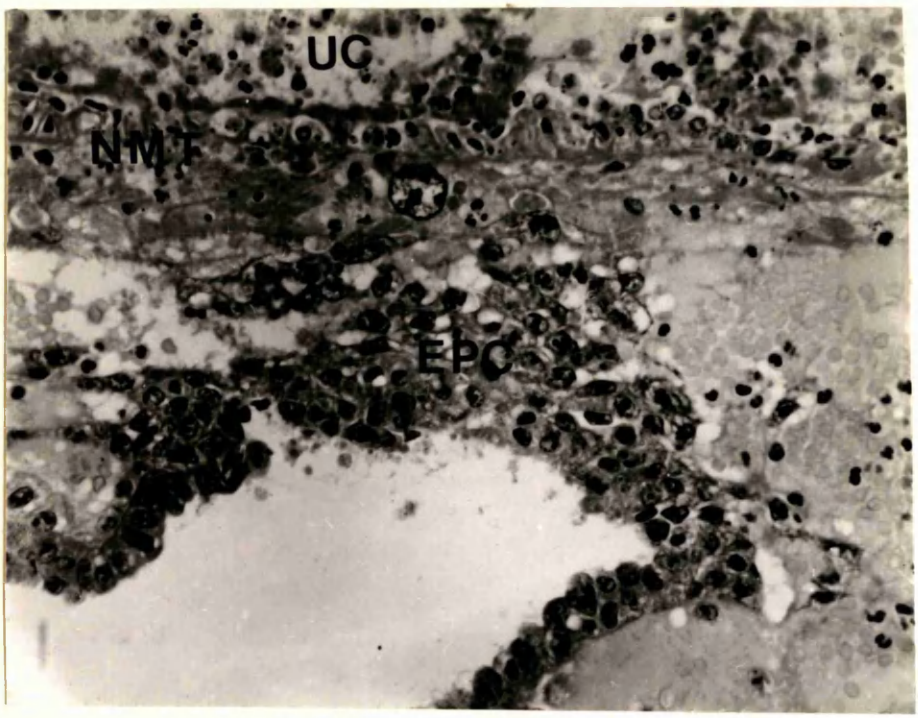

UC
A
B
IC
C

Fig. 80: High power view of the rectangle A in Fig. 79b. The ectoplacental cone consists mainly of untransformed trophoblast cells. A 'typical' secondary trophoblastic giant cell is arrowed. (Uterine cavity - UC; necrotic maternal tissue - NMT; ectoplacental cone - EPC).

(7-day conceptus in situ: 1/10:1:3:6) x 400

Fig. 81: Higher power view of rectangle B, Fig. 79b showing the implantation cavity (IC) of a 7-d conceptus lined by a discontinuous layer of primary giant cells (arrowheads) close to a narrow zone of degenerating decidual cells (asterisks), below which (in the photograph) are healthy, glycogen-filled decidual cells. (Implantation cavity - IC; Reichert's membrane - RM; visceral endoderm - VE).

(7-day conceptus in situ:1/10:1:3:6) x 313


UC
NMT
EPC

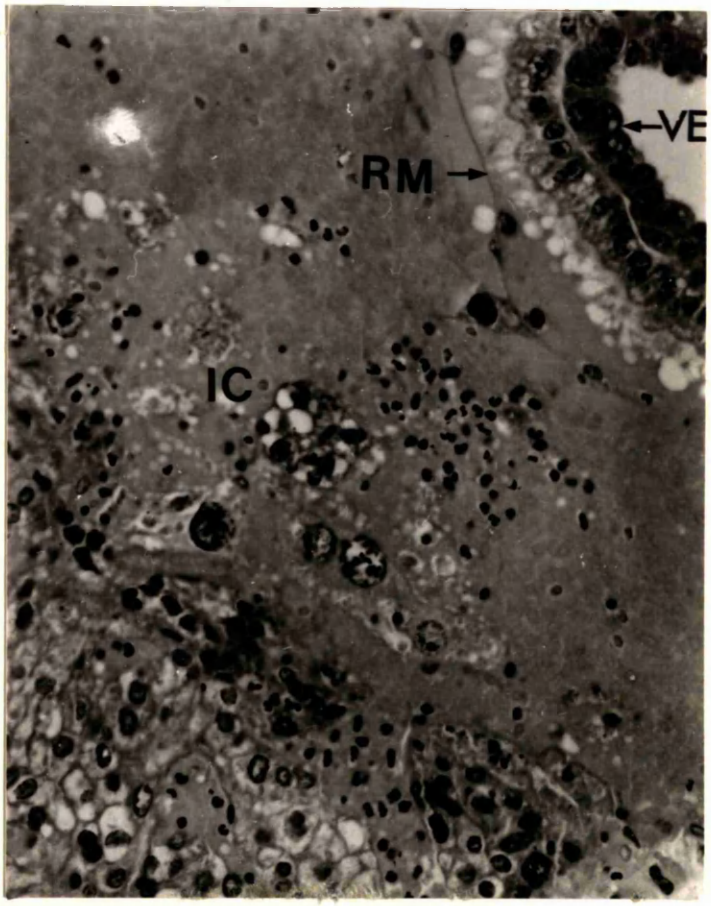

VE
RM
IC

Fig. 82: Higher power view of rectangle C (Fig. 79b), showing, from above down, (i.e. moving antimesometrially):

(1) discontinuous layer of primary giant cells.
(2) narrow zone of necrotic decidua.
(3) thick layer of healthy, glycogen-filled decidual cells, with numerous sinusoidal vessels, one of which opens into implantation cavity (arrowhead).

(7-day conceptus in situ: 1/10:1:3:6) x 250

Fig. 83: Shows a trophoblatic giant cell (arrowhead) within a small thin-walled vessel, probably a vein, at the endometrial-myometrial junction.

(7-day conceptus in situ: 1/10:1:3:1) x 250


1
2
3

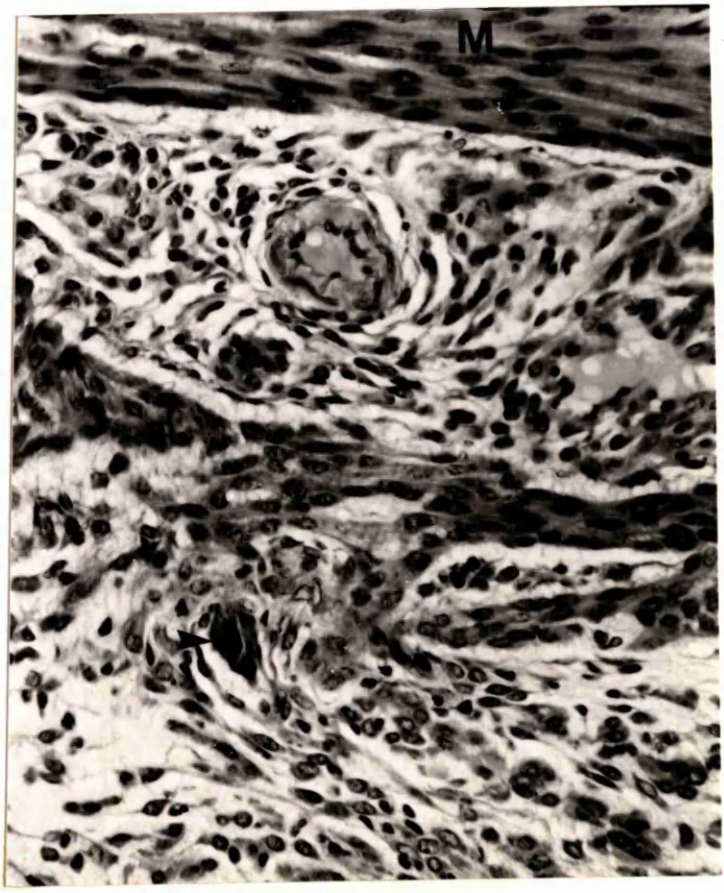
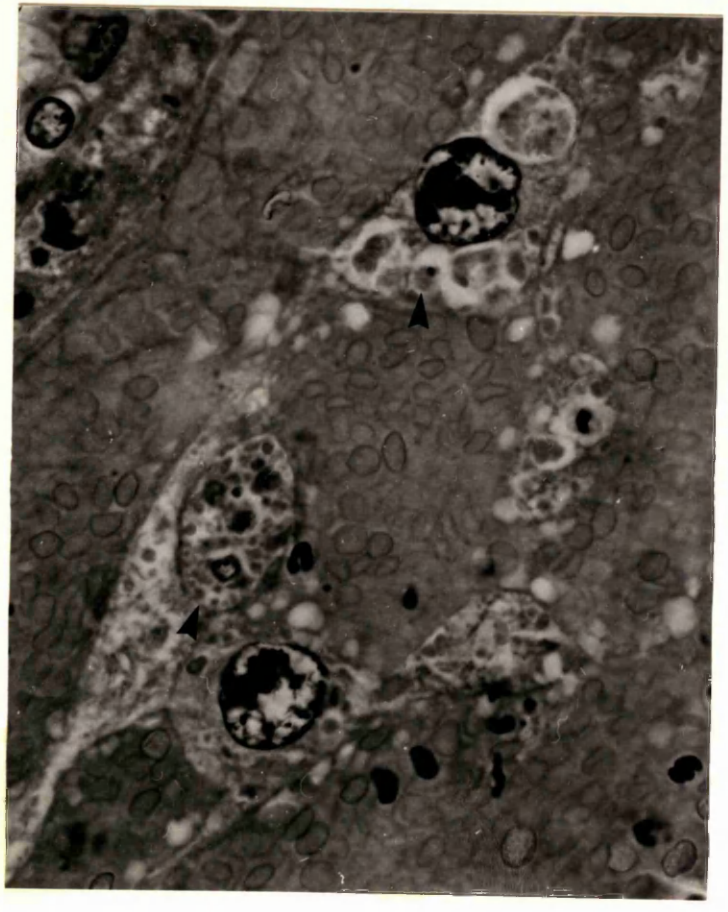

M

Fig. 84: Shows two binucleated trophoblastic giant cells (arrowheads), each lying within small blood vessels, probably arterioles, in the mesometrial decidua.

(7-day conceptus in situ:1/10:1:4:8) x 500

Fig. 85: High power view of 'typical' primary trophoblastic giant cells in the implantation cavity (IC) containing phagocytosed maternal red cells.

(7-day conceptus in situ:1/10:1:3:7) x 800



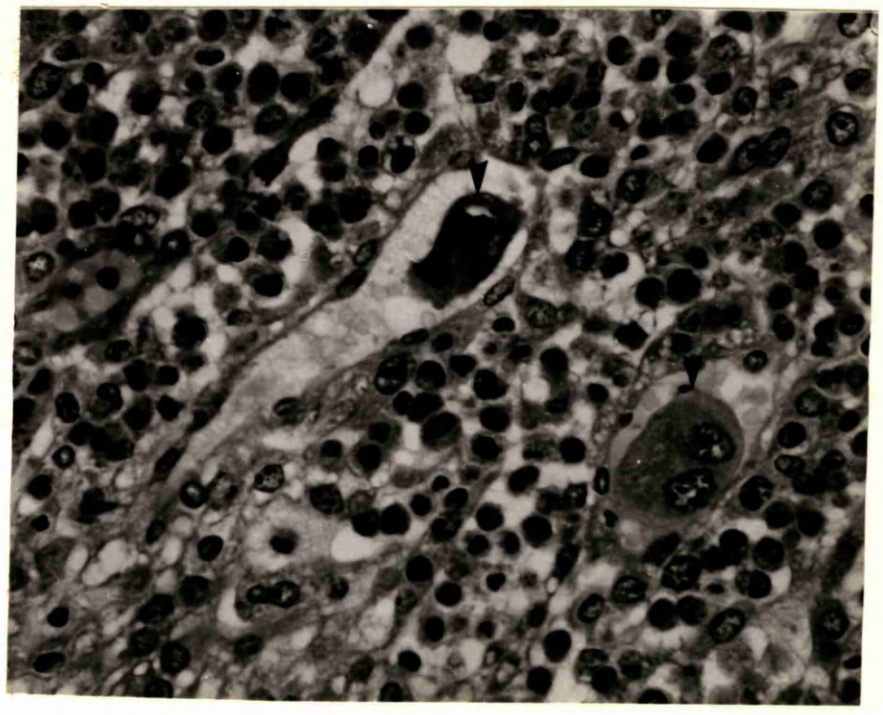
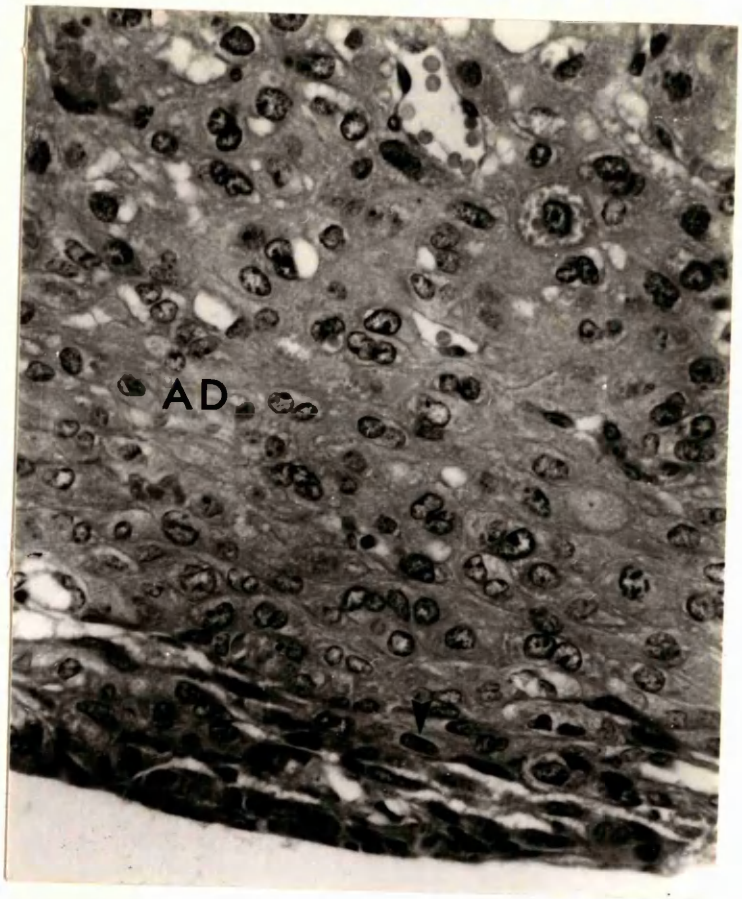


Fig. 86: Higher power view of rectangle B (Fig. 79a) showing part of the antimesometrial decidua. Decidual cells have rather dense non-vacuolated cytoplasm, very different from that of vacuolated (glycogen-filled) decidual cells near the conceptus (Fig. 82).
Note: at the margin of the decidual swelling, there are several spindle-shaped (non-decidualized) stromal cells.

(7-day conceptus in situ: 1/10:1:3:6) x 400

Fig. 87: Shows part of the mesometrial decidua. Most decidual cells are small with dense cytoplasm; a few are larger and vacuolated. Notice numerous polymorphonuclear leucocytes (arrowhead) in a venous sinusoid in this region.

(7-day conceptus in situ: 1/10:1:3:6) x 250


AD

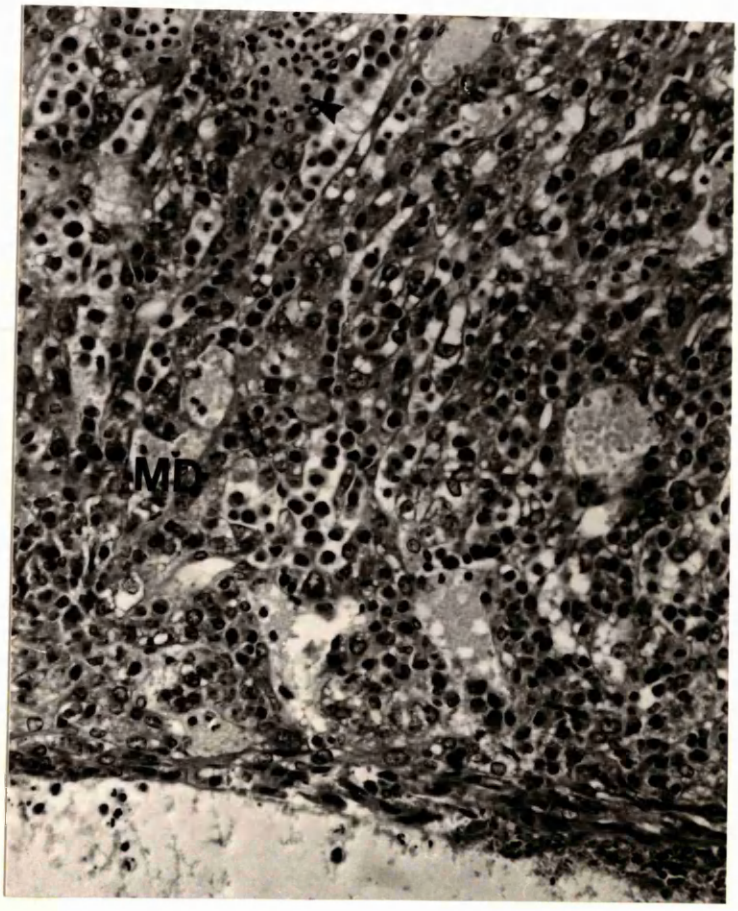

MD

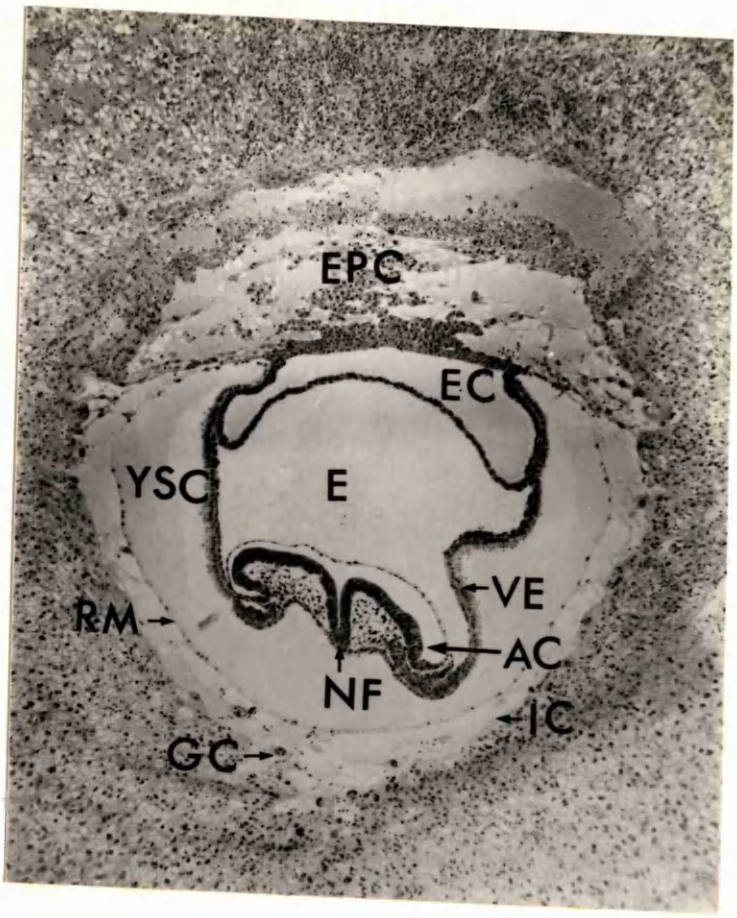
Fig. 88: Shows section of a 7.5 conceptus in situ. Implantation, cavity - IC, line of giant cells - GC, parietal endoderm and Reichert's membrane - RM, yolk sac cavity - YSC, visceral endoderm - VE, amniotic cavity - AC, exocoelom - E, ectoplacental cone - EPC, ectoplacental cavity - EC,

embryonic ectoderm and neural folds - NF).

(7.5-day conceptus in situ:1:3:1:7) x 60

Fig. 89: Shows the ectoplacental cone (EPC) on the mesometrial side of the conceptus consisting: (1) of an epithelial plate containing many layers of compactly arranged small basophilic untransformed trophoblast cells. (2) widely spaced irregular anastomosing cords of larger trophoblast cells.

(7.5-day conceptus: 1/10:1:3:7) x 163


EPC
EC
YSC
E
VE
RM
AC
NF
IC
GC

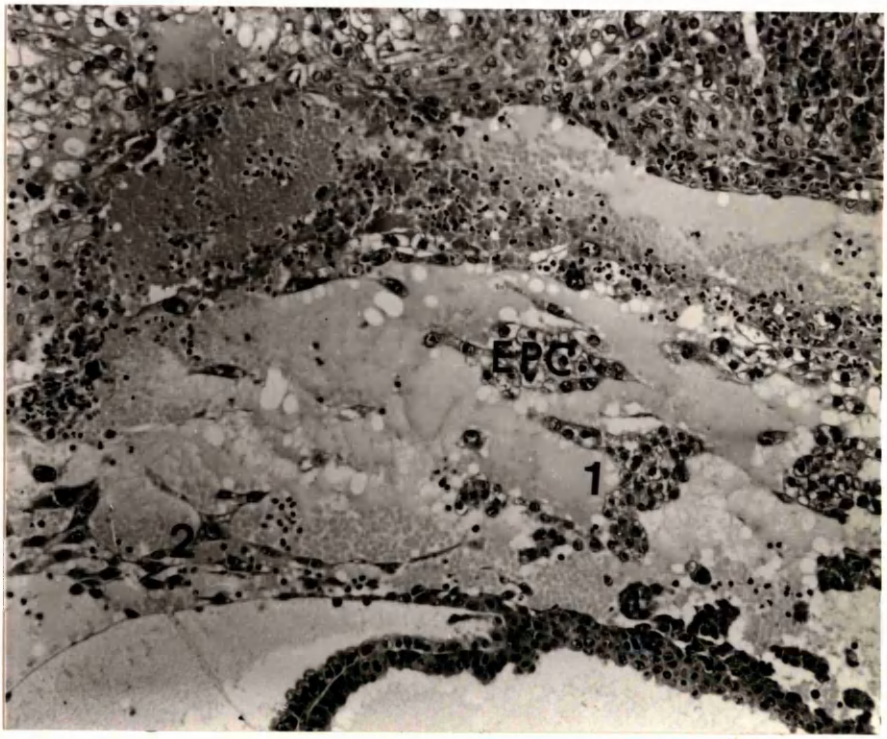

EPC
1
2

Fig. 90: Photomicrograph of a 7.5-day conceptus. Note the ectoplacental cone (EPC) occupying the mesometrial sector of the perimeter of the conceptus and primary giant cells (arrowheads) flattened tangentially to the decidua on the antimesometrial side of the implantation cavity.

(7.5-day conceptus in situ:1/10:1:3:7) x 65


M
EPC
AM

Fig. 91a: Photomicrograph showing primary trophoblastic giant cells (arrowheads) which had penetrated the decidua for about 230μm. For higher power view of rectangle marked A, see Fig. 91b.

(7.5-day conceptus:1/10:1:2:4) x 70

Fig. 91b: Higher power view of rectangle marked A in Fig. 91a shows a primary trophoblastic giant cell (arrowhead) which had penetrated the zone of partially necrotic decidua for about 230μm.

(7.5-day conceptus:1/10:1:2:4) x 320


D
A

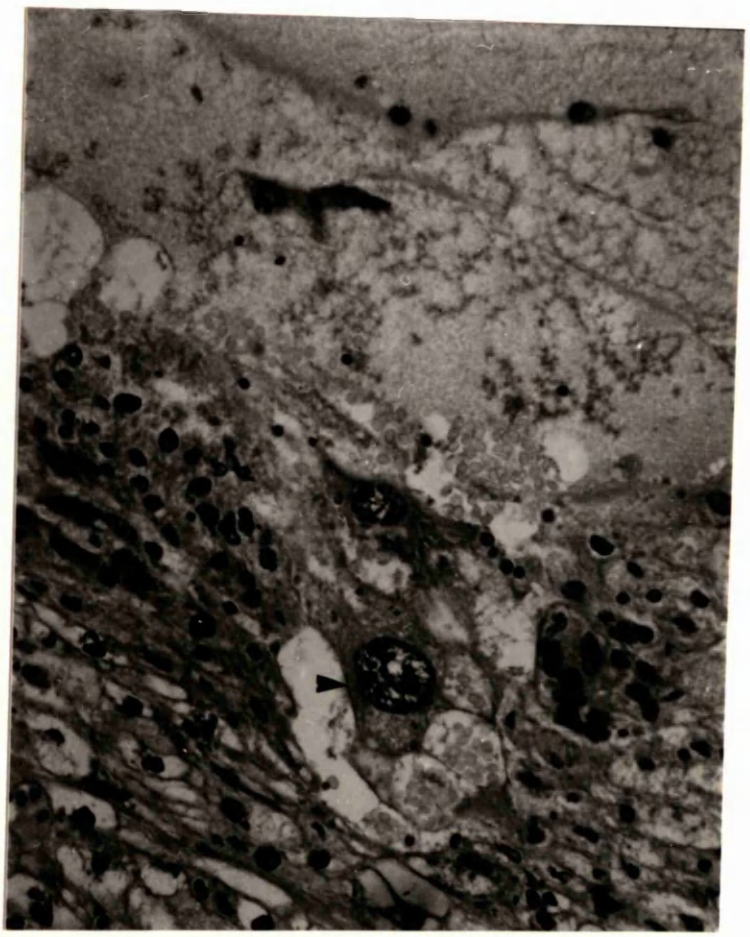
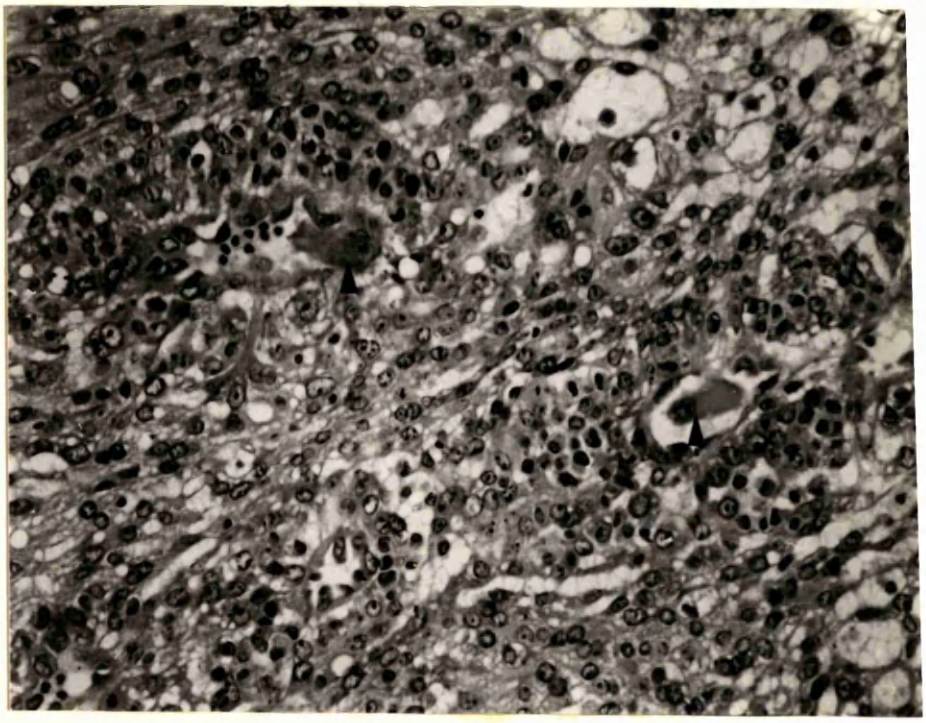


Fig. 92: Photomicrograph showing endovascular spread of trophoblastic giant cells (arrowheads) in the mesometrial decidua.

(7.5-day conceptus:1/10:1:2:9) x 280

Fig. 93: Shows a cluster of 3 (or more) giant cells (the nucleus of only one of them is sectioned) within a spiral arteriole in the mesometrial decidua. The vessel is surrounded by a cuff of compactly arranged decidual cells.

(7.5-day conceptus: 1/10:1:4:4) x 320



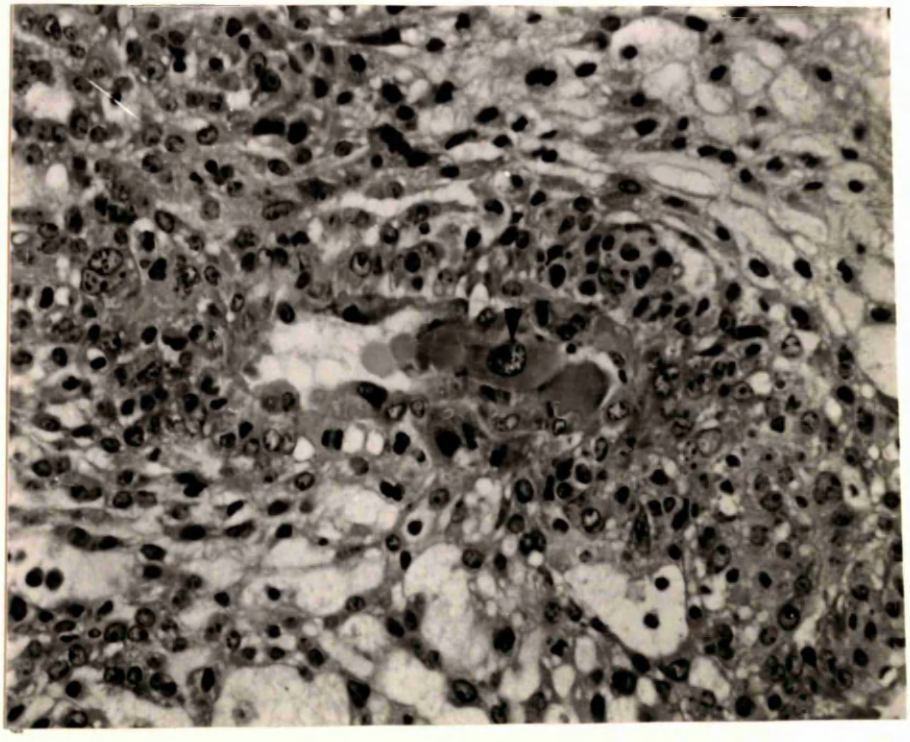
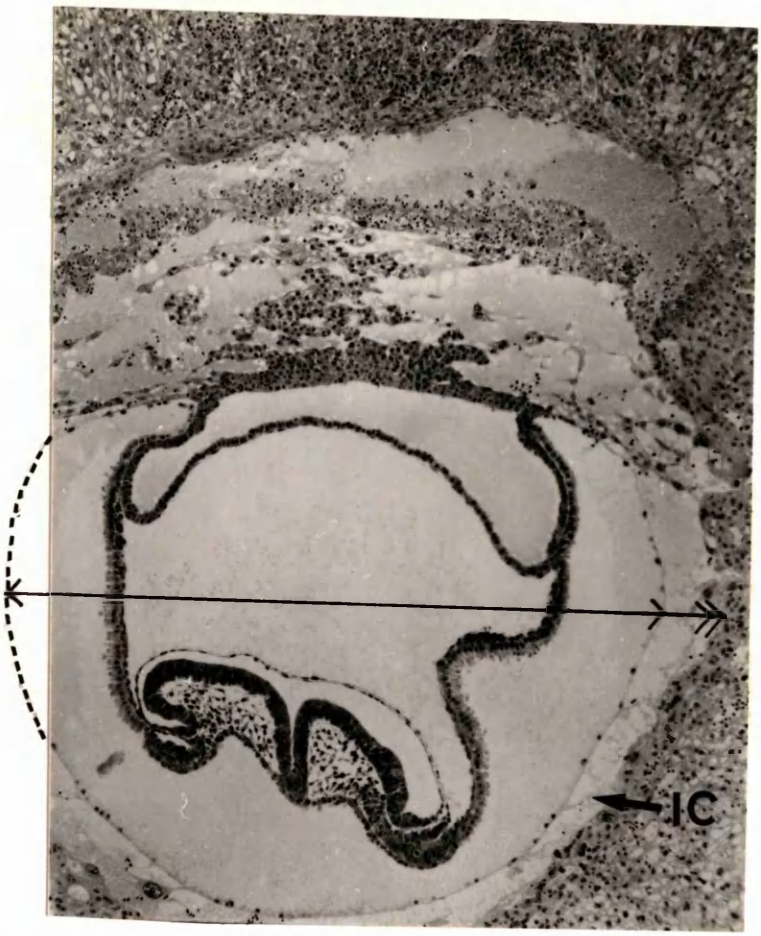


Fig. 94: Shows method of measuring maximum diameter of conceptus (⟷) and of implantation cavity (⟪⟷⟫) at 7.5 days; for comparison with corresponding measurements at 7 days, see Fig. 79b, at the same magnification.

(7.5-day conceptus: 3:1:7) x 80

Fig. 95: Shows necrotic zone of decidua adjacent to the implantation cavity, with some glycogen rich decidual cells below.

(7.5 day conceptus: 3:1:7) x 250


IC

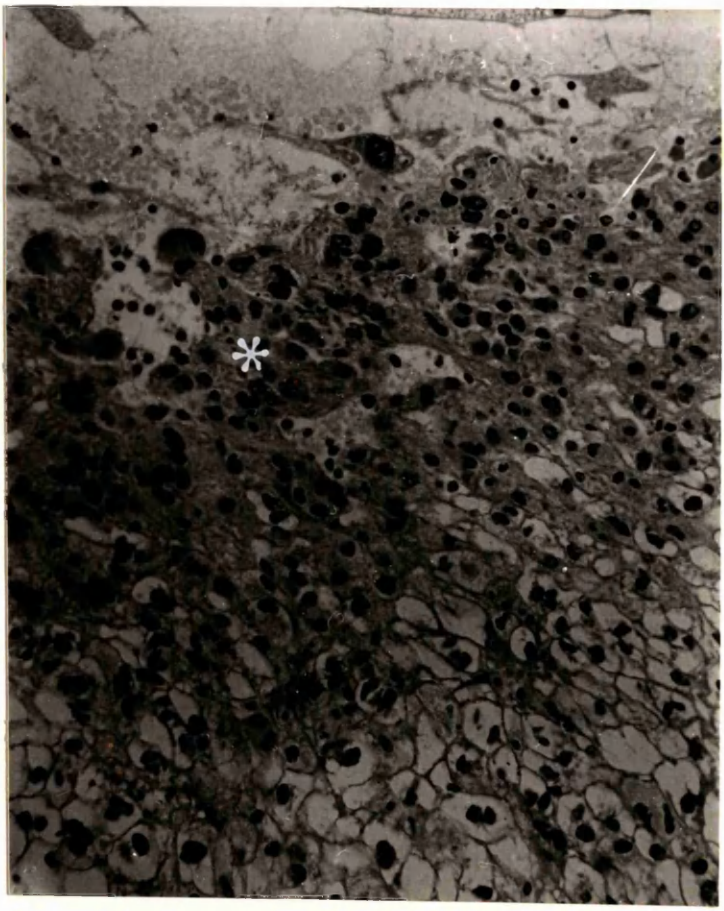
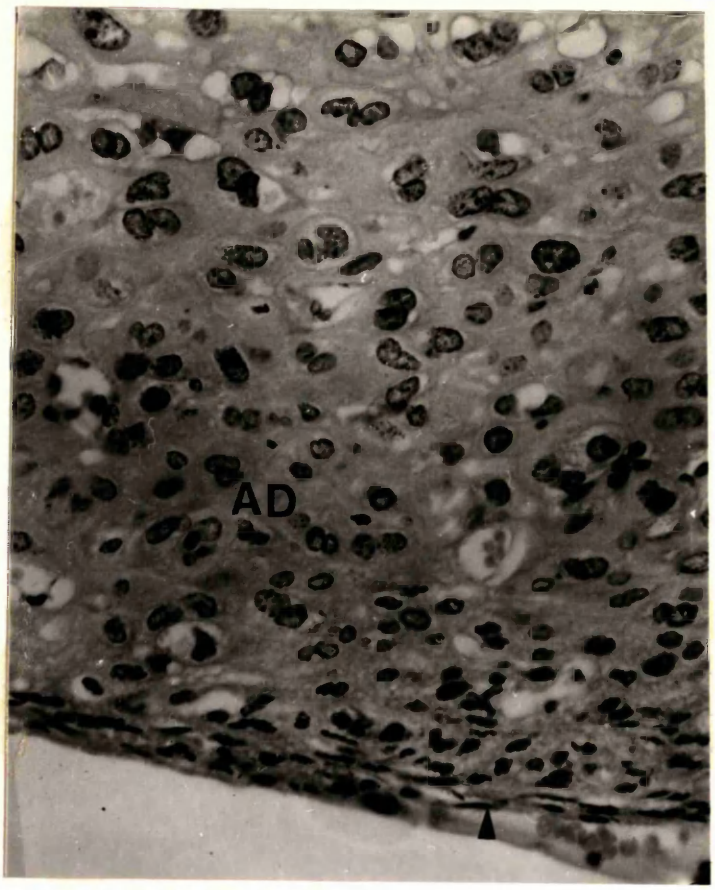

*

Fig. 96: Photomicrograph of the decidua at the periphery of the swelling (antimesometrial pole). Most decidual have dense cytoplasm, few are vacuolated. Note spindle-shaped, non-decidualized stromal cells at margin of swelling.

(7.5-day conceptus: 3:1:7) x 400

Fig. 97: Photomicrograph of mesometrial decidua showing admixture of decidual cells with dense cytoplasm and vacuolated ones. Notice numerous polymorphonuclear leucocytes in venous sinusoids in this region.

(7.5-day conceptus: 3:1:7) x 400


AD

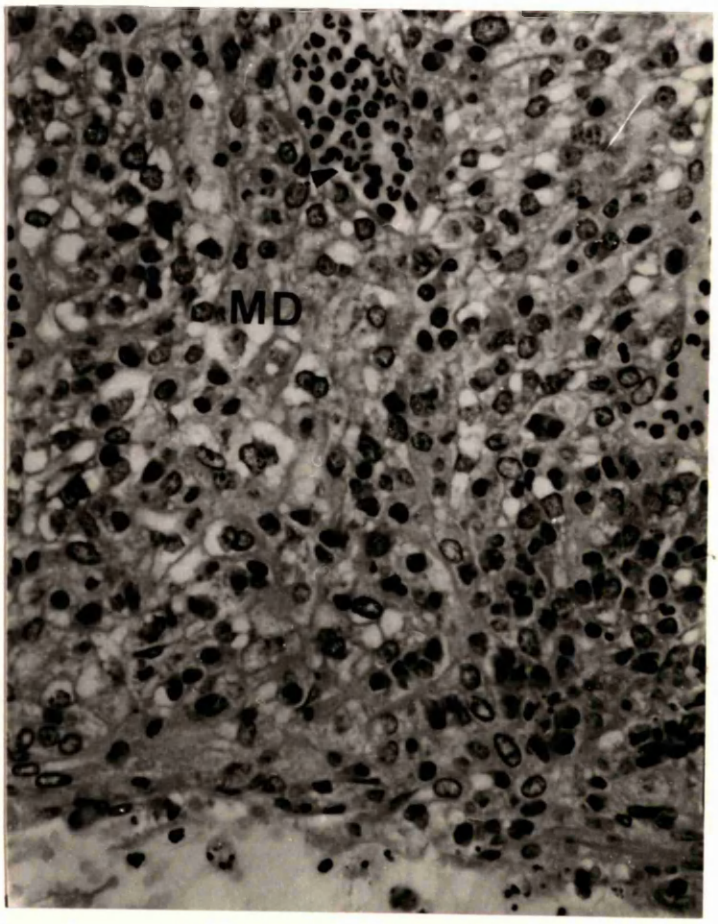
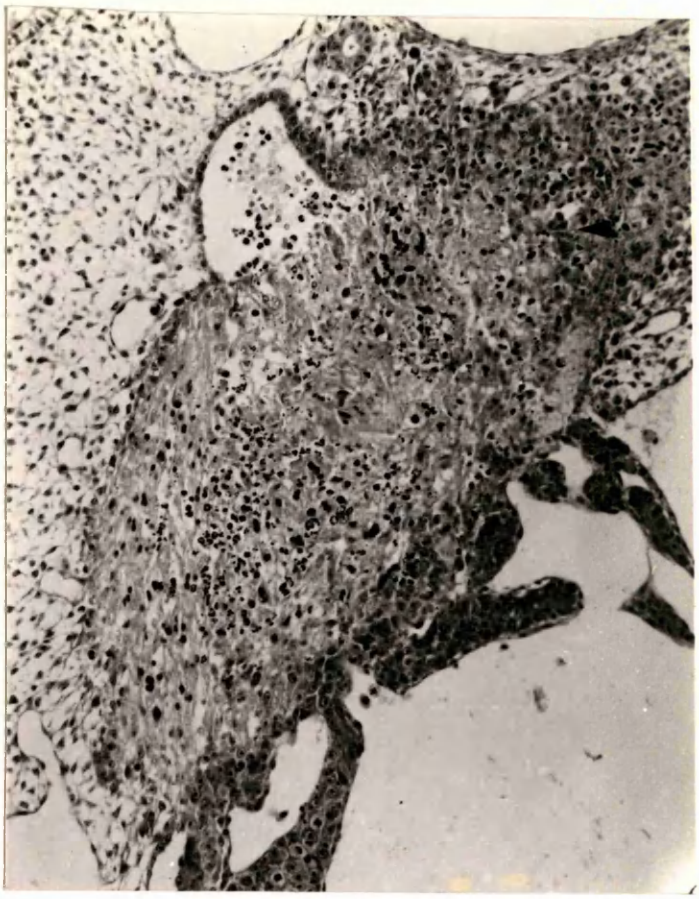

MD

Fig. 98a: Shows a decidual graft (DC4) occupying the upper right coelom. It has encroached upon the dorsal, medial, ventral and lateral margins of the coelom, with loss of the coelomic mesothelium but no damage to blood vessels. Most of the graft cells are necrotic.

(DC4: 2:3:6) x 250

Fig. 98b: Higher power view of a part of Fig. 98a. The mesothelial lining of the coelom has been disrupted. The nature of the thick layer of healthy epithelial cells (asterisks) lying within the dorsal mesogastrium is uncertain, but they do not resemble decidual cells.

(DC4:1/10:1:4:5) x 250



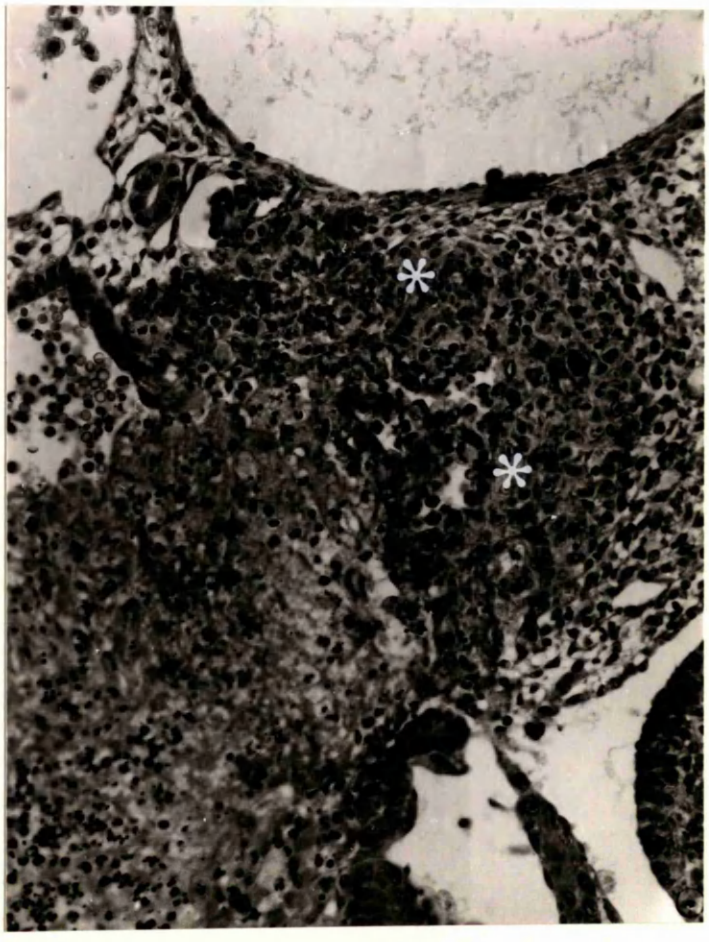
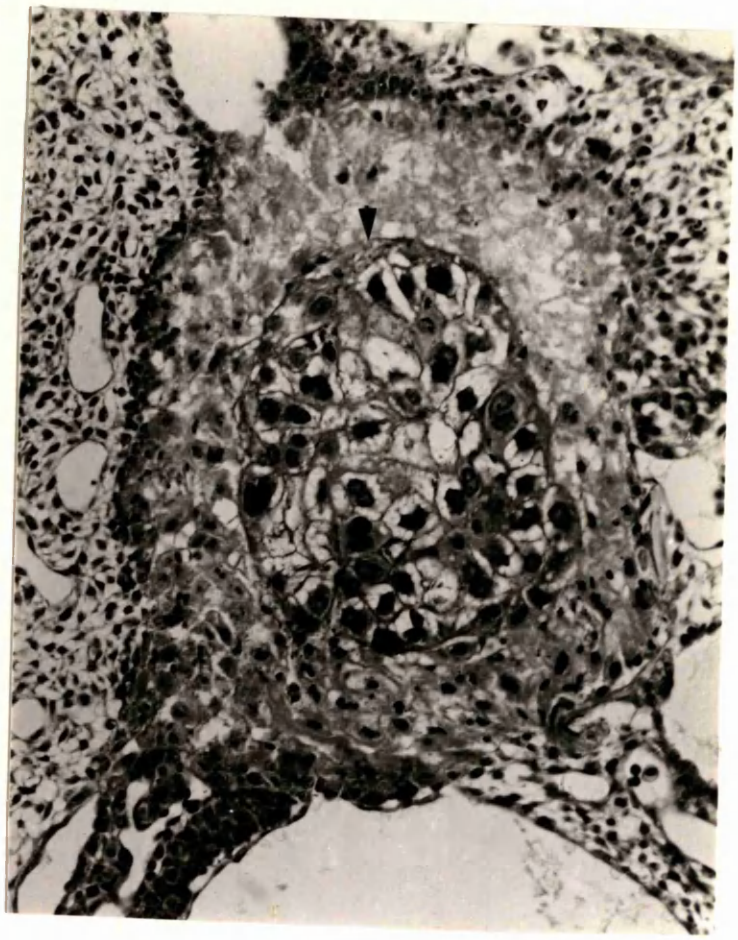


Fig. 99a: Shows a decidual graft (DC3) occupying the upper right coelom. Note: core of healthy decidual cells of the "glycogen-filled" type (arrowheads) at the centre of the graft, surrounded by a layer of necrotic cells, which had encroached upon the chick tissues, leaving no organised mesothelial lining.

(DC3: 1/10:1:4:2) x 250

Fig. 99b: High power view of DC3 in contact with the dorsum of the liver. Notice absence of mesothelium over the liver (arrowheads) in the area of contact. Note: 1. Core of healthy, "glycogen-rich" decidual cells, surrounded by necrotic decidua. 2. absence of mesothelium from dorsal surface of liver.

(DC3: 1/10:1:4:2) x 400



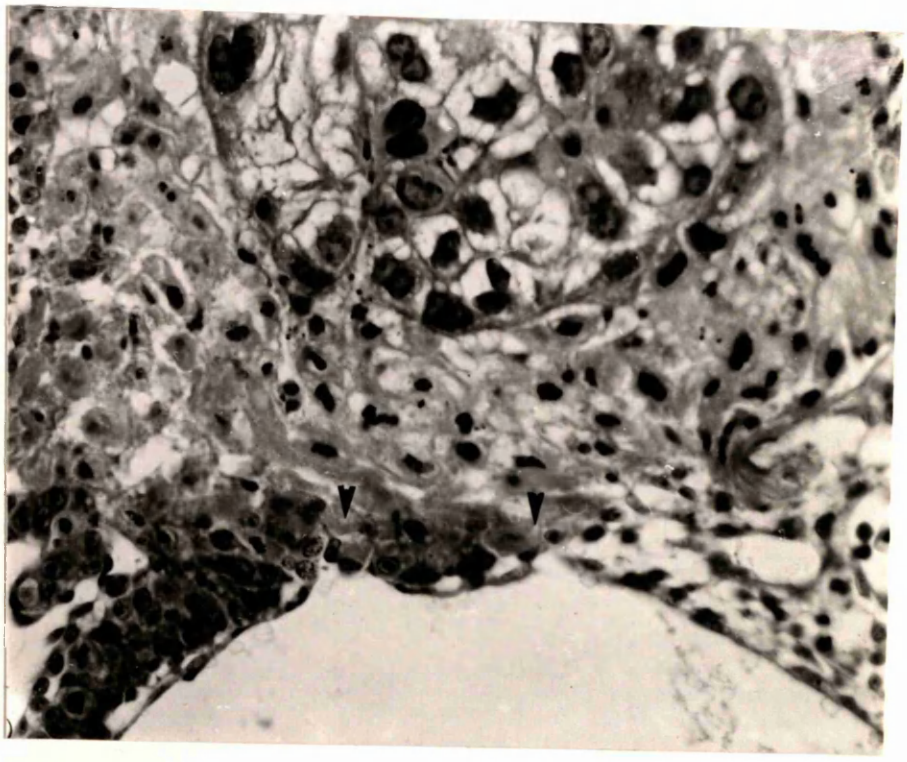
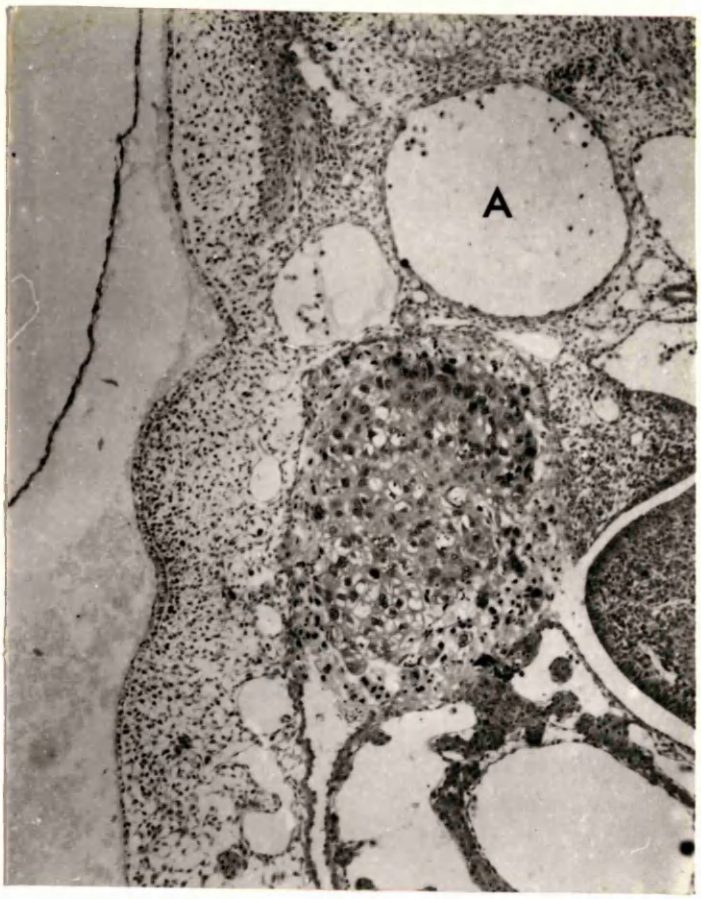


Fig. 100a: Shows decidual graft (DC2), filling the upper right coelom. Most of the cells are healthy but there is a thin peripheral zone of necrosis. The coelomic mesothelium is largely intact laterally and dorsally but absent medially and ventrally.

(DC2: 3:3:4) x 100

Fig. 100b: Higher power view of upper part of Fig. 100a. Note: a mesothelial layer is identifiable over the dorsal wall, but is absent over the dorsal mesogastrium. The graft shows two types of healthy decidual cells: (i) with non-vacuolated dense cytoplasm. (ii) with clear vacuolated cytoplasm and angular, shrunken nuclei.

(DC2: 3:3:4) x 250


A

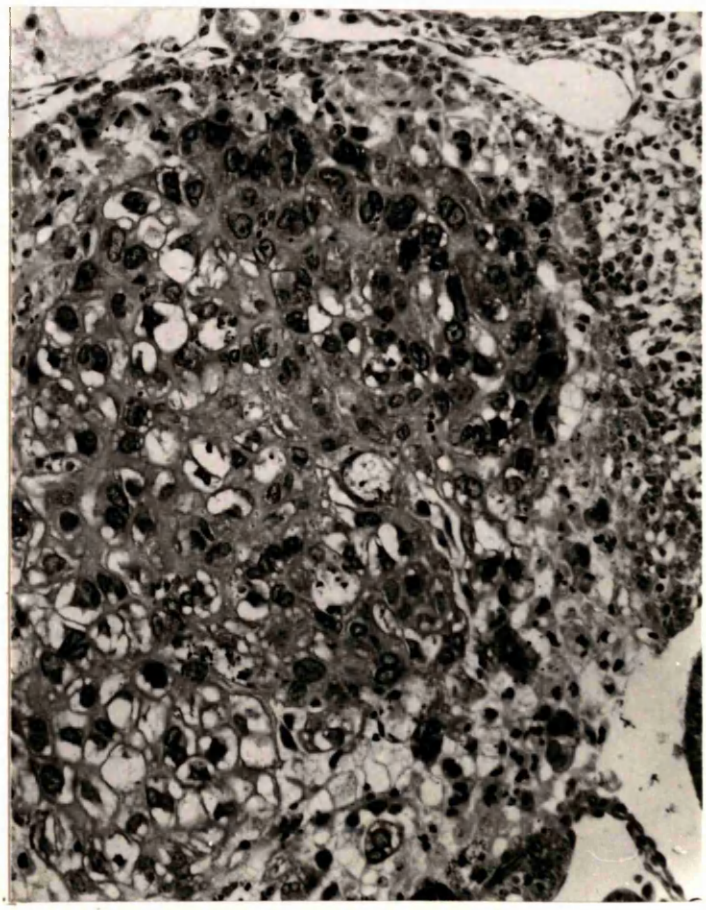


Fig 101: Higher power view of upper part of Fig. 100a showing the decidual graft in contact with the dorsal surface of the liver. Note: mesothelium over the liver is eroded (arrowheads), that over the lateral body wall is intact. Most of the surviving cells of this part of the graft are of the "glycogen-rich" variety.

(DC2: 3:3:4) x 280

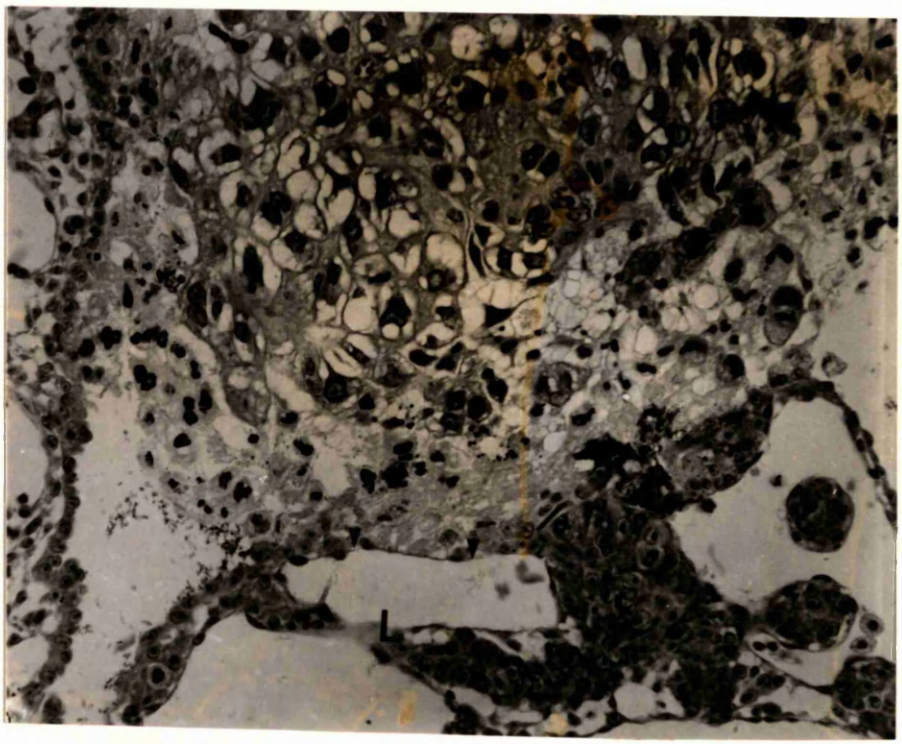
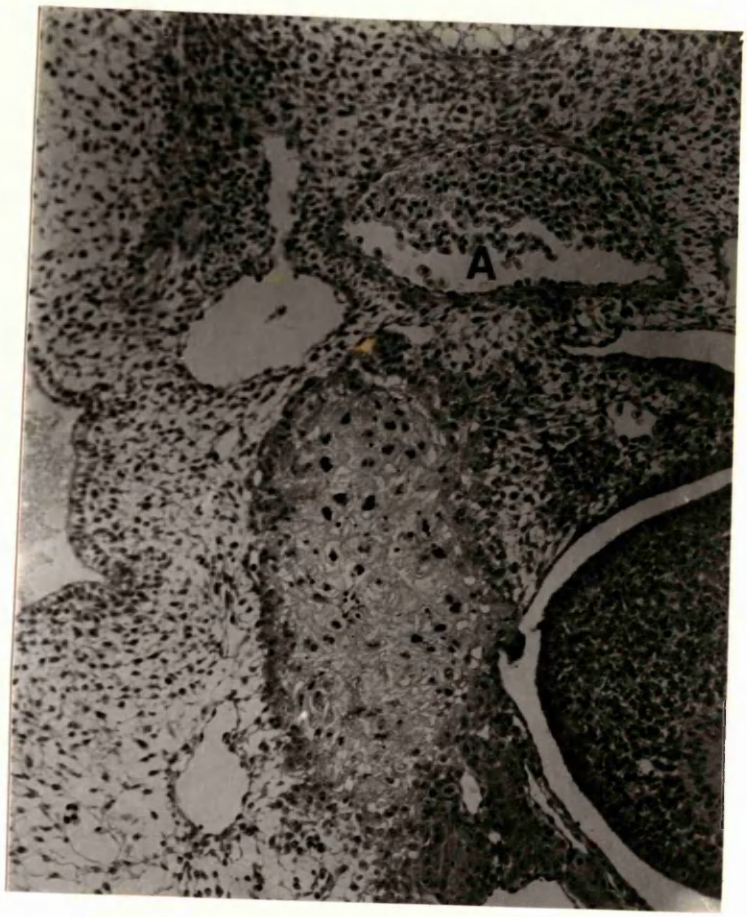


Fig. 102a: Shows decidual graft DC1, which occupied the upper right coelom. Most of its cells were necrotic. Only small areas of coelomic mesothelium survived. Most of the boundary between graft and host consisted of condensed host mesenchyme.

(DC1: 1/10:1:3:1) x 158

Fig. 102b: High power view of the upper part of Fig. 102a showing graft in contact with coelomic mesothelium over the dorsal mesogastrium. Note absence of coelomic mesothelium (arrowheads) over the area of contact.

(DC1: 1/10: 1:3:1) x 500


A

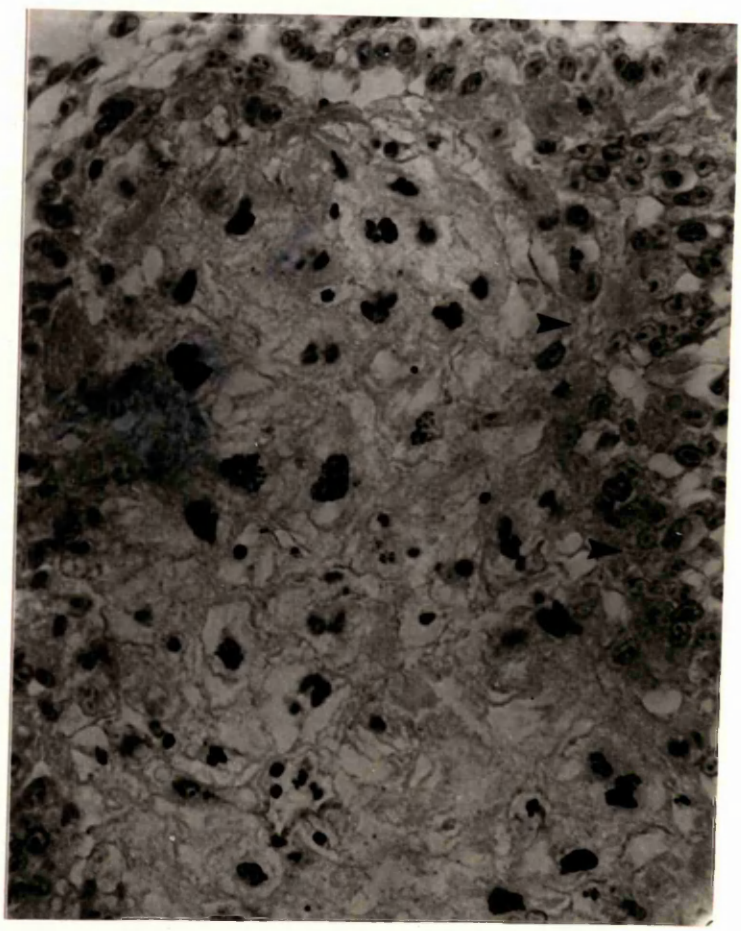
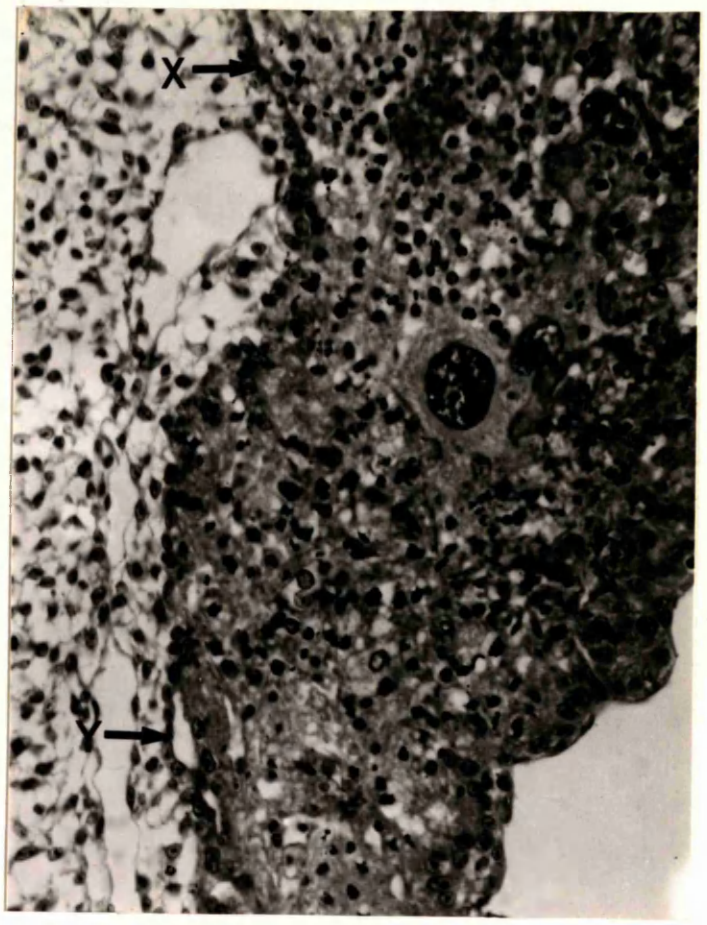


Fig. 103a: Shows part of decidual graft, DC5. Most of the decidual cells are necrotic. Note two healthy giant cells. The coelomic mesothelium survives at X and Y. Elsewhere on the lateral body wall, and over the liver, it is absent.

(DC5: 1/10: 1:4:2) x 400

Fig. 103b: Shows part of decidual graft, DC5. Decidual cells are all necrotic. Much fibrinoid material and many polymorphonuclear leucocytes. One small area of normal mesothelium remains (arrow). Elsewhere it is replaced by a rather disorganized layer of condensed mesenchyme.

(DC5: 1/10:1:4:2) x 400


X
Y

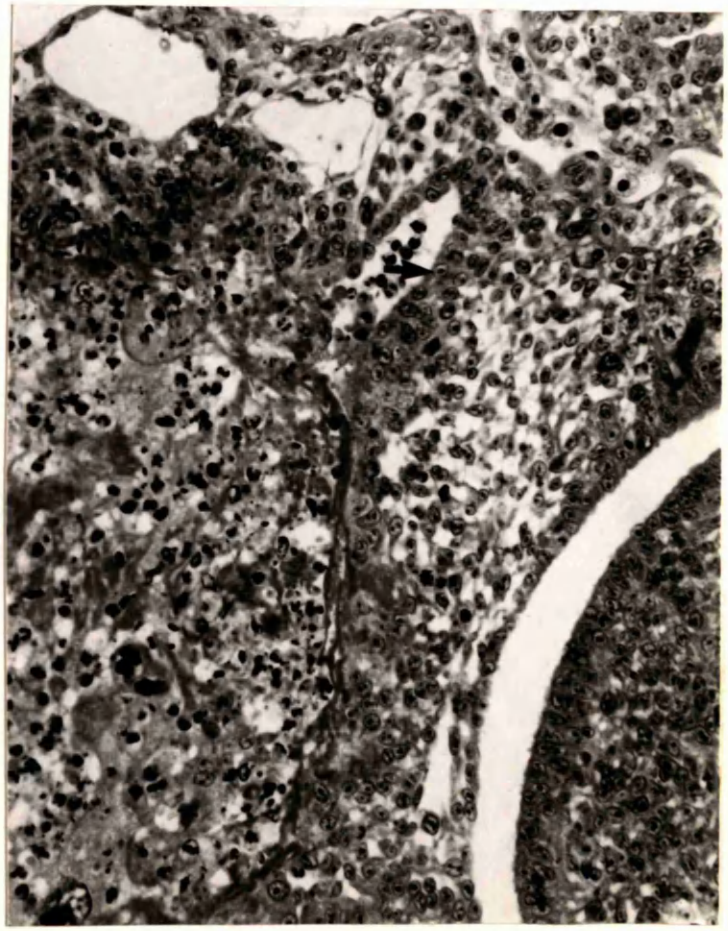
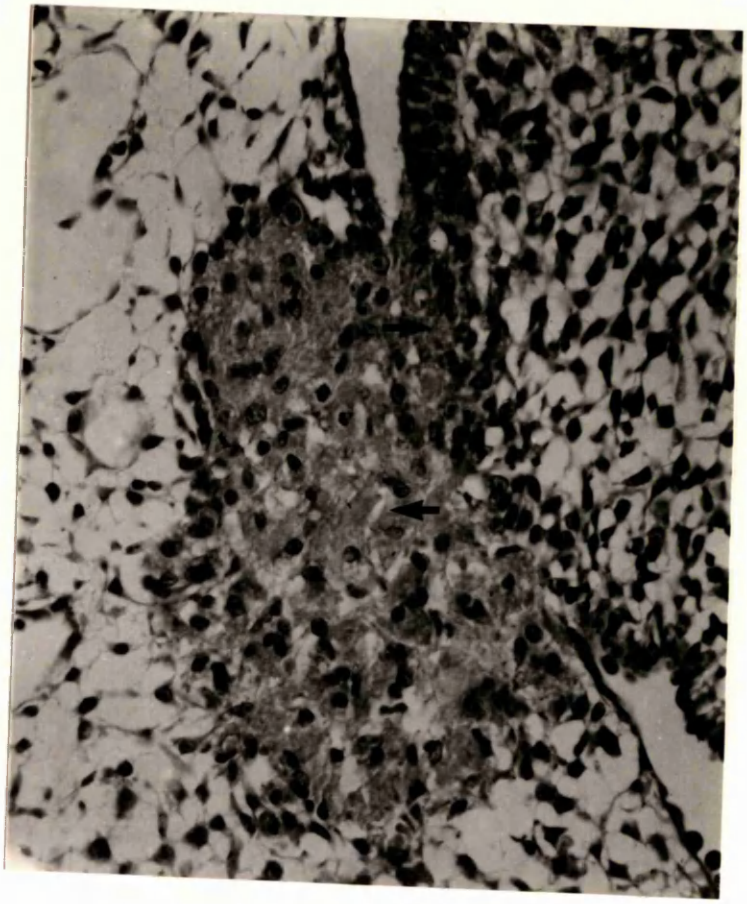

X

Fig. 104: Photomicrograph showing the rostral end of DC7, embedded within the mesenchyme of the lateral body wall. Notice absence of mesothelium on lateral body wall and over the right lung bud, in areas of contact with the graft.

(DC7: 1/10: 1:4:3) x 400

Fig. 105: Caudal end of DC7 shows central core of healthy decidual cells having weakly stained nuclei, with pale and vacuolated cytoplasm, surrounded by a narrow zone of necrotic cells (arrowhead). Note absence of coelomic mesothelium over the lateral body wall, dorsal coelomic bay, dorsal mesogastrium and the dorsal surface of the liver.

(DC7: 1/10:1:4:3) x 125



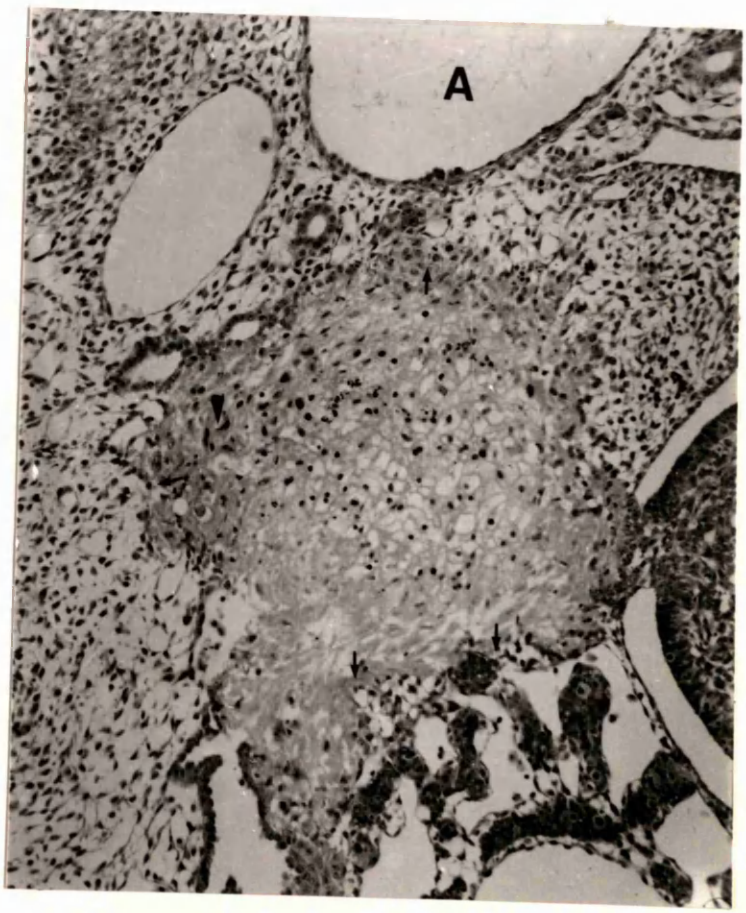
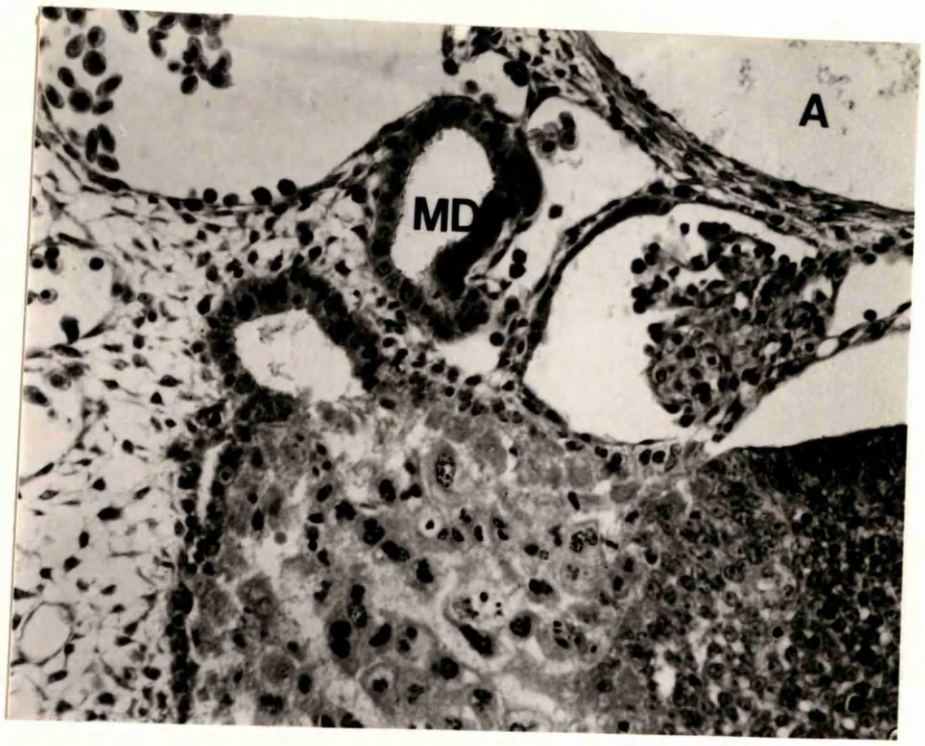

A

Fig. 106: Photomicrograph of DC8 in the upper right coelom. Note: (i) karyorrhexis in some of the nuclei in the dorsal part of the graft. (ii) mesothelium of lateral body wall disorganized; that over dorsal wall atrophic and discontinuous; that over dorsal mesogastrium apparently replaced by thick layer of condensed mesenchyme. The only normal mesothelium is that lining the dorso-lateral remnant of the coelom.

(DC8: 1/10:1:3:4) x 320

Fig. 107: Photomicrograph showing DC8 as a sharply circumscribed ball of mainly healthy decidual cells adjacent to thickened dorsal mesothelium. Note spindle-shaped cells at perimeter of graft, similar to non-decidualized stromal cells at edge of antimesometrial decidua. (Compare with Fig. 86).

(DC8: 1/10:1:3:10) x 400


A
MD

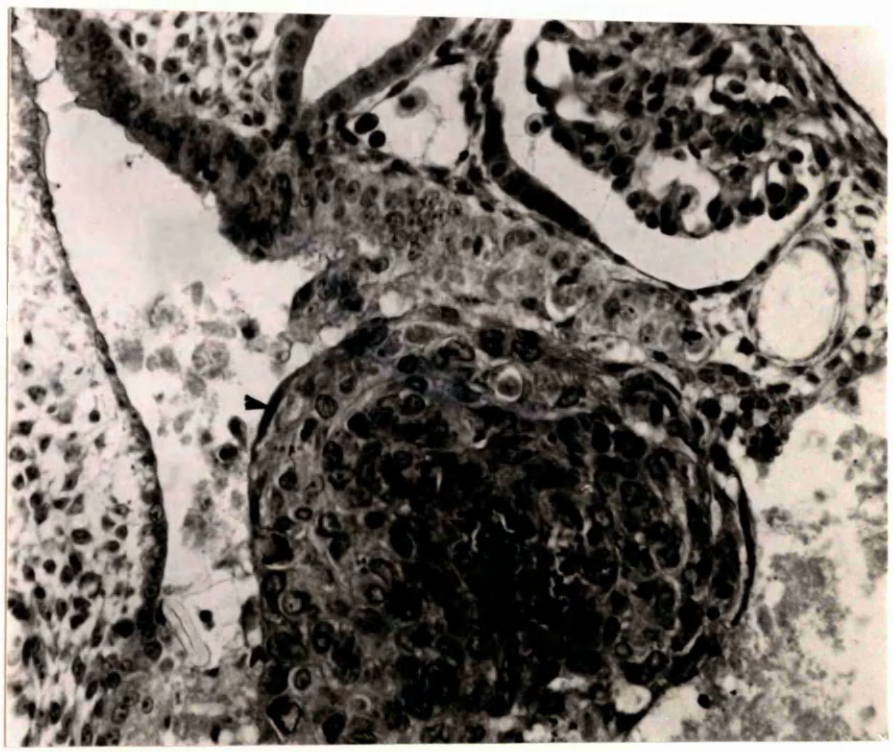
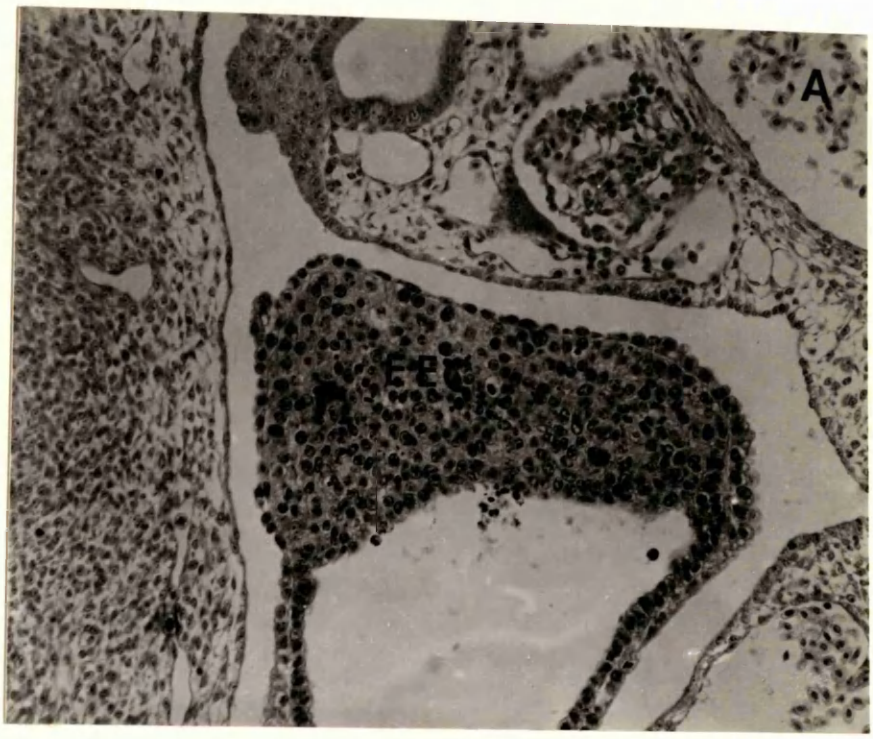


Fig. 108: Photomicrograph showing the abembryonic pole of an egg cylinder graft without the EPC. The compact mass of extraembryonic ectoderm (EEC) is covered by a thin layer of extraembryonic endoderm.

(HM12: 9:1:4) x 400

Fig. 109: High power view of the mass of extra-embryonic ectoderm at the embryonic pole of an egg cylinder graft without EPC. Note: small area of giant cell transformation (arrowheads) at the dorsolateral angle of the graft. The giant cells were covered by a thin layer of endoderm and did not make contact with the coelomic mesothelium.

(HM15: 1:4:9) x 500


A

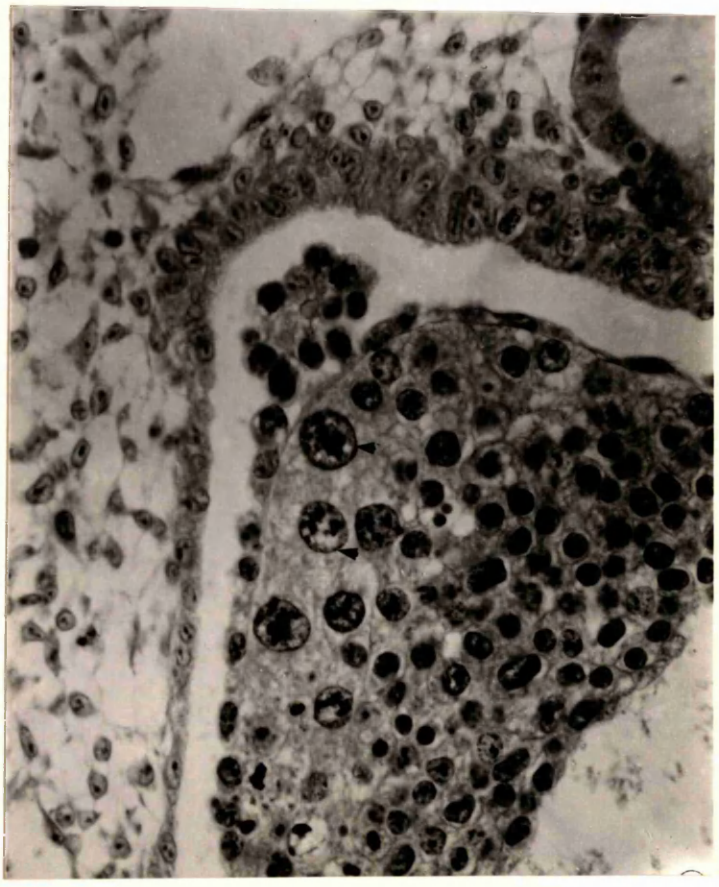
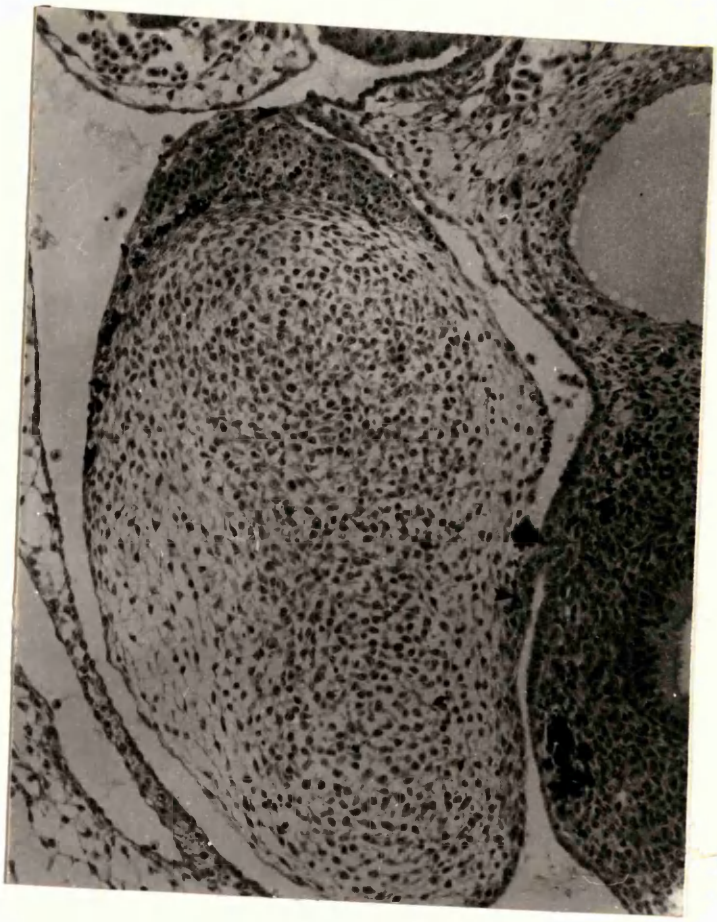


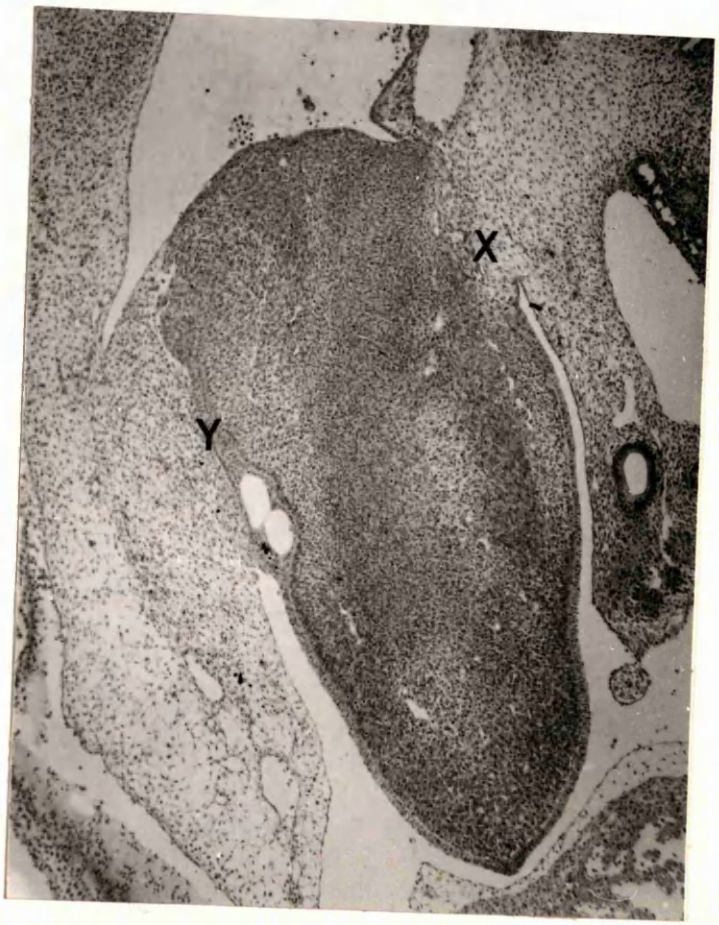
Fig. 110: Shows graft of 5.5d chick limb bud (L.G.C.1) in contact with the host. Note minimal erosion of coelomic mesothelium (arrowheads) over points of contact and healthy epidermal and mesenchymal cells of the graft.

(L.G.C.1: 3:2:10) x 250

Fig. 111: Shows a limb bud graft after 72 hours in the coelom. At X, mesothelium and graft epidermis are missing, allowing contact between the two mesenchymes. At Y, epidermis is in contact with host mesenchyme. There was no evidence of invasion of host by graft cells, no vascular damage and no bleeding.

(L.G.C.2: 4:1:4) x 100




X
Y