

The behaviour of embryonic chick and quail tissues in culture

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SUMMARY

Pieces of tissue were dissected from early chick and quail embryos (Stages XIII and XIV of Eyal-Giladi & Kochav, 1976; and stages 3–5 of Hamburger & Hamilton, 1951).

These tissues were taken from three different regions of the early embryos, and from eight different regions of the older ones, and were derived mainly from the lower layer. Epiblast tissues were also used. The experiments were designed to test the ability of one tissue to penetrate another.

A single tissue was grown in culture in a Falcon dish for 18–24 h until it had formed a coherent sheet of cells (Explant I). A second tissue was then combined with it in one of two ways:

(a) A small piece of tissue (Explant II) was explanted on top of Explant I. In most cases Explant II penetrated through Explant I and spread on the Falcon dish.

(b) Another small piece of tissue (Explant III) was explanted beside (in confrontation with) Explant I. Usually, Explant III penetrated into Explant I rather than *vice versa*.

The results were analysed to see if there were any variations in behaviour of the different tissues. The main result was that important differences were found to exist between certain types of chick and quail cells when grown in culture; the implications of this finding for the widely used technique of xenoplastic grafting are mentioned.

Another result was that Explant I was more likely to be penetrated when the second tissue was placed on top of it (Explant II) than when it was confronted with it (Explant III). The significance of these results is discussed.

INTRODUCTION

During gastrulation in the chick embryo, cells derived for the initial dorsal layer, the epiblast, pass ventrally through the primitive streak and give rise to the definitive endoblast and to the mesoderm (Modak, 1966; Nicolet, 1965, 1970; Rosenquist, 1966, 1972; Vakaet, 1970; Fontaine & Le Douarin, 1977). The original lower layer is a continuous sheet of cells, the central region being called the *hypoblast*, whilst the peripheral region is called the *germ wall*, or area opaca endoderm. The *definitive* endoblast appears to insert into the

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hypoblast whilst the mesoderm remains in the space between the epiblast and the lower layer. Subsequently, the germ wall forms the yolk sac, the hypoblast forms the yolk-sac stalk, and the definitive endoblast forms embryonic endoderm.

The interactions between the hypoblast and definitive endoblast were recently studied by Sanders, Bellairs & Portch (1978), utilizing *in vitro* and time-lapse cinematographic techniques. The major finding was that when a hypoblast explant was grown side by side (confronted) with a definitive endoblast explant, the hypoblast cells became displaced by the definitive endoblast cells. The hypoblast explant tended to fragment into smaller groups of cells many of which migrated around the definitive endoblast, so that the final morphology resembled the situation in the embryo. It was concluded that the readiness with which the hypoblast cells separated from one another might play an important role in the penetration of hypoblast by definitive endoblast, both *in vivo* and *in vitro*.

The purpose of the present investigation is three-fold: First, to bring the two issues into contact in a manner which more closely resembles what appears to be their relationship in the embryo. Thus the definitive endoblast has now been explanted *on top* of an established sheet of the hypoblast to determine whether it can penetrate or insert into it from above as it does *in vivo*. As a control, an additional piece of definitive endoblast has been explanted simultaneously to the side of the hypoblast explant and confronted with it. Second, to extend these experiments to other types of tissue in order to determine what factors promote penetration of one tissue by another under *in vitro* conditions. In future experiments we plan to use malignant cells to investigate their invasive capacity in the same experimental system. Third, we have used heterologous combinations of chick and quail hypoblast and definitive endoblast, in order to investigate the possible differences in the behaviour of tissues from the two organisms. These results indicate that tissues from quail do not always behave in the same way as corresponding chick tissues.

MATERIALS AND METHODS

Hens' eggs (Ross Rangers) were obtained from Ross Poultry (South), U.K. and quail (*Coturnix coturnix japonica*) eggs from Houghton Poultry Research Station, U.K.

The tissues used were taken from two stages of development, an early stage (XIII–XIV of Eyal-Giladi & Kochav, 1976, which is about 6–10 h of incubation) and an older stage (3–5 of Hamburger & Hamilton, 1951, which is about 18–24 h of incubation). The normal tables cited here refer to chick development but have also been used by us as a guide to quail stages. The areas from which the tissues have been taken are shown in Fig. 1. They are as follows:

Early stage (two-layered embryo)

Ventral layer, (a) *hypoblast*, (b) germ wall (area opaca endoderm).

Dorsal layer, epiblast.

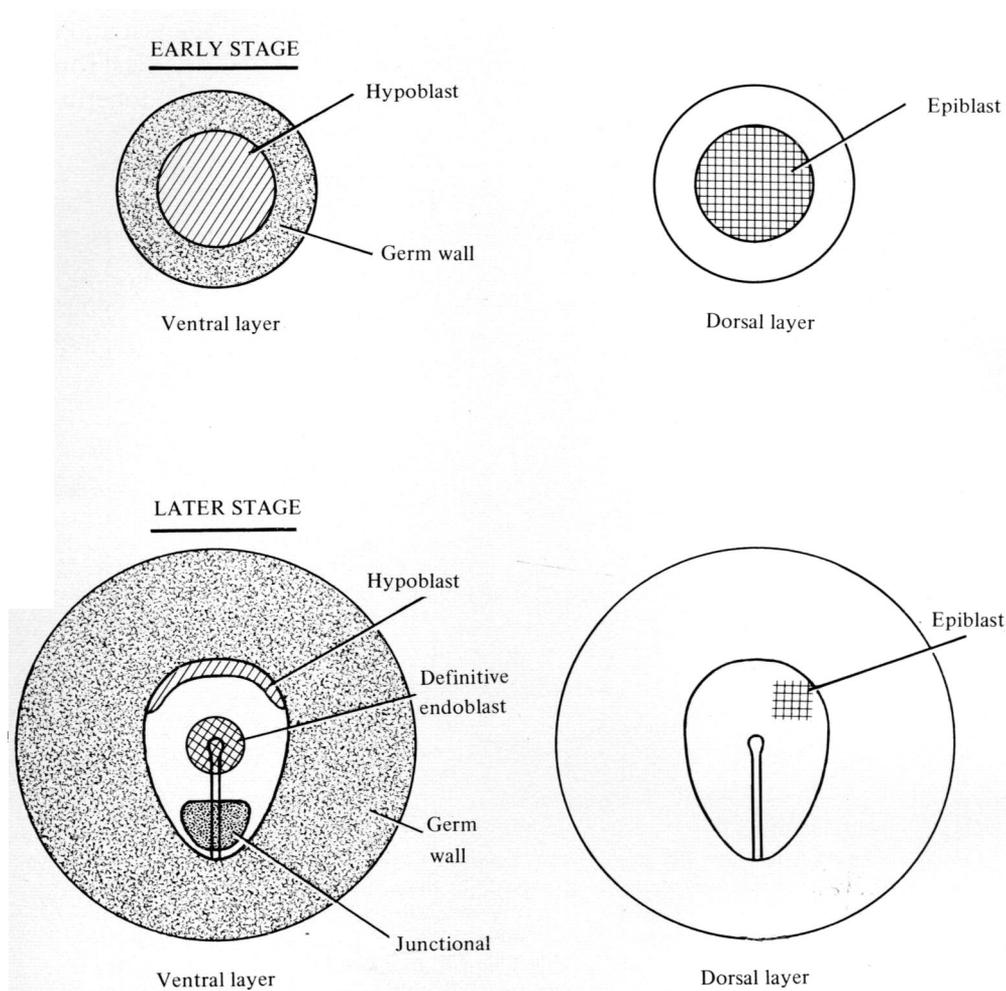


Fig. 1. Diagram to show the regions of the embryo from which tissues were dissected for explantation.

Later stage (three-layered embryo)

Ventral layer, (a) late hypoblast, (b) definitive endoblast, (c) junctional endoblast, (d) germ wall (area opaca endoderm).

Dorsal layer, epiblast.

Much of the above terminology is discussed by Vakaet (1970) & Sanders *et al.* (1978). The designation 'late hypoblast' has been given to the anterior crescent of tissue in the area pellucida of stage-4 to -5 embryos. It is readily distinguished by its foamy appearance and its ease of dissection.

Using these tissues in various combinations, nearly 3000 experiments were carried out over several months to test the ability of one tissue to penetrate an already established sheet of another tissue. In all these combination experi-

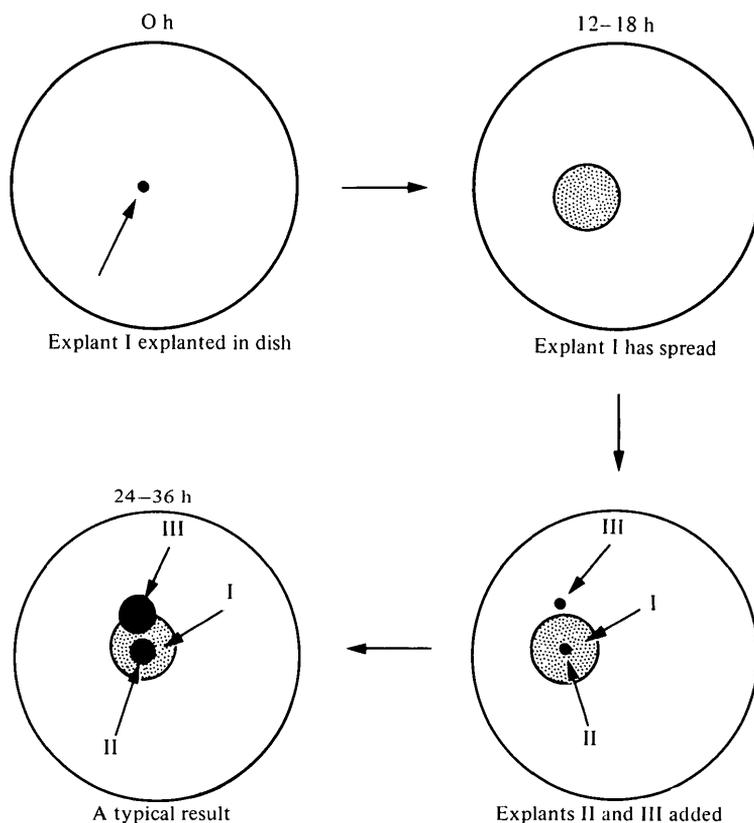


Fig. 2. Diagram to show the relationship between Explant I and Explants II and III in a typical experiment. Explant I is grown on the base of a plastic dish, or occasionally on a glass coverslip, in a drop of culture medium (culture medium not shown). After about 12-18 h, the explant has settled and spread. Explant II is added on top of Explant I. Explant III is placed at the side of Explant I. After a further period of incubation, Explant II and III have penetrated into Explant I. In this example, Explant III is shown as having spread more than Explant II, but this does not always occur.

ments two explants were involved and, apart from those shown in Table 1, they were of homologous tissues. In the first group of experiments the original explant was allowed to spread as a sheet before an additional explant was placed on top of it. In this case the first explant is designated as *Explant I*, whilst the one that is placed on top of it is called *Explant II*.

In the second group of experiments, the original explant is again designated *Explant I*, whilst the additional explant, which is placed as a 'confront' alongside it, is called *Explant III* (see Fig. 2).

In many cases, both Explant II and Explant III were added simultaneously to a single Explant I. In addition, samples of Explant I were grown alone, no further explants being added to them, so that their morphological characteristics could be examined after either one or two days in culture.

The embryos were removed from the yolk and vitelline membrane in Tyrode's solution (maintained at pH 7.0–7.5, with bicarbonate buffer). The pieces of tissue were then dissected with finely ground steel knives or sharpened tungsten needles without the aid of any enzyme treatment. In preparing Explant I, pieces of tissue were explanted directly onto Falcon dishes (Div. Becton Dickinson & Co., U.S.A.) in a drop of culture medium which was made up of 9 ml Earle's 199: 1 ml Foetal Calf Serum (Gibco) and 0.5 ml of stock solution of penicillin and streptomycin (Stock solution: penicillin 5000 units/ml; streptomycin 5000 mcg/ml) filtered through a 0.22 μm millipore filter. Cultures were normally maintained at a high humidity in a CO₂-gassed incubator at 37.5 °C for 12–18 h so that they had attached to the dish and spread out before a second tissue was added. Two pieces of freshly dissected tissue, Explants II and III, which were of similar size to one another were placed in the sitting drop close to Explant I, one being placed on top (Explant II), the other to the side (Explant III). Explants II and III were always smaller than Explant I (see Fig. 2). Dishes containing both chick and quail cultures were then incubated for a further 24 h, after which they were examined, fixed in buffered formal saline for 24 h and subsequently stained with Harris' haematoxylin. They were then mounted in a water mountant ('Aquamount'), using 13 mm diameter coverslips. The dishes were cut down before examination in the light microscope. It was then usually possible on morphological grounds to decide whether one tissue had penetrated the other even in the homologous combinations. A series of control experiments was carried out:

(A) *Controls for age differences of explants* (for rationale see p. 27).

In 71 experiments, Explant II and III were not taken directly from the embryo, but a piece of tissue was first of all grown in culture in exactly the same way as with Explant I. This tissue was then dissected from the culture dish after a period of time and cut into two pieces, one being used as an Explant II on top of a normal Explant I, and the other being placed as a confront (Explant III) to the same Explant I.

(B) *Controls for the role of substrate-attached material*

Sheets of chick cells (usually hypoblast) were cultivated on Falcon plastic Petri dishes for 1 day (36 experiments) or 2 days (34 experiments). These sheets were then removed with tungsten needles and the area on which they had been growing was marked. One piece of freshly dissected early or late chick tissue was then explanted over this area in the same drop of culture medium or in a separate one. The tissue chosen was chick epiblast because it displayed differences in behaviour when used as Explant II and Explant III.

(C) *Size and distance in confront experiments*

It seemed possible that the size of Explant III and its distance from Explant I might be important factors in determining the results. We have therefore taken care with Explants II and III to try and make them of uniform size (although smaller than Explant I), and we have tried always to place Explant III at a standard distance from Explant I.

Time-lapse filming was carried out on some of the cultures using a Nikon model M inverted microscope equipped with dark-field and phase-contrast optics and a 37°C incubator. The Bolex 16 mm cinecamera was driven by a Nikon CFMA time-lapse controller, and was loaded with either Kodak Plus X negative film 7231 or Kodak technical pan film SO115. For filming, the cultures were grown on glass coverslips which were subsequently mounted into filming chambers. It was established that those cultures which were filmed behaved in a similar way on a glass substrate as on a plastic one.

RESULTS

All the explanted tissues exhibited epithelial characteristics in culture, and typically each formed a coherent sheet in which all but the marginal cells were completely surrounded by neighbours. Gaps, which were more or less transient, appeared between cells in the sheet, but time-lapse observation showed that they were of relatively short duration. By about 18 h of culture, the explant had usually spread and formed a monolayer although a remnant of the original tissue sometimes remained as an island in the centre of the explant. This island had usually disappeared by the end of the first day in culture.

The early hypoblast and early germ-wall endoderm in particular, appeared to attach and spread on the substratum rapidly (4–6 h) forming a sheet in which no regionalization was apparent. The other tissues however often showed a clear regionalization after one or two days in culture, which took the form of multilayering or of a vacuolation of certain cells. This multilayering was similar to that shown previously in cultured embryonic ectoderm (Bellairs, Sanders & Portch, 1978). Aligned bands of cells, and swirling patches of cells within the monolayer, were other forms of patterning observed (Fig. 4).

(I) *Major interactions when a second tissue was placed on top of the original explant* (i.e. Explant II on top of Explant I)

Explant II usually penetrated through Explant I and spread on the culture dish (see examples in Figs. 3, 5 and 6) but there were species and tissue variations and these will be discussed below.

(A) *Failure to penetrate.* First, however, let us consider briefly what happened to Explant II in those experiments where it failed to penetrate. There were two main possibilities:

(i) Explant II attached to the upper surface of Explant I but failed to insert into it and remained as a ball of unspread cells (see Fig. 7). It is possible that if the period of incubation had been extended, some of these balls would have inserted themselves into Explant I or penetrated through it.

(ii) Explant II failed to attach to Explant I and was usually found as a ball of cells floating in the culture medium. Failure to attach was more common with epiblast than with other tissues.

(B) *Successful penetration.* We have already seen that in the majority of the experiments, Explant II penetrated through Explant I. In these cultures, Explant II flattened over the plastic culture dish, displacing the cells of Explant I (Figs 3 and 5).

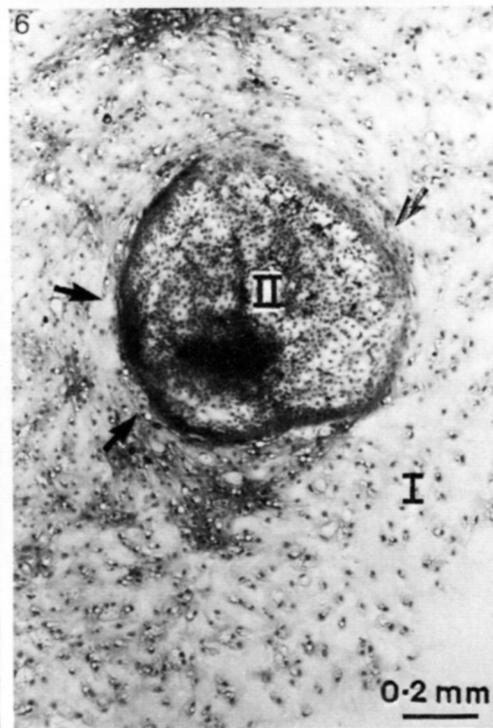
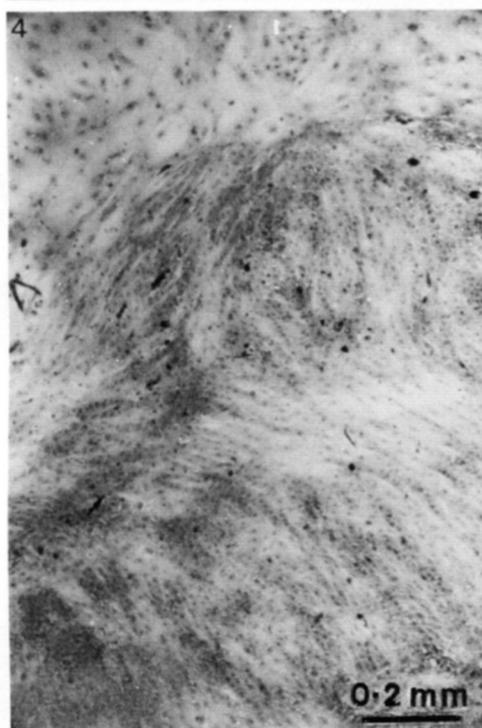
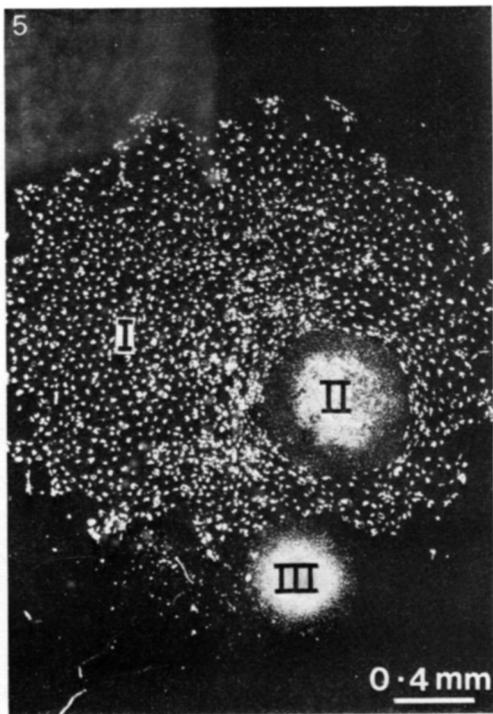
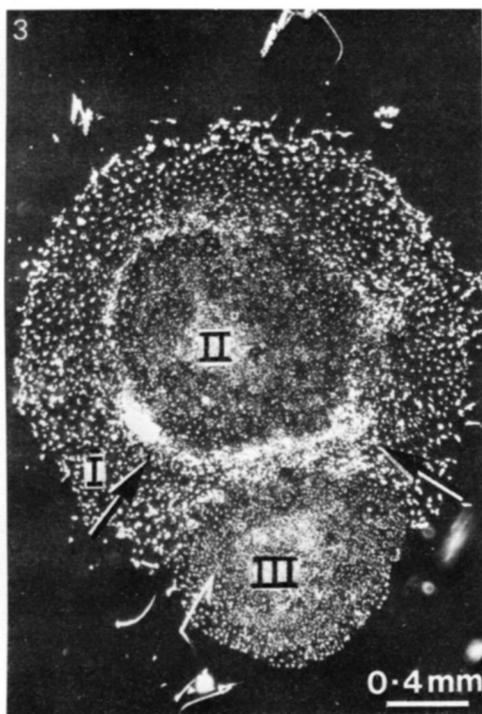
(i) *Species differences*

Table 1 summarizes the results of combining certain chick and quail tissues in both homologous and heterologous associations. In each case, Explant I was composed of hypoblast, either early or late, whereas Explant II consisted of definitive endoblast. The main finding is that Explant II had a much greater chance of penetrating Explant I when the latter was composed of chick rather than of quail tissues. This conclusion is supported by a statistical analysis using 2 – by – 2 contingency χ^2 tests and a 5 % level of significance (comparing ‘penetrated’ with ‘not penetrated’ plus ‘floating’).

Thus if we compare any two sets of results in the table, no significant differences will be found unless Explant I consists of chick tissue in one set and of quail in the other. It is concluded therefore that sheets of chick hypoblast grown *in vitro* are different from sheets of quail hypoblast grown under comparable culture conditions. Examination of Tables 2 and 3 shows that the differences may not be restricted to the combinations shown in Table 1, but may also apply to other early embryonic tissues.

(ii) *Tissue differences*

Tables 2 and 3 show the results of a wide range of homologous tissue combinations. Let us first compare the fate of Explant II (Table 2). All types of tissue appeared to be able to penetrate Explant I but their success in doing so varied slightly. Successful penetration occurred more frequently when chick tissues only were used, than when quail tissues only were used. This is so whether we compare a specific chick tissue with its quail counterpart (as in Table 2) or whether we compare the means. Thus the mean penetration rate in the chick was 95 % \pm 4 whilst in the quail, it was 56 % \pm 16. If we consider the results in relation to Explant I (Table 3) it appears that once again, penetration is more likely to occur when both Explant I and Explant II are composed of chick tissues than when they are both composed of quail tissues.



(II) *Major interactions when a second homologous tissue was placed in confrontation with the original explant (i.e. Explant III confronted Explant I)*

The main question to consider is 'Did Explant III become invaded by Explant I, or *vice versa*?' The answers are contained in Tables 4 and 5. The data shown here does not include those experiments where the two tissues had not been in contact long enough to establish a clear relationship. Three main categories of results are shown, and these are illustrated diagrammatically in Fig. 12 (examples are shown in Figs 3 and 8).

Table 4 shows that the chance of Explant III invading (i.e. penetrating into) Explant I was much greater than that of Explant I penetrating into Explant III, whatever the type of tissue used for Explant III.

Indeed, unless Explant I was composed of early hypoblast, it was unlikely to surround the other tissues. In some cases, neither explant surrounded the other. In other cases however, especially where the explants consisted of either early hypoblast or germ-wall endoderm, cells from the two tissues tended to mingle and form a region of mixed population. In combinations where the explants were composed of more cohesive tissues, such as definitive endoblast, a region of aligned cells separated the two explants. This corresponds to the 'barrier' region described by Sanders *et al.* (1978), and is probably an example of the contact inhibition of locomotion of Abercrombie & Heaysman (1954).

Table 5 shows that the chance of Explant III penetrating into Explant I was much the same, whatever the type of tissue used for Explant I.

(III) *Local interactions between Explant I and Explants II and III*

A number of different situations have been encountered and these will be described separately. However, it should be noted that these were not always distinct, and that two or more might be present in the region where the two explants met.

FIGURES 3-6

Fig. 3. Dark ground photomicrograph of living culture. I = Explant I composed of chick definitive endoblast (stage 4), whilst II and III = Explants II and III respectively, composed of quail definitive endoblast (stage 4). Note that Explant II has penetrated through Explant I and spread on the substrate. Explant I has rolled back at the edge of the hole (arrows). Explant III has penetrated into Explant I. $\times 25$.

Fig. 4. Fixed and stained preparation to show swirling patterns of aligned cells (chick late hypoblast, stage 3). $\times 59$.

Fig. 5. Dark-ground photomicrograph of a living culture. I = Explant I composed of chick hypoblast (stage 3) whilst II and III = Explant II and III respectively, each composed of quail epiblast (stage XIV). $\times 25$.

Fig. 6. Fixed and stained preparation to show the region where Explant II (chick definitive endoblast, stage 4) has penetrated Explant I (quail germ wall, stage 5) and spread. Note the alignment of cells at the boundary between the two tissues (arrows). $\times 46$.

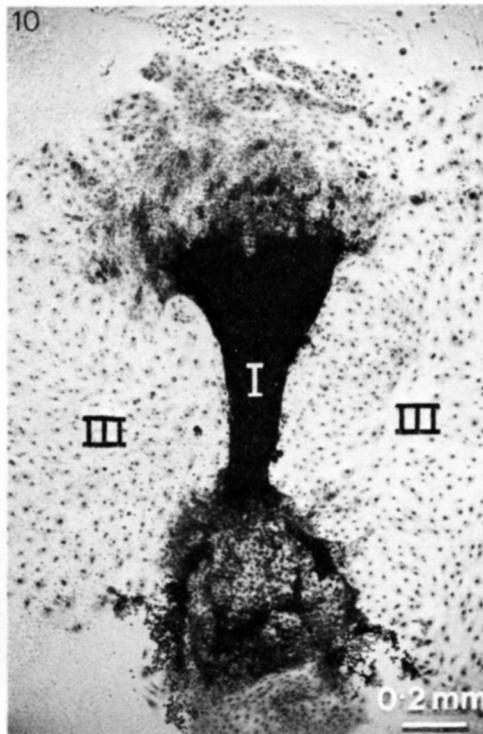
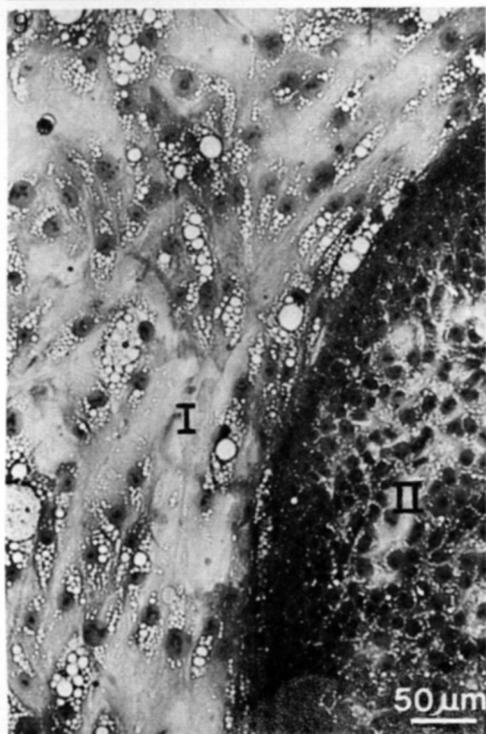
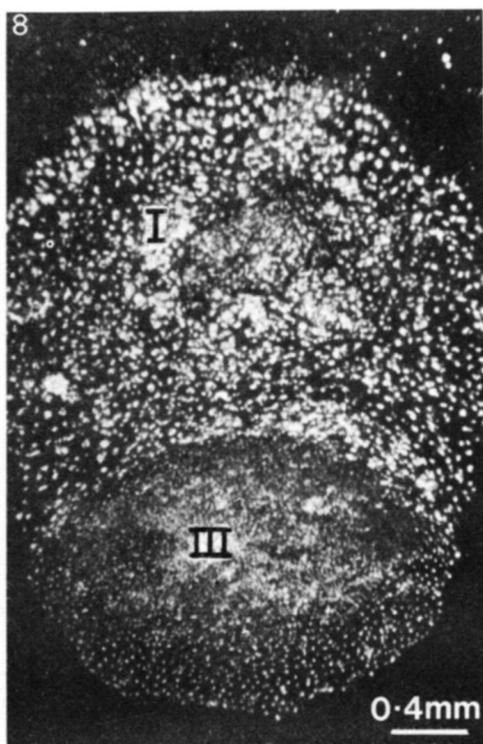
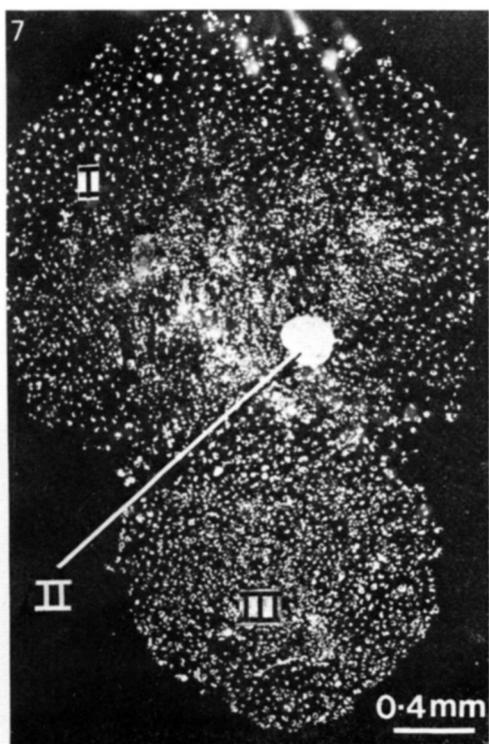


Table 1. Results of experiments using hypoblast and endoblast from chick and quail

Explant II	Explant I		Explant II penetrated Explant I (%)*	Explant II attached to surface of Explant I (%)	Explant II not attached to Explant I (%)	Total number of specimens
Chick endoblast	Chick	Early hypoblast	81	9	9	43
		Late hypoblast	93	0	7	14
Quail endoblast	Chick	Early hypoblast	75	5	20	20
		Late hypoblast	86	14	0	14
Chick endoblast	Quail	Early hypoblast	37	26	37	19
		Late hypoblast	32	45	23	22
Quail endoblast	Quail	Early hypoblast	22	44	33	18
		Late hypoblast	53	13	33	15

* In this condition Explant II penetrated and spread on the underlying substratum.

Table 1. Differences in the behaviour of analogous chick and quail tissues. The main finding is that quail explants are less likely to be invaded than chick ones.

The commonest situation was that in which the cells of Explant I became elongated and re-orientated so that their major axes lay parallel to one another (Figs 6 and 9). Occasionally, the cells at the edge of Explant II were also aligned and lay parallel to those of Explant I. In some cultures, the cells of Explant II had inserted themselves beneath those of Explant I and lay between them and the plastic culture dish (Fig. 13). This underlapping could be seen most clearly by examining the underside of the fixed and stained specimens with a $16\times$ objective. Underlapping was usually restricted to small regions at the edge of the inserted tissues and was often visible as short tongues of cells. They were particularly conspicuous where Explant II was derived from epiblast or definitive endoblast. Similar underlapping tongues were found when mesoderm was placed on top of definitive endoblast cultures (Sanders, 1980). In some cases the cells of Explants II and III mingled with those of Explant I. This was

FIGURES 7-10

Fig. 7. Dark-ground photomicrograph to show Explant II (quail hypoblast, stage XIV) which has attached but failed to spread and has remained a ball of cells on top of Explant I (quail hypoblast, stage 3). Explant III (quail hypoblast, stage XIV) however has spread on the substrate. $\times 25$.

Fig. 8. Dark-ground photomicrograph to show Explant III (quail hypoblast, stage 4) penetrating into Explant I (chick germ wall, stage XII). $\times 25$.

Fig. 9. Fixed and stained preparation to show the border between Explant I (quail germ wall, stage 5) and an inserted Explant II (chick epiblast, stage 4). Note the alignment of the cells of Explant I parallel to the border. $\times 175$.

Fig. 10. Fixed and stained preparation to show a 'bridge' arrangement formed by an Explant I composed of quail epiblast (stage XIV) and two pieces of Explant III (quail germ wall, stage XIV). $\times 36$.

Table 2. *Ability of Explant II to penetrate Explant I and spread on the substrate*
Types of Explant I tissues not shown.

Explant II (Types of tissue)	Both explants from chick		Both explants from quail	
	% penetrated	Total number of specimens = 100%	% penetrated	Total number of specimens = 100%
Early hypoblast	98	42	47	45
Early germ wall	100	31	80	41
Early epiblast	93	68	55	31
Late hypoblast	97	32	28	36
Late germ wall	100	22	60	5
Late epiblast	90	72	78	9
Junctional endoblast	94	33	60	16
Definitive endoblast	89	79	43	37

Tables 2 and 3. These tables show that penetration is more likely to occur when both Explant I and II are composed of chick tissues. Table 2 shows the variation in results obtained by using different tissues as Explant II, whilst Table 3 shows the variation in results obtained by using different tissues as Explant I. Both tables are compiled from the same series of experiments and are therefore complementary.

Table 3. *Effect of tissue type of Explant I on its penetration by Explant II*
Types of Explant II tissue not shown.

Explant I (Types of tissue)	Both explants from chick		Both explants from quail	
	% penetrated	Total number = 100%	% penetrated	Total number = 100%
Early hypoblast	88	86	42	64
Early germ wall	98	43	68	19
Late hypoblast	96	68	65	54
Late germ wall	91	79	59	32
Junctional endoblast	95	55	67	24
Definitive endoblast	98	48	26	27

more common in combinations containing hypoblast or germ wall endoderm and corresponded with the situation reported by Sanders *et al.* (1978).

Another morphological pattern occasionally noted was 'bridging', a feature seen principally with epiblast. A typical example is shown in Fig. 10. Here the epiblast (Explant I) has been rolled up into a bridge by two explants of germ wall (Explant III) which have pushed beneath it.

Penetration of Explant I by Explant II was followed by means of time-lapse phase-contrast microscopy. Examination of these films showed that it was clearly possible to distinguish the two explants. Settling of Explant II on the dorsal surface of the sheet of Explant I was followed in many cases by retraction of the latter allowing the penetration and spreading of Explant II. These steps

Table 4. Ability of Explant III to penetrate Explant I
Types of Explant I tissues not shown

Explant III (Types of tissue)	Both explants chick			Both explants quail		
	Explant III penetrates Explant I	Explant I penetrates Explant III	Total no. = 100%	Explant III penetrates Explant I	Explant I penetrates Explant III	Total no. = 100%
	(%)	(%)		(%)	(%)	
Early hypoblast	49	14	37	56	7	57
Early germ wall	59	0	39	71	3	35
Early epiblast	39	0	44	50	0	26
Late hypoblast	67	0	15	67	8	12
Late germ wall	70	7	27	100	0	3
Late epiblast	37	0	41	57	0	14
Junctional endoblast	80	0	30	100	0	6
Definitive endoblast	86	0	42	88	0	16

Tables 4 and 5. These tables show that in homologous combinations of both chick and quail tissues, Explant III (the confronting tissue) is more likely to penetrate Explant I than *vice versa*. (Percentages where no clear penetration could be distinguished are not shown). Table 4 shows the variation in results obtained by using different tissues as Explant I. Both tables are compiled from the same series of experiments and are therefore complementary.

Table 5. Effect of tissue type of Explant I on its penetration by Explant III
Types of Explant III tissue not shown.

Explant I (Types of tissue)	Chick			Quail		
	Explant III penetrates Explant I	Explant I penetrates Explant III	Total no. = 100%	Explant III penetrates Explant I	Explant I penetrates Explant III	Total no. = 100%
	(%)	(%)		(%)	(%)	
Early hypoblast	68	6	63	58	0	38
Early germ wall	49	0	37	67	8	12
Late hypoblast	62	0	50	67	5	60
Late germ wall	52	0	48	69	6	32
Junctional endoblast	64	0	36	67	0	15
Definitive endoblast	54	7	41	58	8	12

are illustrated by Fig. 11, which shows the penetration of chick endoblast by chick mesoderm, taken from a time-lapse film. The endoblast sheet is seen to be displaced by the spreading of Explant II, and the different tissues remain distinct.

(IV) Control experiments

(A) Age differences between Explant I and Explant II and III. Most of the tissues used as Explant I had been grown in culture for 12–18 h before Explant II and/or III were added to them. Usually therefore they had become firmly attached to the plastic dish; indeed if they had not done so they were discarded.

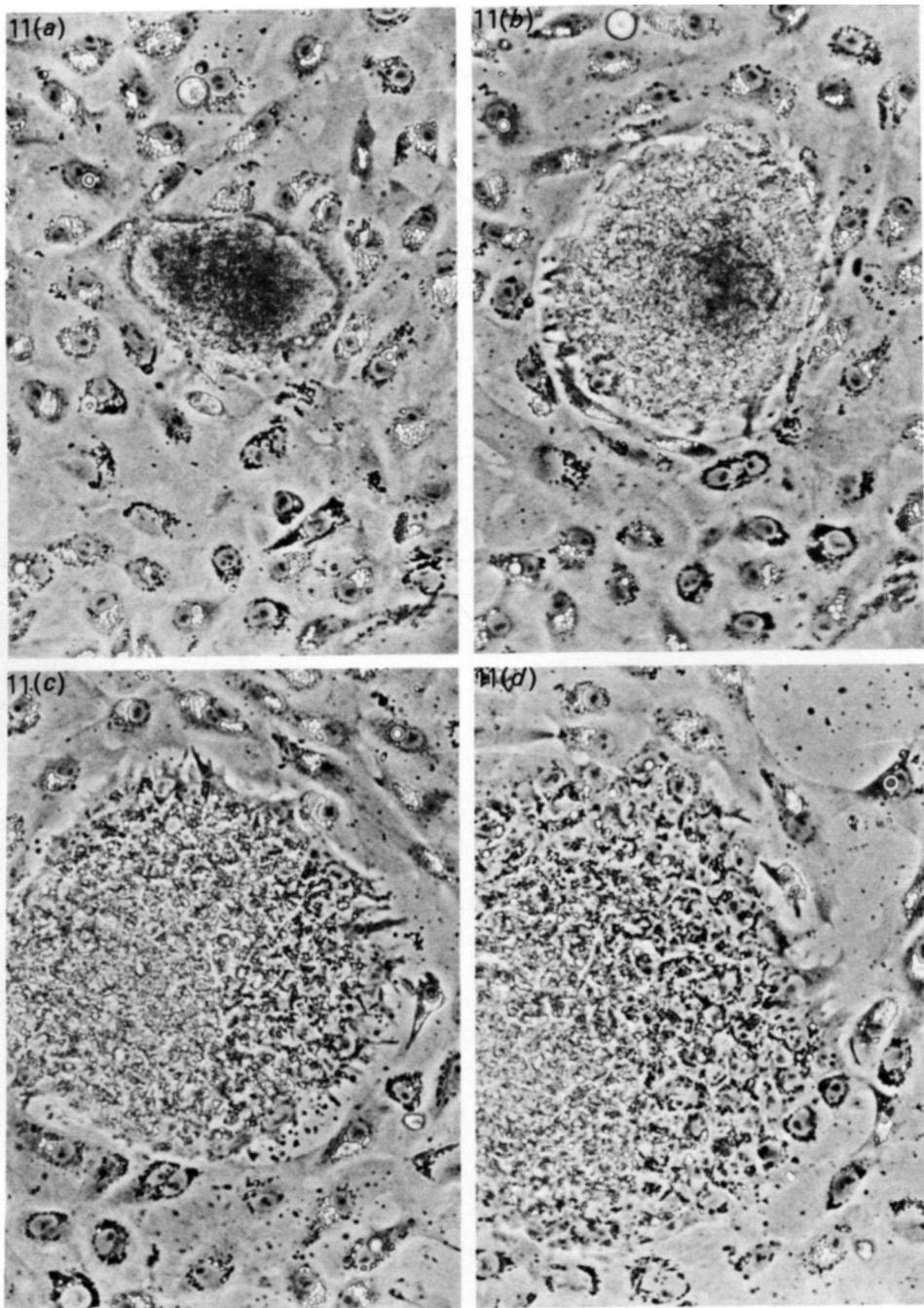


Fig. 11. Frames from a time-lapse film showing the stages of penetration of Explant I by explant II. (a) 0 min, (b) 56 min, (c) 157 min, (d) 260 min. $\times 195$.

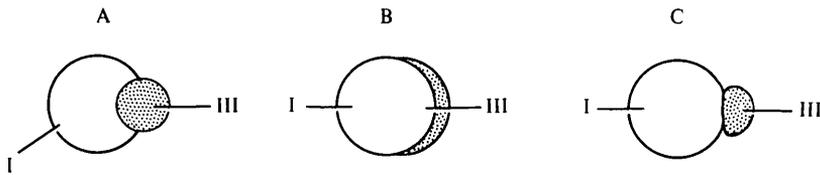


Fig. 12. Diagram to illustrate the possible relationships between Explants I and III. (A) Explant III has penetrated into Explant I. (B) Explant I has penetrated into Explant III. (C) Neither explant has penetrated the other.

The cells of Explant II and III however were normally taken directly from the embryo and were thus chronologically younger. The possibility that the results we obtained might be affected by the difference in ages was tested in a series of 71 control experiments (see Materials and Methods). In 40 of these Explants II and III were grown in culture for the same length of time as Explant I (usually 18 h) before being retransplanted and combined with Explant I. The results obtained from these control experiments were comparable with those found in the main experiment. In the remaining 31 experiments they were maintained in culture for two days before being retransplanted and were therefore one day *older* than Explant I. The settling and spreading of these older tissues was slightly less than of freshly dissected tissue.

In a converse series of experiments, no difference was found when Explant I had been in culture for a reduced length of time prior to the addition of Explant II. Thus, when Explant I consisted of chick hypoblast which had been in culture for only 4 h, before Explant II (chick definitive endoblast) was added to it, the results could not be distinguished from those obtained when Explant I had been in culture for the usual period of 12–18 h.

(B) *Possible role of substrate-attached material.* After Explant II tissues had penetrated Explant I, they migrated over a region of culture dish which had previously been in contact with Explant I. It seemed possible therefore that their behaviour might be affected by extracellular material laid down by Explant I. No difference in behaviour could be found however between chick epiblast tissues explanted onto substrate which had previously supported another explant, and onto substrate which had never supported another explant.

DISCUSSION

Invasion is assessed as the ability of a tissue to penetrate through Explant I when placed on top of it, or to displace Explant I when explanted in confrontation with it. We shall discuss the results under two headings:

(a) *Species differences*

Important differences have been shown to exist between the behaviour of certain types of chick and quail cells when grown in culture.

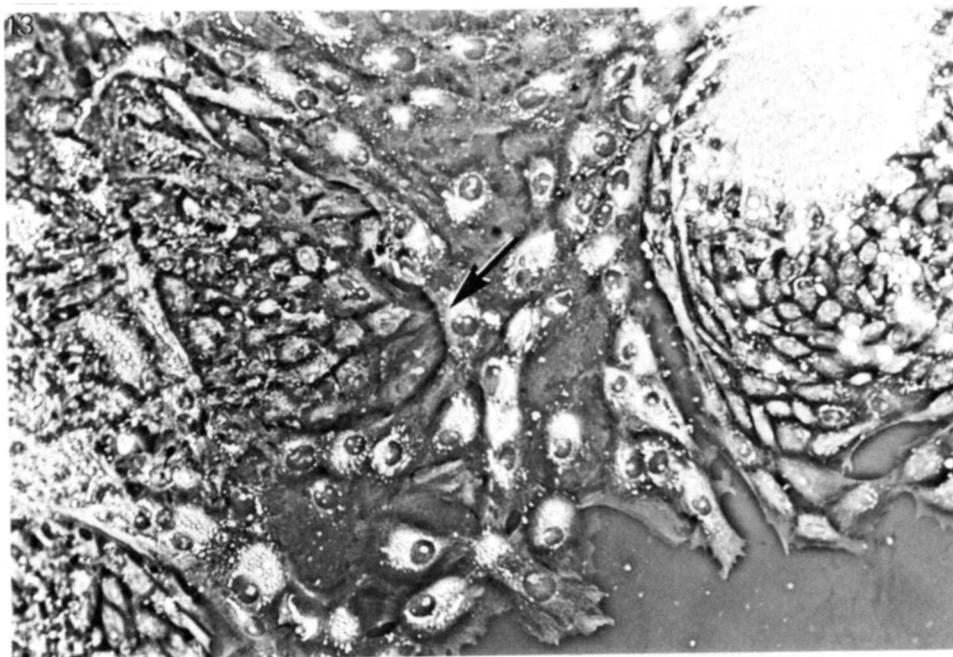


Fig. 13. Relationship between an invading explant and Explant I. The arrow shows a region of clear underlapping by the invading Explant II. $\times 174$. Phase-contrast microscopy.

Although we do not possess a clear understanding of the differences between chick and quail cells, nevertheless, we have gained the impression from handling them that sheets of quail cells are more cohesive than sheets of chick cells (i.e. cells remain attached to one another more readily). If this is so, then it is possible that the cells of Explant II might have more difficulty in penetrating a sheet of quail cells than a sheet of chick cells. It is possible that quail cells are also more adhesive to the substrate than are sheets of chick cells. During insertion the invading tissue may be competing for the same substrate with Explant I. Thus, if Explant I is very strongly adhesive to the substrate, then this would reduce the likelihood of invasion by the other tissue.

These results should not be taken to imply that the general mechanism of gastrulation differs in chick and quail embryos. It is commonly assumed that chick and quail cells are sufficiently similar to be exchanged in heteroplastic grafting (see Le Douarin, 1969), without any need to take species differences into account when assessing the results. Our results suggest however that there are important differences between them *in vitro*, at least in the early tissues. We shall be discussing the wider implications of these findings for Developmental Biology in another publication, but it may be noted here that differences in behaviour between chick and quail tissues have also been described by others, e.g. Chevallier, Kieny & Mauger (1977) who found that when quail somites were grafted into chick limb buds they gave rise exclusively to muscles,

but in the converse experiment when chick somites were grafted into quail limb buds, they often formed tendinous components.

(b) Differences displayed by tissues when used as Explant II or Explant III

The main differences among chick combinations was that a tissue was more likely successfully to invade another if it was placed on top of it (i.e. Explant II) than beside it (i.e. Explant III). This was so for every individual chick tissue (cf. Tables 2 and 4). This difference was particularly marked with the epiblast explants.

There are several possible explanations. The first is that the cells of Explant I may be more readily separated from one another if a second tissue invades from the dorsal side rather than from the lateral edge. We have described that small gaps appear, even when no second explant is present. It seems likely that the cells of Explant II may take advantage of these gaps. The second possibility is that once the cells of Explant II have managed to pass through Explant I and reach the culture dish they find a substrate which has already been coated with extracellular materials secreted by the cells of Explant I. Sanders (1980) has suggested that this is the reason why mesoderm cells become more epithelial and less fibroblastic when used as Explant II, and showed that fibronectin was associated with the substrate.

Another difference between the results obtained when explants were placed on top of, or in confrontation with, Explant I was that the arrangement of some of the cells varied. It has often been shown that when cultures of fibroblasts growing as a monolayer come into contact with one another, the cells where they meet become aligned at right angles to their original direction. Individual cells become elongated and arranged in parallel bands (Elsdale & Bard, 1974). These were termed 'barriers' by Sanders *et al.* (1978) and were found to be characteristic of confronted cultures of definitive endoblast with definitive endoblast. They were however not found in confronts of definitive endoblast with early hypoblast where, instead, the hypoblast cells separated and the definitive endoblast cells penetrated among them.

In the present experiments where Explant II penetrated into Explant I, this alignment usually appeared to be formed from one of the tissues only, and this was found with all types of combinations. The result was in contrast to the alignments found between Explants III and I, which usually involved both tissues.

One reason for this difference in behaviour is that epithelial sheets growing in culture are not adherent to the substrate except at the periphery (Middleton, 1973; Di Pasquale, 1978; Bellairs *et al.* 1978). This non-adhesion of the centre of Explant I to the substrate may also explain why extensive regions of underlapping of Explant I occur.

Relevance of these results to events in the embryo

The young chick embryo consists initially of two layers; the upper or epiblast is formed of cells firmly attached to one another, whilst the lower or hypoblast is composed of more loosely attached cells. The definitive endoblast is formed by the insertion of cells from the upper to the lower layer. In the present experiments we have shown that with the chick material, *whatever tissue forms the lower layer in culture, most cells are able to penetrate it*. Thus both *in vivo* and *in vitro* the lower layer appears to present little resistance to invasion from above. This may be related to the fact that small gaps between the cells are present both in hypoblast in the embryo (Revel, 1974; Sanders *et al.* 1978) and in the explants growing in culture.

Many extracellular materials have now been found in association with most layers of the early chick embryo. These include fibronectin (Critchley, England, Wakely & Hynes, 1979), hyaluronic acid (Solursh, Fisher & Singley, 1979) and basal lamina constituents (Sanders, 1979).

Thus the type of substrate that the invading tissue secretes or encounters may be an important factor in successful insertion *in vivo*.

This technique may therefore be used to study the importance of extracellular material to the behaviour of cell sheets during invasive processes.

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