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Effects of the Substratum on the Migration of Primordial Germ Cells

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Effects of the substratum on the migration of primordial germ cells

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[Plates 1–4]

It is now clear from work on defined cell types on artificial substrates that various chemical and physical inhomogeneities in the substrates can guide cell locomotion. It is also becoming clear that less well defined inhomogeneities in living cell substrates can guide the normal locomotion of embryonic migratory cells *in vivo*.

The primordial germ cells (p.g.cs) of early anuran amphibian embryos are proving a useful model for the study of cell migration. When isolated from the embryo and cultured on living cellular substrate, p.g.cs become oriented by the shapes of the underlying cells or by their stress fibre cytoskeleton, or both.

A combination of scanning and transmission electron microscopy *in vivo* shows a clearly aligned cellular substrate for p.g.c. migration along part of their route. Furthermore, we find that the glycoprotein fibronectin is involved in p.g.c. adhesion, which suggests a link between orientation of the substrate cells and p.g.c. guidance.

Introduction: the use of primordial germ cells in cell migration studies

In the work described here, our principal aim has been to correlate observations of cell behaviour *in vitro* with those during migration of the same cell type *in vivo*. To this end, we have turned our attention to defined populations of embryonic cells, whose migratory history is reasonably well documented.

Embryonic cells are assumed to have a certain set of phenotypic properties, including locomotion, guidance response, and invasiveness. We further assume them to be triggered in some way to start moving towards the site in which they will differentiate, and to stop in the correct position. However, these properties are hard to see directly, except in certain cases (see Trinkaus (1976) for a comprehensive and outstanding review). They are generally inferred from a combination of histology of fixed tissue, and from the more elegant observations made *in vitro* on the behaviour of motile differentiated cells such as fibroblasts (for reviews see Dunn (1980) and this symposium), epithelial cells (see Vasiliev & Gelfand (1981) for review) or leucocytes (Zigmond 1978). The relative ease with which fibroblasts can be cultured has lead to a vast amount of research on their locomotion, including the pioneering work of Abercrombie (see Abercrombie (1980) for review).

In such studies, however, we know little of the migratory history of the cells concerned, making it difficult to refer back to the animal to confirm or deny observations *in vitro*. Our efforts to circumvent this problem have led us to the study of a population of cells in the early anuran amphibian embryo, the primordial germ cells (p.g.cs). These offer two main advantages: their origin and migratory route are well known (Bounoure 1934; Blackler 1958; Whitington & Dixon 1975; Wylie & Heasman 1976), and they are so large and easy to identify that they

can be isolated from their migratory route, and studied *in vitro* (Wylie & Roos 1976). Although this approach carries with it certain difficulties, e.g. the handling of very early embryos, and the paucity of tissue, we feel that it offers a reasonable compromise for a study of cell migration.

The germ lineage of the anuran amphibian *Xenopus laevis*, like that of other vertebrates studied, becomes established at the very earliest stages of embryogenesis. The progenitor cells of the germ line are the p.g.cs, which migrate to the site at which the gonad differentiates. The origin and migratory route appear to differ in different vertebrate groups. In *Xenopus laevis* they arise at the vegetal pole of the blastula, with which they become part of the embryonic endoderm, and eventually become incorporated into the developing gut. Once the gut has adopted a tubular form, the p.g.cs migrate dorsally through it, around the central lumen, and eventually pass into its dorsal mesentery. Here they can be seen quite clearly, even under the dissecting microscope, owing to their large size, and content of refractile yolk platelets.

At this stage of their migratory route, the p.g.cs can be removed from the mesentery by simple disaggregation, and collected in a finely drawn pipette to be introduced into the relevant conditions *in vitro*. Three aspects of such isolated p.g.cs have interested us: their adhesive and motile properties (Heasman, Mohun & Wylie 1967; Heasman & Wylie 1978), their invasiveness (Swan *et al.* 1981), and their guidance (Wylie *et al.* 1979; Heasman & Wylie 1981). This short review will focus on factors that appear to guide the p.g.cs *in vitro*, and observations that suggest that the same factors are at work during normal migration *in vivo*.

Substrate shape and p.g.c. spreading

When isolated *Xenopus* p.g.cs are seeded onto cellular substrates of epithelial-like cells derived from adult *Xenopus* mesenteries, they adhere to these and spread upon them. They then spend a variable amount of time on the surface of the culture, before invading it, when their movements become more difficult to follow. When p.g.cs move on the surface of the cultured cells, they extend a (usually single) phase-dense process (figure 1), which we have rather loosely called a filopodium. This does not always result in movement; however, when it does, the yolky cytoplasm is seen to stream forwards into the filopodium. P.g.cs would be particularly useful in a study of cytoplasmic streaming, because the refractile yolk platelets make an excellent marker. After this forward movement of the cytoplasm, the trailing end withdraws from the substrate, and the p.g.c. rounds up in its new position. Figure 2 shows scanning electron micrographs of a filopodium. These are seen to be rather complex structures, with lamellar areas, lateral microspikes, and a small ruffling area near the tip. The term filopodium is clearly misleading here, and describes their simple appearance under the light microscope. Indeed, it is difficult to think of an adequate term, and perhaps this should await further work on these and other migratory embryonic cells.

It is particularly interesting to note the relation between the direction of spreading and movement of the p.g.c. on the one hand, and the contours of the substrate cells on the other. P.g.c.s always align themselves coaxially with the shapes of the substrate cells if these are obviously bipolar (figure 3), or when the substrate cells are not obviously bipolar, with the orientation of their cytoskeleton (figures 1 and 4). The mesentery cells used as substrates in these studies have particularly well developed microfilamentous stress fibres, with which the p.g.cs always coalign (Wylie *et al.* 1979).

These observations prompted us to look closely at the normal substrate of migration *in vivo*,

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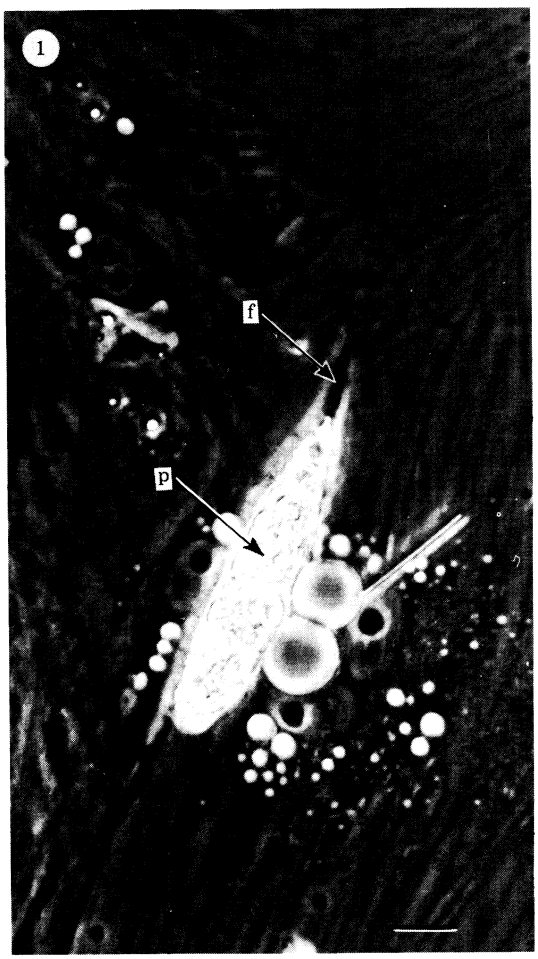

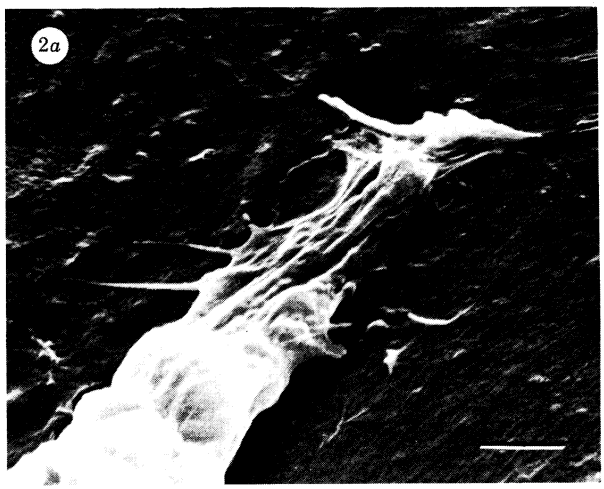

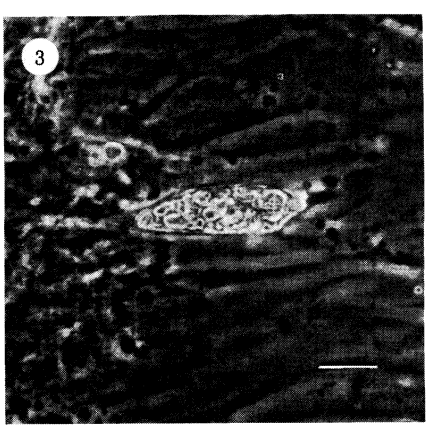

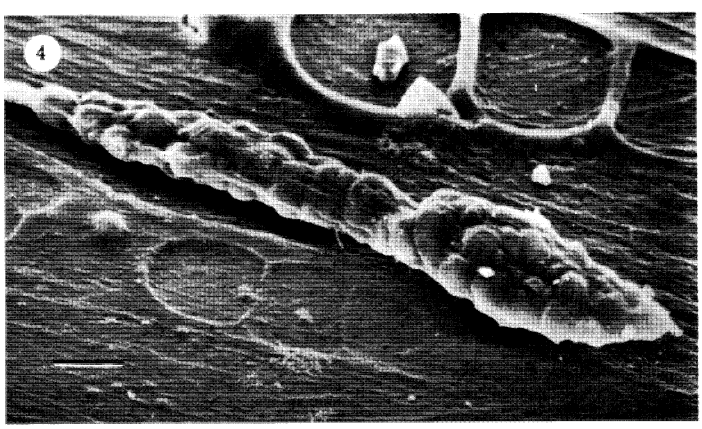

FIGURE 1. Phase-contrast micrograph of a single p.g.c. (p) on a feeder layer of mesentery cells. Note the single dense filopodium (f) and the relation between the direction of p.g.c. elongation and stress fibres of the substrate cell. Bar = 10 μm.

FIGURE 2. Scanning electron micrographs of the same filopodium from two angles. Note lateral microspikes, one of which appears to be following stress fibres of substrate cell just ahead of the p.g.c. Stress fibres are thrown up into ridges in these preparations. Bar = 2 μm.

FIGURE 3. Single p.g.c. on a layer of oriented bipolar cells. Bar = 20 μm.

FIGURE 4. Elongated p.g.c., coaligned with stress fibres of substrate cell, which appear as fine ridges. Bar = 8 μm.

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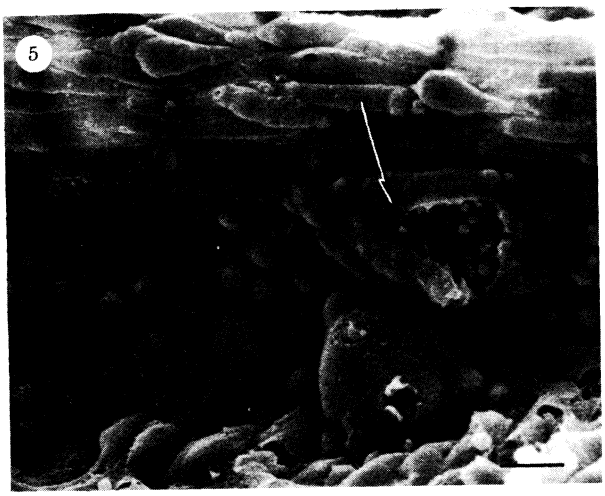

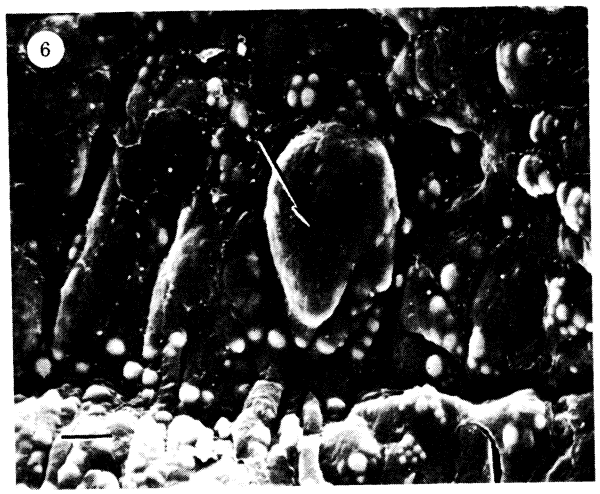

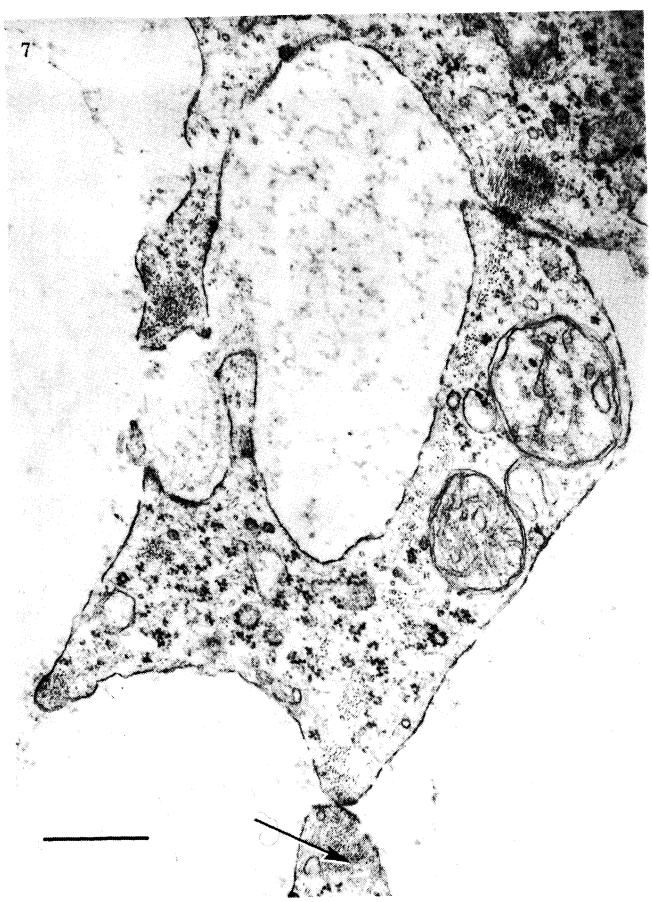

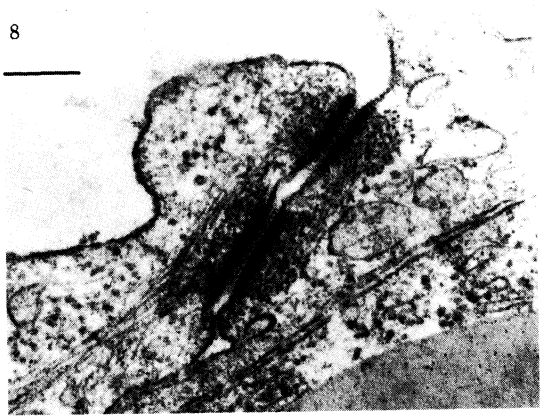

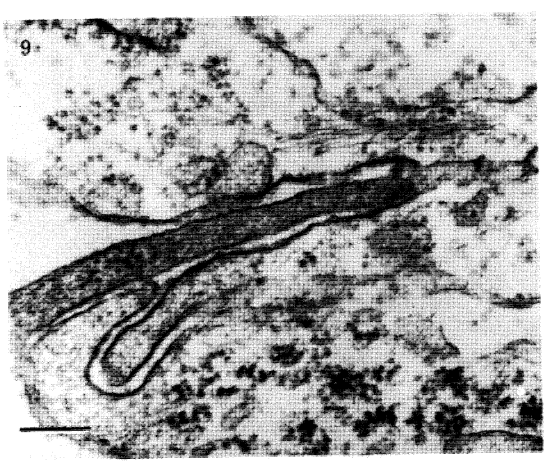

FIGURE 5. P.g.c. inside the mesentery of stage 43 embryo, coaligned with the array of oriented c.e.cs. The p.g.c. (arrowed) is easily recognizable because it has been broken during preparation, and the yolk granules in the cytoplasm are exposed. Bar = 12 μm.

FIGURE 6. By stage 44, most p.g.cs are nearer to the dorsal body wall, and seen coaligning with c.e.cs oriented towards the dorsal body wall. P.g.cs are seen as lumps under the c.e.cs (arrowed). Bar = 12 μm.

FIGURE 7. Transverse section of stage 43 embryo mesentery, showing the extensive filamentous cytoskeleton of the c.e.c. array. Bundles of filaments (cut in cross section) are particularly large near margins of adjacent c.e.cs (arrowed). Bar = 1 μm.

FIGURE 8. Some of the c.e.c. filaments are inserted into desmosomes between c.e.cs. This section is at the site of a change in orientation of c.e.cs. Filaments on either side of the desmosome are thus cut in opposite planes of section. Bar = 0.2 μm.

FIGURE 9. Filopodium of p.g.c., showing microfilamentous core. Filopodia coalign with the c.e.cs and their filament bundles. Bar = 0.2 μm.

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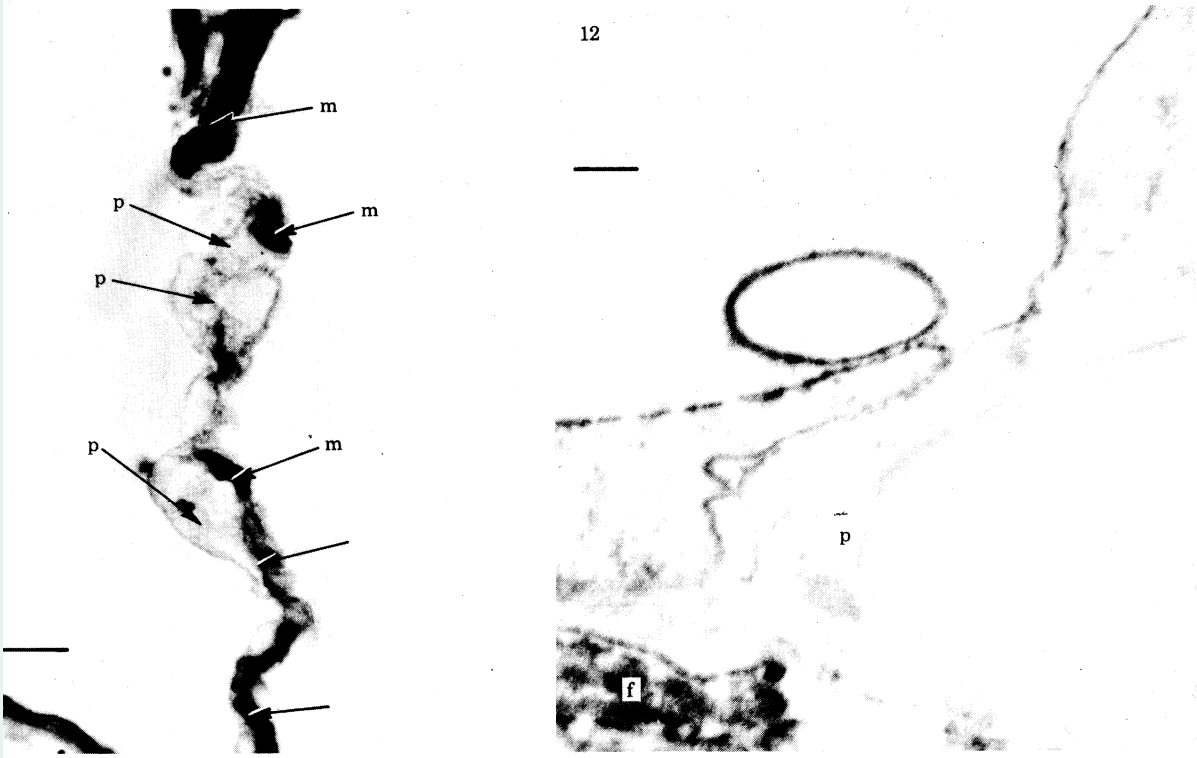

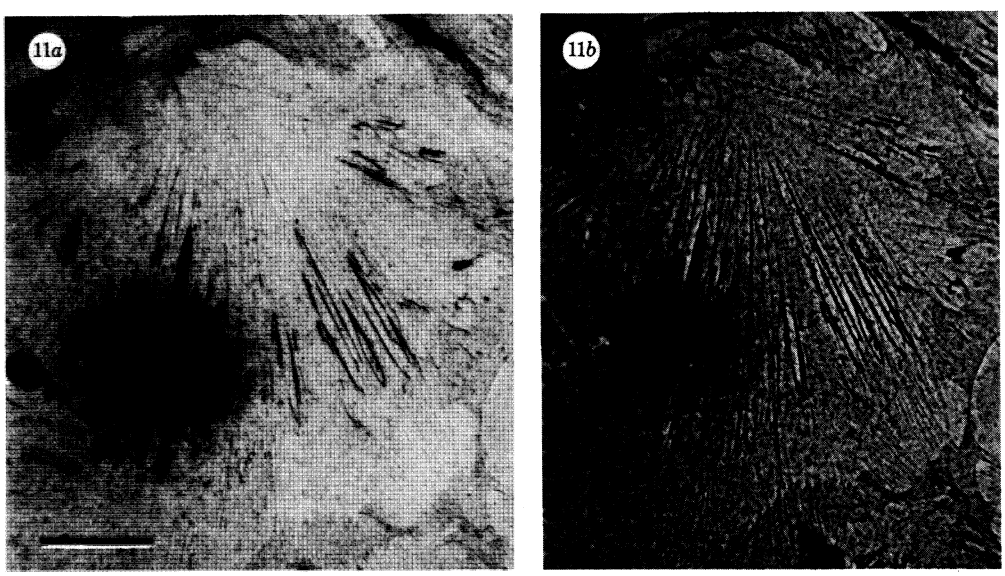

FIGURE 10. Light micrograph of immunoperoxidase-stained mesentery of stage 44 embryo. Anti-fibronectin stains the core of the mesentery (arrowed) and outlines the p.g.cs (p) with a fine golden-brown precipitate. Melanocytes, containing black pigment granules. Bar = 10 μm.

FIGURE 11. Bright-field (*a*) and phase-contrast (*b*) micrographs of the same field of view, showing coalignment of the fibronectin, visible as a dark precipitate in (*a*) by the immunoperoxidase reaction, with the stress fibres, seen in phase-contrast in (*b*). Bar = 30 μm.

FIGURE 12. Electron micrograph, stained by the indirect immunoperoxidase reaction with anti-fibronectin, showing a p.g.c. (p) pushing a process beneath the feeder layer of mesentery cells, and running along the fibronectin-containing fibrillar material beneath (f). Bar = 0.2 μm.

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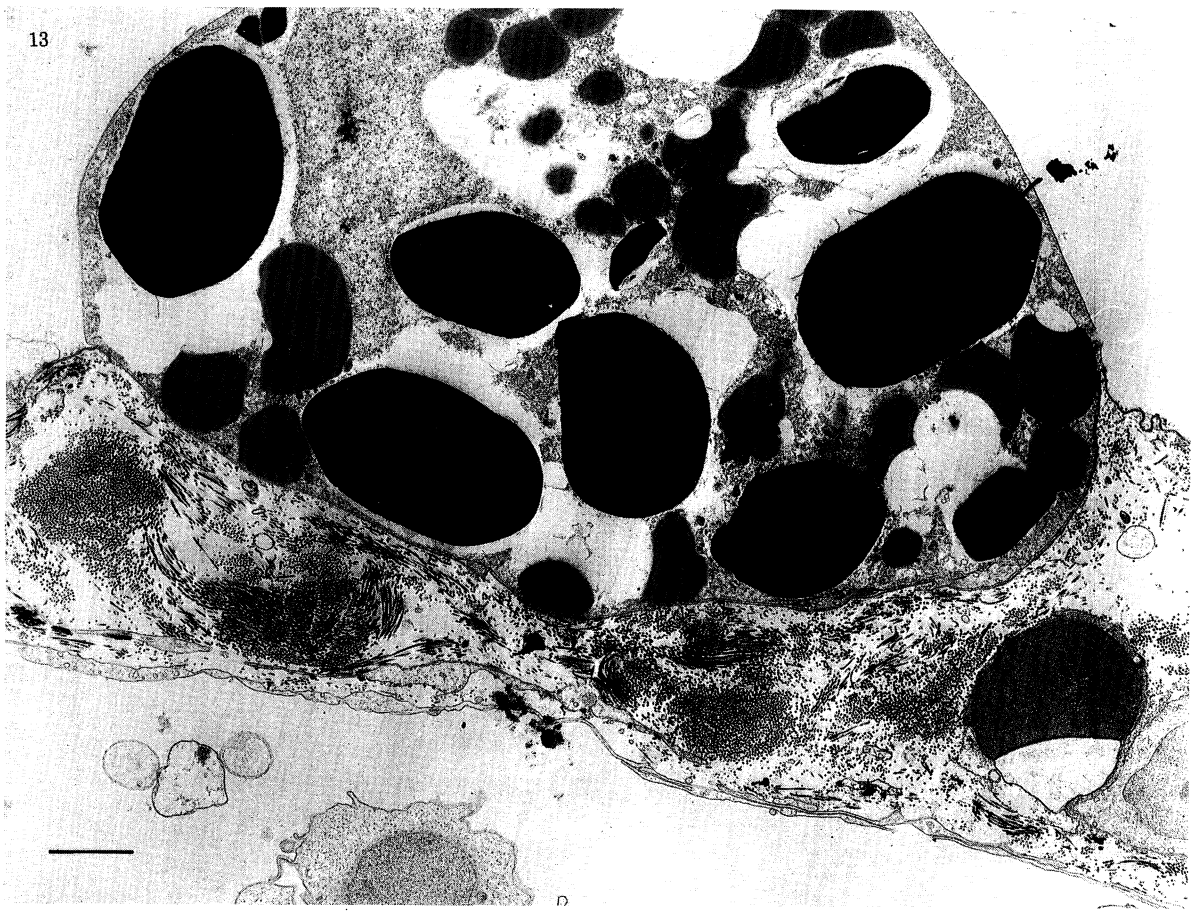

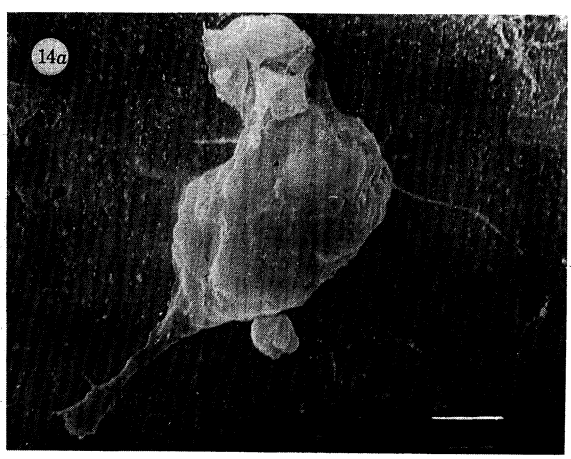

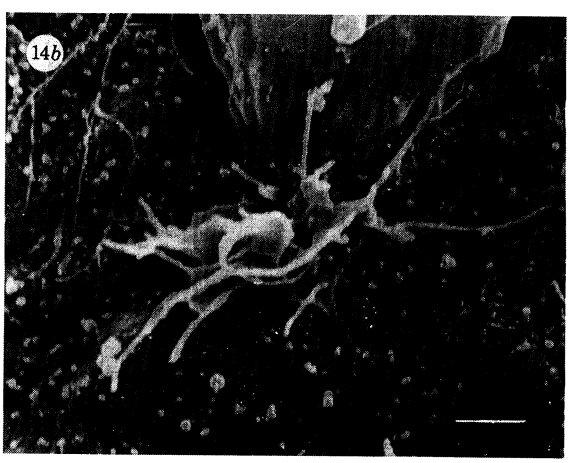

FIGURE 13. Isolated p.g.c. tightly attached to the basement membrane of isolated adult frog mesentery. Note that at the edges of the p.g.c. the basement membrane is ruffled. Bar = 2 μm.

FIGURE 14. (*a*) Isolated *Xenopus* p.g.c. on extracellular matrix of the chick embryo. Bar = 4 μm. (*b*) Higher-power view of a filopodium from a p.g.c. similar to that shown in (*a*). Lamellar areas and microspikes are seen, similar to those in figure 2. Bar = 2 μm.

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paying close attention to two of its features; namely the shapes, and nature of the cytoskeleton, of the substrate cells. The results of this study were reported by Heasman & Wylie (1981) and will only be summarized here. While in the mesentery of the gut, p.g.cs migrate along the basal (inner) surface of the epithelial lining of the mesentery. This simple squamous epithelium is part of, and continuous with, that lining the rest of the coelomic cavity. Since these coelomic epithelial cells (c.e.cs) are the substrate for p.g.c. migration, it is these that are of interest, as well as any intervening extracellular matrix material. The shapes of the c.e.cs can be clearly seen from the outer (coelomic) side of the epithelium by using scanning electron microscopy, as can the p.g.cs beneath them, which are so large that the covering cells are pushed out into visible lumps. When p.g.cs enter the gut mesentery (at the early swimming tadpole stage: stage 43 (Nieuwkoop & Faber 1967)) the c.e.cs are arranged as a characteristic array of highly polarized cells. These run from dorsal body wall to gut tube in a curved course, being arranged obliquely nearest to the gut, and turning dorsally towards the dorsal body wall (Heasman & Wylie 1981). The important point is that wherever elongated p.g.cs are seen in these scanning electron micrographs, they always coalign with the c.e.cs, being directed obliquely nearest the gut (figure 5) and dorsally nearest the dorsal body wall at later stages (figure 6). The p.g.cs thus appear to take an oblique course through the mesentery, directed by the shapes of the c.e.cs.

These shapes appear to be maintained by a complex cytoskeleton, made of obvious bundles of filaments lying in the long axes of the cells. The number and size of these bundles can best be appreciated when the c.e.cs are cut across in transverse section. Figure 7 shows a transmission electron micrograph of such a section. Bundles of filaments are particularly obvious near the edges of the c.e.cs, though smaller bundles can also be seen elsewhere in the cells. The filaments of the c.e.cs are inserted into two sites in their surface membrane; most obvious is their insertion into desmosomes, which join the margins of the c.e.cs (figure 8). Filaments are also inserted into junctions with the p.g.cs (see Heasman & Wylie 1981). These are very similar to the focal contacts seen between fibroblasts and their substrates *in vitro* (Abercrombie *et al.* 1971; Goldman *et al.* 1975). When p.g.cs are cut in longitudinal section, their filopodia are found to coalign with both the shapes of the c.e.cs, and with the filament bundles in their cytoplasm (figure 9).

The impression given by these studies is that the oriented array of c.e.cs forms a relatively rigid framework for p.g.cs to migrate upon. Since p.g.cs *in vitro* appear to be guided in their spreading and movement by very similar inhomogeneities of the cellular substrate, it seems most likely that they could be guided by this framework *in vivo*.

After the arrival of p.g.cs at the dorsal body wall, the axis of orientation of the c.e.cs undergoes a profound change (Wylie *et al.* 1979). At stage 46 (Nieuwkoop & Faber 1967), they show no obvious alignment over the surfaces of the p.g.cs, whereas at stage 48, by which time the gonadal ridge has formed, the c.e.cs over the p.g.c. surfaces have differentiated into a thick 'plait' of interlocking spindle-shaped cells arranged craniocaudally along the dorsal body wall lateral to the mesentery. The p.g.cs are now smaller than previously; they have lost their cytoplasmic yolk, and entered the gonial stage of their differentiation, dominated by cell division. Little is known about the factors that mediate the cessation of migration, and the interaction with a particular part of the coelomic lining to form the developing gonad. We do know that the c.e.cs do differentiate into a gonadal ridge in the apparent absence of p.g.cs, since embryos in which p.g.c migration has been delayed or abolished (by ultraviolet irradiation of the vegetal pole at the fertilized egg stage) still show this change in c.e.c. orientation (Wylie *et al.* 1976).

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The role of fibronectin in p.g.c. migration

It would be interesting to know the molecular basis by which the aligned substrate could influence the direction of p.g.c. migration. An obvious molecule to study in this respect is fibronectin. This is known to be a cell-surface glycoprotein involved in cell adhesion, one that coaligns with the cytoskeleton of certain cell types *in vitro* (see Hynes (1981) for review). It is found at various sites of cell migration in embryos, e.g. chick gastrulae (Critchley *et al.* 1979) chick neural crest (Newgreen & Thiery 1980; Mayer *et al.* 1981) and developing chick cornea (Kurkinen *et al.* 1979). In collaboration with R. O. Hynes, we have raised an anti-*Xenopus* plasma fibronectin, and used it in a series of studies on *Xenopus* p.g.c. migration (Heasman *et al.* 1981). In this work we show the following.

1. Fibronectin is found on the basal surfaces of the c.e.cs of the embryonic mesentery (figure 10). We do not know whether this is arranged as a single layer or in fibrils.
2. When p.g.cs are isolated from the embryonic mesentery, they are found by immunofluorescent staining to be heavily contaminated with fibronectin-containing cells and extracellular debris. When these are removed by trypsinization, fibronectin does not reappear on the surfaces of the p.g.cs, despite the fact that they regain their adhesive, motile and invasive properties. This suggests that p.g.cs do not synthesize fibronectin but may well adhere to that synthesized by their substrate.
3. The cells used as a feeder layer and substrate for migration of the p.g.cs *in vitro* do synthesize large quantities of fibronectin, which is found in the culture medium, on all their surfaces and as dense fibrils beneath them, coaligned with their stress fibres (figure 11). Furthermore, electron microscope immunocytochemistry shows that p.g.cs *in vitro* push cytoplasmic processes beneath the substrate along these fibronectin-containing fibrils (figure 12).
4. If p.g.cs do indeed adhere to the fibronectin made by their substrate cells, then one might expect anti-fibronectin to inhibit this adhesion. This indeed appears to be so. When p.g.cs are seeded onto cellular substrates in increasing concentrations of $F(ab)_2$ fragments of anti-*Xenopus* fibronectin, their adhesion is reduced to 10–30 % of control levels in the dose range 10–100 μg of antibody (see Heasman *et al.* 1981) for further details).

Thus the interaction of p.g.cs with fibronectin derived from their cellular substrate, both *in vitro* and *in vivo*, offers an explanation of their behaviour seen in both situations. The way in which this interaction could guide p.g.cs is not clear. There seems little doubt that various embryonic migratory cells do follow defined pathways established by their cellular or extracellular environments. Examples include the neural crest cells (see Le Douarin (1982) for review), neuron cell processes (Katz & Lasek 1980; Singer *et al.* 1979; Silver & Sidman 1980) and endocardial cushion cells of the developing heart (Markwald *et al.* 1979). Furthermore, it has been shown that in many cases of embryonic cell migration, fibronectin is a feature of this environment.

There seem to be three obvious ways in which fibronectin, perhaps in conjunction with other matrix or cell surface molecules, could influence the direction of migration.

1. By contact, or substrate guidance, whereby the physical contour of the substrate polarizes the cells (Weiss 1935; Dunn & Ebendal 1978). In this case only polarity is achieved, leaving directionality to be established by some other means.
2. As a gradient of adhesiveness (Carter 1967; Harris 1973).

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3. By chemotaxis. This mechanism has been demonstrated in *Dictyostelium* (Konijn *et al.* 1967), neurons (Menesini-Chen *et al.* 1978) and leucocytes (Zigmond 1978), and has been suggested for p.g.cs in chick embryos (Dubois 1964) and mammalian embryos (Rogulska *et al.* 1971). There has also been a recent suggestion that fibronectin may act as a chemotactic agent in the migration of neural crest cells (Greenberg *et al.* 1981).

If fibronectin is involved in the adhesion of p.g.cs to their normal terrain, then it would be interesting to study their response to other naturally occurring and artificial substrates rich in fibronectin. We have studied several of these, two of which provided interesting results.

The behaviour of p.g.cs on basement membranes of adult tissue

When isolated p.g.cs are seeded onto adult epithelial-covered organs *in vitro* (we have primarily used adult gut mesentery) they first adhere to the apical (coelomic) surface of the epithelium, and then invade it, causing the epithelial cells to lose their attachment to the basement membrane, and round up. The p.g.cs then adhere tightly to the basement membrane thus exposed (figure 13). The p.g.cs cause the basement membrane to ruffle, and to ride up partly over the surface of the p.g.c. This is presumably due to a pull exerted by the p.g.c. It is possible that the ability of the p.g.cs to adhere to substrata, and then pull themselves past these adhesion, is fundamental to their normal migration. We do not know yet whether p.g.cs can penetrate basement membranes. This information will have useful implications for their guidance, since it is possible that basement membranes may serve to contain migrating cells in embryos, if they cannot migrate through them.

The behaviour of isolated p.g.cs on explanted chick embryos

In collaboration with M. A. England, we have recently introduced *Xenopus* p.g.cs onto part of the migratory route of chick-embryo p.g.cs. At the primitive streak stage of chick development, their p.g.cs are associated with extracellular matrix material, rich in fibronectin, between the two layers of the embryonic disc. This material is primarily the basement membrane of the upper ectoderm layer, thickened into a fibrous band arranged as a crescent around the cranial end of the embryo (Critchley *et al.* 1979; Wakely & England 1979). This area can be exposed in cultured chick embryos, by removal of the lower, endodermal layer of the embryonic disc. Isolated *Xenopus* p.g.cs adhere well to this, and migrate upon it (figure 14) (Wylie & England, unpublished data). This may be a particularly useful system for studying the orientation of p.g.cs by the fibrous band, and for testing the effects on adhesion and migration of immunological probes against the p.g.cs themselves and extracellular and cell surface molecules with which they interact during migration.

In summary, therefore, p.g.cs represent an attractive system with which to study the mechanism of cell motility and guidance of embryonic cells. The fact that they can be isolated from the embryo in a pure form should allow the analysis of both their own cell surface molecules that mediate such behaviour, as well as those of their normal substrate.

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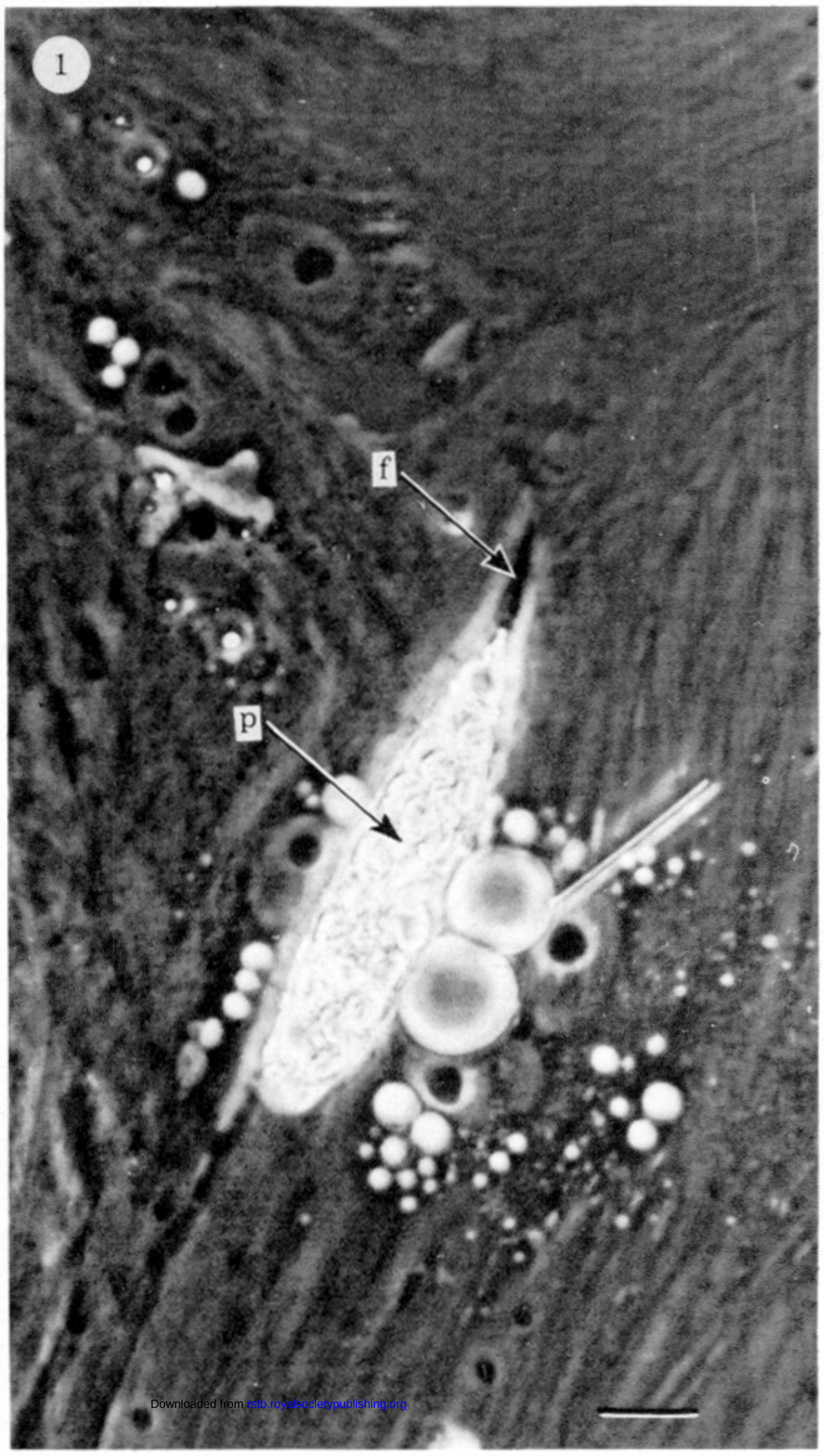
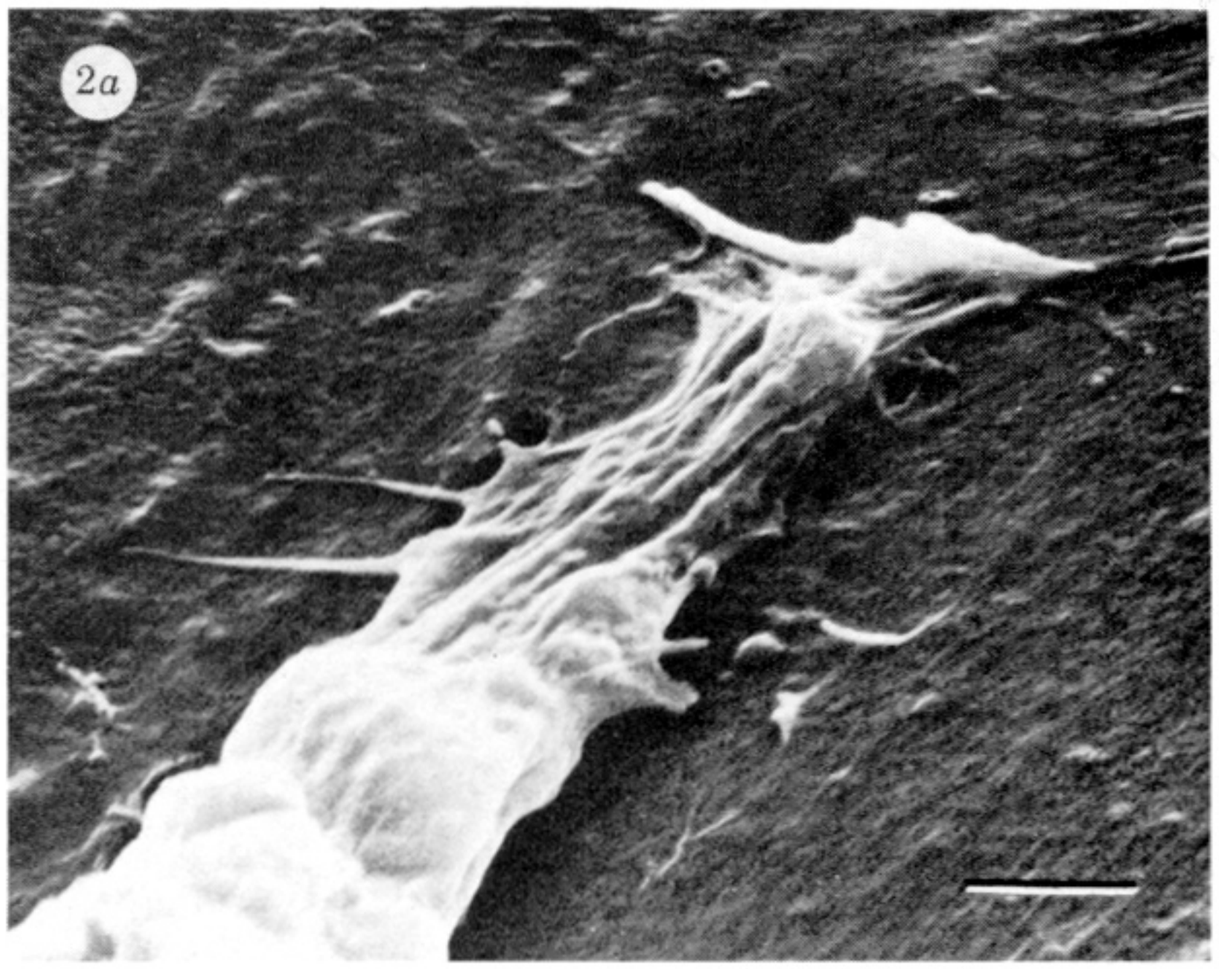
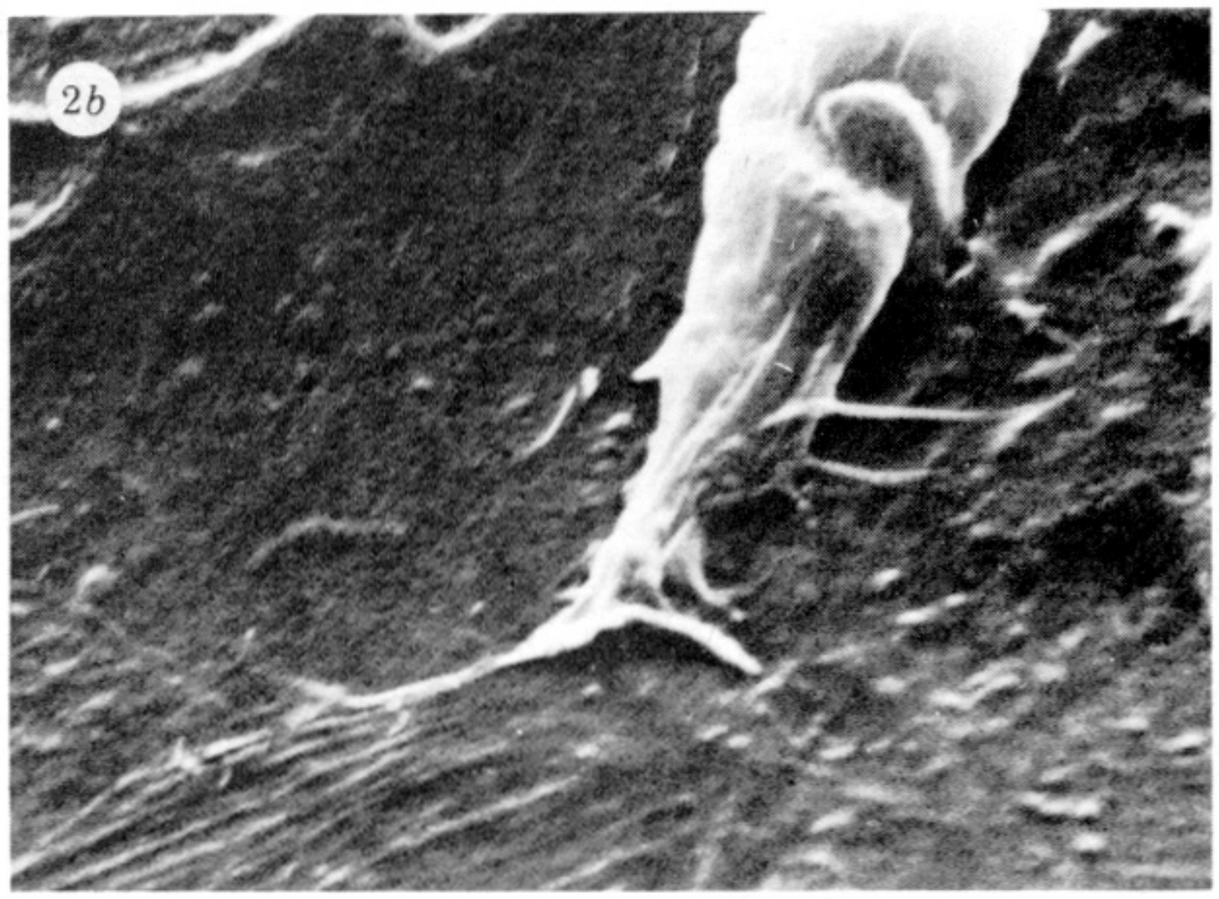
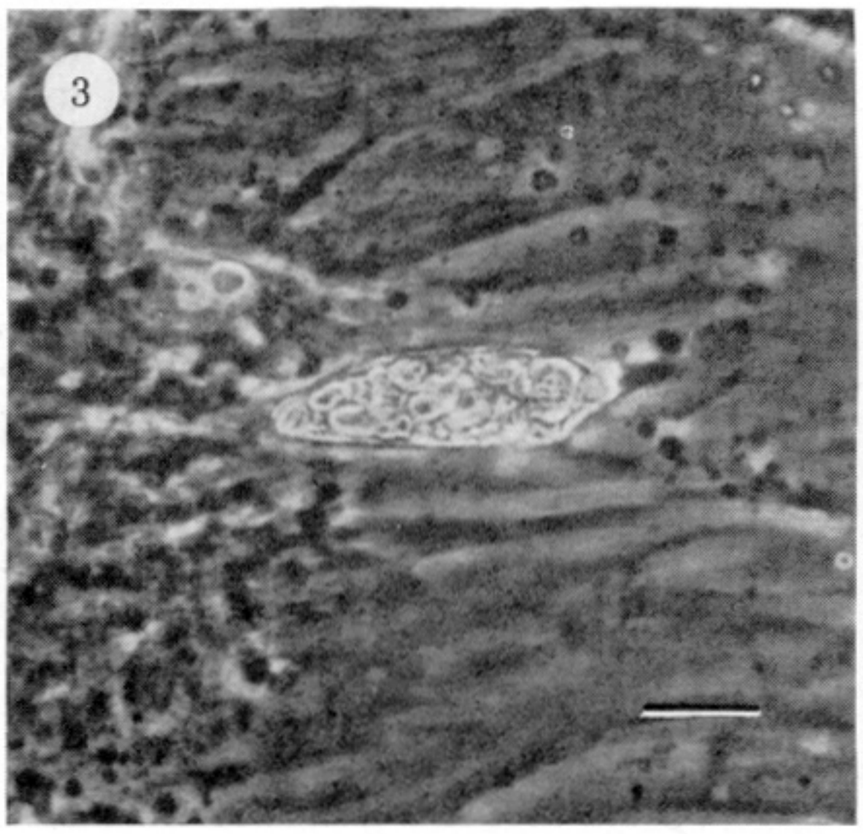








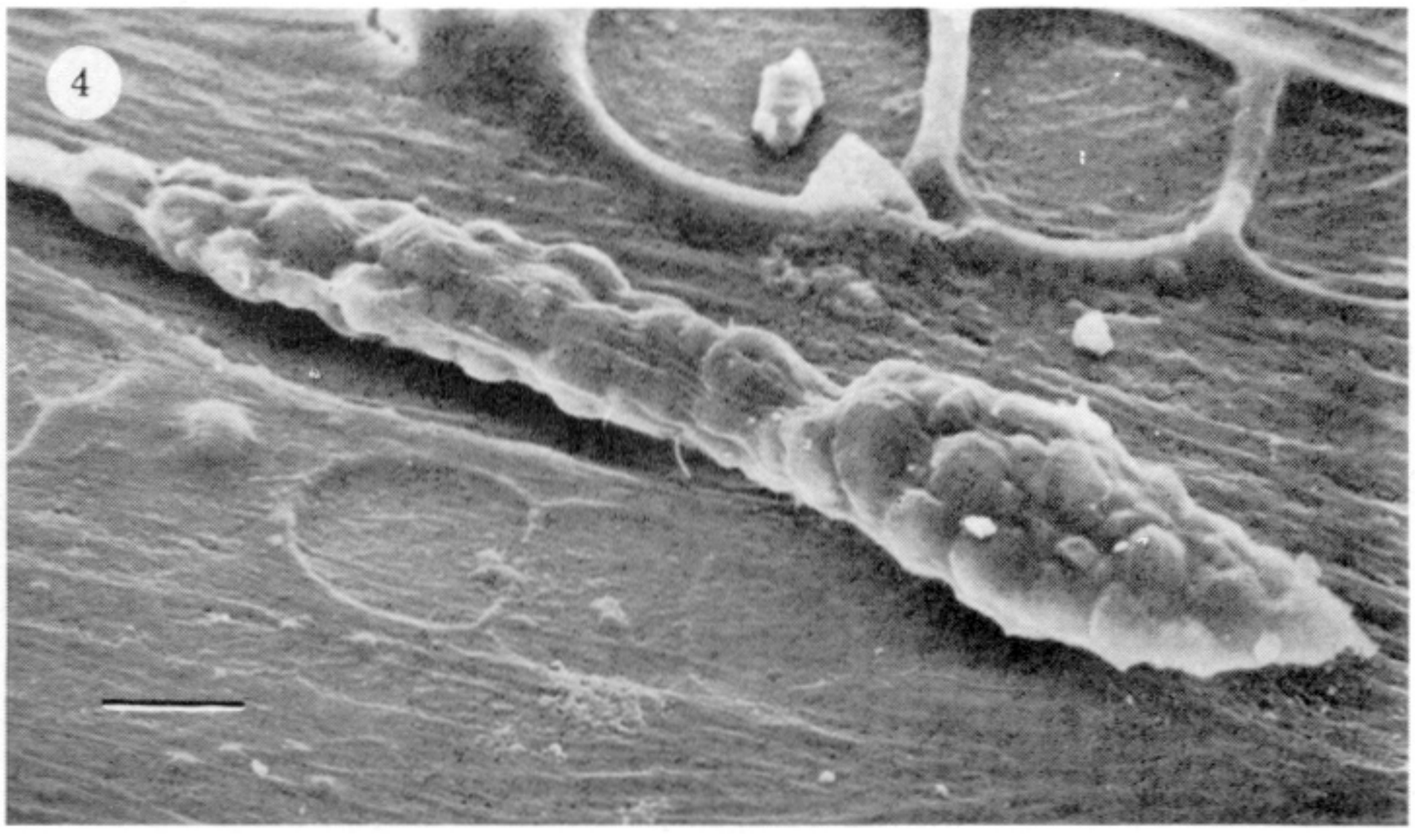
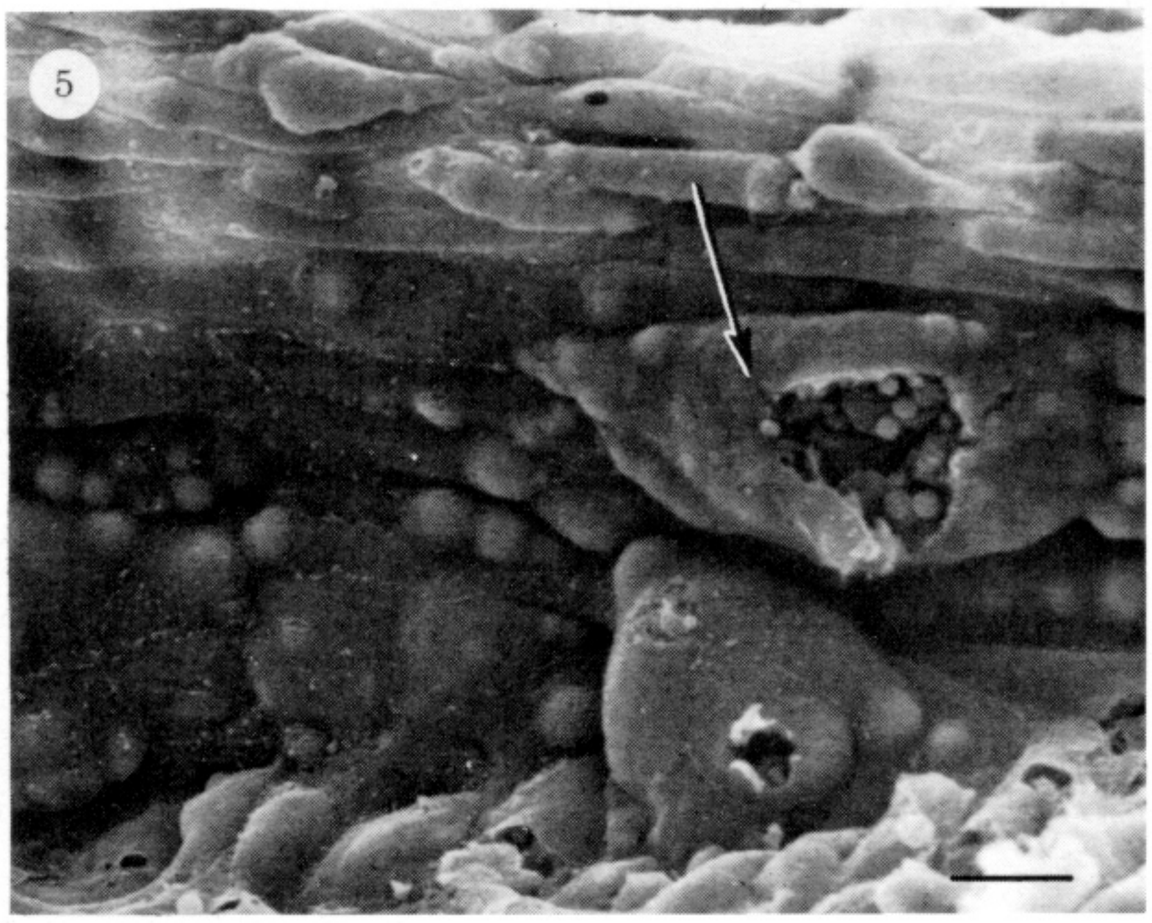


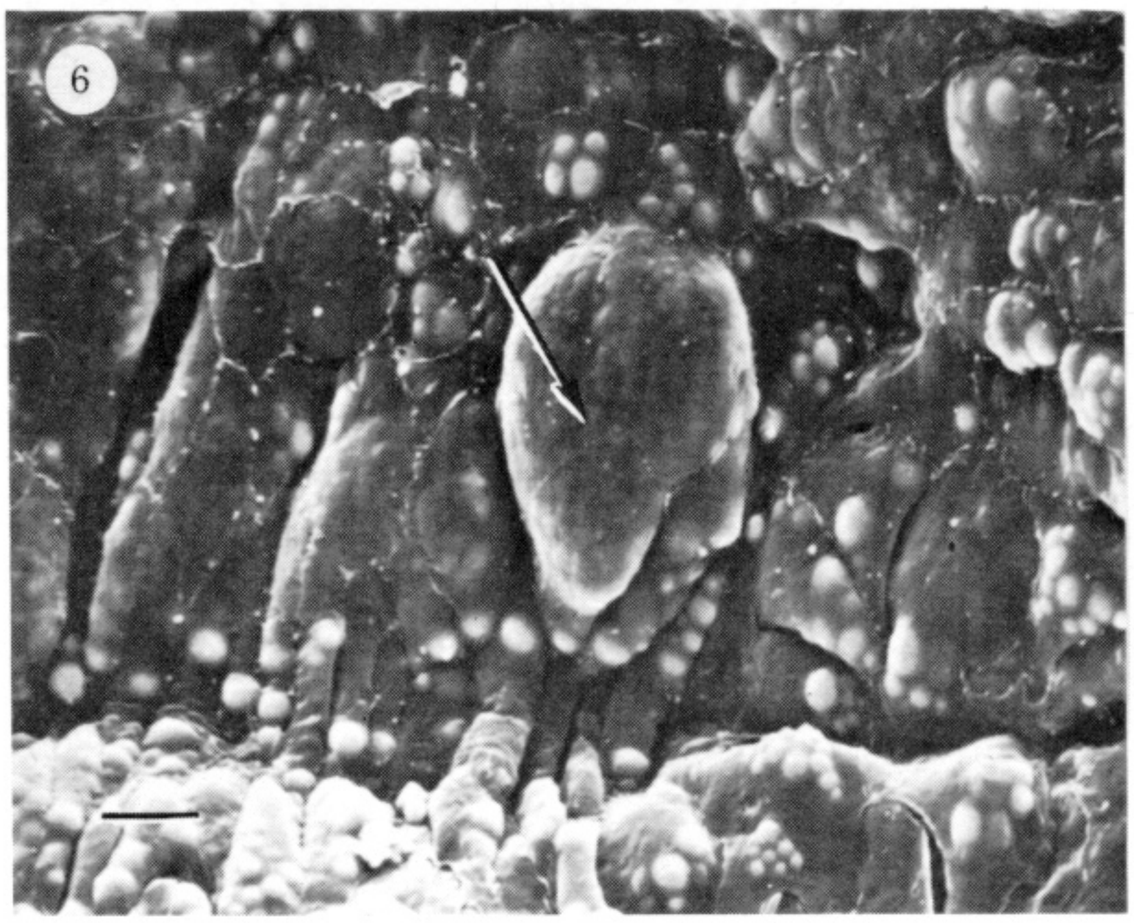
FIGURE 1. Phase-contrast micrograph of a single p.g.c. (p) on a feeder layer of mesentery cells. Note the single dense filopodium (f) and the relation between the direction of p.g.c. elongation and stress fibres of the substrate cell. Bar = 10 μm.

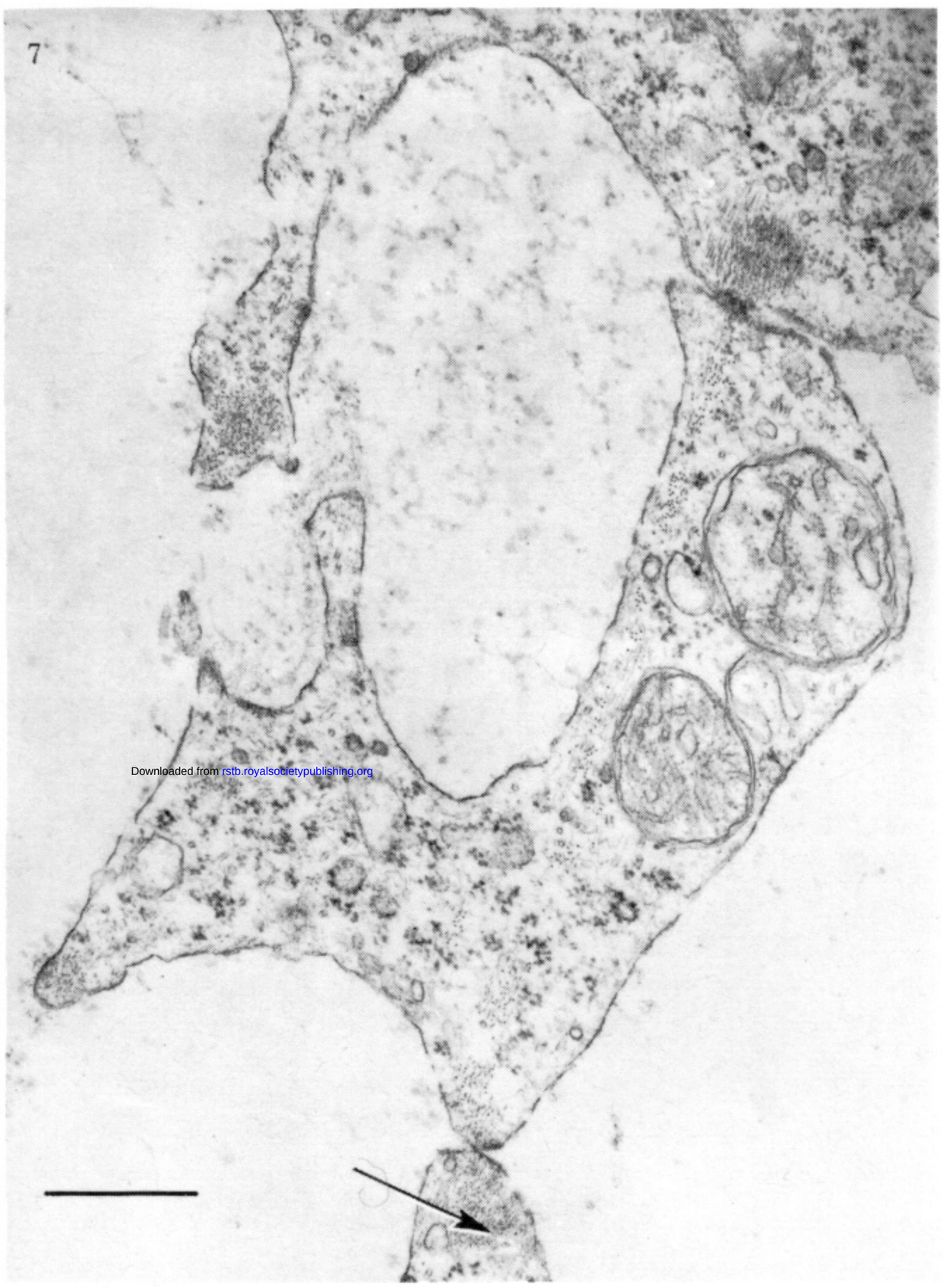
FIGURE 2. Scanning electron micrographs of the same filopodium from two angles. Note lateral microspikes, one of which appears to be following stress fibres of substrate cell just ahead of the p.g.c. Stress fibres are thrown up into ridges in these preparations. Bar = 2 μm.

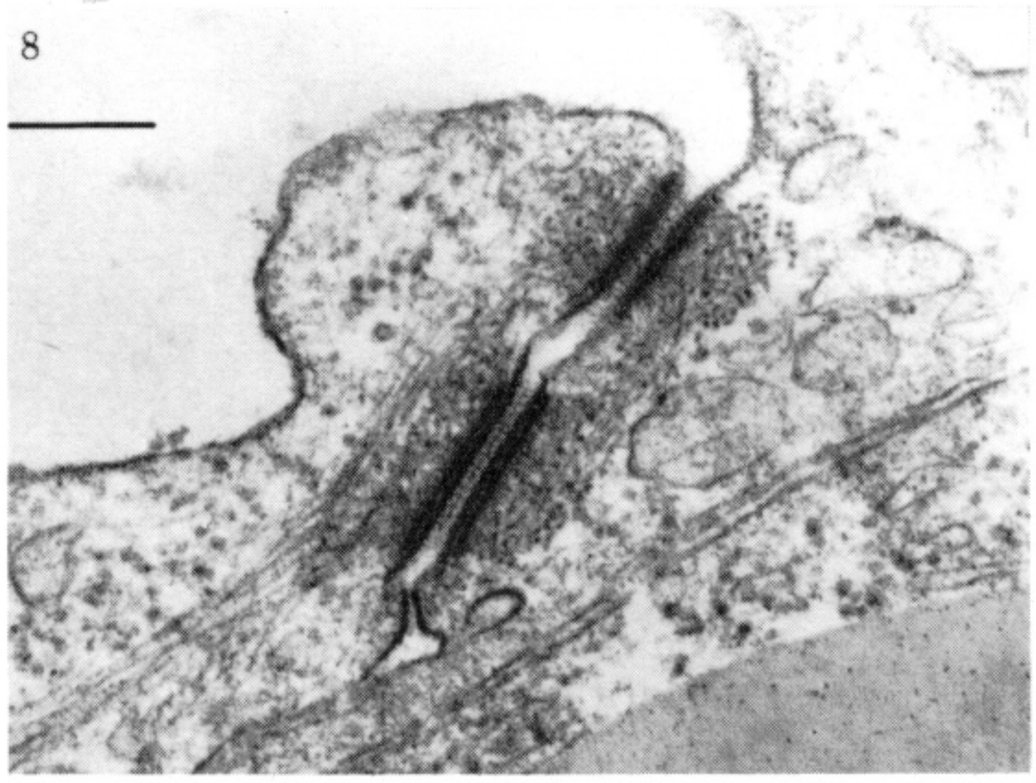
FIGURE 3. Single p.g.c. on a layer of oriented bipolar cells. Bar = 20 μm.

FIGURE 4. Elongated p.g.c., coaligned with stress fibres of substrate cell, which appear as fine ridges. Bar = 8 μm.









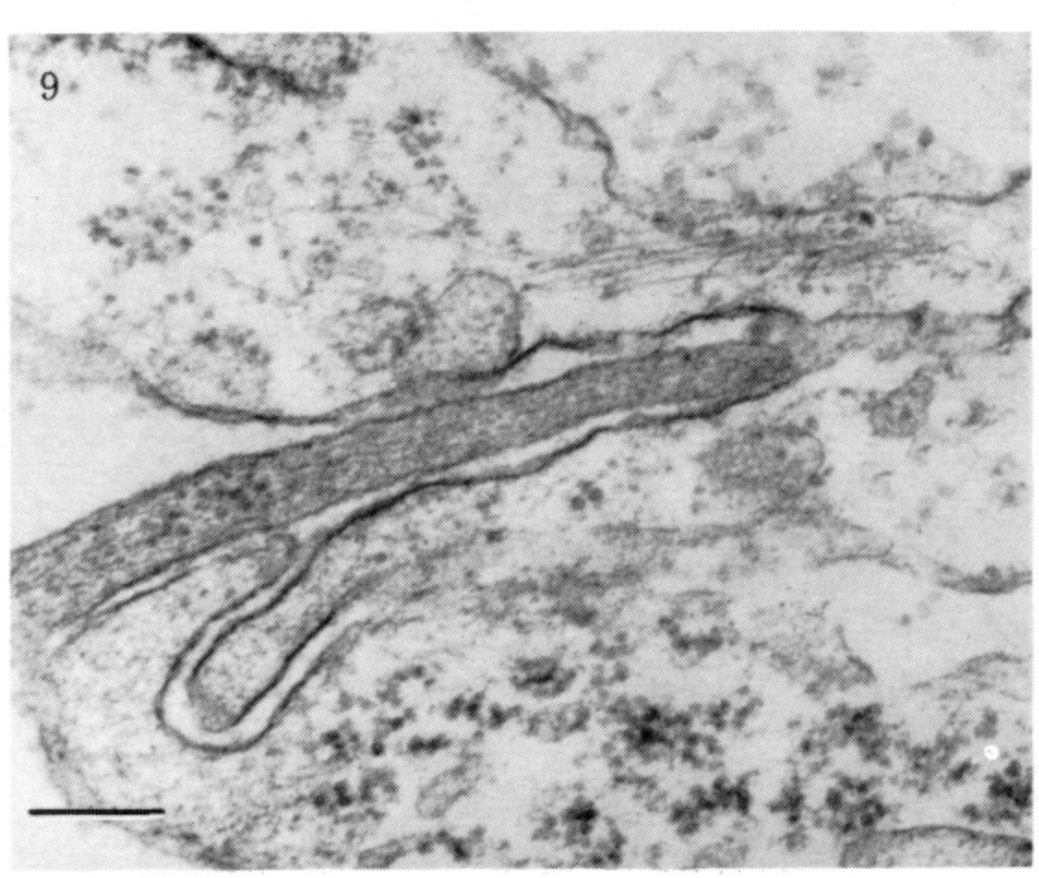


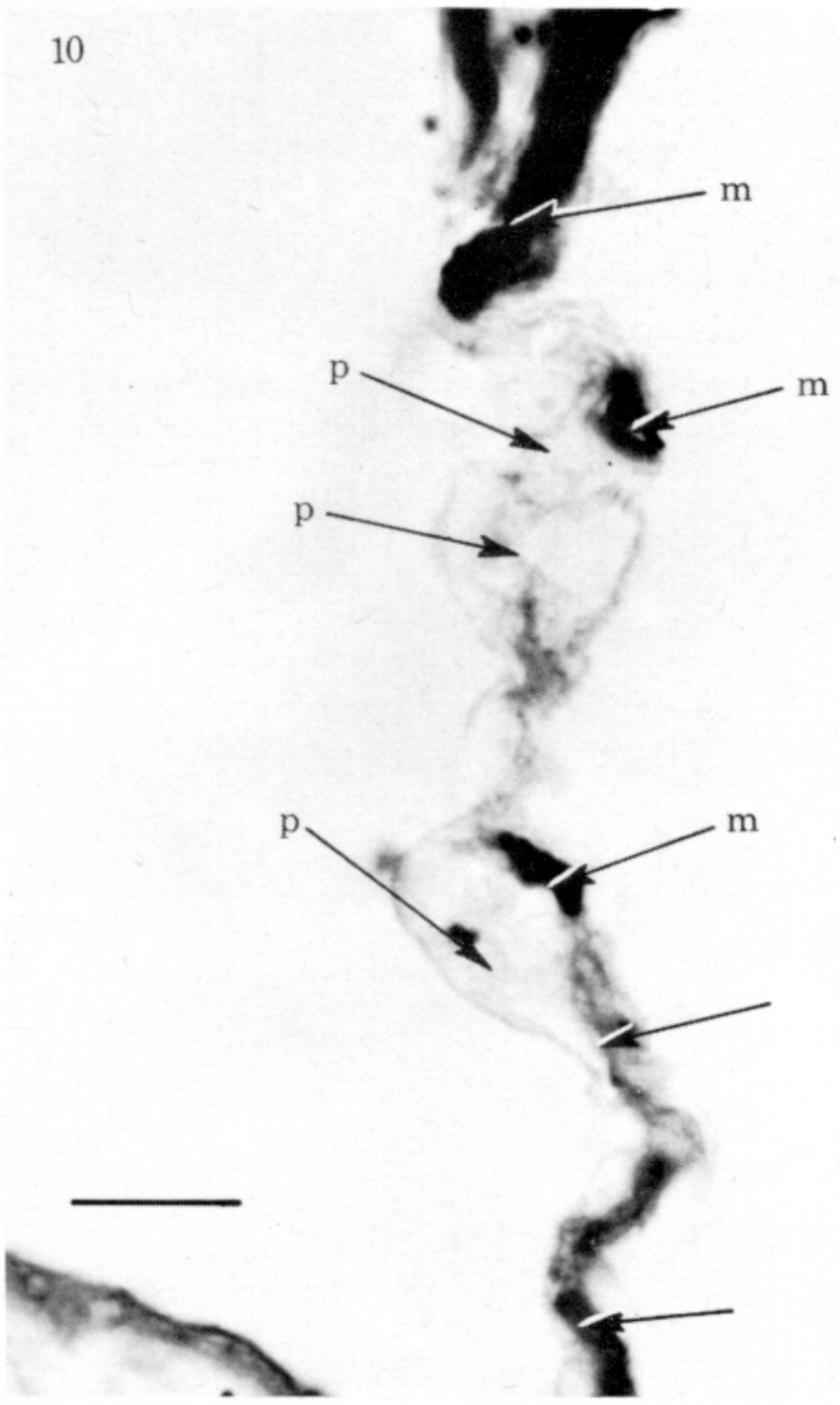
FIGURE 5. P.g.c. inside the mesentery of stage 43 embryo, coaligned with the array of oriented c.e.cs. The p.g.c. (arrowed) is easily recognizable because it has been broken during preparation, and the yolk granules in the cytoplasm are exposed. Bar = 12 μm.

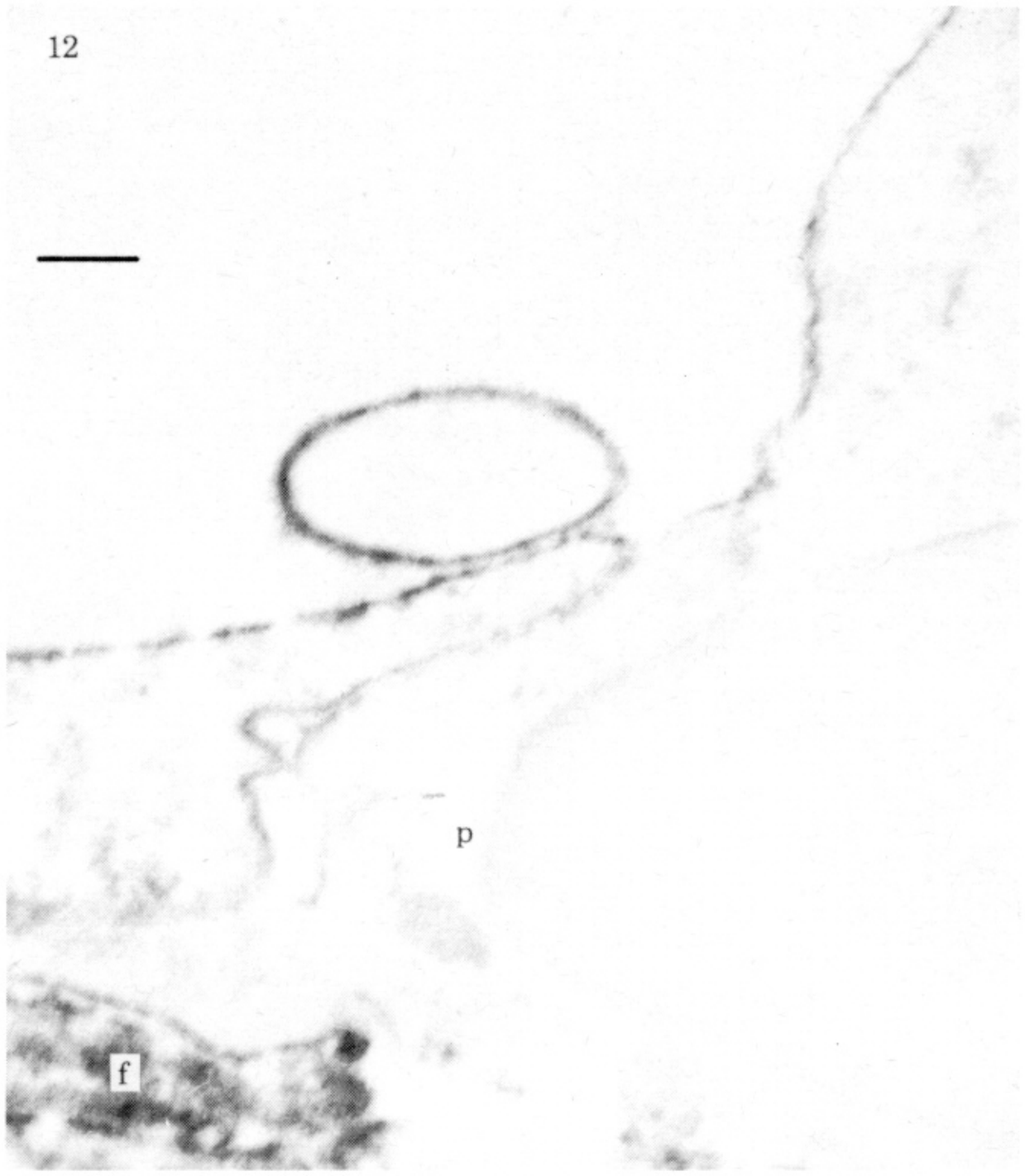
FIGURE 6. By stage 44, most p.g.cs are nearer to the dorsal body wall, and seen coaligning with c.e.cs oriented towards the dorsal body wall. P.g.cs are seen as lumps under the c.e.cs (arrowed). Bar = 12 μm.

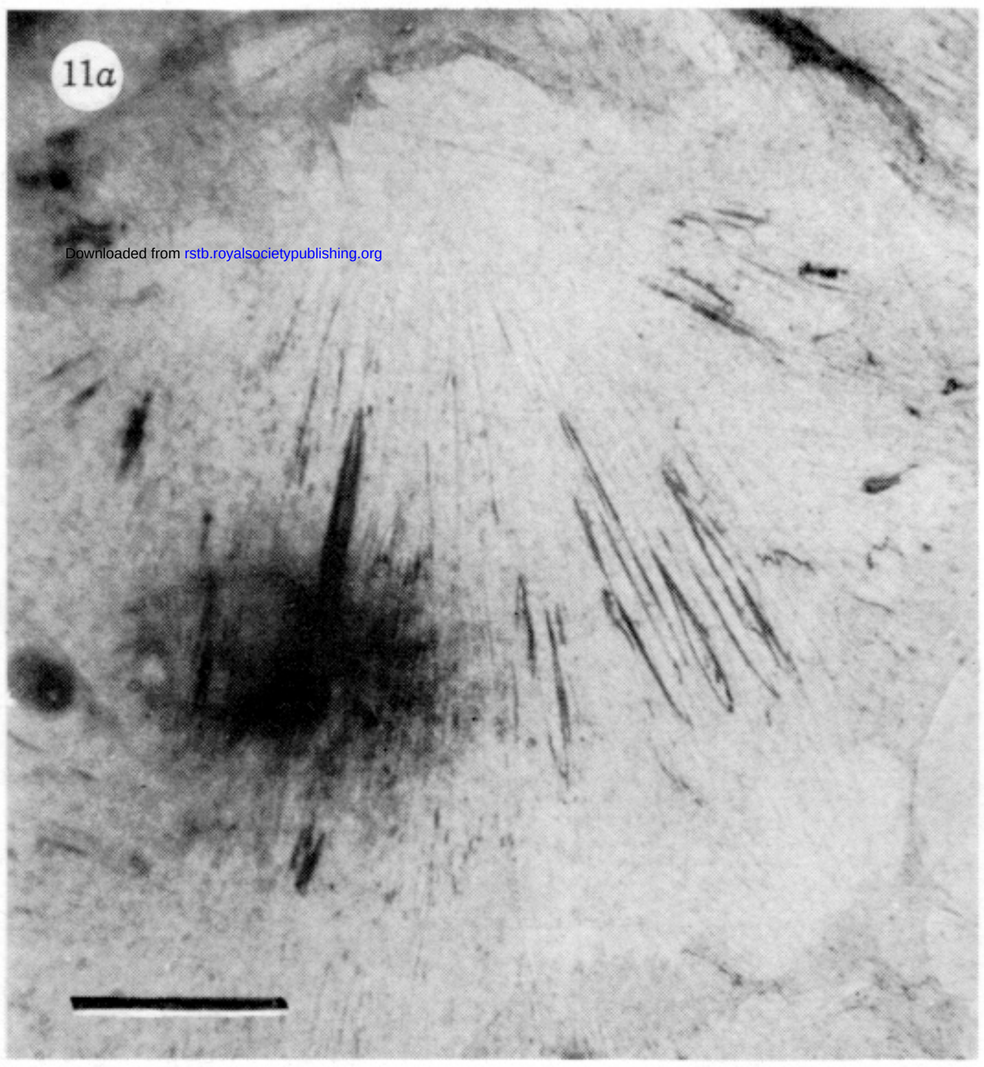
FIGURE 7. Transverse section of stage 43 embryo mesentery, showing the extensive filamentous cytoskeleton of the c.e.c. array. Bundles of filaments (cut in cross section) are particularly large near margins of adjacent c.e.cs (arrowed). Bar = 1 μm.

FIGURE 8. Some of the c.e.c. filaments are inserted into desmosomes between c.e.cs. This section is at the site of a change in orientation of c.e.cs. Filaments on either side of the desmosome are thus cut in opposite planes of section. Bar = 0.2 μm.

FIGURE 9. Filopodium of p.g.c., showing microfilamentous core. Filopodia coalign with the c.e.cs and their filament bundles. Bar = 0.2 μm.







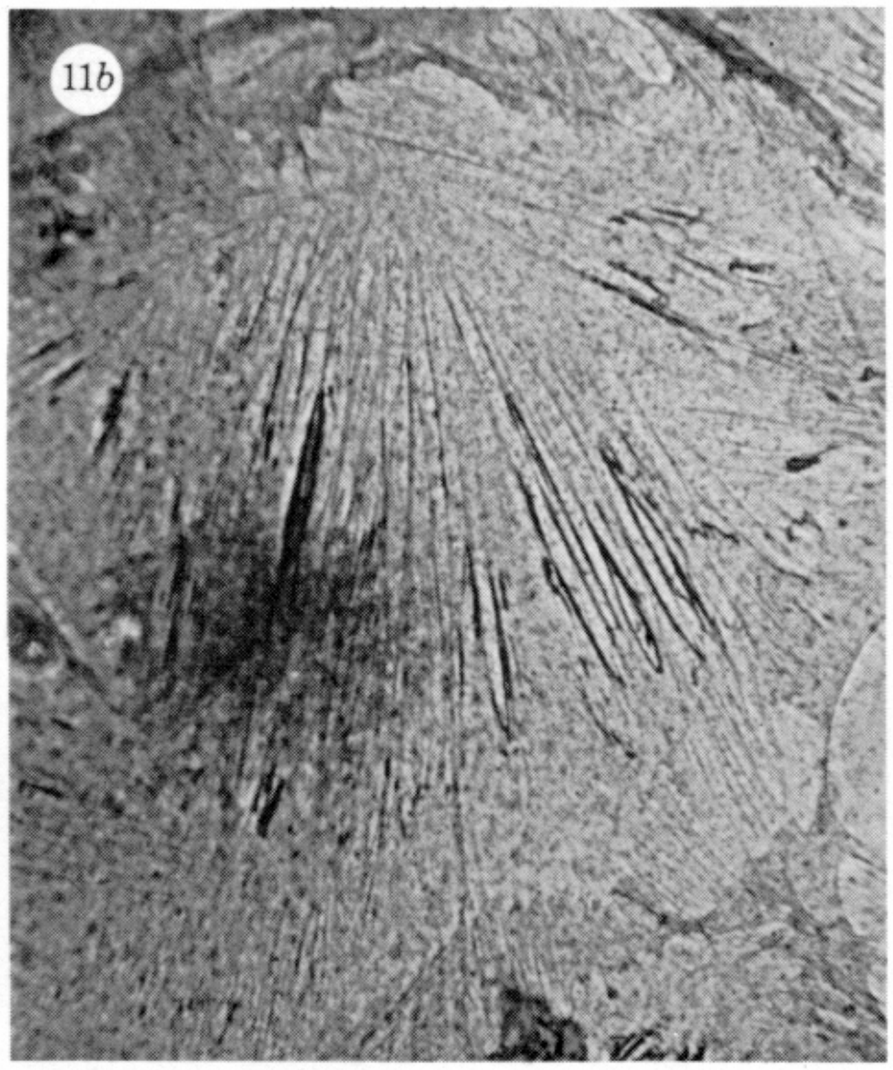


FIGURE 10. Light micrograph of immunoperoxidase-stained mesentery of stage 44 embryo. Anti-fibronectin stains the core of the mesentery (arrowed) and outlines the p.g.cs (p) with a fine golden-brown precipitate. Melanocytes, containing black pigment granules. Bar = 10 μm.

FIGURE 11. Bright-field (*a*) and phase-contrast (*b*) micrographs of the same field of view, showing coalignment of the fibronectin, visible as a dark precipitate in (*a*) by the immunoperoxidase reaction, with the stress fibres, seen in phase-contrast in (*b*). Bar = 30 μm.

FIGURE 12. Electron micrograph, stained by the indirect immunoperoxidase reaction with anti-fibronectin, showing a p.g.c. (p) pushing a process beneath the feeder layer of mesentery cells, and running along the fibronectin-containing fibrillar material beneath (f). Bar = 0.2 μm.

FIGURE 13. Isolated p.g.c. tightly attached to the basement membrane of isolated adult frog mesentery. Note that at the edges of the p.g.c. the basement membrane is ruffled. Bar = 2 μm.

FIGURE 14. (*a*) Isolated *Xenopus* p.g.c. on extracellular matrix of the chick embryo. Bar = 4 μm. (*b*) Higher-power view of a filopodium from a p.g.c. similar to that shown in (*a*). Lamellar areas and microspikes are seen, similar to those in figure 2. Bar = 2 μm.