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Cinemicrographic study of the cell movement in the primitive-streak-stage mouse embryo

N. NAKATSUJI[1,2], M. H. L. SNOW[2]

[1]*Division of Developmental Biology, Meiji Institute of Health Science, 540 Naruda, Odawara 250, Japan*

[2]*MRC Mammalian Development Unit, University College, Wolfson House, 4 Stephenson Way, London NW1 2HE, UK*

AND C. C. WYLIE

Department of Anatomy, St George's Hospital Medical School, Cranmer Terrace, Tooting, London SW17 0RE, UK

SUMMARY

Migration of the mesoderm cells in the primitive-streak-stage mouse embryo was directly studied by cinemicrography using whole embryo culture and Nomarski differential interference contrast optics. Relative transparency and small size of the early mouse embryos enabled direct observation of the individual cells and their cell processes. Seven-day-old mouse embryos were isolated and cultured in a small chamber in a medium consisting of 50 % rat serum and 50 % Dulbecco's modified minimum essential medium. The mesoderm cells move away from the primitive streak in both anterior and antimesometrial (distal) directions at a mean velocity of 46 $\mu m\,h^{-1}$. They extend cell processes and constantly change cell shape. They do not translocate extensively as isolated single cells, but usually maintain attachment to other mesoderm cells. They show frequent cell division preceded by rounding up of the cell bodies, and accompanied by vigorous blebbing before and after cytokinesis. This study shows that it is possible to examine the motility of embryonic cells inside the mammalian embryo by direct observation if the embryo is small and transparent enough for the use of the Nomarski optics.

INTRODUCTION

How embryonic cells move inside the embryo during morphogenetic movements is an important problem in developmental biology which requires more attention than has so far been paid (for reviews, see Trinkaus, 1976, 1984). Direct observation of cell movement inside the embryo, however, is not possible in most cases. The few fortunate examples include the relatively transparent embryos of sea urchin (Dan & Okazaki, 1956; Gustafson & Kinnander, 1956), teleost (Trinkaus, 1973; Trinkaus & Erickson, 1983) and nematodes (e.g. Deppe *et al.* 1978). In the case of opaque embryos such as amphibians and chick, the morphogenetic cell movements have been studied using fixed samples and various forms of microscopy, or by following carbon particles or vital dye markers applied to the

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surface (e.g. Malan, 1953; Spratt & Haas, 1965; Daniel & Olson, 1966). Additional information has been obtained by correlating *in vitro* and *in vivo* studies, in which culture conditions allowed the isolated embryonic cells to behave in a similar fashion to that *in vivo* (Heasman, Mohun & Wylie, 1977; Heasman *et al.* 1981; Wylie & Heasman, 1982; Nakatsuji & Johnson, 1982, 1983; Nakatsuji, 1984).

Early mouse embryos are relatively transparent because of the small amount of yolk. However, they are not accessible *in situ*, due to the enveloping uterine tissues. Methods of culturing postimplantation embryos have been considerably improved recently for mouse embryos (Hsu, 1979; Tam & Snow, 1980; Chen & Hsu, 1982) as well as rat embryos (for reviews, see New, 1973, 1978). Nomarski differential interference contrast optics (DIC) enable images of optical sections inside three-dimensional cell masses to be seen, provided they are transparent enough. Utilizing these methods, we have attempted to look directly at the migration of the mesoderm cells inside the whole embryo and record it by time-lapse cinematograpy. The hypothesis that the mesoderm cells migrate from the primitive streak in both anterior and antimesometrial (distal) directions has been based on the examination of the fixed samples (Batten & Haar, 1979; Tam & Meier, 1982; for a review, see Beddington, 1983). Whether they indeed migrate actively or passively during the course of gastrulation has not yet been settled (Poelmann, 1981). Our time-lapse films in this study show that the mesoderm cells are motile and migrate actively away from the primitive streak.

MATERIALS AND METHODS

Randomly bred Q strain mice were kept in a room with a 12 h light (7 a.m. to 7 p.m.), 12 h dark cycle. The noon of the day when the vaginal plug was found was designated as 0·5 day of pregnancy. For cinemicrography, 7·5-day-old embryos were dissected out from the uterus and decidua. Reichert's membrane and ectoplacental cone trophectoderm were removed. These embryos were mostly at the middle primitive-streak stage, in which the mesoderm cell layer was already formed in most parts of the egg cylinder. To obtain earlier stages, random-bred MF1 strain mice were kept in a room of reversed light–dark cycle. Embryos were dissected out at 7 days. In these early primitive-streak-stage embryos, there still was an area free of mesoderm cells between the epiblast and primary endoderm layers. Two different strains of mouse were used in these experiments because they develop at different rates. MF1 embryos develop faster than Q strain. This fact maximized our chances of finding embryos at precisely the right stage, namely when the presumed mesoderm migration was still progressing.

Methods of whole-embryo culture were based on those described in Tam & Snow (1980). A few modifications were made to allow cinematography. *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid (Hepes) was used as a buffer, to avoid a pH rise during cinematography in the absence of gassing. The medium was 50 % rat serum prepared by the methods described in Tam & Snow (1980) on the day prior to the experiment, and 50 % Dulbecco's modified minimum essential medium (DMEM) containing 20 mM-Hepes (Flow Laboratories) supplemented by sodium pyruvate, L-glutamine and antibiotics as described in Tam & Snow (1980).

The isolated embryos were cultured in a chamber that consisted of a base glass plate with an approximate thickness of 1 mm and a lid with a glass cover slip. The distance between the base glass plate and the top cover slip can be adjusted by a screw thread. Thus the embryos were cultured sandwiched between two glass plates that very slightly compress the embryo to keep it still during cinematography.

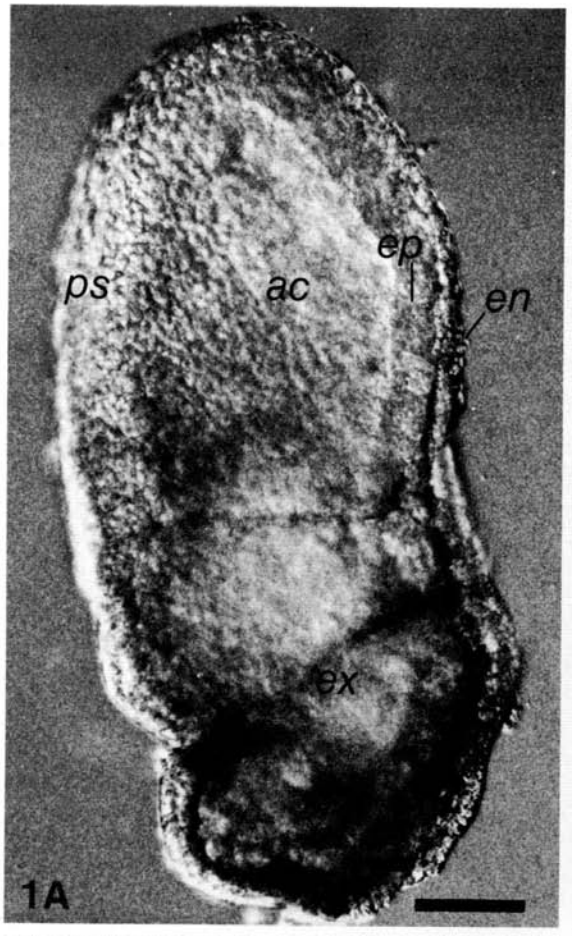
The prepared chamber was set on a warm stage (Microtec Warm Stage) to keep it at 37 °C. This in turn was mounted on the mechanical stage of a Zeiss photomicroscope III equipped with

DIC and a long working distance condenser lens. An objective lens of ×40 was used to record cell migration. Kodak Plus-X films were used for the time-lapse cinematography using a Bolex 16 mm camera controlled with Paillard-Wild Variotimer system (MBF-A, B and C). Interval time was set at 15 s, with exposure time of 0·5 s. Duration of the filming used for analysis was limited to 5 h, because the small volume of medium available to the embryo prevents normal development for longer periods. In several films taken for more than 12 h, degenerative appearances such as general shrinkage of the cell processes and continuous blebbing without cell division started almost simultaneously in many cells around 10 h from the start of filming. We believe that the cells inside embryos showed normal behaviour at least during the initial 5 h from the start of filming, because preliminary experiments showed that the 7-day-old embryos kept for 5 h in the chamber for cinematography developed to more advanced embryos with neural plates if they were transferred into a culture dish and then maintained in an incubator for one day. Another observation that indicated that development was proceeding actually during the filming was that size of the whole embryo showed certain growth by the end of filming. Time-lapse films were analysed by projecting frames at 2 min intervals and marking the approximate centre of the cell body of the mesoderm cells. These marks were connected with straight lines to give the cell trails as shown in Fig. 3. A 16 mm film projector–analyser (NAC Co., Japan) was used for such analysis. The total length of the zigzag cell trail for each 30 min was measured with a KONTRON IBAS I image analysis system.

RESULTS

Quality and resolution of the optical image is dependent critically on the thickness of the embryo. In this sense, the results on 7-day-old mouse embryos are marginal. Frequently, slight compression of the embryo by the upper cover slip of the chamber dramatically improves the optical image, but this is offset by the possibility that such compression might affect the normal development of the embryo. Fig. 1 shows prints from 35 mm still photographs. Fig. 1A shows a whole embryo at lower magnification. Usually, the embryo lies in the chamber sideways with right or left side down. We can recognize the side of the image with the primitive streak, because the already established mesoderm cell layer near the primitive streak obscures the inner border of the epiblast layer. Also, when the focal plane is altered, we can recognize two cell layers (epiblast and primary endoderm) with the mesoderm cells between them near the primitive streak, whereas the opposite side lacks the mesoderm cell layer.

When the focal plane is moved into such a cultured embryo from above, the first cell layer encountered is the primary endoderm (Fig. 1B). This is an epithelium-like cell layer consisting of very flat cells connected to each other very tightly with clear cell borders. The cells contain many vacuoles, whose number and size increase towards the mesometrial (proximal) end of the egg cylinder. As the focal plane is slightly lowered, mesoderm cells with large intercellular spaces are seen (Fig. 1C). These cells show widely varying shapes, and possess several types of cell process, including frequent slender filopodia, rounded lobopodia, and flattened lamellipodia. These processes are attached to the inner surface of the endoderm cell layer or outer surface of the epiblast layer. Further down can be seen the epiblast layer (Fig. 1D). This appears as a tightly packed epithelium-like cell layer, whose cells appear smaller than in the endoderm layer because of their columnar shape. Each cell contains a round organelle, probably the nucleus. The resolution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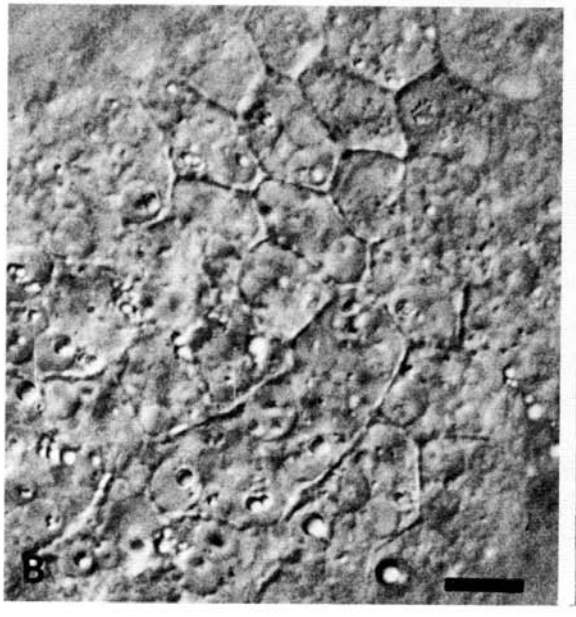
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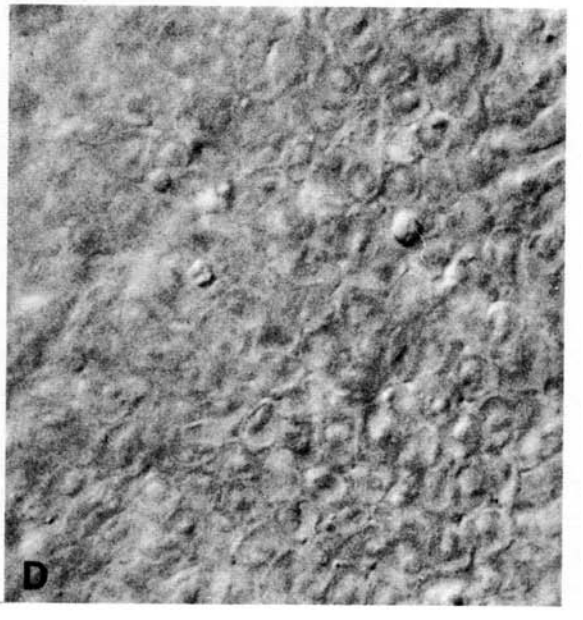
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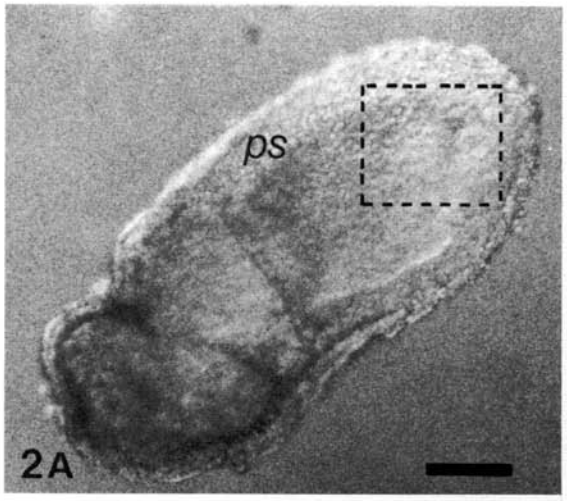
decreases when looking at the deeper layer of the cell mass, thus the primary endoderm shows the best resolution, and the epiblast the worst. The resolution of the mesoderm layer is good enough to recognize each cell and its cell processes.

Fig. 2 shows prints from a 16 mm film. This sequence shows movement of the cells and cell division in the anterior and antimesometrial part of an early primitive-streak-stage embryo, where there were still remaining spaces free of mesoderm cells. Mesoderm cells translocate as individual cells, but frequently they maintain attachment with one or two other cells, and move in the same direction. The shape of whole cells and their processes constantly change during migration. Cells move in a zigzag fashion (Fig. 3), but the direction of movement is mostly away from the primitive streak in anterior and antimesometrial directions (Figs 3, 4). Each cell can be followed in the film usually for at least 30 min and sometimes for 2 h. However, the cells eventually escape from the selected focal plane of the film. Measurements of the lengths of the cell trails for each 30 min give a rate of $46 \pm 10\,\mu m\,h^{-1}$ (mean and standard deviation of 116 cell trails). Sometimes, an area with slower cell movement exists next to an area with faster cell migration. Rapid migration usually occurs in the areas of lower population density of mesoderm cells and larger spaces between cells. On the other hand, slower cell migration appears in the regions where the mesoderm cell layer is already established and cells are packed tightly. For example, Fig. 3 contains an area with slow cell migration and the remaining area has faster cell movement. The direction of the movement is uniformly away from the primitive-streak region, but cells do not move in parallel fashion. Instead, they take trails of varying zigzag shape. Fig. 4 shows the result of movement for 30 min. Again, it shows a strong tendency toward the anterior and antimesometrial directions, but with substantial variation.

Cells of the primary endoderm layer or epiblast layer do not show any individual movements. In several films, these layers appear besides the mesoderm cells (such as in Fig. 1C) because of the convex shape of the embryo surface. In both cell layers, cells appear to be tightly connected with neighbouring cells, and translocate only slowly and smoothly as a cell sheet when the whole embryo shows growth in size during the filming, but never move independently as individual cells. These films also show that the mesoderm cells are migrating relative to the

Fig. 1. Still photographs of a 7·5-day-old mouse embryo isolated and cultured in the chamber for cinemicrography. DIC optics were used for all the pictures. (A) A low magnification view of the whole embryo. Scale bar, 0·1 mm. *ac*, amniotic cavity; *en*, primary endoderm; *ep*, epiblast; *ex*, extraembryonic tissues; *ps*, primitive streak. The inner border of the epiblast layer is obscured by the mesoderm cells in the area close to the primitive streak. (B–D) Higher magnification views when the focal plane was gradually lowered from B to D. (B–D) The same magnification; scale bar in B, 10 μm. B shows the primary endoderm layer consisting of very flat cells with distinct cell borders. They contain many vacuoles whose size and number increase in the mesometrial (proximal) region. C shows several mesoderm cells with large intercellular spaces. An arrowhead indicates a lobopodium-like protrusion. Double arrowhead indi cates a filopodium. *en*, primary endoderm layer. D shows the epiblast layer. The cell size appears smaller than other layers. The nucleus can be recognized inside each cell.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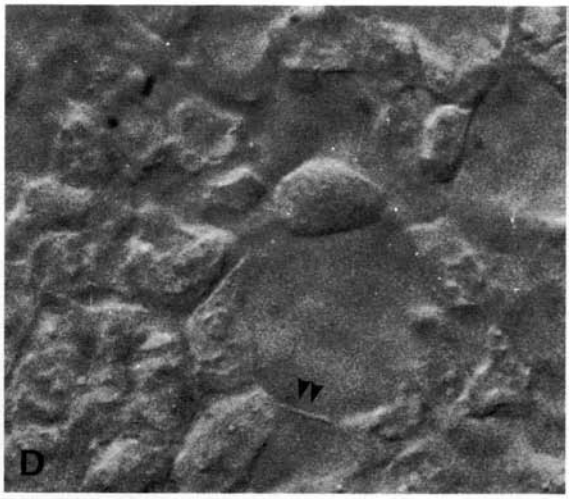
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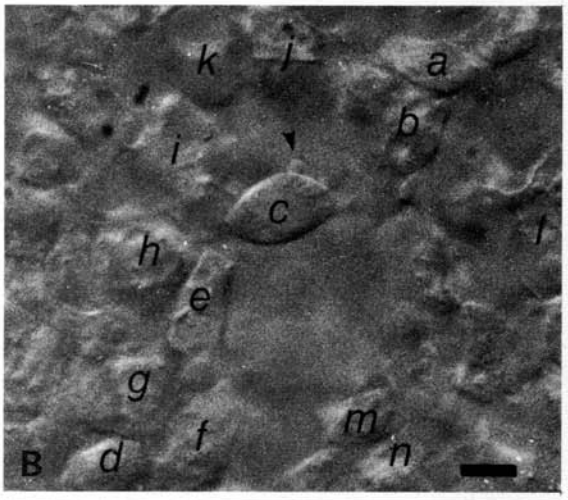
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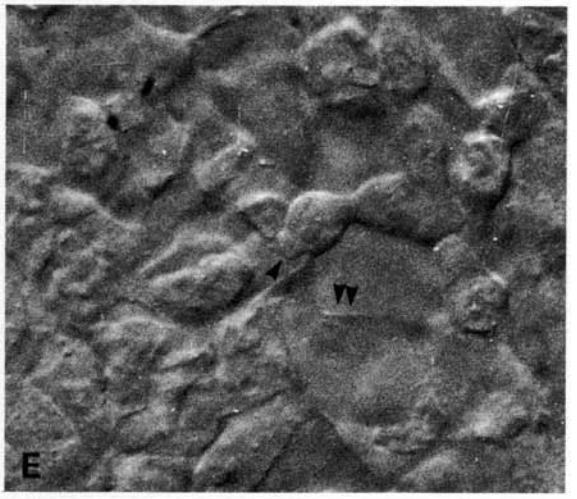
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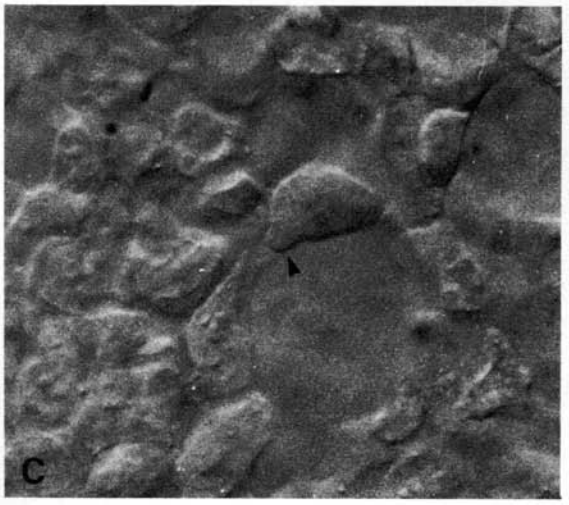
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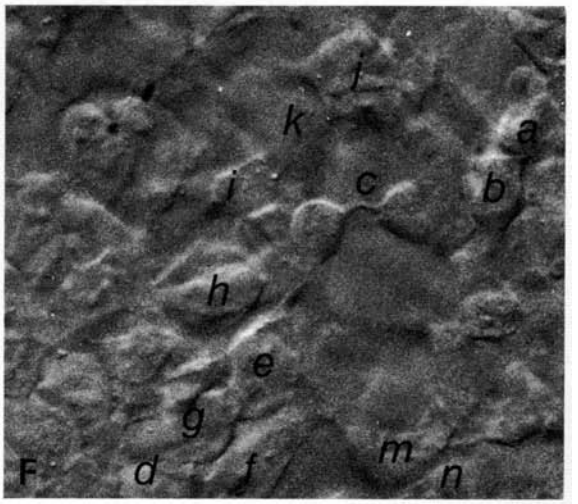
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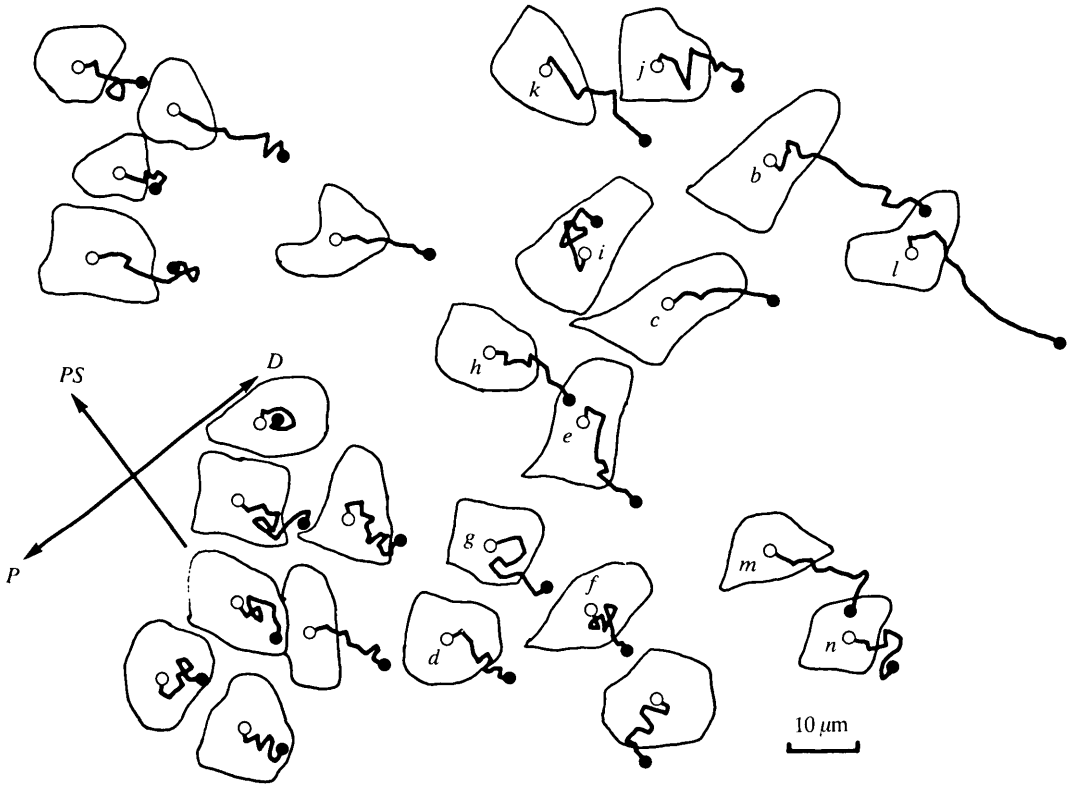

Fig. 3. Cell trails for 30 min obtained from the same segment of the film shown in Fig. 2. The labels *b–l* correspond to the labelled cells in Fig. 2B,F. Open circles show starting points of the cell centre. Solid circles, finishing points. *PS*, direction toward the primitive streak; *P*, proximal (mesometrial) direction; *D*, distal (antimesometrial) direction.

epiblast and the primary endoderm layers, and so exclude the possibility that growth or distortion of the whole embryo produce an artificial apparent movement of the mesoderm cells.

One interesting feature of these films is cell division. Fig. 2 shows one example. We analysed time courses of eight such sequences. They start as the cell body becomes rounded from the previously more angular cell shape with processes.

Fig. 2. Prints made from 16 mm time-lapse films. (A) Low magnification view of a 7-day-old embryo in the same orientation as B–F. Scale bar, 0·1 mm. The square indicates the approximate area shown in B–F. *ps*, primitive streak. (B–F) A sequence from a film showing cell division of a mesoderm cell (*c*). Scale bar, 10 μm. Cells in the top and right part of the picture move to the right and bottom direction (such as cells labelled as *a* and *b*), while cells in the left-bottom part (such as cell *d*) show only small movement. Arrowheads in B and C indicate blebs formed during the rounding up of the cell body before the cytokinesis. Double arrowheads in D and E indicate filopodia. The cytokinesis of cell *c* starts in E and almost finishes in F. The arrowhead in E points to a bleb formed after the cytokinesis started. Cells labelled as *b–l* correspond to the cell trails in Fig. 3. Times from B are 9·0 min (C), 10·2 min (D), 19·7 min (E) and 23·0 min (F).

Very shortly, vigorous blebbing occurs around the cell body until a furrow appears around the equator. Cytokinesis starts 20 to 30 min after rounding up of the cell body. Blebbing starts again during telophase as sister cells move away from each other (30 to 40 min after the rounding up). The sister cells resume normal cell shape with cell processes after 40 to 50 min from the first sign of the rounding up and blebbing.

DISCUSSION

The present study shows that it is possible to look at and record the cell migration inside the mouse primitive-streak-stage embryo by use of whole-embryo culture and DIC optics. This optical system is helped by the relative transparency and small size of the 7-day-old mouse embryo. Such direct observation may be possible for other morphogenetic movements in mammalian embryos.

The mesoderm cells in the primitive-streak-stage mouse embryo migrate as individual cells, not as a coherent cell sheet. Constant changes of cell shape and protrusion of cell processes strongly suggest that the observed migration is due to active locomotion by the mesoderm cells. The direction of migration is mostly away from the primitive streak and toward the anterior and antimesometrial directions. Cells do not move either in parallel fashion or in unison. They show considerable variability in both rate and direction of movement. The variability and individuality of the mesoderm cell movements are not shown by the epiblast

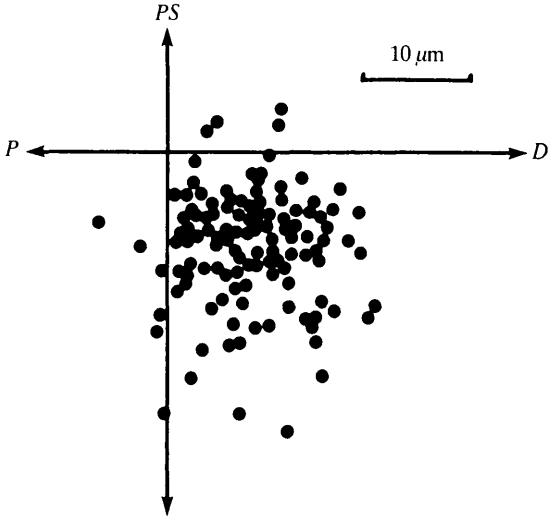

Fig. 4. Distribution of the finishing points of the cell trails for 30 min, such as shown in Fig. 3, when the starting point is superimposed on the origin of the coordinate. *PS*, *P* and *D* indicate directions as described in Fig. 3. The most points distribute within the quadrant between the distal (antimesometrial) direction and that away from the primitive streak.

and the primary endoderm layers, suggesting that the observed movements of the mesoderm cells are real and not an artifact caused by growth or distortion of the whole embryo. These results are consistent with the hypothesis deduced from the examination of fixed samples by sectioning and scanning electron microscopy (Spiegelman & Bennett, 1974; Batten & Haar, 1979; Tam & Meier, 1982). The average rate of the movement (46 $\mu m\, h^{-1}$) is enough for a mesoderm cell to travel from the primitive streak to other areas of the embryo, which has an approximate diameter of 0·3 mm at this stage, within one day (from 6·5 days to 7·5 days old). The mesoderm cells show faster movements in regions where there are large intercellular spaces. In regions already crowded with mesoderm cells, they move more slowly and more in unison with other mesoderm cells. One reason for such differences may be contact inhibition of movement that would direct cells toward the free spaces. The mesoderm cells show frequent cell divisions while they are migrating away from the primitive streak. Such cell divisions are accompanied by extensive blebbing, both before and after cytokinesis. It is well known that cultured tissue cells show similar blebbing during cell division (Porter, Prescott & Frye, 1973; Harris, 1973). The frequent cell division observed in these films is consistent with the high rate of cell proliferation reported at this stage of development (Snow, 1977, 1978).

Most of the successful films we took were of middle primitive-streak-stage embryos. In these embryos, in contrast to the early primitive-streak-stage embryos shown in Figs 2, 3, the mesoderm cell layer is already established in most parts of the embryo, and not much free space for the mesoderm cells remains. In these films, the mesoderm cells move relatively slowly in a similar fashion to the cells in the lower-left part of Fig. 3. Therefore, it would be more interesting to take films of earlier embryos with larger mesoderm-free areas. However, the difficulty of achieving normal development of the embryo increases dramatically around this stage. For example, it was extremely difficult to culture 6·5-day embryos, which showed success rates of only 20 % in culture dishes in our preliminary experiments. Since the chamber for filming is much less optimal than the Petri dish, we chose 7-day-old embryos for the best chance of recording the movements of mesoderm cells.

Even so, it turned out to be difficult to maintain normal development whilst at the same time obtaining reasonable optical conditions. Therefore, it would be extremely helpful if one could improve the culture conditions that can be used for the time-lapse cinematography or video recording. For the study of mesoderm formation and migration, the best stage is the very early primitive-streak stage in which the mesoderm cells have just emerged from the epiblast and started their migration between the epiblast and the primary endoderm layer.

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