sections, indicating that the fixation reduced only slightly the antigenicity of the myosin-like protein. The present results indicate that the parathyroid principal cells seem to be not kinetically passive but active, because they contain contractile elements; they might be able to produce discrete movements in the living organism.

The effective role of acto-myosin-like proteins in nonmuscular cells is not yet fully clear. In some types of cells, an actin-like protein is thought to take part in the microfilamentous structures seen close to cell membrane. Very little is know about the site and the functional role of the myosin-like protein.

Previous papers by our laboratory[9,10,13] have demonstrated the presence of a myosin-like protein in cells very rich in microfilamentous structures. Studies by the electron microscope have demonstrated that the cytoplasm of parathyroid principal cells contains microtubules and microfilaments[15-17]. The microtubule-microfilament system in some endocrine glands play a role in the secretory activity of several kinds of cells[18-20]. The correspondence between the immunofluorescence and the E.M. observations make it reasonable to suppose that the myosin-like protein could take part in the structure of the microtubular-microfilamentous cellular system.

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Evidence for changes in cell shape from a 2-dimensional to a 3-dimensional substrate

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Summary. Chick embryo mesoderm cells were explanted to culture systems in vivo and in vitro and their subsequent movements were correlated with the external morphology as studied by SEM. In vitro cell movements are exaggerations of normal in vivo movements where a 2-dimensional substrate is encountered rather than a 3-dimensional environment.

Cell movement in vitro has been extensively studied in attempts to understand movement in vivo[1-3]. Various substrates, media and environmental conditions have been devised which allow cells to survive for long periods in culture. Numerous studies[4,5] have reported that various cell types in different culture systems have similar patterns of movement; lamellipodia, leading lamellae and ruffling activity. When new methods allowed further examination of cells in vivo it was reported by many experimenters that the characteristic morphological features of cell movement in vitro were largely absent from tissues in vivo[6].

Further studies using collagen in culture systems have shown that fibroblasts assume a bipolar spindle form on this substrate but do not invade the interior lattice[7].

We report here a series of experiments to demonstrate whether the differences between the 2 systems relate to the 2-dimensional substrate provided in an in vitro system and the 3-dimensional substrate in vivo. All specimens were examined on the advancing cell edge and on the internal aspect of the mass of cells. White Leghorn chick embryo mesoderm cells stage 4[8] were studied in ovo; in wounded New Culture systems[9]; in vivo on a cellular substrate; in vitro on glass coverslips and on Sterispon Absorbable Gelatin Sponge. In ovo specimens were incubated at 37.5 °C until stage 4. Other stage 4 embryos were mounted as for New Culture and an incision 0.2–0.3 mm long was made in the area pellucida endoderm adjacent to the area opaca border on the level of Hensen's node. 3 embryos were re-incubated at 37.5 °C and the healing wound examined at 30 min; 1 h and 2 h. In another group mesoderm cells to be grown on a cellular substrate were introduced onto the area opaca ventral ectoderm layer of a host chick embryo stage 4 mounted as for New Culture.

The overlying endoderm layer had previously been surgically removed in an area 0.5 mm × 0.5 mm. Host embryos were re-incubated at 37.5 °C for 1 h. Mesoderm cells were also cultured in vitro on glass coverslips and Sterispon Absorbable Gelatin Sponge BP (Allen and Hanburys Ltd., London) at 37.5 °C in TC 199 containing 10% serum, penicillin, streptomycin. All specimens were fixed in Karnovsky's fluid[10], buffered in cacodylate[11], osmicated in a 2% solution and dehydrated in a graded series of ethanol until 100%. They were then transferred to 100% acetone; critical point dried by acetone replacement with liquid CO_2, mounted on Cambridge stubs with colloidal silver paint and coated with 20 nm of gold. The specimens were examined in an ISI 60 scanning electron microscope.

Mesoderm cells in ovo on the advancing edge follow the ectoderm substrate first. They have numerous filopodia on the leading cell edge. Mesoderm cells in ovo internal to the layer are stellate and numerous filopodia on each cell contact approximately 8–12 adjacent cells in the mesoderm,

ectoderm and endoderm layers. Occasional small ruffles are present on a mesoderm cell (figure 1). Although numerous investigators[12-14] have demonstrated the general direction of movement of groups of cells in the chick embryo, no precise method has been described where an individual cell's movement can be determined by its external morphology. The problem is even more complex in a moving sheet of cells[15] where cell interrelationships, for example, the angle of initial contact, must also be considered.

In all of our experimental specimens the direction of cell movement was known. Mesoderm cells identical to those in ovo on the leading edge were observed in the wounded New Culture systems. Cells adjacent to the wound moved by initially extending lobopodia and ruffled membranes into space. Ruffles contacting the substrate flattened out onto the surface, those not establishing contact remained twisted in space, and frequently their tips formed blunt, knobbed ends. The trailing edge of the cells was not visible through the wound.

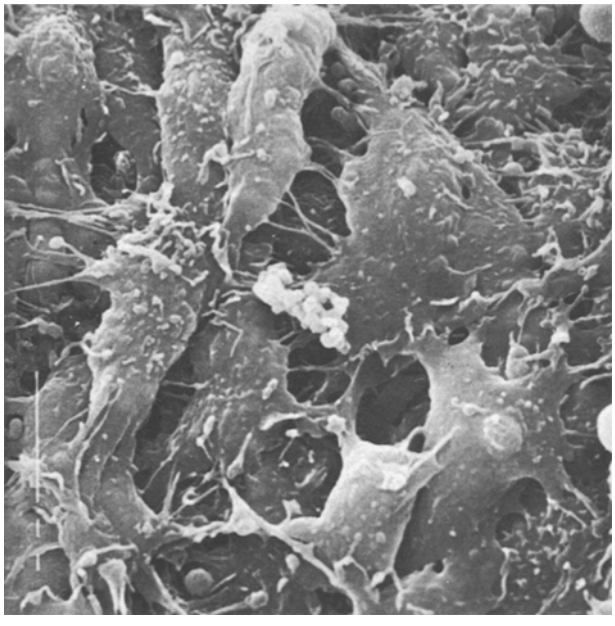

Fig. 1. Stage 4 chick embryo mesoderm cells in vivo. Bar represents 10 µm.

Mesoderm cells transplanted as a group onto ventral area opaca ectoderm were similar morphologically along their leading edge to those in the wounded embryo. The general cell shape was pointed with numerous lobopodia and ruffles on the leading edge. Those lobopodia establishing contact with the substrate were enlarged by cytoplasm streaming into the region until a webbed appearance was established around the cell's perimeter. The trailing edge was tapered and blunt. It frequently was raised above the level of the substrate and in contact with adjacent cells. In addition to studying the mesoderm cells moving out from the transplanted group of cells, we also studied the cells internally situated in the group and they too were morphologically identical to those observed in ovo internally in the mesoderm layer.

Mesoderm cells cultured on glass coverslips flattened onto the substrate (figure 2) and the characteristic features of in vitro cell movement described by many authors were present. Ruffling leading edges were present on numerous specimens. In contrast those cells transplanted to Sterispon sponges penetrated the sponge by following the individual gelatin fibres of the sponge until the fibres crossed one another (figure 3). Near these junctions, the mesoderm cells which appeared morphologically similar to those cells grown in petri dishes assumed the appearance of in vivo specimens. This change was not only apparent generally near the junction of fibres but individual cells at one end were flattened and at the other end were displayed as in vivo contacting multiple substrates.

This in vitro system was truly 3-dimensional using the invasion of the substrate by the cells as a criterion of its similarity to an in vivo system. Although the substrate used by Elsdale and Bard[7] provided a 3-dimensional surface it

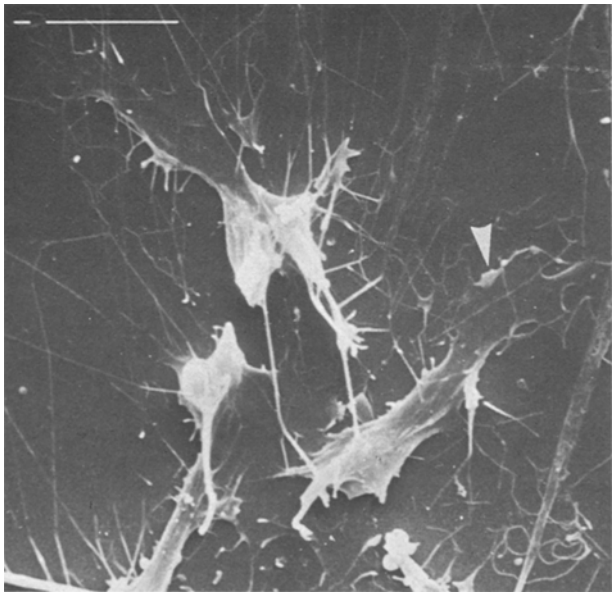

Fig. 2. Stage 4 chick mesoderm cells cultured on glass. The cells are flattened onto the substrate and characteristic features of in vitro cell movement are present (e.g. ruffles arrowed). Bar represents 10 µm.

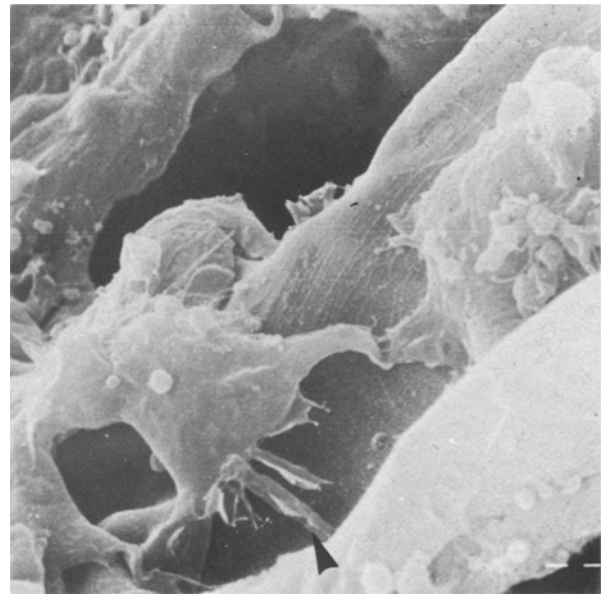

Fig. 3. Stage 4 chick mesoderm cells cultured on Sterispon sponges. Where the gelatin fibres of the sponge cross one another, the mesoderm cells assume the appearance of in vivo specimens (arrowed). Bar represents 1 µm.

was not 3-dimensional in an invasive situation similar to in vivo models. The cells had a 3-dimensional 'floor' to move across, but lacked the 3-dimensional 'ceiling' necessary to simulate the in vivo situation.

Several authors[16,17] have reported that a cell will respond differently on different substrates and that age and the state of differentiation[18] affect cell movement. Our observations show that cells respond differently in 2-dimensional and 3-dimensional systems. We would confirm that the classical described features of cell movement in vitro are present in vivo but are greatly diminished. The morphological features normally associated with in vitro cell movement, we feel, are exaggerations of normal movements encountered in ovo because of the differences encountered between a 2-dimensional and 3-dimensional environment.

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Promotion of epithelial keratinization by N-methyl-N'-nitro-N-nitrosoguanidine in rat forestomach in organ culture[1]

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Summary. The effect of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) on epithelial differentiation of fetal rat forestomach was investigated in organ culture. When forestomach tissues removed from 16.5-day fetuses were treated with 5 µg and 3 µg of MNNG per ml for 1 h, epithelial keratinization was observed after 4 and 5 days, respectively, whereas it occurred after 6 days in control cultures. A clear dose-response relationship was found in the promotion of epithelial keratinization by MNNG.

N-Methyl-N'-nitro-N-nitrosoguanidine (MNNG) has been known since the report of Mandell and Greenberg[3], to be a powerful mutagen in microorganisms and viruses and it has also been widely used as a potent chemical carcinogen. When given to animals in their drinking water, MNNG induces malignant tumors in the digestive tract, especially in the glandular stomach and forestomach[4]. The reaction of MNNG with biological materials has been extensively investigated at the molecular level[5]. We report here that MNNG promotes epithelial keratinization in fetal rat forestomach in organ culture.

Materials and methods. Forestomachs were removed from the fetuses of inbred Fischer 344/DuCrj rats (Charles River Japan Inc., Japan) at 16.5 days of gestation. MNNG (Aldrich Chemical Co., USA) was dissolved in doubly distilled water (DDW) at appropriate concentrations, and 0.5 ml of the solution was added to 4.5 ml of Hanks' solution in dishes with the tissue fragments. Only DDW was added to control dishes. After incubation with MNNG for 1 h at 37 °C in the dark, tissue fragments were washed 3 times with Hanks' solution, soaked for 2 h in Hanks' solution containing 50% fetal calf serum, and finally

Effect of MNNG on the time-course of epithelial differentiation in organ culture

Concentration of MNNG (µg/ml)	Epithelial differentiation	Incubation time (days) 1	2	3	4	5	6	7
5	Not keratinized	4/4	6/6	12/18	1/17	0	0	0
	Partly keratinized	0	0	5/18	4/17	1/19	0	0
	Keratinized	0	0	1/18	12/17	18/19	6/6	13/13
3	Not keratinized	-	-	7/7	3/8	2/7	0	0
	Partly keratinized	-	-	0	3/8	2/7	0	0
	Keratinized	-	-	0	2/8	3/7	6/6	3/3
1	Not keratinized	-	-	2/2	5/5	3/6	0	0
	Partly keratinized	-	-	0	0	3/6	3/5	0
	Keratinized	-	-	0	0	0	2/5	5/5
Control	Not keratinized	3/3	2/2	14/14	17/17	11/18	1/10	0
	Partly keratinized	0	0	0	0	6/18	1/10	1/10
	Keratinized	0	0	0	0	1/18	8/10	9/10

-, Not examined.