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Scanning electron microscopy of the aggregation of head mesoderm cells from chick embryos

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Summary. Head mesoderm cells from chick embryos at different stages of development were dissociated and cultured on plastic coverslips. In all cultures several cellular aggregates were described by means of scanning electron microscopy. Isolated cells present filopodia and lamellipodia. However, when mesoderm cells make contact with one another the filopodial and lamellipodial activity in the contact cellular edge disappear. Thus, the cells into cellular clusters do not present projections. The clusters were circular and bidimensional in character. The scanning electron microscopic observations showed that it is the type 1 variant of "contact inhibition of locomotion" which occurs. By means of these mechanisms the bidimensional aggregates are formed and cellular overlapping is not present. Since the behaviour of the mesoderm cells "in vitro" in some way could be comparable to their behaviour "in situ", the results here observed are discussed in relation to the conduct of mesoderm cells "in vivo".

Key words: Aggregates - Mesoderm - SEM

Introduction

When embryonic cells are dissociated they become motile and can reaggregate (Moscona and Moscona, 1952; Stanisstreet and Jumah, 1982; Kurais and Stanisstreet, 1984; Chamorro, 1985; Foucaud and Gombos, 1985; Chamorro et al., 1986a). Recently, the features of spreading and adhesion of chick embryo mesoderm cells have been studied (Chamorro, 1985; Chamorro et al., 1986b), since they might provide clues to the mechanisms involved in normal morphogenesis (Stanisstreet and Jumah, 1982). Studies of the aggregation of early embryonic cells are of interest because it is likely that at least some of the cellular mechanisms operating during the reaggregation and sorting of embryonic cells are the same as those which effect normal morphogenesis (Kurais and Stanisstreet, 1984). Evidence for the correlation of the properties of cells "in vitro" and "in situ" comes from the results of studies on the properties of cells from genetically deficient hybrid embryos which arrest at gastrulation or gastrulate abnormally. Cells from such hybrid embryos show altered properties "in vitro" (Jumah and Stanisstreet, 1980). Similarly, cells isolated from embryos prevented from undergoing normal gastrulation by biochemical insult do not show the properties shown by cells from normal embryos (Kurais and Stanisstreet, 1980b). In the same sense, the types of cell movement shown "in vitro", the proportion of cells exhibiting movement and the morphological characteristics vary with the stage of the embryo from which the cells are taken (Kurais and Stanisstreet, 1980a; Chamorro, 1985).

The scanning electron microscope has revealed much about the morphological characteristics of isolated and aggregated embryonic cells (Stanisstreet and Jumah, 1982; Nakatsuji and Johnson, 1983; Chamorro et al., 1984; Arias et al., 1984; Paz et al., 1984; Chamorro, 1985) since the scanning electron microscopy (SEM) provides three-dimensional images at high resolution and allows observation and quantification of fine cellular projections (Le Blanc and Brick, 1981; Chamorro et al., 1986b).

Despite the usefulness of the scanning electron microscope in studies of embryonic cells and the use of cell aggregation as a model to elucidate the properties of cells important to embryogenesis, the process of the aggregation of mesoderm cells from chick embryo has not been morphologically studied by SEM.

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In previous reports, by using such microscopy, the morphological characteristics showed by reaggregates of neuroectoderm cells cultured in liquid medium have been studied (Arias et al., 1984). The cells of the reaggregate surface showed numerous filopodia, microvilli and beaded-threads but few lamellipodia. These results were interpreted in relation with sorting movements and cellular aggregation process. The more important morphological features of aggregates of chick neuroectoderm cells cultured on plastic substratum have also been described (Chamorro et al., 1984). Many of the isolated cells were rounded and frequently presented filopodia, blebs and lamellipodia. Nevertheless, the cells incorporated into the aggregates present a featureless surface and they are lacking filopodia and lamellipodia. The present paper describes the reaggregation of chick embryo head mesoderm cells cultured on plastic substratum by means of SEM.

Materials and methods

Fertile White Leghorn eggs were incubated to stages 6, 8, 10 and 12 (Hamburger and Hamilton, 1951). Cephalic areas (prenodal region at stage 6 and presomitic area at stages 8, 10 and 12) (Chamorro, 1986 a,b) were dissected and treated with 2% trypsin (Difco) and 0.3% carboxymethylcellulose in Ca^{2+} - and Mg^{2+} - free buffered Tyrode's solution at 37°C for 15 minutes. Addition of carboxymethylcellulose helps prevent damage to cell membranes during exposure to trypsin (Cole, 1971). The mesoderm was separated by gentle pipetting. Then, this layer was rinsed in Ca^{2+} - and Mg^{2+} - free buffered Tyrode's solution and incubated at 37°C for 15 minutes with 0.5% trypsin and 0.3% carboxymethylcellulose in the same solution. These mesoderm areas were rinsed and pippeted until total dissociation. The cells (10^6 /ml) were cultured on plastic coverslips into Leighton tubes with 1 ml of medium (minimal medium Eagle, 10% fetal calf serum, 6% glutamine, 100 U.I./ml penicillin, 100 µgr/ml streptomycin) for 24 hr at 37°C. The coverslips (9 x 35 mm) used as substratum were previously washed several times in ethanol, rewashed thoroughly with tap water and rinsed with an aqueous solution of alkaline detergent (Extran, Merck). Subsequently they were newly washed in tap water, rinsed with deionized and demineralised water and dried in a sterilizing oven.

Four experiences were carried out obtaining finally a sample of 16 cultures onto coverslips for each stage. Each coverslip was divided into four equal parts for SEM observation.

For scanning electron microscopy, the cultures were fixed with 1% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) at 37°C for 1 hr. Then they were washed three times in the same buffer, postfixed for 1 hr in 1% OsO_4 at room temperature and washed in the same way. The samples were dehydrated in increasing concentrations of ethanol and infiltrated with amylacetate. They were dried by replacing amylacetate with liquid CO_2 in a critical-point drying apparatus (CPD 010, Balzers), mounted on aluminium stubs and sputer-coated with gold-palladium. The cultures were observed with a Jeol 35 C scanning electron microscope at 20 kV.

Results

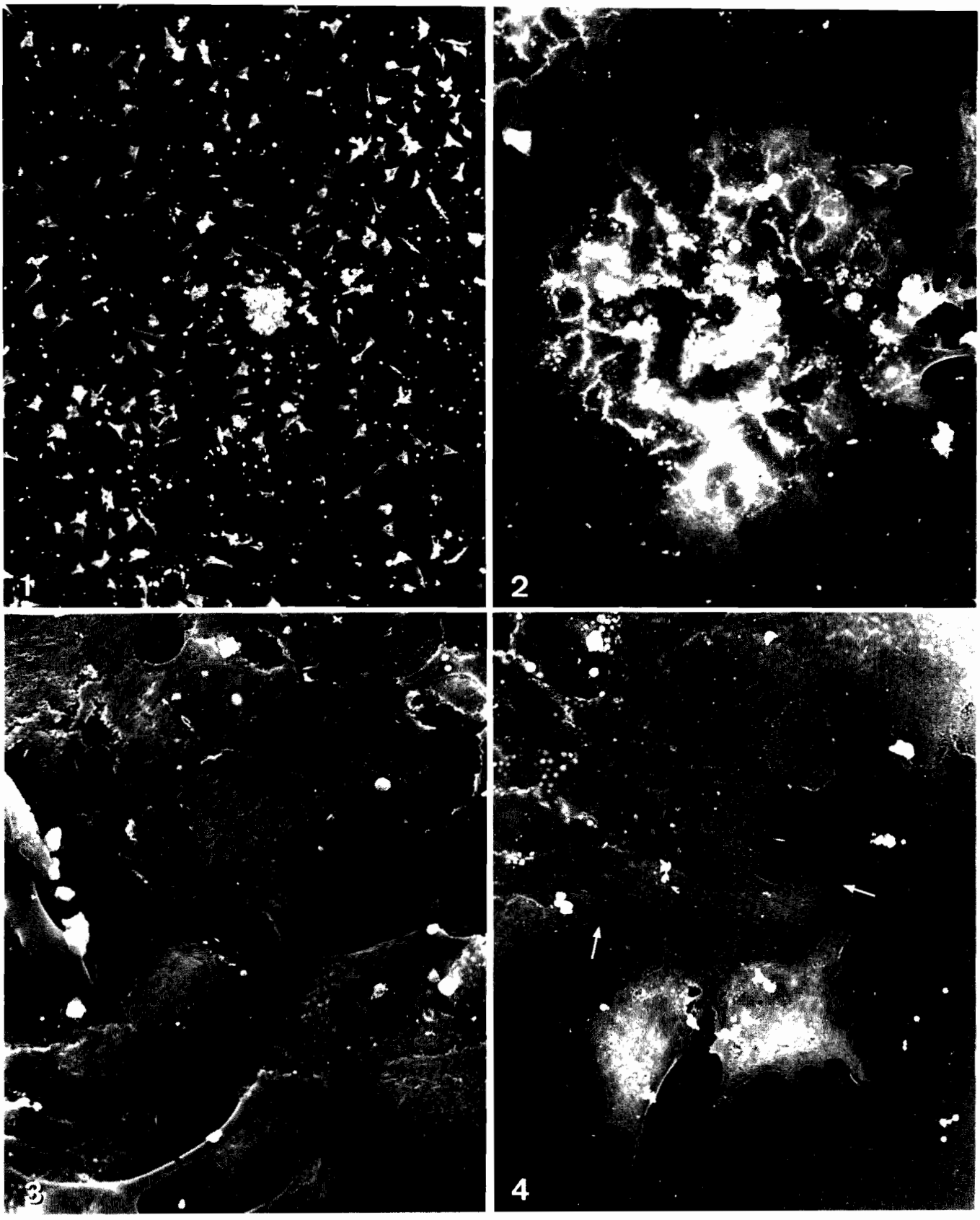
In all cultures, beside numerous attached isolated cells, several aggregates were observed on the coverslips (Fig. 1). Isolated cells were multipolar and occasionally bipolar, but were often triangular. Furthermore, the majority of the attached cells were very flattened onto the coverslip. The characteristics of these cells have been previously described in detail (Chamorro et al., 1986b).

The results of the scanning electron microscopic observations showed that cells form several clusters (Fig. 2) onto the coverslip, probably due to the cellular locomotion and proliferation (Fig. 3). These aggregates were preferentially located at central regions of the coverslip. The cellular aggregates observed in the cultures of the mesoderm cells from the four developmental stages present similar morphological features, although the stage 6 mesoderm cells showed poorer outgrowth and minor number of aggregates than the other three.

The size of the aggregates was variable, from small aggregates (wich were formed by a low number of cells) to large aggregates (which were formed by a high number of cells) of 1 mm in diameter. Although the cluster's outline was irregular, the aggregates generally presented a circular-like aspect (Fig. 2), which was more frequent when they had an intermediate size. However, the large aggregates presented a more irregular aspect, which could be due to their formation. In this sense, the large aggregates might appear as a result of the joining up of two or more small aggregates.

A remarkable fact is that in all cultures the clusters showed a bidimensional structure. It was possible to observe, with some clarity, the edge of each cell in the aggregate (see Fig. 2). These cells presented an edge without cellular projections such as filopodia or lamellipodia. However, filopodia and lamellipodia were present in the free cellular edge of the aggregate's peripheric cells (Fig. 4).

As has been mentioned above the isolated cells showed numerous filopodia and lamellipodia in the cellular edge (Fig. 5). However, when mesoderm cells in culture made contact the disappearance of the filopodial and lamellipodial activity in the contact cellular edge was observed (Fig. 6).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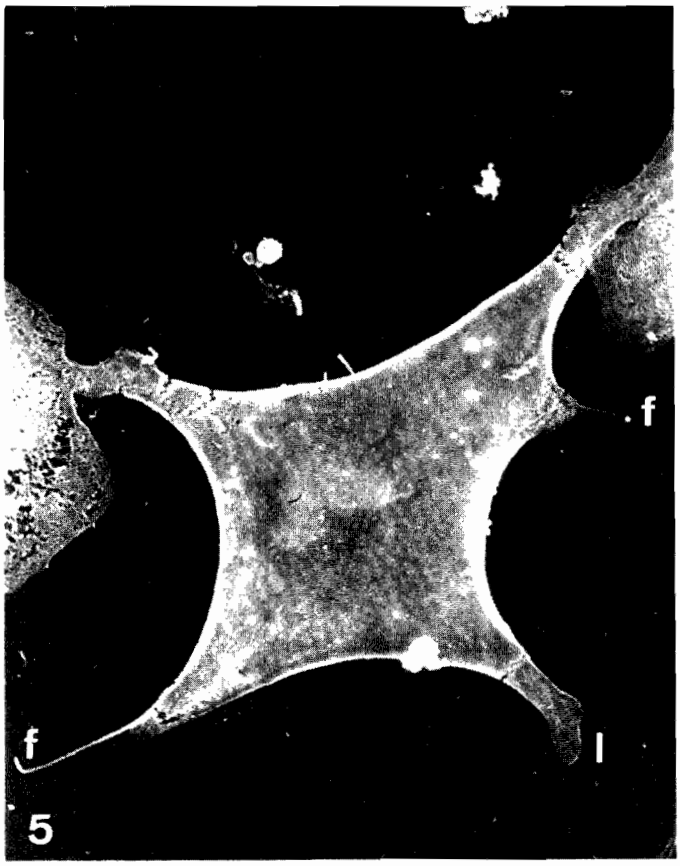

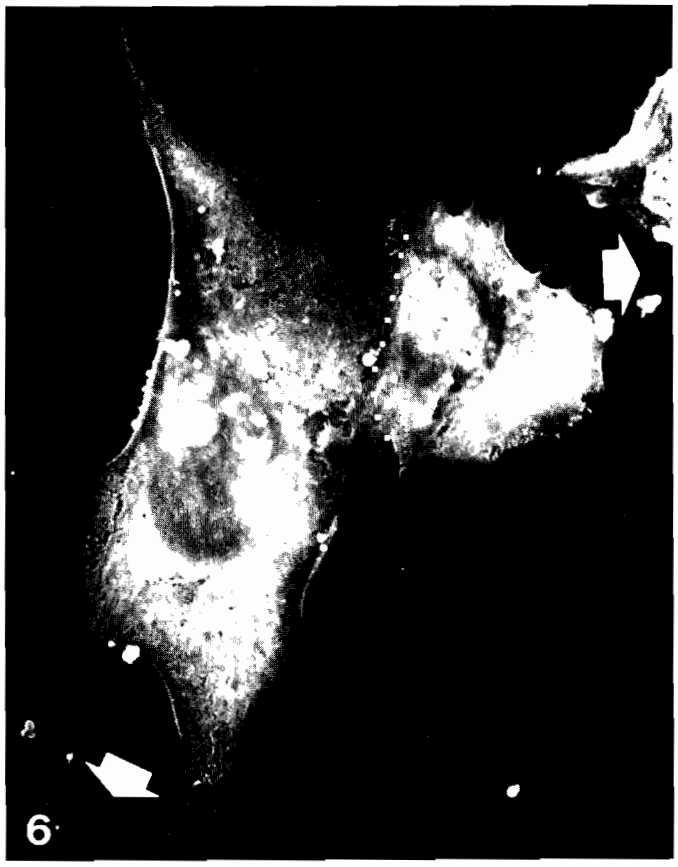

Fig. 1. Scanning electron micrograph (SEM) of chick embryo head mesoderm cells after 24 hours in culture. Note the presence of a cellular aggregate. x 120

Fig. 2. Aggregate of mesoderm cells obtained from cephalic areas of stage 8 chick embryos. Note the bidimensional character and the circular-like aspect. x 550

Fig. 3. SEM micrograph showing cellular coalescence previous to aggregate formation. The absence of cellular projections is observable. x 2,150

Fig. 4. Edge of an aggregate. The free cellular edge of the peripheric cells presents numerous filopodia (arrows) x 1,720

Fig. 5. Mesoderm cell showing filopodia (f) and a small lamellipodium (l). In the contact sites with other cells, contact inhibition of locomotory projections is observed. x 4,320

Fig. 6. Two mesoderm cells in culture make contact. The arrows indicate the direction probably taken by the cells after the contact. In this micrograph the contact line between both cells is also suggested (dotted line). x 2,000

Discussion

When cells in culture make contact with one another there may be an observable alteration in the behaviour of one or both members of the pair. This may take the form of a cessation of lamellipodial activity and forward locomotion, and a retraction from the point of contact resulting in the formation of a monolayer of cells and the avoidance of multilayering. This "contact inhibition of locomotion" (type 1) has been previously described in detail (Abercrombie, 1970; Sanders and Prasad, 1981) and this phenomenon could explain the formation of bidimensional cellular clusters described in this work. Nevertheless, it has been shown previously that mesoderm cells from other embryo areas seemed not to exhibit contact inhibition of locomotion when these cells were cultured in hydrated collagen gels (Sanders and Prasad, 1983). Time lapse-films showed that cells contacted one another freely, but without consequent change of direction or contact paralysis, and cells were frequently observed to move unimpeded over or under one another. In our results it is not possible to observe cellular locomotion or change of direction, but the scanning electron microscopic observations showed the existence of "contact paralysis" since, when mesoderm cells contacted one another, the locomotory cellular projections such as filopodia and lamellipodia disappeared. By means of this mechanism the aggregates are formed and cellular overlapping is not present. The free cellular edge of the cells located in the aggregate periphery presents numerous filopodia and lamellipodia. However, locomotory projections are neither present in the inner cellular edge of the peripheric cells nor in the aggregate inner cells. This fact, which is invariably observed demonstrates that contact paralysis of head mesoderm cells "in vitro" takes place.

A variant of "contact inhibition of locomotion" has been identified (Sanders and Prasad, 1981) and termed "type 2" contact inhibition in which contact paralysis is absent, but nuclear overlapping and multi-layering of cells is nevertheless still not seen. The result of this behaviour is a monolayer in which lamellipodial activity continues and cells are observed to mill about in the sheet. In aggregates of head mesoderm cells contact paralysis was observed and lamellipodial activity was not present. This fact indicates that it is the type 1 variant of "contact inhibition of locomotion" which occurs.

The existence of several steps in cell adhesion has already been observed (Foucaud and Gombos, 1985). The aggregation of embryonal retinal cells, one of the most studied systems of recognition between vertebrate cells, occurs according to two succesive steps: (1) reversible, and formally expresed by the authors as an equilibrium, followed by (2) an irreversible adhesion reaction. According to these mechanism the inside cells of aggregates observed by us are at the second step. In aggregates of embryonal retina cells, McClay et al. (1981) have shown that the adhesion forces involved in step 1 are of the order of magnitude of non-specific low energy interactions, while much stronger interactions intervence in step 2.

Studies on the locomotion of fibroblasts "in vitro" have revealed that cells attach to, and move upon, solid substrata by means of hyaline extensions from the cell periphery, known as ruffling lamellae or lamellipodia (Abercrombie et al., 1970; DiPasquale, 1975). Also, for fibroblasts and epithelial cells, cell contact restricts cell movement by contact paralysis of the ruffled membrane (DiPasquale, 1975; Johnson, 1976). Several authors have suggested that the contact inhibition of cell locomotion may also serve as an important guiding force for morphogenetic cell movements (Trelstad et al., 1967). In this sense, although there is no experimental evidence presented to support the notion, it has been suggested that the lateral emigration of mesoderm cells from the primitive streak in chick embryos is also a phenomenon which results from the tendency of cells to occupy spaces or to spread only when there is a free edge (Trelstad et al., 1967; Ebendal, 1976). In the same sense we must make two considerations: first, the mesoderm cells "in vivo" migrate as part of the morphogenetic movements by using the inner surface of the ectoderm (amphibian, Nakatsuji and Johnson, 1983) or epiblast (chick, Ebendal, 1976) as bidimensional substrata, that in some way could be comparable to the plane culture substrata (Ebendal, 1976; Chamorro et al., 1986b); second, evidence for the correlation of the properties of cells "in vitro" and "in situ" comes from results of studies by numerous investigators (Jumah and Stanisstreet, 1980; Kurais and Stanisstreet, 1980a, b; LeBlanc and Brick, 1981; Chamorro, 1985).

Bearing in mind these considerations, the present results might, in some way, corroborate the concept of "contact inhibition of locomotion" as an important factor for orientating head mesoderm cells during neurulation.

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