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Amoeboid movement: A review and proposal of a 'membrane ratchet' model

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Summary. Diverse cell types, including Amoebae, leukocytes, embryonic cells and tumour cells move about on solid surfaces to accomplish such activities as feeding, bacterial destruction, embryological development and metastasis. Theories of the mechanism of this movement are reviewed and a model is proposed which invokes the existence of specific, laterally mobile, transmembranous structures in the cell membrane, which are reversibly adhesive for both the contractile apparatus of the cell internally, and the substratum externally. By this model, the movement of all these cell types can be explained.
Key words. Amoeboid movement; Amoebae; polymorphonuclear leukocytes; cells.

Introduction

Amoeboid movement is a motion performed by single cells whilst adherent to solid surfaces and is exhibited by free-living protozoa, especially Amoebae, by leukocytes and to a lesser extent by embryonic cells, tumour cells and metazoan cells in tissue culture. A most satisfying theory of amoeboid movement would be one which could apply to all these cell types. However, despite numerous suggestions in reviews[1–7], monographs[8,9], and symposia[10–16], no such single mechanism has been described.

This paper summarises the phenomena of amoeboid movement and theories of its mechanism, as well as proposing a new model based on reversible adhesive transmembranous structures acting in a manner analagous to a ratchet.

Features of amoeboid cells

Polarity. The universal characteristic of amoeboid movement is the formation of an asymmetric protrusion of the cell body[17,18]. This protrusion, (the 'pseudopod'), is always at the front of the advancing cell and without it, the cell is incapable of movement. The shape of pseudopods of Amoebae can be short or long, and cylindrical or flattened according to species[19] while those of leukocytes and cultured tissue cells usually appear as thin lamellae. Pseudopods arise from any aspect of the cell body and when multiple, behave independently of one another[2]. The tips of pseudopods frequently exhibit cytoplasmic granule-free zones referred to as 'hyaline caps'[2,17] for Amoebae or 'veils'[2] for tissue-cultured cells. These zones contain more water than the remainder of the cell and probably form by syneresis from contracting cytoplasm[20,21].

The volume of the hyaline caps of Amoebae can be increased by sudden release of excessive hydrostatic pressure on the cell[22] and their volume is reduced by immersing the cell in hypertonic media[23]. If the plasma membrane and granular cytoplasm of a hyaline cap are forced together mechanically, the pseudopod tends to disappear and reappear elsewhere on the cell body[24].

The tail end of the locomoting cell sometimes forms a distinct region ('uropod') of the cell body, but is of variable shape and size[19]. According to 'rear contraction' models of amoeboid movement[25,26], it is the main site of cytoplasmic contraction, but other work[27] has shown that formation of pseudopods, as well as cell movement, occur independently of events in this region of the cell.

Numerous attempts have been made to determine whether the physical properties (viscosity and elasticity) of the cytoplasm are different at either end of the polarised motile cell[2,6], with conflicting results, probably due to technical factors[6]. One fact established by micropipette studies of membrane-cytoplasm-complex deformation at the front and rear regions of both amoebae[6] and neutrophils[28] is that the tail regions of these cells are more elastic than the pseudopodal regions despite the tail regions having a lower water content. These observations are in apparent conflict with the usual properties of gels, in which elasticity falls with reduced water content.

Streaming. The cytoplasm of some types of cells performing amoeboid movement shows the flow of apparently liquid or 'sol' cytoplasm (endoplasm) through cylinders of more solid or 'gel' cytoplasm (ectoplasm) into advancing pseudopods and out of retracting ones. While streaming is frequently observed in Amoebae, its occurrence in leukocytes is disputed[1,29,30] and it does not occur in *Hyalodiscus*[5] or in tissue-cultured cells.

Whether these 'sol' and 'gel' appearances indicate differences in the consistency of cytoplasm, the effects of thixotrophy (reduced viscosity with increased mechanical agitation) or are due to the presence of contractile fibres in only the apparently 'gel' zone is unclear[2,6] (see also above). Streaming and contractility can be demonstrated in 'naked' (i.e. isolated from cell membrane) preparations of Amoeba cytoplasm[31].

The cell as a whole. Details of the morphological appearances of whole cells performing amoeboid movement are controversial. Many authors describe the movement as 'crawling'[32,33] while others have referred to 'rolling'[34,35], or 'walking'[36,37] movements. In some studies of leukocytes and tissue cultured cells, contraction waves[38–40] or 'ruffles'[7,18,41–43] have been described, while in other studies, whole cells have been seen to undergo alternating extensions and contractions[5,44,45]. Much of this diversity of opinion relates to differing conceptions of the behaviour of the cell surface during amoeboid movement. Particles, such as soot and carmine applied to the surface of Amoebae have been reported to move forward relative to the cell body, so that the whole cell surface appears to move in the manner of the tread of a military tank[34,35,46]. However, fine glass rods laid transversely across Amoebae move backwards[25,47], and studies of various surface 'labels' on metazoan cells have confirmed this rearward movement (referred to as 'capping'[48–51]). Several authors have attempted to explain this rearward movement by suggesting that cell surface must be continuously formed at the front of the cell and resorbed at the rear[25,48]. However, neither the necessarily high turnover, nor traffic, of membrane required by this hypothesis has been demonstrated[5].

Another feature of amoeboid motion is that it can occur when the entire circumference of the cell is engaged by the surface on which the cell is moving. This is demonstrated by Amoebae advancing in fine capillary tubes[17,52] or leukocytes penetrating the narrow interstices of microporous cellulose acetate membranes[53,54]. This characteristic appears to be incompatible with a rolling behaviour of the whole cell surface.

Adhesiveness. Amoeboid movement is dependent on a degree of adhesiveness of the outer cell surface for the underlying substratum. The aspects of adhesiveness which are directly relevant to theories of locomotion are:

1) whether the total intensity of adhesiveness of the whole surface is variable,

2) whether the adhesiveness of cell surface in contact with substratum is diffuse or focal in distribution,

3) whether individual sites on the membrane change their adhesiveness cyclically with time during locomotion, and

4) whether adhesiveness of mature cells is mediated by specific membrane molecules or is a general property of cell surface (distinct from the specific adhesion sites, which have been invoked as a mechanism of morphogenesis[55–59]).

With respect to Amoebae, adhesiveness was reported to be of intermediate intensity, diffuse in distribution and constant with time[60]. In *Acanthamoeba castellani*, the ventral platform of the cell body apparently has these properties, although the tips of the acanthopodia of these organisms provide extra focal adhesions[61]. The possible temporal reversibility of adhesiveness of these sites has been mentioned by few authors[62,63]. With neutrophil leukocytes, it has long been recognised that the overall intensity of adhesiveness is influenced by the nature, roughness, wettability and electric charge of the substratum, as well as ambient protein (especially fibronectin) and Ca^{++} content of the medium[64,65]. Because of these factors, comparison of published reports is difficult. Focal adhesiveness of neutrophils to substratum has been described by some authors[45] but denied by others[66,67]. The possibility that 'specific' and 'non-specific' adhesion sites might exist on the surfaces of neutrophils has been proposed[68,69] and several allegedly specific adhesion molecules have been described[70–72]. However, the importance of these structures relative to 'general' adhesion sites[73] is unclear. Temporal reversibility of neutrophil adhesion sites has received little attention. With tissue cultured cells, the importance of substratum factors as well as composition of medium for total adhesiveness is also well recognised[74–79]. Grinnel[63] emphasised 'active' versus 'passive' total cell adhesiveness in the presence and absence of protein respectively. Tissue cultured cells are also generally considered to exhibit focal adhesion sites[60,80–82], especially at the tips of their pseudopods[83]. 'Specific' and 'non-specific' adhesion sites were discussed by Taylor[74] while 'close' as distinct from 'focal' adhesions have been distinguished by Grinnell and Geiger[84]. For myogenic and fibroblastic cells in culture, specific membrane adhesion antigens have been described[85]. The fact and possible mechanisms of temporal reversibility of adhesion sites of tissue cultured cells has been briefly discussed by Curtis[57] and Grinnell[63].

Amoeboid movement can be reduced both by excessive cell-

substratum adhesion (as in leukocytes incubated without protein in their medium[67,68,86]) and possibly by hypoadhesiveness to substratum[87,88].

Spreading refers to the concentric extension of cell cytoplasm on a surface, apparently limited only by the surface area of cell membrane[42]. It is not seen in Amoebae, but can be induced in leukocytes by exclusion of protein from the medium[67], and is the usual appearance of tissue cells cultured in vitro. Spread neutrophil leukocytes can adopt amoeboid shape (i.e. become 'oriented',[89,90]) even when actual movement of the cell relative to the substrate is abolished by hyperadhesiveness.

Chemotaxis is exhibited by most amoeboid cells and is defined as directed movement according to an ambient chemical concentration gradient[42]. From studies of leukocytes migrating in plasma clots, McCutcheon[91] concluded that chemotaxis occurs without change of speed of spontaneous locomotion and this finding was supported by Ramsey[92]. Other workers studying leukocyte migration on glass and in cellulose acetate membranes, however, have concluded that chemotaxis is usually associated with increased speed of locomotion ('chemokinesis'[42]).

Phagocytosis is regularly performed by Amoebae and neutrophil leukocytes, but can also be shown by tissue cultured cells[84]. The process involves contact of the particle with the cell surface, and then division of the pseudopod into two parts. These parts then flow around the particle and fuse on its opposite side. There is no evidence that the particle is pulled into the cell because the pseudopod membrane attached to the particle remains stationary while the divided pseudopod continues to advance[30,93,94].

Theories of a amoeboid movement

According to de Bruyn[1], the earliest attempts to explain the phenomena of amoeboid motion invoked contractility of cytoplasm. In the second half of the nineteenth century, surface tension theories were popular, but were eventually discarded, mainly because the necessary peripheral rearward currents of cytoplasm could not be demonstrated[2]. 'Sol-gel' theories, beginning with the work of Hyman[95] suggested that the conversion of cytoplasm from the one physical form to another provided motive force for locomotion by virtue of the expansion, contractility or elasticity of the gel component. The mechanism of conversion of cytoplasm from 'gel' form to 'sol' was considered to result from local intracellular acidification, dehydration or thixotrophy[1].

The main current theories of amoeboid movement are firstly: the contractile ectoplasmic tube theory[96], in which the motive force is provided by contraction of the outer, relatively stationary 'gel' cytoplasm probably by an actin[97–101] or actomyosin mechanism[102–106]. However, whether the site of contraction might be in the whole outer cytoplasm ('bulk contraction'[47,96]) in the rear[25,26] or front[2] region of the cell has not been resolved. Secondly, the membrane contraction theory[7,41] proposes that a submembranous network of filaments inserted into the membrane might contract rhythmically producing undulating waves (seen as ruffles) on the cell surface, which could move the cell forward in a fashion analogous to the gastropedal waves of molluscs[107]. This theory requires waves of surface detachment followed by cell extension and surface re-attachment followed by cell contraction to occur with respect to the substratum. Thirdly, the focal adhesion with sliding filament theory[108] proposes that bundles of actin or actomyosin filaments insert into sites on the inner aspect of the cell membrane, which are in turn linked to focal external substratum binding sites. Contraction of the bundles then pull the cell along. Like the membrane contraction theory, this model implies that the focal binding sites are more strongly adhesive for substratum than the rest of the cell surface during cytoplasmic contraction, but less strongly adhesive at other times.

Various shortcomings of each of these theories have been described (table). Thus the contractile ectoplasmic tube theory, which proposes that 'sol' cytoplasm streams forward to the open end of the tube (the pseudopod) does not explain amoeboid movement in cells which do not exhibit such streaming (*Hyalodiscus*[6] and tissue cultured cells). Furthermore the movement of cells in confined spaces appears to be consistent only with continuous formation of cell surface at the front of the cell, together with resorption at the rear, for which little supportive evidence has been found[5].

Comparison of current models of amoeboid movement

	Contractile ectoplasmic tube	Surface contraction waves	Filament bundles with focal membranous attachments	'Membrane ratchet'
Cell polarity	Pseudopod is open end of tube	Not explained	Not explained	Essential; pseudopod is site of highest water content
Cytoplasmic streaming	Essential	Compatible but not essential	Compatible but not essential	Compatible but not essential
Contractility of isolated cytoplasm	Compatible	Probably incompatible	Probably incompatible	Compatible
Behaviour of cell surface	*A* continuous rolling *or* *B* formation at front with resorption at rear	Waves of detachment/ extension/attachment/ contraction	Focal detachment/ extension/attachment/ contraction	Specific transmembranous adhesion sites only move back and forth laterally in membrane
Movement of particles attached to surface	Forward: consistent with *A*, backward: consistent with *B*	Not explained	Not explained	According to nature and state of binding site involved
Movement in confined spaces	Compatible with *B*	Compatible	Compatible	Compatible
Adhesiveness	Uniform 'intermediate' intensity	Assumes reversibility for most of membrane	Assumes reversibility of focal sites	Invokes reversibility of 'specific' transmembranous adhesion sites by a 'third factor'
Spreading	Assumed not relevant	Not explained	Not explained	Invokes excessive non-specific adhesion
Chemotaxis	Factor possibly 'weakens' ectoplasm of pseudopod	Factor possibly activates contractile apparatus or modifies adhesiveness	Factor possibly activates contractile apparatus or modifies adhesiveness	Factor possibly increaseses rate of activation of specific transmembranous adhesion sites
Phagocytosis	Not explained	Not explained	Not explained	Particle fixes to site on cell surface by adhesion to adjacent particle binding sites as well as specific and non-specific adhesion sites

The surface contraction wave theory suffers from the lack of evidence of such waves in Amoebae, and also offers no explanation of the necessity and morphology of the pseudopod[109]. The fact that isolated cytoplasm[31] can contract and stream also implies that attachment to cell membrane is not necessary for the activities of the cytoplasmic contractile apparatus, although membranes can apparently assist actin filament assembly in vitro[110]. Similarly the filament-bundle/focal membrane attachment theory does not explain the necessity for the pseudopod in amoeboid movement and that membrane adhesion is not necessary for apparently normal cytoplasmic contraction[31].

The role of water in amoeboid movement has received little attention. Among the early sol-gel theories of amoeboid movement (see de Bruyn[1]) was the suggestion by Pantin[23] that cytoplasm became liquid at the front of the cell because extracellular water was imbibed there, while cytoplasm became more solid at the tail because water was excreted from this site. However, this theory could not be supported because it would not explain liquid cytoplasm in gel cylinders in elongated pseudopods, and no such exchange of water between the exterior and the cytoplasm of amoebae could be demonstrated. Kavanau[111] proposed that matrix was 'pumped' forward in the cell, but did not discuss the role of cell membrane. Allen and coworkers (reviewed[27]), provided evidence that in Amoebae, the pseudopod has a higher water content than the rear of the cell, implying the existence of a constant gradient of water concentration in the locomoting cell. Water content of amoeboid cells has been mentioned by Bovee and Jahn[96] and in two symposia[112,113] but no recent suggestions have been made of its possible role in amoeboid movement.

Most of the current works on the mechanism of amoeboid movement concerns the possible physical[114], and biochemical bases of cytoplasmic contraction, especially the roles of actin[97-101] and actomyosin[102-106] complexes. Cations, especially Ca^{++} have been widely considered to play a role in cytoplasmic contractility[96,115-117], although certain recent studies have found no change in cytoplasmic free Ca^{++} during either actomyosin contraction[118,119] or phagocytosis[120]. Transmembranous fluxes of Na^+ and K^+ have been suggested as important in initiating locomotion in neutrophils[121].

Considerable ultrastructural evidence has been obtained for submembraneous webs of actin and myosin, intracytoplasmic bundles of actin filaments[122-125] as well as for the adhesion of these structures to inner cell membrane. However, a single model linking these studies with the known phenomena of amoeboid movement in all cell types has not been described.

Description of proposed model

Components. The necessary elements of the proposed model are as follows:

1) The motile force is derived from a contractile apparatus distributed through the whole granular cytoplasm, as envisaged by Bovee and Jahn[96]and Grebecki[47]. The precise mechanism of the contractile apparatus (i.e. actomyosin or actin alone) is not defined.

2) The contractile apparatus engages the inner aspects of specific transmembranous structures. These structures are at the same time externally adhesive for substratum in a manner similar to that suggested by Huxley[108]. Whether these structures are identical with any of the known actin-to-membrane binding molecules[126] or other specific membrane receptors, such as those on *Dictyostelium*[127], neutrophil leukocytes[70-72,128] or myoblasts and fibroblasts[85] is not determined.

3) A gradient of water concentration exists in the motile cell, being highest at the tip of the pseudopod, lower in the adjacent granular cytoplasm and lowest at the rear of the cell, as proposed by Allen[27,52]. This gradient is maintained by continuous syneresis from contracting cytoplasm so that a constant flow of water from the tail through the middle of the cell to the tip occurs during locomotion[20,21].

4) The internal and external adhesiveness of the specific transmembranous structures is reversible, and is activated at the front of the cell by a relatively high concentration of a 'third factor' and inactivated at the rear of the cell by a lower concentration of the same factor.

5) The gradient of 'third factor' implied in (4) is created because 'third factor' is either water itself or a cation or other small molecule carried forwards into the hyaline cap with water expressed by the contracting cytoplasm (see above).

6) The specific transmembranous adhesion structures, when either active or inactive are laterally mobile in the cell membrane according to the fluid mosaic model of Singer and Nicholson[129], in the manner already discussed in relation to locomoting cells[130].

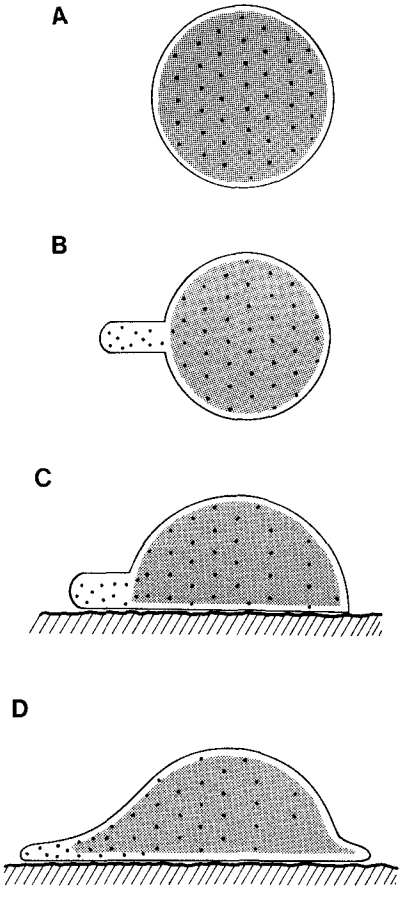

Figure 1. Mechanism of pseudopod formation according to the 'membrane ratchet' model. *A* Inactive and unattached cell is spherical with cytoplasmic contractile apparatus bound to inner cell membrane. 'Third factor' (either water or a small molecule distributed in it; indicated as dots) is uniformly dispersed in the cytoplasm. *B* Contraction of cytoplasm reduces the volume of the spherical part of the cell and creates a dehiscence of cytoplasm-membrane binding at a random point. Water flows into this space. n.b. a 5% reduction of an initial cell diameter of 10 μm produces a pseudopod volume of approximately 75 μm^3. *C* If the contracted cell adheres to a surface, and becomes hemispherical, the new diameter of the contracted part of the cell is approximately 120% of the diameter of the original, uncontracted sphere. *D* Actual shape of locomoting cell depends, in addition, on the consistency of cytoplasm, the rigidity of cell coat and adhesiveness of membrane for substratum.

7) Non-specific substratum binding sites exist on the cell membrane which have no internal component and are not freely mobile in the cell membrane. In Amoebae, these sites are totally masked by its thick cell coat[131], and in leukocytes and tissue culture cells are partly masked by plasma protein, according to the views of Grinnell[63], Schreiner and Hopen[68], and Taylor[74].

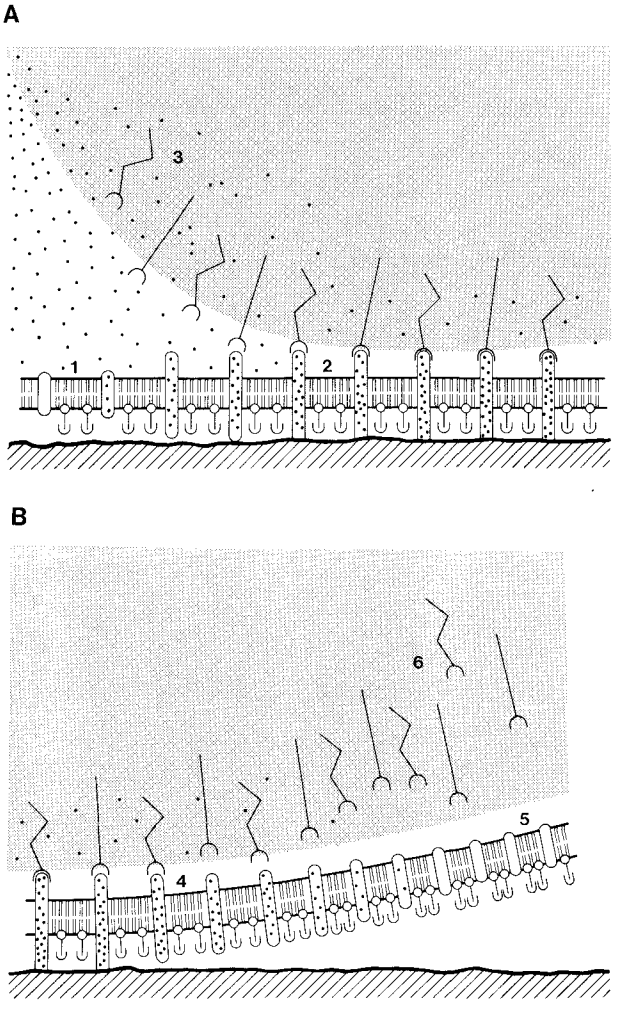

Figure 2. Mechanism of amoeboid movement according to the 'membrane ratchet' model. *A* Margin of pseudopod. 1) In the hyaline tip of the pseudopod, inactive specific transmembranous structures are activated by 'third factor' which is either water, or a cation or a small molecule carried with it (Structures drawn as though they are activated by hydration, but this is not necessarily invoked). 2) At the margin of the hyaline cap, sites on the cytoplasmic contractile apparatus attach to activated transmembranous structure. Subsequent cytoplasmic contractions pull the cytoplasm forward relative to the substratum. (Apparatus drawn as intermittently contracting filaments, but this is not necessarily invoked). 3) Fresh cytoplasmic contractile apparatus takes the place of membrane bound apparatus which is now fixed externally to substratum. *B* Rear of cell. 4) Active specific transmembranous adhesion structures are inactivated by depletion of 'third factor', probably brought about by diffusion from specific adhesion structures into relatively dehydrated cytoplasm at rear of cell. 5) Completely inactive specific transmembranous adhesion structures return to the front of cell laterally through membrane. 6) Detached cytoplasmic contractile apparatus returns to the front through the middle of the cell. This movement is probably assisted by a forward flow of cytoplasmic water in the same part of the cell. n.b. ⌡ represents reversible specific transmembranous adhesion structure; ♀ represents non-specific membrane adhesion structure masked externally by protein as for leukocytes in plasma.

Mechanism of the model

Formation of pseudopod (fig. 1). It is proposed that in the inactive cell (e.g. at 4 °C), the 'third factor' is distributed uniformly through the cytoplasm, the contractile apparatus is flaccid but engaged to specific membrane adhesion structures. With activation of the cell (as by raising temperature to physiological range) the cytoplasm contracts. This contraction causes a rise of intra cytoplasmic pressure, which results in a focal dehiscence of cytoplasm-inner membrane binding. Cytoplasmic water then flows freely into the site of dehiscence, forming the hyaline cap zone of the pseudopod. This flow releases raised intra cytoplasmic pressure, although the cytoplasmic contractile apparatus is still engaged to the inner cell membrane at other sites. This proposal is compatible with the relationship of cytoplasm to membrane as described by Allen[2]. No adhesion of apparatus to membrane is possible at the tip, because of the physical separation caused by the collected water, but the specific structures in this zone are nevertheless activated by 'third factor' (either water itself or possibly a cation carried with it, see above).

Locomotion (fig. 2). At the junction between the hyaline tip and the cytoplasm of the cell body, the randomly contractile cytoplasmic apparatus engages the active specific transmembranous adhesion structures. The cytoplasm is then pulled towards the specific structures because the latter are bound to substratum externally. The cell therefore moves in the direction of the pseudopod. The engaged adhesion structures are progressively displaced in the membrane from the front to the rear of the cell by cytoplasm arriving through the middle of the cell body.

At the rear of the cell, disengagement of contractile apparatus from transmembranous structures occurs, because 'third factor' either is water which diffuses out of the apparatus-membrane site complex into the relatively dehydrated adjacent cytoplasm or is an ion or small molecule carried with such water (see above). The inactive specific membrane structures flow laterally in the membrane to the front of the cell to be re-activated by the relatively high concentration of 'third factor', and the disengaged cytoplasmic apparatus returns through the middle of the cell to the pseudopod.

Morphological sequence. The initial result of early action of the mechanism is simple extension of the pseudopod because specific membrane adhesion structures at the tail of the cell still adhere to the substratum. When the pseudopod achieves sufficient size, the cell body and tail region become relatively dehydrated and the rear of the cell is deprived of so much 'third factor' that the specific transmembranous sites in this region are inactivated. This allows detachment of the tail of the cell from substratum, which is then free to move along behind the pseudopod.

Discussion

The present model does not address the biochemical nature of cytoplasmic contractility[97–106]. Similarly, the identity of the specific membrane adhesion structures is not defined, but could well be the reported actin-membrane binding molecules[123, 126] or complexes thereof. The present paper does, however, present a model of amoeboid movement which is compatible with, and can explain the known observations of amoeboid cells (table).

The universal requirement for the pseudopod, its more hydrated (hyaline) tip and the fact that the cell always advances in its direction is because the pseudopod's margins are the site of both activation and engagement of specific cytoplasm-membrane-substrate binding. The opposite end of the cell is the site of inactivation of such binding, allowing the cell body to follow the pseudopod.

The shape of the pseudopod of any cell is seen to be affected by: 1) the volume of its contracted cytoplasm relative to the

area of cell membrane, 2) the consistency of the cytoplasm (hyaline and granular) and 3) the deformability of the cell membrane and coat (glycocalyx).

The rate of locomotion of the cell is seen to be affected by: 4) the amount of contractile protein relative to non contractile protein in the cytoplasm; and 5) the contribution of specific compared to non-specific membrane structures to total cell adhesion. The results of these variables are therefore that, at one extreme, Amoebae having limited cell membrane compared to cell volume, high relative contractile protein content, fairly rigid cell coat (possibly due to thick glycocalyx) and intermediate total adhesiveness of which most is due to specific adhesion (non specific adhesion being masked by cell coat) will have short, cylindrical pseudopods and be capable of rapid locomotion. At the other extreme, tissue-cultured cell having abundant cell membrane compared to cell volume, low relative contractile protein content, supple cell coats and strong total adhesiveness to substratum, of which most is non-specific, will exhibit large flattened pseudopods and sluggish movement. Neutrophil leukocytes will behave in an intermediate manner according to conditions, such as protein content of medium, of incubation.

The 'hyaline cap' is seen as visible water accumulated in the pseudopod, occurring when the contractile apparatus is strong enough to express sufficient water and create a high water gradient in the cell. It is suggested that 'hyaline caps' are not seen in electron microscopic studies[131] because the necessary preparative techniques dehydrate the cells and destroy the zone.

The apparent discrepancy between greater water content of the cytoplasm and lower elasticity of the cytoplasm-cell membrane complex (demonstrated by cell deformation techniques) of the pseudopod compared to the rear of the cell[6] may be due to relatively increased hydration having an effect of increasing turgor and hence rigidity of the cell membrane in this part of the cell. This explanation would be especially appropriate if the putative 'third factor' is water itself rather than some other molecule.

True gel formation of cortical cytoplasm ('ectoplasm') is not invoked by the present theory. The relatively stationary appearance of the cortex of cytoplasm of certain cells is attributed to the engagement of contractile elements of the cytoplasm by the adjacent transmembranous structures, which immobilises cell organelles. This is compatible with the fact that the most prominent 'gel' appearance can be seen in cell types which move most rapidly, and hence presumably have the highest relative cytoplasmic proportion of contractile apparatus (see above). Disengaged cytoplasm is free to flow ('stream') and appears as 'sol' cytoplasm. 'Gel-sol transformation' occurring at the rear of Amoebae is thus interpreted as resulting from disengagement of cytoplasmic contractile apparatus from inner sites of transmembranous structures. A degree of actomyosin collapse[103, 132] or F-actin solation[98, 133] might accompany this disengagement, but is not necessarily invoked.

The ability of the cell to move through confined spaces without new membrane formation is explained because the activated specific membrane molecules of locomoting cells contribute most of the total adherence for substratum, and inactive specific molecules can flow forwards in the membrane on all sides to the pseudopodal region[130].

The various reports of movements of particles on the surface of amoeboid cells[34, 35, 48] can be explained by their binding to different structures. Thus binding to active specific transmembranous adhesion structures will result in their rearward movement, to inactive specific sites in forward movement, and to non-specific sites in no definite movement of the particle or label[48, 134].

'Ruffles'[18] are seen as a manifestation of buckling of supple cell membrane which is not attached to substratum, but which is engaged internally by the contractile cytoplasmic apparatus as has been proposed by Harris[48]. Hence ruffles are not seen in cells such as Amoebae, having presumably more rigid cell surfaces due to thick glycocalyx[131].

Waves of detachment/extension/attachment/contraction by the cell surface and cytoplasm in the manner of the gastropedal wave of a mollusc[107] is not invoked by the present theory.

Loss of motility with spreading as occurs with leukocytes deprived of protein in their medium is seen as the result of insufficent masking of non-specific membrane binding sites[72], so that the entire surface engages the substratum and overwhelms the effects of specific transmembranous adhesion structures (see above). This corresponds to 'passive' adhesion as discussed by Grinnell[63]. The absence of this phenomenon with Amoebae is ascribed to their thick cell coat[131] masking non-specific adhesion sites.

'Orientation' of spread neutrophil leukocytes[89] is considered to be observable on the free surface of the cell because the specific transmembranous adhesion sites are not overwhelmed by excess non-specific substrate adhesiveness, and the cell on this side can therefore react normally.

Chemotaxis could result from slightly increased rate of specific transmembranous structure activation at the pseudopod by chemotactic factor. This would have the effect of increasing the relative number of active specific sites on the side of the pseudopod exposed to the higher concentration of chemotactic factor. The cytoplasmic contractile apparatus would therefore be more strongly engaged on that side, and hence the cell would turn in that direction. This would not necessarily change the rate of cell locomotion (since the cytoplasmic contractile apparatus itself would be unaffected) and hence be consistent with the observations of McCutcheon[91] and Ramsey[92]. Alternatively, chemotactic factors could enhance adhesiveness of the specific transmembranous sites to substratum, and thus turn the cell in the direction of higher concentration of factor ('haptotactic' mechanism of Carter[135, 136]). This might, however, tend to impede the rate of cell locomotion, but such slowing is not a feature of chemotaxis[42].

Phagocytosis could be explained by the binding of the particle to particle-specific receptors (e.g. Fc and complement receptors) as well as to immediately adjacent non-specific and specific adhesive membrane structures. The particle specific receptors would then be held immobile in the membrane together with the specific adhesion molecules and cytoplasmic contractile apparatus attached to them. The unattached divided hyaline tips of the pseudopods would then move freely about the stationary piece of membrane to engulf the particle consistent with the 'zipper' analogy previously described[93].

Testing of the model

The proposed model is compatible with the microscopic observations of amoeboid cells, but would be greatly supported if the water content of the cell membrane of the locomoting cell could be demonstrated to be higher at the front of the cell compared to the rear, as this would confirm the explanation that membrane turgor is the basis of the apparent conflict between lower cytoplasmic water content and greater elasticity of the tail regions of cells. It would also tend to identify water as the 'third factor'. Additional support for the model would be provided if the putative specific transmembranous adhesion structures could be isolated. At present, great advances are being made in the techniques for separating cell membranes and purifying their components, so that isolation of the putative structures is theoretically possible. After separation, the identification of the specific structures would then depend on demonstrating their reversible adhesiveness

to solid substrata. This purely biochemical approach, however, may present formidable problems, since the nature of the 'third factor' is unknown (despite the speculation concerning water), the exposed internal end of the structure might bind irreversibly to substrata, and large amounts of the structure might be lost on the surfaces of laboratory-ware in processing. An alternative avenue of investigation might be to examine antibodies prepared against the membranes of amoeboid cells for their ability to inhibit cell motility, but not reduce non-specific adhesion or viability. The structures bearing the corresponding antigens could then be investigated. Studies of this general nature (but with other objectives) have already been reported with respect to neutrophils[72, 137].

Conclusion

The model of amoeboid movement described in this paper is consistent with the agreed biological and morphological features of the phenomenon. While the precise biochemical nature of its components are not identified, this account may nevertheless be of value in the interpretation of data concerning membrane adhesion, the cytoplasmic contractile apparatus and the supposed influences of water and cations on the locomotion of cells.

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Full Papers

Evidence for an olfactory receptor which responds to nicotine – nicotine as an odorant

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Summary. The tobacco alkaloid (S)(−)-nicotine, when applied as a vapour to an in vitro head preparation, stimulates the olfactory epithelium in three strains of rats and to a lesser extent in two strains of mice. The electro-olfactogram (EOG) generated by nicotine has similar characteristics to the EOGs produced by known odorants. The nicotine EOG increases with increasing concentration of nicotine vapour (1–100 nM) applied to the olfactory epithelium.
Differential reduction of the nicotine EOG by the lectin concanavalin A is seen in Wistar and Lister Hooded rats. The reduction of the nicotine EOG by concanavalin A is prevented by adding alpha-methyl-D-mannoside to the lectin superfusion medium. This suggests that there is a glyco-moiety associated with at least one olfactory receptor responding to nicotine.
Our results suggest that rat olfactory epithelium has receptor sites for nicotine. Nicotine is an unusual compound because it shows both odorant and pharmacological properties.
Key words. Nicotine; odorant; electro-olfactogram; olfaction; receptor; rat.

Introduction

The biochemical properties of nicotine and in particular its effects as an agonist for the nicotinic acetylcholine receptor, have been extensively reviewed in the literature[2,3]. Specific binding of nicotine to human[4], mouse[5,6] and rat brain[7–9], to rat liver[10] and to human leucocyte membranes[11] has been demonstrated. Nicotine is known to be the primary satisfaction factor for tobacco and to influence the flavour of the smoke considerably[12]. It occurs at high levels in tobacco products, typically 1.8 mg of nicotine per cigarette[3]. Considering the latter and the amount of work carried out with nicotine, it is surprising that there are no detailed studies reported on nicotine as a stimulant for the olfactory epithelium.
We calculate the saturated vapour phase concentration above pure nicotine to be 3.6 mM at 20 °C, which is likely to produce perceivable concentrations in the olfactory mucosa.