SUMMARY

As part of a search for chemical agents able to promote the fusion of mouse A9 fibroblasts, oleylamine, a positively charged compound, has been investigated for its fusogenic properties. In the pH range 5.5-7.5 and in the presence of dextran, fibroblast polykaryons were produced on treatment of monolayers of cultured cells with oleylamine dispersed directly in a modified Eagle’s medium at concentrations of not less than 0.11 mg/ml. Electron microscopy demonstrated the absence of a dividing plasma membrane between the constituent nuclei of the polykaryons, and showed clustering of other subcellular organelles around their original parent nuclei.

Fusion, which was preceded by rounding and swelling of the cells, occurred between cells in contact after 10-15 min. Oleylamine in lipid droplets containing glyceryl mono- and dioleate also caused swelling and fusion but to a lesser extent. Phosphatidylcholine appeared to have an inhibitory effect on oleylamine-induced fusion: lecithin liposomes containing oleylamine were only weakly fusogenic. The fusion process, but not the preceding swelling, was calcium-dependent; fusion was inhibited by low concentrations of lanthanum ions.

While oleylamine inhibited cell division in monolayer cultures and prevented adhesion of fibroblasts in suspension to glass coverslips, oleylamine in lipid droplets was less toxic and is thus potentially more useful in this form for interspecific hybridization experiments.

INTRODUCTION

Since the discovery that viruses can be used for the fusion of various cell types to produce multinucleated homokaryons and interspecific hybrids (Okada, 1961; Harris & Watkins, 1965), some advances have been made in the use of chemical agents for cell fusion. Lysolecithin has been used to induce fusion but with concomitant degradation of the treated cells (Howell & Lucy, 1969; Poole, Howell & Lucy, 1970). Changes in incubation conditions and presentation of lysolecithin in a lipid emulsion retarded disintegration of the membranes of the resultant multinucleated hen erythrocytes (Ahkong, Cramp, Fisher, Howell & Lucy, 1972). In the same work, clones of hybrid mouse-hamster fibroblasts were obtained following treatment with lysolecithin but at only twice the frequency of the spontaneous fusion rate. Similar results were obtained using a mouse-human cell system following lysolecithin treatment, which was found to have maximum effect at pH 8.0 (Croce, Koprowski & Eagle, 1972).

Agents other than lysolecithin, such as retinol, glyceryl monooleate, oleic acid and surface-active adjuvants have been shown to have fusogenic properties with hen erythrocytes (Lucy, Ahkong, Cramp, Fisher & Howell, 1971; Ahkong, Cramp, Fisher,
Howell, Tampion & Lucy, 1972). Glyceryl monooleate promotes fusion of erythrocytes and mouse fibroblasts and significantly increases the number of hybridizations resulting from fusions of mouse and hamster fibroblasts (Lucy et al. 1971).

Cultured fibroblasts appeared to be consistently more resistant to fusion by chemical agents than erythrocytes, although recently it has been claimed that a 30-fold increase in the number of hybrid clones has been achieved following treatment with lysophosphatidylcholine in monolayers (Keay, Weiss, Circulis & Wildi, 1972). The experiments described in this paper are part of a search for agents which promote extensive fusion of fibroblasts and may eventually be used for the ready formation of interspecific hybrids.

In this laboratory, stearylamine and to a greater extent oleylamine have been shown to have potent fusogenic properties with hen erythrocytes (Q. F. Ahkong & J. A. Lucy, unpublished work). Stearylamine has been used in the formation of liposome structures (Bangham, Standish & Watkins, 1965) indicating an action on membrane lipids which may be relevant to fusogenic properties. Furthermore, liposomes containing 10% stearylamine were readily taken up by the amoeba, *Acanthamoeba castellanii* (Korn, 1970). In the present work oleylamine has been used to induce the fusion of fibroblasts in tissue culture. Oleylamine was used in preference to stearylamine in this study because of its liquidity at incubation temperatures and because of its identical hydrocarbon chain to oleic acid, a known fusogenic agent.

The importance of calcium ions in virus-induced cell fusion (Okada & Murayama, 1966; Yanovsky & Loyter, 1972) and chemically induced fusion (Lucy et al. 1971) has been established. Lanthanum ions are known to oppose the effects of calcium in nervous tissue (Miledi, 1971) and have been shown to be potent inhibitors of the calcium-dependent release of histamine from antigen-stimulated mast cells (Foreman & Mongar, 1972). In fusion experiments with erythrocytes the concentration of Dextran 60C and the pH of the medium were shown to be important factors in modifying the effectiveness of fusogenic agents (Ahkong, Fisher, Tampion & Lucy, 1973). Oleylamine-induced fusion has therefore been investigated at different pH values in the presence of calcium and lanthanum ions, and of Dextran 60C.

**MATERIALS AND METHODS**

Mouse A9 fibroblasts, kindly supplied by Dr J. H. Roscoe, Department of Botany and Microbiology, University College, London, were cultured as monolayers in Dulbecco's modified Eagle's medium/10% foetal calf serum in an atmosphere of 90% air/10% CO2.

For experimental purposes, cells were trypsinized (0.04% trypsin in 0.02% EDTA) and replated on sterile coverslips (22 x 22 mm) at a concentration of 5 x 10^3 cells per coverslip and cultured for 20 h in plastic Petri dishes (90 x 15 mm) until the cells had spread on the glass, but were not confluent.

Prior to incubation, coverslips were washed 3 times in modified Eagle's medium (NaCl, 116 mM; KCl, 5.5 mM; MgSO4·7H2O, 0.8 mM; CaCl2·2H2O, 5 mM; glucose, 10 mM; Tris, 10 mM - adjusted to pH 7.4 with 1 N HCl; 200 000 units of benzylpenicillin and 50 mg of streptomycin sulphate per litre).
Oleylamine-induced cell fusion

Oleylamine dispersions

Oleylamine (Koch-Light Ltd, Colnbrook, Bucks., U.K.) was distilled under vacuum before use in fusion experiments.

Dispensations of oleylamine (0.042–0.67 mg/ml) were prepared by sonicisation in modified Eagle’s medium for 2 min using a 150-W ultrasonic disintegrator fitted with a 19-cm diameter titanium probe (M.S.E. Ltd, London, U.K.). In later experiments satisfactory results were also obtained by dispersing oleylamine from a solution in ethanol such that the final concentration of ethanol in the medium never exceeded 1.6 %.

Dispensations of oleylamine were prepared in media containing Dextran 60 C, mol. wt 77 800 (Sigma Chemical Co., St Louis, U.S.A.), at concentrations ranging from 0 to 48 mg/ml. All media were sterilized by Millipore filtration (0.22 μm pore size, Millipore Filter Corp., Bedford, U.S.A.); the dispersions remained sterile following sonicisation.

Incubation conditions

Monolayers of fibroblasts on coverslips were incubated at 37 °C in Petri dishes (90 x 15 mm) with 6 ml oleylamine dispersion, or in identical media without oleylamine as controls. Five coverslips were used in each incubation, one of which was observed for fusion by phase-contrast microscopy using a cavity slide containing a small volume of fusing medium with a Zeiss Standard WL microscope fitted with a heated stage maintained at 37 °C.

Two coverslips were fixed and stained at appropriate intervals during the fusion process, usually 15 or 40 min after the start of the incubation. The cells were fixed with Zenker’s acid fixative (20 vol. Zenker’s fixative + 1 vol. glacial acetic acid) for 20 min, followed by 30 min in neutral Zenker’s fixative and then finally stained in Harris’s haematoxylin. The remaining coverslips were washed free of incubation medium with several washes in Dulbecco’s medium/10 % foetal calf serum. They were then incubated in this medium and inspected daily for multinucleated cells and cell division.

Incubations with fibroblasts in suspension

Cell suspensions were prepared by treating monolayers of cells in glass culture vessels with a solution of 0.04 % trypsin in 0.02 % EDTA until cells loosened from the glass. The cells were then washed in Dulbecco’s medium/10 % foetal calf serum twice and finally in medium without serum. 2 x 10^6 Cells were suspended in 2 ml modified Eagle’s medium, pH 7.4, containing 24 mg/ml Dextran 60 C and 0.042–0.167 mg of dispersed oleylamine per ml. After 1, 15 and 40 min of incubation, 0.4 ml of the cell suspension was washed 3 times with Dulbecco’s medium; the cells were dispersed in Dulbecco’s medium/10 % foetal calf serum and cultured in Petri dishes (90 x 15 mm) containing coverslips. During incubation samples were taken on microscope slides for observation under phase-contrast microscopy. Some fibroblast suspensions were incubated in media containing 0.167 mg/ml oleylamine and 10 % (v/v) dimethylsulphoxide.

Incubations with oleylamine dispersed in lipid particles

Mixtures of oleylamine and other lipids in chloroform were dried under N2 and, after addition of modified Eagle’s medium, pH 7.4, were allowed to swell for 15 min. The subsequent procedure was the same as that described above for oleylamine alone. Mixed particles of glyceryl dioleate (K & K Rare and Fine Chemicals Ltd, New York, U.S.A.), glyceryl monooleate (Sigma Chemical Co.) and oleylamine were prepared. In some experiments liposomes containing egg phosphatidylcholine (Lipid Products, Redhill, Surrey, U.K.) and oleylamine were used. These preparations were used within 15 min of preparation.

Factors affecting oleylamine-induced fusion

pH. When pH levels other than 7.4 were required, 10 mM maleic acid was included in the modified Eagle’s medium and adjustment to the appropriate pH made with 1 N HCl or NaOH.

Divalent ions. In some cases, monolayers were pre-washed in modified Eagle’s medium, pH 6.5, containing no divalent ions but including 10 mM EDTA. Incubations were performed
with oleylamine (0-167 mg/ml) in medium containing 10 mM EDTA and no divalent ions and the cells were observed for 15 min after which they were fixed. The effect of adding 15 mM Ca\(^{2+}\) and 0-8 mM Mg\(^{2+}\) to cells which had already been incubated for 5 min with oleylamine in the presence of 10 mM EDTA was also studied.

**Lanthanum.** Fibroblast monolayers were incubated with 0-002, 0-02 and 2 mM lanthanum chloride in modified Eagle’s medium, pH 6-5, containing 5 mM Ca\(^{2+}\), 0-8 mM Mg\(^{2+}\) and 0-167 mg oleylamine/ml. In some incubations cells were pre-washed with medium containing 10 mM EDTA, followed by a wash with 2 mM La\(^{3+}\) and then incubated with oleylamine in a medium containing 2 mM La\(^{3+}\), 5 mM Ca\(^{2+}\) and 0-8 mM Mg\(^{2+}\).

**Electron microscopy**

Control and oleylamine-treated cells (0-167 mg oleylamine and 30 mg Dextran 60 C per ml modified Eagle’s medium, pH 7-4) were fixed in 1 % glutaraldehyde (ice-cold 0-2 M cacodylate buffer) for 30 min (Glaubert, 1965), washed thoroughly with water, and then postfixed for a further 30 min in 1 % osmium tetroxide/ruthenium red (Pate & Ordal, 1967). The cells were dehydrated through 10, 30, 50, 70 and 90 % ethanol, 15 min in each and finally twice in absolute ethanol. The coverslips were transferred from propylene oxide to Araldite epoxy resin containing propylene oxide and finally embedded in Araldite after heating at 60°C overnight in foil dishes. The foil was stripped off the blocks which were trimmed and immersed in liquid N\(_2\) to remove the coverslips (a modification of the method by Chang, 1971). Sections were cut with an LKB Ultratome III and after staining with lead citrate, viewed under an AEI EM 6b electron microscope with an accelerating voltage of 60 kV and micrographs taken at an initial magnification of \(\times 3500\) to \(\times 11000\).

**RESULTS**

**Oleylamine-induced fusion of fibroblast monolayers**

The response of A9 fibroblast monolayers to incubation with oleylamine was characterized under the phase-contrast microscope by a sequence of events leading to fusion of the cells into multinucleated syncitia (Fig. 1). Although at no time during the process were the cells released from the coverslip, the first stage involved a rounding of the cells approximately 3 min after the incubation commenced. The rounded cells then swelled forming clearly defined spaces immediately beneath the membrane and also concomitant accumulation of organelles around the nucleus (Fig. 1). After 10-15 min swollen cells, which were in close contact, fused to form polykaryons. The dividing membranes between the cells ‘popped’ with great speed, presumably having reached a critical point due to mechanical stress or chemical change. The fused cells remained swollen and the organelle clusters surrounding the nuclei did not intermingle, even after the cells were washed free of oleylamine.

Electron micrographs of oleylamine-treated cells confirm that fusion of fibroblasts occurred, the cytoplasmic connexions between the cells showing no trace of a dividing membrane (Fig. 2). As observed under phase-contrast, a separation of the organelles from each fibroblast constituting the polykaryon occurred. The region in which fusion occurred thus contained cytoplasm free from subcellular organelles. Signs of nuclear damage were evident from the condensation of chromatin, but the nucleoli were still visible and the nuclear membrane appeared to be intact. Oleylamine caused disorganization of the endoplasmic reticulum and also rounding of the mitochondria (Fig. 3). Another unusual feature was the presence of large membranous vesicles surrounding weakly staining areas. These may have arisen as a result of a reorganiza-
Oleylamine-induced cell fusion

The extent of swelling and fusion observed depended on the concentration of oleylamine and on the presence of Dextran 60C. Although swelling was seen at a concentration of only 0.055 mg oleylamine/ml, a significant number of fusions were not apparent until 0.110 mg/ml was used. Swelling and fusion occurred most frequently in the less-densely populated areas of the coverslip, but at high concentrations of oleylamine (0.67 mg/ml) almost all the cells were involved. High concentrations of oleylamine accelerated the fusion process and the eventual disruption of the cells. In the absence of dextran, lysis of the fibroblasts was rapid, but at a concentration of 48 mg dextran/ml, swelling was diminished and no fusion occurred. An intermediate concentration of 30 mg dextran/ml was found to permit swelling and fusion, and to afford protection against lysis for periods of 20 min or more, thereby permitting further study of the fusion process. The presence of 10% foetal calf serum in the medium only slightly inhibited the fusion of fibroblasts. Fibroblasts brought into suspension with trypsin were rapidly lysed by oleylamine, swelling and fusion occurring much less frequently.

Dispersion of oleylamine in particulate lipid systems

Oleylamine has obvious qualities as a fusogenic agent but because of its lytic properties and toxicity (see below) modifications in its application were needed and the efficacy of oleylamine dispersed in liposomes or lipid droplets was therefore investigated.

Fibroblasts in suspension

(1) Phosphatidylcholine liposomes. Fibroblasts were incubated with liposomes containing 1 mg phosphatidylcholine and 0.33 mg oleylamine per ml medium. When compared to cells exposed to oleylamine alone, swelling and fusion were diminished, indicating an inhibitory effect of phosphatidylcholine on fusion, but not on lysis.

(2) Lipid dispersions. Incubations with 2.67 mg glyceryl dioleate, 0.33 mg phosphatidylcholine and 0.33 mg oleylamine per ml medium induced clumping of the cells and some fusion and also reduced the lytic effect of oleylamine.

Fibroblasts in monolayers

Since fibroblasts adhere to glass even after oleylamine treatment, monolayers were treated with dispersions containing low concentrations of oleylamine. A combination of 1.67 mg glyceryl dioleate, 0.166 mg oleylamine, with or without 0.33 mg phosphatidylcholine per ml, caused only limited swelling and very infrequent fusion. It was also observed that washing oleylamine-treated monolayers with phosphatidylcholine liposomes (1 mg/ml medium) reduced lysis following swelling and fusion.

Further investigations were made with even lower concentrations of oleylamine in lipid particles which included glyceryl monooleate, a known fusogenic agent for hen
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erythrocytes (Lucy et al. 1971). Glyceryl monooleate forms unstable dispersions at high concentrations and therefore glyceryl dioleate was also included. Monolayers of fibroblasts were incubated with dispersions of 0.83 mg glyceryl monooleate, 0.83 mg glyceryl dioleate, and 0.084 or 0.042 mg oleylamine per ml medium. Again swelling and fusion were observed at both concentrations of oleylamine whereas dispersions without oleylamine caused rounding and a small degree of swelling but no fusion of the fibroblasts.

Other factors influencing oleylamine-induced fusion

**pH.** Monolayer cultures of fibroblasts were exposed to oleylamine (0.167 mg/ml) in modified Eagle’s medium buffer with Tris/maleic acid at pH 5.5, 6.5, 7.5 and 8.5 at a dextran concentration of 30 mg/ml. Extensive fusion was observed under phase contrast at pH 5.5, 6.5 and 7.5, 7-10 min after the beginning of the incubation. Over this range, pH did not have a significant effect, but at pH 8.5, cells appeared to be in poor condition and showed distinctly less swelling and fusion than at low pH values.

**Divalent ions.** In the presence of Ca$^{2+}$ and Mg$^{2+}$ when the membranes of swollen adjacent cells came into contact as a result of oleylamine treatment, the separating membranes collapsed giving rise to a single membrane surrounding the 2 or more nuclei (Fig. 4B, c). If the cells were pre-washed for 5 min with medium containing 10 mM EDTA but no divalent ions, followed by oleylamine treatment (0.167 mg/ml) again in the absence of Ca$^{2+}$ and Mg$^{2+}$, then fusion did not take place to any significant degree, although the rounding and swelling processes were not inhibited (Fig. 4D).

However considerably less fusion occurred when Ca$^{2+}$ was omitted from the medium and Mg$^{2+}$ was present, even without EDTA in the medium (Fig. 4E). Incubation of the cells in the absence of Mg$^{2+}$ but in the presence of Ca$^{2+}$ did not reduce the ability of the fibroblasts to fuse (Fig. 4F). In incubations with oleylamine for 5 min in the absence of divalent ions but with 10 mM EDTA, fibroblast fusion was partially restored by the addition of 15 mM Ca$^{2+}$. This may indicate that Ca$^{2+}$ is important at the early stages of exposure to oleylamine in order to effect fusion.

**Lanthanum ions.** Monolayers were incubated with oleylamine (0.167 mg/ml) in the presence of divalent ions and also La$^{3+}$ at 2, 0.02 and 0.002 mM. Lanthanum ions (2 mM) inhibited the fusion of fibroblasts but permitted swelling, thus reproducing the effects of EDTA in the absence of divalent ions (Fig. 4G). This inhibitory effect was only marginally evident at 0.02 mM and absent at 0.002 mM. Lanthanum can therefore antagonize the effects of calcium ions in promoting oleylamine-induced fusion, even at lower molar concentrations than that of calcium.

Viability of fibroblasts following oleylamine treatment

At concentrations of oleylamine required to promote cell fusion (0.167 mg/ml) or at lower concentrations (0.084 mg/ml) where swelling only was observed, cell division was halted in cells grown as a monolayer. Cells treated in suspension lost their ability to adhere to glass and to divide. These effects may be due to the disruptive action of oleylamine on the cell membranes or possibly a toxic effect on metabolic processes.
Oleylamine-induced cell fusion

The presence of 10% dimethylsulphoxide or foetal calf serum in the incubation medium did not protect the cells against these effects of oleylamine. Fibroblasts treated in suspension with oleylamine in the form of liposomes or lipid particles were also unable to adhere to glass coverslips. However fibroblasts grown in monolayers and treated with mixed lipid particles containing 0.084 mg oleylamine, 0.84 mg glycercyl dioleate and 0.84 mg glycercyl monooleate did divide and became confluent after treatment. Therefore oleylamine may be more useful as a fusogenic co-agent, even though the extent of fusion was less than with oleylamine alone. Hybridization experiments are necessary to determine whether fused cells obtained by using oleylamine can give rise to viable heterokaryons.

DISCUSSION

Oleylamine is an effective agent in promoting the fusion of A9 fibroblasts to form polynucleate homokaryons. At high concentrations of oleylamine most cells in monolayer cultures are involved in fusion or the preliminary stages associated with fusion. Oleylamine was less effective with cells in suspension. These results are encouraging in that they show that extensive chemically induced fusion of fibroblasts is feasible despite the fact that oleylamine ultimately had an inhibitory or toxic effect on cell division. It is also still possible that this or similar compounds may be employed in hybridization experiments in which viable hybrids are required.

In many respects the events leading to fusion of the fibroblasts resemble those resulting in fusion of avian erythrocytes induced by other chemical agents (Ahkong et al. 1973). In the erythrocyte an initial quiescent phase is followed by swelling, some loss of haemoglobin and finally fusion of cells. In the fibroblast there is no obvious loss of cytoplasm or organelles in the early stages, but the appearance of large clear spaces under phase-contrast microscopy suggests a rapid influx of water into the cells particularly where the large protrusions begin to form. As in the erythrocyte only swollen cells eventually fuse, usually when 2 adjacent membranes covering the regions of swelling come into contact.

In the fusion process there were no obvious microvilli, the presence of which facilitates virus-induced fusion (Schneeberger & Harris, 1966), but membrane distension may have had a comparable effect, exposing larger areas of membrane lipid that may enhance the coalescence of the adjacent membranes. Electron-microscopic evidence strongly suggests that oleylamine has a profound effect on the cytomembranes indicating rapid penetration of the plasma membrane to produce internal changes in the cell. However, for cell fusion the effects on the plasma membrane are of major interest. The positive charge of oleylamine may neutralize the net negative charge on the outer surface of the plasma membrane, thus reducing repulsive forces which decrease the chance of fusion occurring. On the other hand, insertion of oleylamine molecules in the membrane bilayer could alter the fluidity of the membrane or bring about a reorientation of its constituent lipids. Such effects may give rise to the increased permeability of the membrane, leading to the swelling that is a prerequisite of fusion.

The apparent toxicity of oleylamine to fibroblasts may in part be a separate phenomenon from its fusogenic properties. A study on the toxicity of streptolysin to leuco-
cytops revealed a similar sequence of events to those described for oleylamine, i.e. rounding and liquefaction of the cytoplasm as evidenced by the increased Brownian movement of the cytoplasmic granules (Hirsch, Birnheimer & Weissmann, 1963). However, no mention was made by Hirsch et al. of leucocyte fusion: if it had occurred this would have been of particular interest since these cells are known to be resistant to virus-induced fusion. It was suggested that the toxicity of streptolysin was the result of lysosomal disruption.

The swelling of fibroblasts following oleylamine treatment must be distinguished from the process of stalagmosis and stalagmoptysis in which the swellings or 'blebs' become spherical and bud off from the parent cell. Such effects were observed in Ehrlich ascites tumour cells treated with autoxidation products of polyunsaturated compounds and were considered to be different both from the processes observed in hypotonic media and from those resulting from the lytic action of detergents (Schauenstein, 1967).

The observed diminution of the effectiveness of oleylamine at pH 8.5 contrasts with the results of Croce et al. (1972) which suggest that at pH 8 hybridization with viruses or lysolecithin takes place more frequently than at lower pH. This difference may be due to the net positive charge of oleylamine but in experiments on erythrocyte fusion lower pH values were also more effective with anionic fusogenic agents (Lucy et al. 1971; Ahkong et al. 1973).

The facilitation of the fusion process by dextran is currently not understood. Conceivably dextran may help to stabilize the treated membrane or it may simply counter the osmotic influx of water.

The apparent requirement for calcium ions is typical of many membrane-dependent processes. Calcium is necessary for certain stages in virally-induced fusion of cultured cells (Okada & Murayama, 1966), and also for the actions of fusogenic chemical agents (Lucy et al. 1971; Ahkong et al. 1973). Although the swelling stage was independent of divalent ions, the fusion itself was distinctly enhanced by calcium in our experiments. Yanovsky & Loyter (1972) have suggested a dual function for Ca2+ in virus-induced fusion. Both Ca2+ and Mn2+ reduce lysis of chicken erythrocytes by viruses (Toister & Loyter, 1970) and therefore it was proposed that these ions promote fusion by stabilizing the membranes. In the case of oleylamine-induced fusion, significantly more lysis of fibroblasts occurred in the absence of Ca2+, lending support to this proposition. A second proposed effect of Ca2+ is the inhibition of the activity of ATPase (Na+, K+) thereby reducing the utilization of ATP, which may also help stabilize the membrane (Yanovsky & Loyter, 1972). Poste & Allison (1971) have drawn attention to the importance of calcium ions in secretion and have put forward a hypothesis for membrane fusion involving a calcium-dependent ATPase. Lanthanum ions block the calcium-dependent component of antigen-stimulated histamine release from mast cells at very low concentrations, reaching an optimum inhibition at 5 x 10^-7 M in the presence of 1.8 x 10^-5 M Ca2+ (Foreman & Mongar, 1972). In the absence of antigen, a small calcium-independent release of histamine was observed, which was stimulated by 3 x 10^-5 M La3+. In oleylamine-induced cell fusion, the greatest effect of La3+ was at 2 x 10^-3 M, but inhibition was apparent to
some extent at $2 \times 10^{-5}$ M, adding further weight to the evidence that this fusion process is a calcium-dependent phenomenon.

From the evidence presented above, it appears that oleylamine-induced cell fusion is governed by conditions that are common to other dynamic membrane phenomena. Further investigations of oleylamine and related compounds may be useful in relation to the production of viable, interspecific cell hybrids.

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REFERENCES


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Fig. 1. Photomicrographs (phase-contrast x 700) of mouse A9 fibroblasts cultured as monolayers. A, cells after treatment with oleylamine (0.167 mg/ml modified Eagle's medium pH 7.4 containing 30 mg/ml Dextran 60 C) for 15 min. Different stages observed in cell fusion are indicated by arrows; w, a rounded and swollen cell showing a clear space beneath the membrane and an accumulation of organelles around the nucleus; x, 2 swollen cells in close contact; y, a homokaryon containing 3 nuclei each of which is closely surrounded by subcellular organelles of the corresponding parent cell; and z, fused cells undergoing lysis, in some cases liberating free nuclei. B, A9 fibroblasts incubated in modified Eagle's medium containing 30 mg/ml Dextran 60 C.
Fig. 2. Electron micrographs of mouse A9 fibroblasts fixed on coverslips. $\times 10500$. 
A, fused cells observed following incubation for 15 min in modified Eagle's medium pH 7.4 containing 0.167 mg/ml oleylamine and 30 mg/ml Dextran 60 C; b, cytoplasmic bridge between the 2 cells showing the absence of a dividing plasma membrane and of organelles in the region of the bridge; c, condensed chromatin; n, nucleus; nm, nuclear membrane. b, a cell treated under the same conditions as in A, except that oleylamine was absent from the incubation medium.
Fig. 3. Electron micrographs of mouse A9 fibroblasts fixed on coverslips. × 22,000. 
A, part of a cell observed following incubation for 15 min in modified Eagle's medium 
pH 7.4 containing 0.167 mg/ml oleylamine and 30 mg/ml Dextran 60; m, damaged 
mitochondrion; n, nucleus; r, disorganized endoplasmic reticulum; v, membrane- 
bound vesicle. B, part of a cell treated under the same conditions as in A, except that 
oleylamine was absent from the incubation medium.
Fig. 4. Photomicrographs of mouse A9 fibroblasts fixed on coverslips with Zenker's fixative and stained with Harris's haematoxylin. Cells were treated with oleylamine at a concentration of 0.167 mg/ml in B-G.

A, cells incubated in modified Eagle's medium (containing 5 mM Ca\(^{2+}\) and 0.8 mM Mg\(^{2+}\)) pH 7.4 containing Dextran 60 C. \(\times 300\).

B, fused cells observed after incubation for 20 min as in A, but with added oleylamine. \(\times 300\).

C, as for B, but at higher power (\(\times 700\)).

D, cells pretreated with 10 mM EDTA and then treated with oleylamine in a medium containing no Ca\(^{2+}\) or Mg\(^{2+}\) ions; no fusion occurred. \(\times 700\).

E, cells treated with oleylamine in the absence of calcium ions but in the presence of Mg\(^{2+}\) (0.8 mM); few fusions occurred. \(\times 700\).

F, fused cells observed after treatment with oleylamine in the absence of Mg\(^{2+}\) but in the presence of Ca\(^{2+}\) (5 mM). \(\times 700\).

G, cells treated with oleylamine in modified Eagle's medium (containing 5 mM Ca\(^{2+}\) and 0.8 mM Mg\(^{2+}\)) with added La\(^{3+}\) (2 mM). Cell fusion was inhibited. \(\times 700\).
Oleylamine-induced cell fusion