Zusammenfassung. Nachweis, dass Vorbehandlung der Larven von *Spodoptera littoralis* (Boisduval) mit subtoxischen Dosen von Gossypol die Toxizität der lokal applizierten Insektizide Leptophos, Cyolan, Zectran und Endrin reduziert. Es wird angenommen, dass Gossypol die Toxizität dieser Insektizide neutralisiert, indem es deren Detoxifizierung durch die Larven stimuliert.

M. B. ABOU-DONIA[15], FARIDA TAMAN, NABILA M. BAKERY and A. H. EL-SEBAE

Department of Plant Protection, College of Agriculture, Alexandria University, Alexandria (Egypt), 19 April 1974.

[15] Present address: Department of Physiology and Pharmacology, Duke University Medical Center, Durham (N.C. 27710, USA).

Immunological Evidence for the Presence of Smooth Muscle-Type Contractile Fibres in Mouse Macrophages[1]

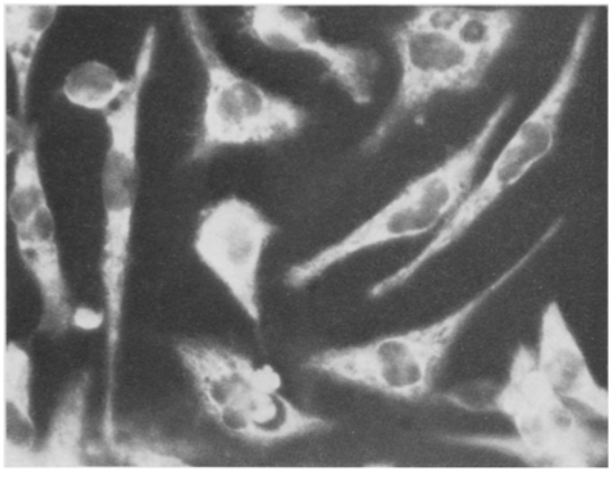
Proteins with properties similar to smooth muscle actomyosin, myosin and actin have been isolated from non-muscular cells such as rat sarcoma cells[2], blood platelets[3], leukocytes[4], and fibroblasts[5,6]. The presence of microfibrils in monocytes with dimensions similar to actin fibrils was first demonstrated by de PETRIS et al.[7], and it was later suggested that these are contractile[8].

It was recently shown by the senior author that antibodies prepared against actomyosins from smooth muscle, although species non-specific, are muscle-type specific and are capable of reacting with contractile elements from non-muscular cells[9,10]. We assumed that the use of such antibodies tagged with fluorescein might assist in localization of contractile fibrils in macrophages.

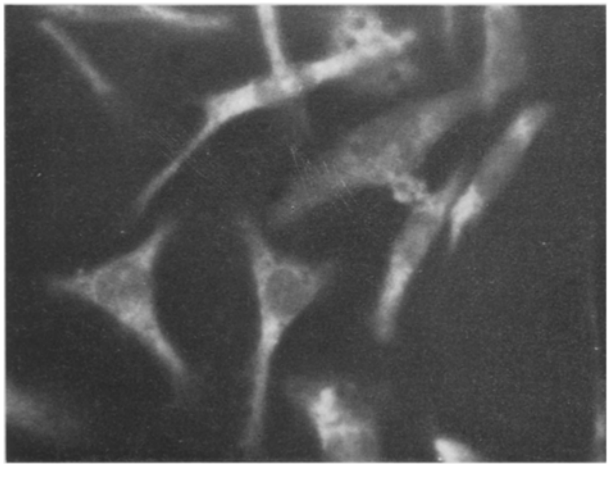
Mouse peritoneal macrophages were harvested and allowed to attach to glass slides according to a modification of GILL and COLE's method[11]. After 16 h of incubation (37 °C, 5% CO_2 in air) the cells were fixed for 30 sec in icecold methanol, and then incubated with the γ-globulin enriched fraction of fluorescein-tagged anti-chicken gizzard (smooth muscle) myosin, anti-chicken cardiac (striated) muscle myosin and anti-chicken actin. (The latter was previously shown not to be muscle-type specific[12].) Bright cytoplasmic staining, excluding the nuclear region, was observed when antisera against smooth muscle myosin or actin were applied (Figure 1a). Antibody against striated muscle myosin did not stain beyond the usual background obtained with non-immune control globulin (Figure 1b).

When isolated and washed macrophages were incubated in the presence of fluoresceinated antibodies against smooth and striated muscle myosins or non-specific γ-globulin, the cells were attached and showed no signs of toxicity after 16 h. Diffuse cytoplasmic fluorescent staining was not observed with any of the three antisera; however, many of the cells showed fluorescent staining of small vesicles near the surface of the cell (Figure 2).

a)



b)



← Fig. 1. Methanol-fixed mouse macrophages stained with fluoresceinated antiserum against: a) chicken actin: diffuse cytoplasmic fluorescence. ×1,000. b) chicken striated cardiac muscle myosin: background fluorescence only. ×1,000.

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From these preliminary experiments we conclude that mouse peritoneal macrophages have in their cytoplasm contractile elements resembling smooth muscle, which can be visualized by immunofluorescent staining with anti-smooth muscle myosin and anti-actin, but only after methanol fixation of the cells. Unfixed macrophages cannot be stained with these antisera. The granular fluorescence seen after prolonged exposure of cultured macrophages to specific as well as non-specific fluoresceinated antisera is due to pinocytotic uptake of the labeled globulin. It was noted that the antibodies directed against contractile proteins will not interfere with the normal attachment and retention of the macrophages on the glass surface. Since this attachment is dependent on the motility of the macrophage, one can conclude that the contractile elements are not inactivated by the antisera and, probably, are not localized in the external layer of the cell membrane. Movement may be caused by cytoplasmic fibrils which can be stained by specific antisera only after the integrity of the cell membrane has been destroyed by fixation.

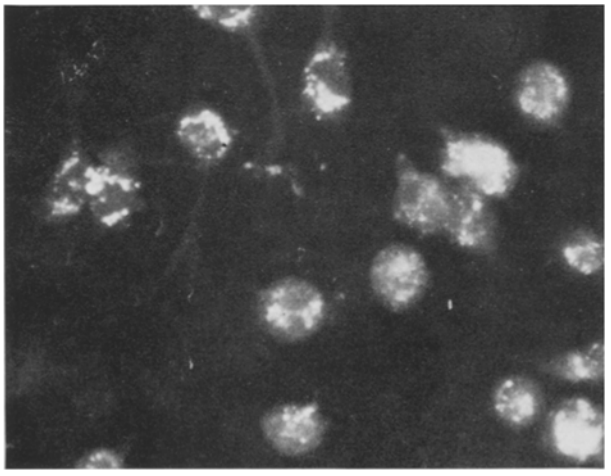

Fig. 2. Unfixed mouse macrophages after 16 h incubation with fluoresceinated non-specific γ-globulin: fluorescence of pinocytotic vacuoles. $\times 1{,}000$.

Zusammenfassung. Im Cytoplasma von Makrophagen aus der Peritonealflüssigkeit von Mäusen finden sich kontraktile Elemente, die mit fluoreszenzmarkierten Antikörpern gegen Myosin glatter Muskeln und gegen Aktin nachzuweisen sind.

UTE GRÖSCHEL-STEWART[13] and D. GRÖSCHEL

Universitäts-Frauenklinik, D–87 Würzburg (Federal Republic of Germany), and University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston (Texas 77025, USA), 17 April 1974.

[13] This work was supported by a research and a travel grant to U.G.-S. from the Deutsche Forschungsgemeinschaft.

In vitro Formation of Calcite Concretions

The formation of calcite ($CaCO_3$) by living organisms has been the subject of many investigations[1–3]. Few reports have appeared which enable us to judge how biological forms of calcite in coccoliths, foraminifera deposits, urinary and pancreatic calculi, and mulluscan shells, can be formed with such high degree of organization from both crystallites and organic matter[1]. Laboratory methods for growing calcite succeeded to grow reasonable sizes of calcite single crystals[4,5]. Attempts to grow structured artificial concretions similar to those formed in nature, met so far with little success. This report describes, for the first time, the conditions by which we were able to grow calcite concretions in vitro, having similarity in architecture and organization to coccoliths and chambered tests of foraminifera.

Calcite was grown in a gel system from interaction between calcium chloride and ammonium carbonate. The theory of crystallization in gel, structure of the gel, mechanism of nucleation and quality of crystals are well-known from the work of HENISCH[6]. In this work, the growth medium was prepared from purified calfskin gelatin (Eastman Kodak). 55 g of gelatin were dissolved in 1 l of bi-distilled water by gentle heating. The solution was cooled and 1 ml of formaldehyde solution (37%) was added. The pH of the gel was 4.7. Slow diffusion of the reacting ions was achieved by carefully layering 10 ml of calcium chloride dihydrate solution (10 mg/ml) at the top of the gelatin gel. At the other end of the gel surface, 40 ml of ammonium carbonate solution (6.5 mg/ml) were added (Figure 1). The growth medium was incubated in

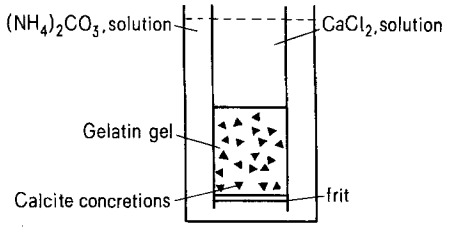

Fig. 1. Calcite growth in tubes with fritted disks.

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