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.

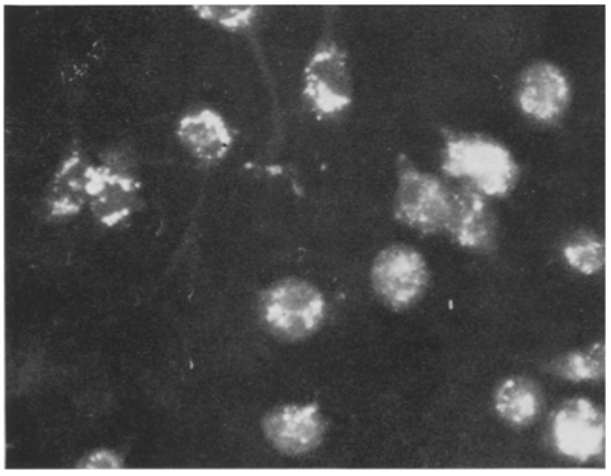

Fig. 2. Unfixed mouse macrophages after 16 h incubation with fluoresceinated non-specific $\gamma$-globulin: fluorescence of pinocytotic vacuoles. $\times 1{,}000$.

*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

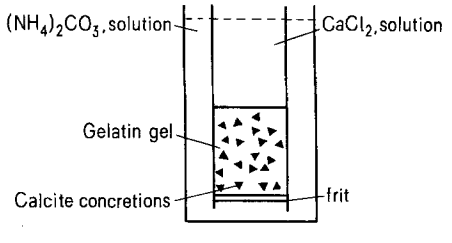


Fig. 1. Calcite growth in tubes with fritted disks.

[1] J. J. ALLEVA, F. R. ALLEVA and B. E. FRY, Science *174*, 601 (1971).
[2] E. BOQUET, A. BORONAT and A. RAMOS-CORMENZANA, Nature, Lond. *246*, 527 (1973).
[3] L. J. GREENFIELD, Ann. N.Y. Acad. Sci. *109*, 23 (1963).
[4] Č. BARTA, J. ŽEMLIČKA and V. RENÉ, J. cryst. Growth *10*, 158 (1971).
[5] P. M. GRUZENSKY, in *Crystal Growth*, Proceeding of an International Conference on Crystal Growth, Boston 1966 (Ed. H. S. PEISER, Symposium Publications Division, Pergamon Press, New York 1967), p. 365.
[6] H. K. HENISCH, *Crystal Growth in Gels* (Pennsylvania State University Press, University Park and London 1970).

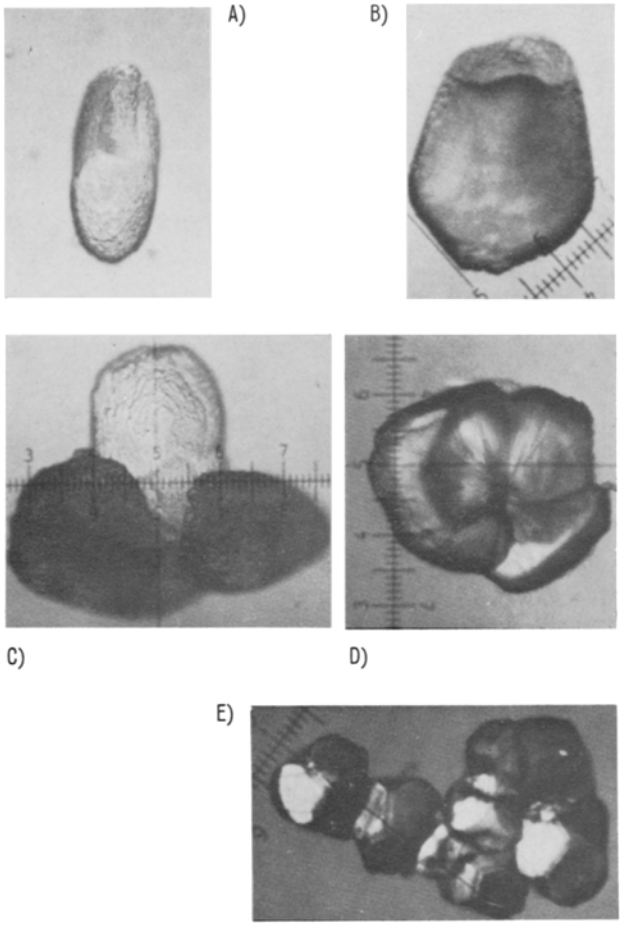


Fig. 2. Different forms of calcite concretions grown in vitro (×125). A), B) and C) calcite grown in gelatin gel system containing formaldehyde; D) calcite grown in gelatin gel system in the absence of formaldehyde; E) individual calcite crystals obtained with a 5-fold increase of calcium and carbonate ions.

the dark at 25 °C for 30 days. Calcium carbonate obtained from this reaction was separated from the gel medium and identified physico-chemically as calcite by its solubility in HCl, by IR-spectroscopy and by differential thermal analysis. Comparison with known calcite crystals provided confirmation.

The microscopic examination of calcite in polarized light revealed an extraordinary architectural build up of small discrete crystallites of calcite. Figure 2 shows the type of concretions obtained in the gel system. A) and B) show 'basket' shaped, and C) elliptical shaped structures similar to certain types of coccoliths described in the literature[7], with the exception that these forms are larger in size (ranging from 100 to 400 μm). If formaldehyde was removed from the gelatin medium, we obtained forms similar to some calcified tests reported in foraminifera species[7] (Figure 2, D). The size of these mineral deposits permitted detailed study of the surface under the optical microscope and showed side by side aggregation of single crystal units. In separate growth experiments, we studied the effect of higher concentration of the reacting ions. It was found that, when the concentration of both Calcium chloride and Ammonium carbonate was increased 5 times to 50 mg/ml for calcium chloride and 32.5 mg/ml for ammonium carbonate, these forms disappeared completely and separate deposits of individual crystals of calcite were formed (Figure 2, E).

We describe this finding in view of earlier reports that the production of highly structured calcite by living organism[7,8] must imply some measures of biological control. In view of these in vitro findings, we feel that the formation of calcite and its concretions in nature is not directly controlled by the cell. It is simply a chemical event dictated and governed by both the diffusion kinetics and the environmental conditions. The gelatin substrate described in this study is highly involved in the calcification process. It served as a favourable organic matrix controlling nucleation, growth and orientation of calcium carbonate crystallites. The proper understanding of these conditions will offer new possibilities of how a living organism manufactures hard structures[9].

*Résumé.* Des concrétions de calcite identiques à celles produites par des organismes vivants ont été développées dans un gel de gélatine. La méthode décrite offre une matrice organique adéquate qui permet de contrôler la nucléation, la croissance et l'orientation des cristaux de calcite dans ces structures organisées.

R. TAWASHI, S. BISAILLON and K. WOLTER

*Faculty of Pharmacy, University of Montreal, C.P. 6128, Montreal 101 (P. Quebec, Canada), 28 March 1974.*

[7] F. G. E. PAUTARD, in *Biological Calcification. Cellular and Molecular Aspects* (Ed. H. SCHRAER; Appleton-Century-Crofts, Educational Division, Meredith Corporation, New York 1970), p. 105.
[8] G. R. CLARK II, Science *183*, 968 (1974).
[9] Acknowledgments: The authors are thankful to M. J. PAUWELS for his technical assistance. This work was supported by the Medical Research Council of Canada.

## Restorative Effect of Cyclic AMP on the Bioelectric Processes of Calcium Deprived Ganglia

It has been observed that cyclic AMP may restore the excitability of $Ca^{2+}$-deprived nerve tissue[1-3]. The effects of cyclic AMP on some bioelectric processes of $Ca^{2+}$-deprived ganglia of the cockroach and the frog have been ascertained in the present study in order to gain some insight into the mechanism of action of cyclic AMP. The results seem to suggest that one of the molecular mechanisms through which cyclic AMP may restore tissue excitability in generation of hyperpolarization through promotion of transmembrane transport of $Ca^{2+}$.

*Methods.* Bioelectric processes have been recorded from the abdominal ganglia of the cockroach following the methods of SPIRA et al.[4] and the paravertebral

[1] C. TORDA and D. O. SITTLER, Fedn Proc. *30*, 2629 (1971).
[2] C. TORDA, in *Advances in Cyclic Nucleotide Research I* (Eds. P. GREENGARD, R. PAOLETTI and G. A. ROBISON, Raven, New York 1972), p. 589.
[3] S. M. CRAIN and E. D. POLLACK, J. Neurobiol. *4*, 321 (1973).
[4] M. E. SPIRA, I. PARNAS and F. BERGMANN, J. exp. Biol. *50*, 615, 628, 633 (1969).