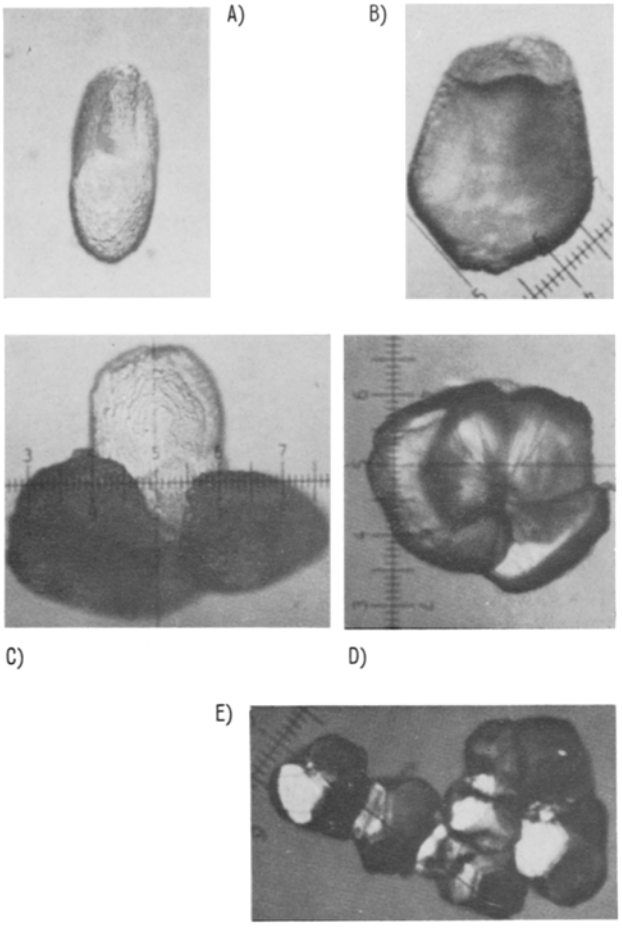

Fig. 2. Different forms of calcite concretions grown in vitro (×125). A), B) and C) calcite grown in gelatin gel system containing formaldehyde; D) calcite grown in gelatin gel system in the absence of formaldehyde; E) individual calcite crystals obtained with a 5-fold increase of calcium and carbonate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

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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

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sympathetic chain of bullfrog following a combination[5] of previously described methods[6–8]. The cockroach (*Periplaneta americana*) was pinned down to a waxed Petri dish. Two lateral longitudinal incisions were made and the dorsal half of the animal (together with the intestines) was cautiously removed, leaving the nerve cord attached to the ventral body wall. The ganglion chain was freed and was mounted on wax in a small lucite chamber. Fine copper-hook electrodes, insulated except at the tips, were inserted between the ganglia. The nerve was stimulated with short square pulses (of 0.05 msec duration) and the responses were recorded by the aid of a Tektronix 561 type cathode-ray oscilloscope. The preparation was moistened with the bathing solution described by YAMASAKI and NARAHASHI[9], either containing Ca^{2+}, or the Ca^{2+} was substituted by Na^+. Cyclic AMP was dissolved in the Ca^{2+}-free bathing solution. (Method of intracellular recordings was the same as described for the frog.)

The paravertebral sympathetic chain of the bullfrog was suspended in a lucite chamber[5] designed by RIKER[8]. The 6th ramus communicans (containing only B fibres) was stimulated by square pulses (of 0.05 msec duration) through fine copper-hook electrodes. The 10th spinal nerve was used as the postsynaptic neuron. Bioelectric processes were recorded through intracellular glass microelectrodes (0.5 μm tip diameter, from 10 to 20 MΩ tip resistance), filled with 3 *M* KCl. The bathing fluid[5] either contained Ca^{2+}, or the Ca^{2+} was substituted by Na^+. Cyclic AMP (Sigma Chemical Co.) was dissolved in Ca^{2+}-free bathing solution. Intracellular microinjections were given by the combined use of pressure and electrophoresis[10]. To measure membrane potentials pulses of constant current were delivered through the recording microelectrode from a bridge arrangement[11,12]. The electric constants of the resting postsynaptic membrane were measured by applying depolarizing and hyperpolarizing square pulses of various intensities through the intracellular recording electrodes. 5×10^{-10} Å applied current usually generated depolarizing and hyperpolarizing potentials of a similar magnitude and time course[5]. The intensity of presynaptic stimuli that generated a comparable spike amplitude for at least 5 min when applied with 1/sec frequency was ascertained. Thereafter, the preparations were immersed into Ca^{2+}-free bathing fluid, changing the fluid until the above described intensity of presynaptic stimuli failed to elicit postsynaptic spikes for several minutes. Thereafter, the bathing fluid was either changed to one containing cyclic AMP in various concentrations, or to a fluid with normal Ca^{2+} content, or remained in the Ca^{2+}-free bathing fluid and cyclic AMP was administered by intracellular microinjections. The effects of the above described presynaptic stimuli on spike formation were tested in the various preparations.

The effects of cyclic AMP on the activity of diphosphoinositide kinase[5,13] and on Ca^{2+} transport[14–17] have also been studied in vitro obtaining essentially similar results[13,14].

Results. Immersion into the Ca^{2+}-free bathing fluid often increased for a few minutes the excitability of the postsynaptic neuron both in the cockroach and in the frog. Within a few minutes spike formation stopped. Reimmersion of the two types of ganglia into either a) Ca^{2+} containing bathing fluid, b) Ca^{2+}-free bathing fluid containing cyclic AMP in various concentrations, or c) intracellular microinjections of cyclic AMP in increasing concentrations (starting with 1 nm) resulted in a gradual recovery of spike formation (Figure 1). Cyclic AMP in concentration of 1×10^{-7} (or $^{-8}$) significantly decreased both the latency of the reversal by Ca^{2+} and the amount of Ca^{2+} that was required to reverse the inhibition of spike formation. Immersion into a Ca^{2+}-free bathing fluid containing cyclic AMP in amounts of 1×10^{-6} *M* sufficed to reverse the inhibition.

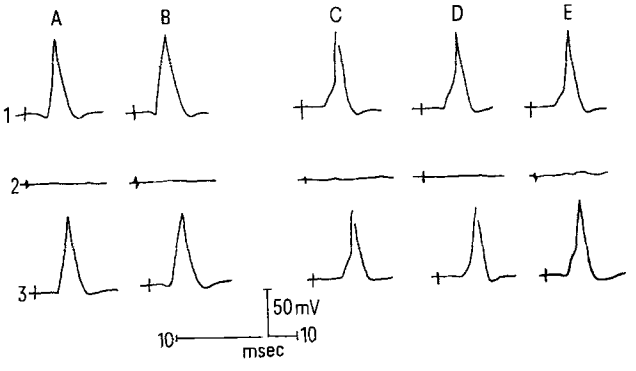

Fig. 1. Reversal of inhibition due to Ca^{2+} deprivation by cyclic AMP (intracellular recording). A) Abdominal ganglion of cockroach (a representative record taken from 100 experiments). 1. Postsynaptic spike generated by presynaptic stimulation (controls). 2. Immersion in Ca^{2+}-free bathing solution. 3. Immersion in cyclic AMP (1×10^{-6} *M*) dissolved in Ca^{2+}-free bathing solution. B) 1. Same as A1–2. Same as A2–3. Immersion in Ca^{2+} containing bathing fluid. C) Sympathetic ganglion of frog (a representative record taken from 120 experiments). 1. Same as 1A–2. Same as A2–3. Same as A3. D) 1. Same as C1–2. Same as C2–3. Same as B3. E) 1. Same as D1–2. Same as D2–3. Intracellular microinjection of cyclic AMP.

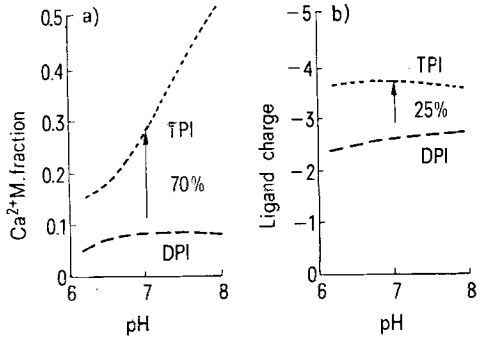

Fig. 2. Effect of phosphorylation of diphosphoinositide to triphosphoinositide on ligand charge and on Binding of Ca^{2+}. Ligand charge: increases about 25% at pH 7. Ca^{2+} bindign: increases about 70% at pH 7.

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Resting membrane potential measurements (frog)

Procedure	No. of experiments	Resting membrane potential ± S.E.
Control	100	− 65 ± 1.7 (critical potential − 39 + 2.0)
Ca^{2+}-free	98	− 36 ± 1.9
Cyclic AMP (1×10^{-6} *M*)	33	− 59 ± 2.3
Cyclic AMP (injection)	33	− 63 ± 3.7
Ca^{2+} containing bathing solution	33	− 66 ± 2.9

Previous researchers[3] assumed that cyclic AMP restores spike formation by increasing the release of acetylcholine from the presynaptic neurons. It occurred, therefore, to ascertain whether cyclic AMP has any direct effect on the postsynaptic neuron. The observed effects of cyclic AMP on the resting potential of the postsynaptic neuron of the bullfrog immersed in Ca^{2+}-free bathing solution have been summarized in the Table. The resting membrane potential of the neurons with normal Ca^{2+} content averaged −65 mV. Immersion into Ca^{2+}-free bathing fluid reduced the resting membrane potential, and thus increased the excitability of the postsynaptic neuron at first. When the resting potential passed the critical potential for spike formation (average of −39 mV), postsynaptic spiking stopped. Cyclic AMP (by intracellular microinjections, or added to Ca^{2+}-free bathing fluid) gradually decreased the resting membrane potential. Spiking recurred as soon as −39 mV was reached. These results suggest that cyclic AMP restores the excitability of Ca^{2+} deprived postsynaptic neuron by a direct action on the postsynaptic membrane.

In vitro biochemical experiments confirmed previously reported results about the increase[14,18] of Ca^{2+}-binding and ligand charge by phosphorylation of diphosphoinositide to triphosphoinositide due to added diphosphoinositide-kinase (Figure 2)[19]. Also, cyclic AMP activates diphosphoinositide kinase[5,13,20]. By binding to cyclic AMP, the regulatory subunit of diphosphoinositide kinase releases the catalytic subunit to phosphorylate diphosphoinositide to triphosphoinositide.

Discussion. The results suggest that the inhibition of postsynaptic spiking during deprivation of Ca^{2+} is due to rising the membrane resting potential between 0 and −39 mV. The reversal of this inhibition may result from hyperpolarization due to reimmersion into Ca^{2+} or to added cyclic AMP. The recurrence of spike formation may also result from increased presynaptic release of acetylcholine due to cyclic AMP[3,21–24].

One of the molecular mechanisms through which cyclic AMP may generate membrane hyperpolarization with a short latency has already been identified[5,18]. Cyclic AMP may use the regulatory subunit of diphosphoinositide kinase[5,13] as one of its postsynaptic receptors. When bound to cyclic AMP the regulatory subunit releases the catalytic one to phosphorylate diphosphoinositide to triphosphoinositide. This process results in a quantitatively predetermined increase of membrane-bound Ca^{2+} and ligand charge[5,14,18,20]. These changes suffice to alter the membrane potential to hyperpolarization[5,25–29].

Zusammenfassung. Ca^{2+}-Entzug hemmt die Bildung von Spikes in postsynaptischen Neuronen, vermutlich durch Änderung des Ruhepotentials. Die Hemmung wird aufgehoben durch Zugabe von zyklischem AMP oder Ca^{2+}. Diese Beobachtung könnte durch Hyperpolarisation der postsynaptischen Membran und Zunahme der praesynaptischen Azetylcholin-Freisetzung eine Erklärung finden.

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Oncornavirus Released from Long-Term Cultures of Human Leukemic Cells

Several attempts have been made to demonstrate the presence of oncornaviruses in human leukemic materials, both morphologically[1–3] and biochemically[4,5], or by combination of both methods[6–8]. Although oncornaviruses were revealed in the cultures tested, no continuous cell lines were obtained by the authors, except of J96 cell line, that, however, has been suspected to be contaminated with HeLa cells[9] producing Mason-Pfizer-like virus[10]. Therefore, basing on our previous observations[2,8], we attempted to obtain cell systems permanently producing oncornavirus type C from human leukemic cells[11].

Materials and methods. Heparinized blood samples were taken from patients with various forms of leukemia. Cell suspension, 6–7 million cells/ml was grown in medium 199