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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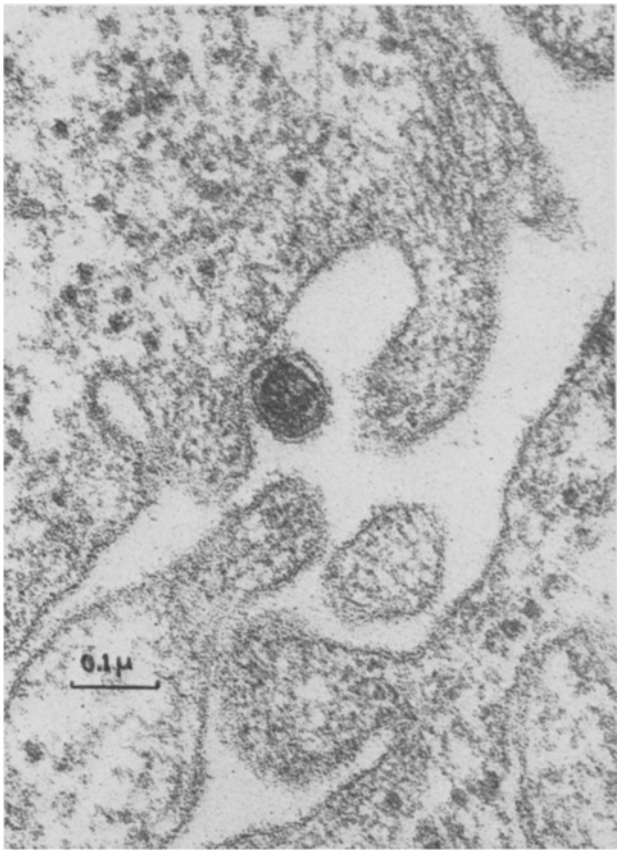
Oncornavirus Released from Long-Term Cultures of Human Leukemic Cells

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].

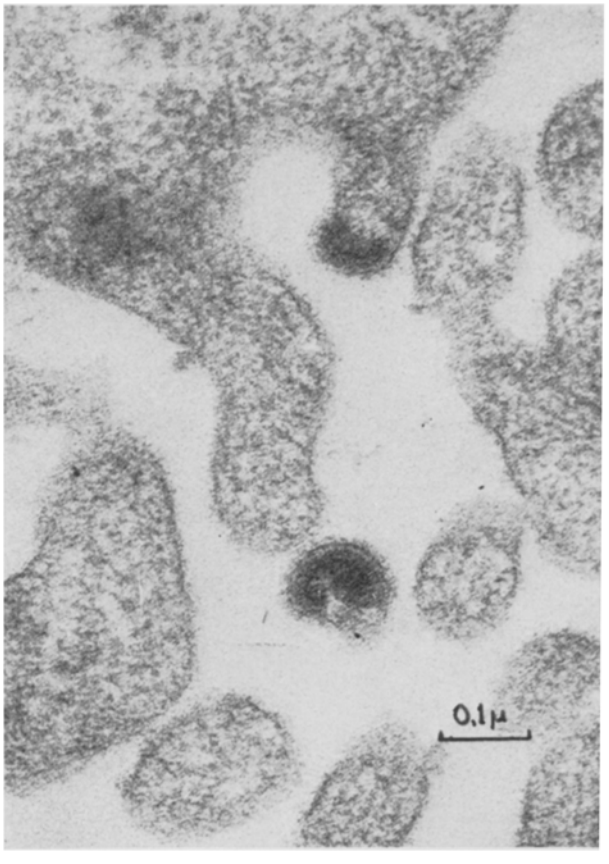
Materials and methods. Heparinized blood samples were taken from patients with various forms of leukemia. Cell suspension, 6–7 million cells/ml was grown in medium 199

Fig. 1. Electron micrographs of particles present in a primary leukocyte culture from a leukemic patient (A) and in human embryo fibroblasts inoculated with the virus from the previously shown leukocyte cultures after at the first (B) and sixth (C) passages. μ = μm.



a)

b)



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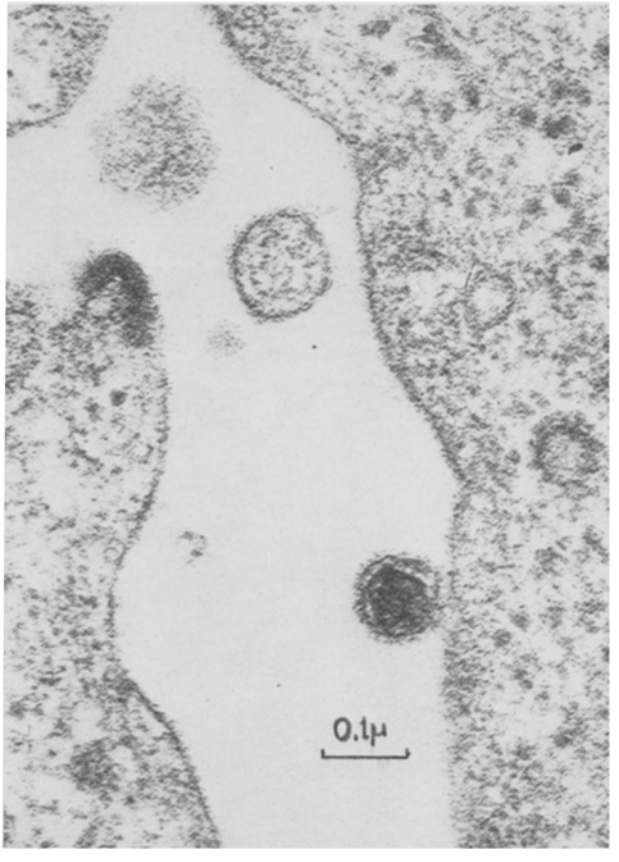

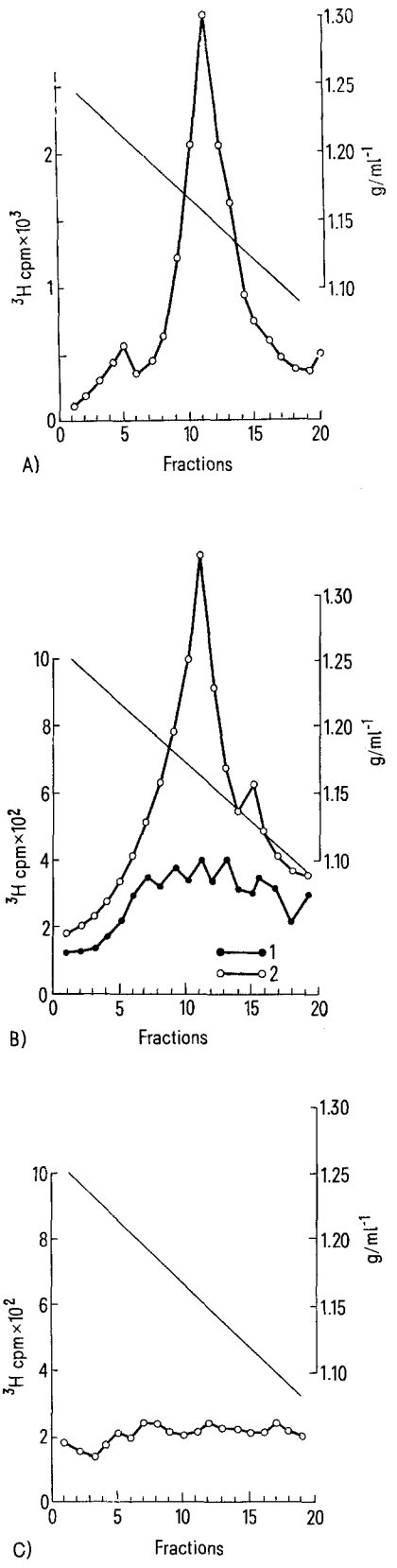

Fig. 2. Isolation of particles from culture medium of a primary leukocyte culture of a leukemic patient (A), from the culture of human embryo fibroblasts inoculated with the virus from the leukocyte culture (B) treated (1) and non-treated (2) with actinomycin D (0.5 μg/ml), and from the non-inoculated culture of human embryo fibroblasts (C).

with 10% bovine serum. The medium was changed every 5–7 days. Ultrathin sections of the cells were studied in an electron microscope. One C-particle positive leukocyte culture was selected for further study. The culture medium from it was clarified and added (1:5) to a monolayer of primary culture of human embryo fibroblasts grown in Eagle's medium. Reimplantation of human embryo fibroblasts were made every 5–7 days.

The following criteria were used for revealing oncornavirus in the culture tested: electron microscopy of ultrathin sections of the cells, the presence of particles with the density of 1.16 g/ml, the presence of high molecular weight (60–70 S) RNA and reverse transcriptase in the particles. The techniques of isolation of oncornaviruses from tissue cultures, and of study of their biophysical and biochemical properties, are described in detail elsewhere[6].

Results and discussion. Figure 1 presents electron micrographs of virus particles revealed in primary leucocyte culture from a leukemic patient and in human embryo fibroblast culture inoculated with the material from leukocytes. It is seen that both the primary culture and the secondary culture contain C-type particles characteristic for oncornaviruses. No such particles were found in control culture of human embryo fibroblasts from the same source, not inoculated with the material from leukemic leucocytes.

The culture of human embryo fibroblasts producing C-type particles was labelled with ^{3}H-uridine, the virus was sedimented from the culture medium and analyzed by equilibrium centrifugation[6] in sucrose density gradients (Figure 2). It is seen that particles are released from the cells into culture medium band at the density of 1.16 g/ml (Figures 2A and B). No particles of this kind are produced by the culture treated with actinomycin D (Figure 2B) and by the control, non-inoculated culture (Figure 2C).

RNA was extracted from particles with the density of 1.16 g/ml and analyzed by velocity centrifugation[6] in sucrose density gradients. Figure 3 shows that the particles contain high molecular weight (70S) RNA that is characteristic for oncornaviruses.

The culture of human diploid cells inoculated with the virus from the leukocyte culture was assayed for the presence of reverse transcriptase reaction associated with 70S RNA[12]. It is seen from Figure 4A that short-term

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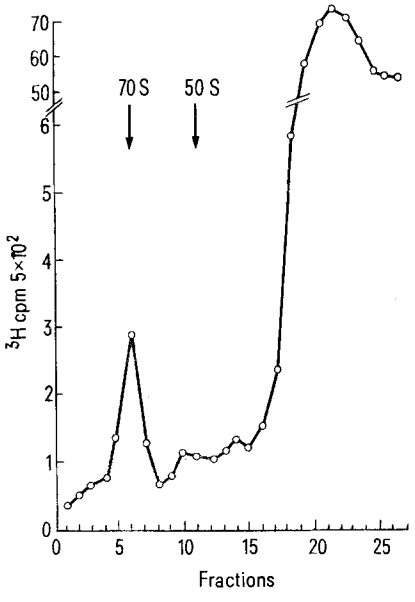

Fig. 3. Sedimentogram of ^{3}H-labelled RNA extracted from particles with the density of 1.16 g/ml produced by the culture of human embryo fibroblasts. The position of the marker 50S P^{32} labelled Sendai virus RNA is shown by the arrow.

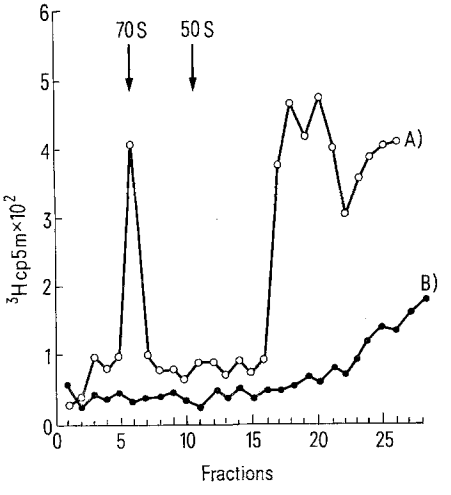

Fig. 4. Sedimentogram of 70S RNA labelled with small fragments of newly synthesized DNA from the culture of human embryo fibroblasts inoculated with the virus from the leukocyte culture (A) and of RNA from the non-inoculated culture (B).

reaction reveals high molecular weights RNA labeled with small fragments of newly syngthesized DNA in the culture, while such structures are absent in non-inoculated human diploid cells (Figure 4).

Our data not only confirm previous observations on presence of C-particles in leukemic leukocyte cultures, but also show the possibility of transmitting the virus to human diploid cells. This opens the way for a broader study of the virus, because the human diploid cells provide a large-scale production of the virus necessary for immunological and molecular-biological studies.

БЫВОДЫ. При выращивании клеток белой крови от больных лейкемией были обнаружены внутриклеточные и внеклеточные вирионы С-типа, характерные для лейковирусов. Материал одной из таких культур был перевит на диплоидные клетки человека. Выделенный вирус имеет основные характеристики онкорнавируса (плотность I, 16г/мл, РНК с константой седиментации 70 S, обратнотранскриптазная активность).

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Latencies in a Thermosensitive Pathway

In mammals, most skin 'warm' receptors and some skin 'cold' receptors are innervated peripherally by unmyelinated C fibres[1] but it is not known what types of fibre are involved in the central projections from these receptors. Some measurements have been made in the cat of conduction velocities in a pathway leading to thalamic cells which respond to skin heating[2]. The response was conducted predominantly by A δ fibres but the pathway in question was not specifically thermosensitive because the thalamic cells responded to both thermal and mechanical stimulation of the skin.

We have now measured latencies at units in the entry zone of the dorsal horn and the relay nucleus in the ventrobasal thalamus in a specifically thermosensitive pathway projecting from the scrotum to the somatosensory cortex of the rat. We used a refinement[3] of the method suggested by Martin and Manning[4] in which the thermal receptors are stimulated by the heat of a photographic flash. Rats were anaesthetized with urethane. A ring-shaped xenon discharge tube, rated at 1 kJ, was mounted 100 mm from the shaved scrotal skin.

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