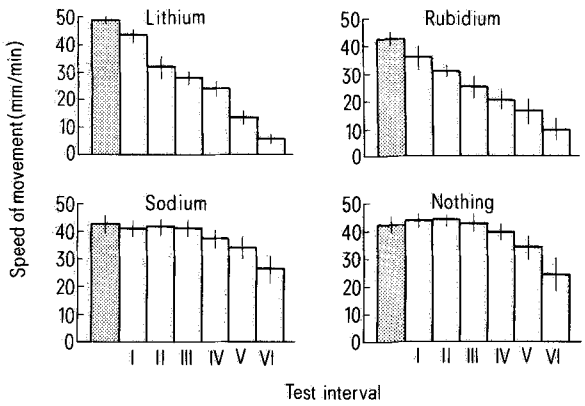
The concentration of lithium at the center and the edge of Petri dishes to which LiCl had been added ranged between 60 and 70 mmoles and 40 and 45 mmoles, respectively. Visual inspection of planaria during tests provided no signs of chemotactic locomotion, but the usual elongated shape of planaria changed to an oval form with ruffled edges in those treated with LiCl or RbCl. No changes were evident in the other 2 groups.

The table shows the concentration of sodium, potassium, lithium and rubidium in planaria after the 30 min test.

Discussion. The present findings show that lithium and rubidium influence the movement of planaria. Most previous studies show lithium and rubidium to influence behavior in opposite ways[6,9,10]. In the present study, however, lithium and rubidium both had an activity-suppressant action. We suppose that the gliding locomotion shown by planaria in our test was governed by exploratory, food-seeking and photophobic responses. Thus, lithium and rubidium appear to have similar actions on behavior mediated by these stimuli in planaria.

Previous studies show that the gliding locomotion of planaria is governed by the movement of cilia[5] and the secretion of mucus[11,12], processes that probably require energy supplied by ATP[13]. In addition, biogenic amines seem to be involved in locomotion of planaria[14]. Lithium and rubidium are also known to influence enzymes involved in ATP production[15,16] as well as the metabolism of some monoamines[17,18]. Perhaps effects of lithium and rubidium on ATP-dependent energy systems and monoamines play a role in their activity-suppressant actions in planaria. Further studies are clearly needed to determine whether these speculations are correct. Nevertheless, the present findings support the notion that phylogenetically low animals may be of use in studies on basic effects of monovalent cations on behavioral processes.



Effect of lithium, rubidium, sodium or nothing (control) on the speed of locomotion of planaria *(Dendrocoelum lacteum)*. The speed of movement was first recorded under baseline conditions (stippled bars). Then solutions of LiCl, RbCl or NaCl (195 mmoles) were added to the 10 ml medium for 30 min at a rate of 6 ml per h during which time the locomotion of each planaria was recorded for 1 min every 5 min. Test intervals I, II, III, IV, V and VI correspond to observations made 5, 10, 15, 20, 25 and 30 min, respectively, after baseline measurements. Each bar corresponds to the mean ± SEM for 12 planaria.

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Inhibition of DNA synthesis in erythroleukemic cells by a liver protein fraction

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Summary. The liver inhibitory factor (LIF) extracted inhibits in vitro the proliferation of erythroleukemic cells. This inhibition takes place 24 h after LIF treatment and is promptly reversible on removal of the factor. ^{3}H-thymidine autoradiography shows that the effect is probably due to a blockage of the cells at the end of G_1.

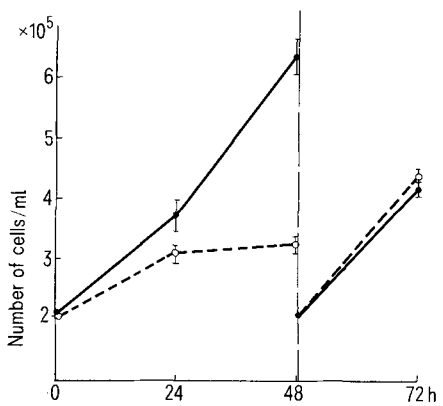
A liver protein fraction has been isolated which inhibits hepatoma cells in vitro[2], and delays hepatic regeneration in vivo[1-3]. This inhibitory substance (LIF) also blocks the proliferation of various kind of tumoral cells within 24 h; it has been shown that this effect is not due to a toxic influence on the cells[2]. The present work was undertaken to

test the action of this rat liver inhibitory factor on viral tumoral cell lines, namely Friend erythroleukemic cells (clone L 745).

Materials and methods. The cells, diluted to a concentration of 2×10^5/ml, were grown in Dulbecco's medium supplemented with 10% foetal calf serum. In the experimental preparation the inhibitory protein(s), which separated as the 3rd peak after filtration on acrylamide-agarose gel according to the method described by Higueret et al.[1], were added at a concentration of 1 µg/ml. An equivalent amount of proteins, separated as the 2nd peak, was added to the controls. The proteins (200 µg/ml) were dissolved in phosphate buffer pH 7.2 and were sterilized by filtration.

In some experiments ^{3}H-thymidine (Radiochemical Centre, Amersham, sp. act. 2000 mCi/mmole) was added to the medium, at a concentration of 0.01 µCi/ml for different period of time; at the end of these periods the cells were washed 3 times with the same medium free of serum and diluted in a new complete culture medium.

For autoradiography a drop of the culture was placed on a slide covered with gelatin and the cells allowed to sediment for a few minutes; after fixation with 70% alcohol according to the method described by Lewensohn and Ringborg[4], the slides were coated with stripping film (Kodak AR 10) and exposed for 20 days.



Inhibition of erythroleukemic cell proliferation in the presence of an inhibitory factor extracted from the liver. The cells are diluted at a concentration of 200,000/ml and their concentration was determined after 24 and 48 h. In the experimental cells 1 µg/ml of LIF was added to the culture medium; in controls an equal amount of liver inactive proteins. The growth of the experimental cells, which is similar to that of the controls during the 1st 24 h, decreases between 24 and 48 h. After this period the cells, both control and experimental, were again diluted at the initial concentration and the medium was replaced with new medium free of proteins. The inhibited cells reacquire their ability to divide at the same rate as that of the controls. Each value is the mean of 5 experiments ± SD. — Control, - - - - liver inhibitory factor.

Results and discussion. In agreement with previous results from our laboratory[5], we found that LIF treatment for 48 h blocks cell growth and that this effect is reversible since the cells retain their ability to proliferate when they are brought back to LIF free medium (fig.). In order to clarify the mechanisms of this reversible antiproliferative effect, the cells were left to grow in the presence of ^{3}H-thymidine for 24 h. In the controls both the percentage of labeled cells (about 75%) and the grain number/cell (about 13%) are the same after 24 h and 48 h. In the LIF exposed cells the labeling index is about equal to that of the controls during the 1st 24 h; however, if the radioactive precursor is present in the medium between 24 and 48 h after LIF exposure the number of labeled cells is halved with respect to that of the controls (34%). In contrast, the rate of incorporation expressed by the grain number/cell does not show any variation (table).

In order to investigate the kinetics of the LIF inhibition, cells, which were grown in the presence of ^{3}H-thymidine between 24 and 48 h, were newly diluted at a concentration of 200,000/ml and placed in new medium. The control cells divide once, as shown by the fact that the percentage of labeled cells does not change, whereas the number of grains/cell is nearly halved. This means that all cells (labeled or not) divide and the cellular cycle is about 24 h.

The same reduction in number of grains/cell can be seen in LIF pretreated cells; however, the percentage of labeled cells decreases significantly compared with 48 h (table). This finding indicates either that some of the cells labeled during the period between 24 and 48 h in the presence of the LIF die, or that they are prevented by LIF from entering the S phase, so that, as soon as LIF is removed, the unlabeled cells enter the S phase immediately, causing a greater dilution of the labeled ones. This 2nd hypothesis is testable, since in this case one should expect the number of

Incorporation of ^{3}H-thymidine in the erythroleukemic cells treated with an inhibitory factor extracted from the liver

Time of exposure to ^{3}H-thymidine (h)	Time of culture after which the cells are processed for autoradiography (h)		Number of cells counted	Number of labeled cells	Percentage of labeled cells		Number of grains/cell
					%	Means ± SD	
0-24	24	C_1	998	750	75.15	76.60	15
		C_2	1,025	800	78.05		15
		E_1	1,021	750	73.46	75.87	15
		E_2	1,022	800	78.28		14
24-48	48	C_1	1,016	745	73.33		10
		C_2	1,029	725	70.46	73.47 ± 3.08*	11
		C_3	1,044	800	76.63		10
		E_1	1,018	326	32.02		11
		E_2	1,055	364	34.50	33.77 ± 1.52**	10
		E_3	1,005	350	34.80		10
24-48	72	C_1	1,003	690	68.80		7
		C_2	1,025	625	60.97	66.26 ± 4.58*	7
		C_3	1,014	700	69.03		7
		E_1	1,018	202	19.84		7
		E_2	1,085	275	25.34	21.99 ± 2.93**	7
		E_3	1,080	225	20.80		8
48-72	72	C	1,037	575	55.45		8
		E	1,001	743	74.22		12

C, cells not treated with LIF; E, cells treated with LIF from 0 to 48 h. * NS; ** $p < 0.05$.

cells labeled with ^{3}H-thymidine, between 48 and 72 h, to be greater in LIF pretreated cells than in controls. To this end cells grown for 48 h with or without LIF were newly diluted and placed in a new medium containing ^{3}H-thymidine. As predicted by the 2nd hypothesis the percentage of labeled cells is higher in LIF treated cells with respect to the control (table).

The mechanisms by which the cells are prevented from entering the S phase are still unknown. It is worth noting that the antiproliferative effect appears with a latency of at least 24 h. Since this latency cannot be attributed to a low membrane permeability to LIF, as shown by the fact that the restoration of the proliferative activity follows immediately upon LIF removal, a time consuming process must be involved, the nature of which remains to be determined. As shown by Pietu et al.[3], LIF inhibits DNA synthesis by preventing the increase in DNA polymerase. It may be speculated that a similar action could be responsible for the effect we have observed on erythroleukemic cells.

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Monoclonal antibodies to metallothionein from Cd^{2+}-resistant Chinese hamster lung fibroblasts

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Summary. Four monoclonal antibodies of the mouse against metallothioneins (MTs) from Cd^{2+}-resistant fibroblasts of the Chinese hamster lung (Cd^r-CHL) have been prepared. Each one of the antibodies showed a unique cross-reactivity pattern when tested against MTs from the livers of several mammals and from yeast.

Metallothionein (MT), a low molecular weight protein with a high content of Cd, does not contain aromatic amino acids, and about 1/3 of its amino acid content is cysteine. Detailed analysis of MTs revealed that 2 components (MT-1 and MT-2) of similar amino acid composition and molecular weight are produced (for a review, see Nordberg and Kojima[2]). An immunoassay for MT offered great advantages in terms of specificity, sensitivity, and precision, but the conventional antisera obtained against MTs show cross-reactivity between the 2 molecular species and among MTs derived from various animals[3-8]. Monoclonal antibodies against each component of MTs should overcome these disadvantages of conventional antisera[9]. In this paper, we report the establishment of hybridoma lines producing monoclonal antibodies reactive to MTs, and describe their reactivities to MTs of several mammals and yeast.

Materials and methods. Chinese hamster lung fibroblastic cells made resistant to 50 μM or 100 μM Cd^{2+} (50 μM or 100 μM Cd^r-CHL) were used as the source of MTs. The cells were made resistant to $CdCl_2$ by increasing the concentration of $CdCl_2$ in the culture medium stepwise, beginning from 5 μM, at intervals of 1–2 weeks. Heat-stable cytoplasmic fraction was applied to Sephadex G-75 and subsequently to a DEAE-Sephadex column[4]. The 2 resulting peaks of Cd were separately pooled and lyophilized. Fusion of the spleen cells of a BALB/c mouse immunized with MT-2 of Cd^r-CHL and NS-1 cells was carried out as described by Oi and Herzenberg[10]. Hybridomas were selected by ELISA using MT-1 and MT-2 of Cd^r-CHL as antigens[11]. Immunoglobulin subclass was determined by the double diffusion method[12]. Rabbit antisera against purified mouse IgA, IgG_1, IgG_{2a}, IgG_{2b}, IgG_3, and IgM were purchased from Miles Laboratory. Interspecies cross-reactivity of monoclonal antibodies was examined by ELISA using various MTs as antigens.

Results and discussion. Almost 90% of Cd of the heat-stable supernatant of 100 μM Cd^r-CHL was associated with a low molecular weight peak exhibiting high absorption at 254 nm with low absorbance at 280 nm (fig., a). This MT fraction was applied to a DEAE-Sephadex column and separated into MT-1 and MT-2 (fig., b). The elution pattern of heat-stable cytoplasmic supernatant of 50 μM Cd^r-CHL was not significantly different from that of 100 μM Cd^r-CHL. Control CHL cells gave no MT peak in the Sephadex G-75 elution pattern. Lyophilized preparation of MT-1 and MT-2 each showed a single band in polyacrylamide slab gel electrophoresis. Preliminary amino acid

Immunoglobulin subclass and interspecies cross-reactivity pattern of monoclonal antibodies

Subclass	ACM-1 IgM	ACM-2 IgG_{2b}	ACM-3 IgM	ACM-4 IgG_{2b}
Cd^r-CHL MT-1	+	−	±	−
2	+	+	+	+
Mouse L MT-1	+	−	±	−
2	+	+	+	±
Rabbit L MT-1	+	+	+	−
2	+	+	+	−
Pig L MT	+	+	+	−
Bovine L MT	+	−	−	−
Monkey L MT-I	+	+	+	−
II	+	+	+	−
III	+	+	+	−
IV	+	+	+	−
V	+	+	+	−
Yeast MT	−	−	−	−

Preparation of MT: Cd^r-CHL MT-1 and 2, see 'Materials and methods'. Mouse L-MT-1 and 2[13]. Rabbit L-MT-1 and 2[14]. Monkey L-MT-I, II, III, IV and V[15]. Yeast MT[16]. Pig L-MT; the MT fraction on Sephadex G-75 gel filtration was prepared from the liver of Cd-treated pig according to the method described by Kimura et al.[14]. Bovine L-MT: the MT was prepared from the bovine liver (unpublished). Abbreviation: L; liver.