Our present results and those of the earlier authors are consistent with the view that streaming does not depend upon an internal hydrostatic pressure gradient. There remains the possibility that local pressure gradients (for example, those caused by frontal contraction) might be present. The fact that pseudopod extension is initiated at the pseudopod tip (*1, 20–22*), as well as the photoelastic cycle at pseudopod tips (*2*), argue that the motive force is applied as tension to the viscoelastic endoplasm at pseudopod tips. Available evidence suggests that the frontal contraction model applies at least to the "*Chaos-Amoeba* group" (*23*). Griffin (*24*) has suggested that anteriorly flattened species of amoebae (such as *Amoeba verrucosa* and *A. striata*) are driven by a similar contraction. The views of Abé (*25*) concerning *A. striata* would be similar if it should turn out that "gel formation" and cytoplasmic contraction are one and the same process at the front ends of amoebae.

As Griffin (*26*) has pointed out, a totally different mechanism of pseudopod extension is evident in the giant herbivorous amoeba, *Pelomyxa palustris*. Here the extremely simple monopodial movement is consistent with a positive pressure gradient model. Many other forms of pseudopods show interesting functional and ultrastructural diversity, which may reflect different mechanisms of pseudopod extension and retraction. The results presented here should not be generalized to all other types of cell movement. They do indicate, however, a reasonable experimental approach to answering the question of whether hydrostatic pressure is, or is not, the motive force for a given biological movement phenomenon involving cytoplasmic streaming.

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Suppression of Urethan-Induced Lung Adenomas in Mice Treated with Trehalose-6,6-Dimycolate (Cord Factor) and Living Bacillus Calmette Guérin

Abstract. *Trehalose-6,6-dimycolate (cord factor), a glycolipid from mycobacteria, suppressed the development of urethan-induced tumors in the lungs of mice to a similar degree as living* Mycobacterium bovis *(strain BCG) bacilli. The inhibition was apparently due to the host cellular reaction caused locally by cord factor.*

Experimental intravenous infection with bacillus Calmette Guérin (BCG) induces granuloma formation in the lungs, spleen, and liver of mice, increases antibody formation against unrelated antigens, and enhances the activity of the reticuloendothelial system (*1*). Nonspecific resistance to heterologous bacterial infections as well as to grafted tumors of animals sensitized with BCG is apparently related to the above changes (*2*). Relevant to these activities are also the observations that experimental leukemia can be cured in some cases (*3*), that remissions in human leukemia can be prolonged with BCG (*4*), and that, according to the results of a recent statistical study, leukemia mortality is about two times less among BCG-vaccinated than among nonvaccinated subjects (*5*). It has become evident that a chemically defined glycolipid from tubercle bacilli, trehalose-6,6-dimycolate (cord factor), is able to elicit a typical granulomatous response in the lungs of mice after an intravenous injection of amounts as small as 1 to 5 μg. The cellular composition was indistinguishable from that in granulomas formed after an infection with living BCG. Repeated injections of cord factor induced a more extensive cellular response than did one injection of the same total amount of cord factor, a reaction very similar to that which appears after repeated injections of intact tubercle bacilli and which is known as the accelerated tubercle formation (*6, 7*). Macrophages from mice first treated intraperitoneally with cord factor and another mycobacterial fraction (wax D) showed increased acid phosphatase activity. Such animals were resistant to infection with heterologous bacteria and Ehrlich ascites cells (unpublished data).

Cord factor (5 to 10 μg) when injected into the foot pads of mice, induced histological changes similar to changes that followed injections of living BCG. Both materials induced, in the draining lymph nodes, hyperplasia of the lymphoid tissue in the paracortical zone, accumulation of macrophages, and formation of granulomas composed of macrophages, epitheloid cells, and lymphocytes (*8*). Because no

cellular reaction was evident in the internal organs such as liver, spleen, and lungs, it was possible to show that the increased antibody response to an unrelated antigen—which was evident in such animals—is of a local character, dependent on the above-mentioned cellular reaction (*9*).

We have assumed that the activities of living BCG are connected with the host cellular reaction caused by the multiplying bacilli at the site of lodgment. Since cord factor causes a similar cellular reaction it might be expected to act as BCG under the same experimental conditions. We now show that formation of lung adenomas induced by urethan was suppressed in mice in which a strong granulomatous response was maintained in the lungs by an intravenous BCG infection or by intravenous injections of relatively small amounts of cord factor (*6*). Albino mice of a local strain were used in the experiment. Preparations of cord factor and wax D as well as their emulsions for injections into the animals have been described (*6*). The suspension of living BCG was prepared from a 7-day culture of BCG in Dubos broth base medium with Tween 80. The cultures were seeded from ampules of vaccine prepared by the Serum Institute of Copenhagen and intended for vaccination of humans.

Groups of 15 mice were used in the experiment. Group 1 was injected intravenously with 0.2 ml of a suspension of BCG; group 2 was injected intravenously three times with 0.2 ml of an emulsion, containing 10 μg of cord factor from *Mycobacterium kansasii*. The interval between the first and the second injection was 7 days; between the second and third it was 5 days. Group 3 was injected three times intravenously with 0.2 ml of emulsion containing 10 μg of wax D from *M. tuberculosis* strain Peurois. Group 4 was injected three times intravenously with 0.2 ml of an emulsion containing 10 μg of cord factor (from *M. kansasii*) and 10 μg of wax D from strain Peurois at the same time as group 2. Group 5 (control emulsion) was injected three times intravenously with 0.2 ml of emulsion alone. Mice of group 6 were normal mice not given preliminary treatment. Seventeen days after the start of the experiment the mice of all groups were injected intraperitoneally with urethan (1 mg per gram of body weight) made up as a 10 percent solution in saline. Two days after the injection of urethan, groups 2, 3, and 4 were injected intravenously with 5 μg of cord factor, wax D, or a mixture of both, respectively, twice each week for 7 weeks. The control-emulsion group was accordingly treated with emulsion alone. The animals were killed 7 weeks after the urethan was injected. The presence of the tumors in the lungs and the cellular response were evaluated microscopically; usually three to five sections were measured, the surfaces of which were also examined. The granulomas composed of epitheloid cells, macrophages, and lymphoid cells were present in all lungs of mice treated with BCG, cord factor, or cord factor plus wax D (Table 1).

Table 1. Influence of living *M. tuberculosis bovis* (strain BCG), cord factor and cord factor plus wax D (*11*) on urethan-induced lung adenomas in mice. In groups 1, 2, 3, 4, and 5 one mouse died before the injection of urethan.

Group	Treatment	Mice in group (No.)	Tumor-bearing mice		Sections of lungs			
			No.	Percent	Sections examined (No.)	Total surface (mm^2)	Tumors (No.)	Tumors per 100 mm^2
1	BCG*	14	3	21	46	793.26	5	0.63†
2	Cord factor	15	4	26	51	916.94	7	0.76†
3	Wax D	14	8	57	45	749.73	20	2.67
4	Cord factor plus wax D	14	4	28	52	840.77	4	0.48†
5	Emulsion	14	10	71	50	869.01	18	2.07
6	None	15	9	60	64	808.52	17	2.10

* The suspension of BCG had an optical density of 80 Klett units (about 6×10^6 per milliliter). † Statistically significant as compared with the emulsion group and the group of normal mice; by the rank order test $P < .01$, by the test of Pittman $P < .01$ (*12*).

There was a significant suppression of urethan-induced tumors in the groups treated with BCG, cord factor, or a mixture of cord factor and wax D. The differences between these groups and the control groups are statistically significant. Wax D alone did not affect the development of tumors, but in combination with cord factor it seemed to add to the effect exerted by the cord factor. Although the differences between these groups are statistically not significant, mixtures of cord factor and wax D in similar experiments with Ehrlich ascites cells had a stronger inhibitory effect than cord factor alone (unpublished data). The results of our study indicate that the development of tumors induced by urethan is inhibited by the cells that accumulated in the lungs. If BCG or cord factor were injected intraperitoneally, tumor cells would probably grow because of the scarce cellular reaction in the lungs after an intraperitoneal injection (*6*). Although such an experiment has not been carried out, the above assumption is supported indirectly by the results of another one which we performed with Ehrlich ascites cells (Table 2). Thus, an intravenous infection of mice with BCG bacilli has a very weak effect, if any, on the subsequent growth of Ehrlich ascites cells in the peritoneal cavity. However, the growth of the tumor cells is strongly suppressed at the same site after a previous intraperitoneal infection with BCG.

The suppression of urethan-induced emerging tumor cells in the lung tissue is apparently due to the encounter with host cells that evolve during infection with BCG bacilli or treatment with cord factor. This is also true with Ehrlich ascites cells.

Our interpretation is supported by recent reports according to which injection of living BCG bacilli into es-

Table 2. Relations between site of administration of living BCG and subsequent growth of Ehrlich ascites cells (EAC) in the peritoneal cavity of mice. The optical density of the suspension of bacilli was 90 Klett units, and the volume injected was 0.2 ml. Cells (1.5×10^6 EAC) were injected intraperitoneally 10 days after injection of BCG. Seven days afterward, the tumor cells were washed out of the peritoneal cavity, and the volume of packed cells was established; i.v., intraveneous injection; i.p., intraperitoneal injection.

Mouse No.	Volume of packed EAC cells (milliliter per mouse)		
	Control	BCG i.v.	BCG i.p.
1	0.77	0.64	0.17
2	0.71	0.44	0.03
3	0.63	0.42	0.02
4	0.40	0.35	0.01
5	0.33	0.33	0.01
6	0.27	0.22	0.01
7	0.20	0.17	0.01
8	0.15	0.02	0.01
9	0.01	0.01	0.01
10	0.01	0.01	0.01
Average	0.35	0.26	0.03*

* The difference between the intravenous and intraperitoneal groups is highly significant by the rank order test, $P < .001$.

tablished intradermal tumors in guinea pigs caused tumor regression and prevented the development of metastases (*10*). Our result demonstrates the possibility of suppression of primary tumor formation in mice through induction of granulomatous cellular response with a chemically defined substance. Whether the cellular response evoked by cord factor has an immunological basis is under investigation.

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Isolation of Filaments from Brain

Abstract. *A method is presented for the isolation of filaments of 90-angstrom diameter from the white matter of bovine brain by first floating the myelinated axons in a centrifugal field and then fractionating the axons on a series of density gradients. This results in a fraction that contains two types of bundles of filaments but few other constituents. The filaments are stable over a wide range of temperatures and at both low and high ionic strength. Their density and their resistance to digestion by ribonuclease and deoxyribonuclease indicate that they are primarily protein. The molecular weight of the subunit is approximately 60,000. The protein does not comigrate with microtubule protein and does not bind cholcicine or nucleotides.*

Neurons and astroglia are both exceptionally rich in intracellular fibrous organelles. Microtubules are present in both types of cells, although far more abundant in neurons, and have been extensively studied from both the morphological and biochemical standpoint (*1*). The neuronal and glial microtubules do not differ significantly from the microtubules of other cells and tissues. Neurofilaments and glial filaments are from 60 to 100 nm in diameter and appear to run in bundles within cells. High-resolution examination with the electron microscope has revealed morphological differences between the two types of filaments, with the glial filament being narrower and being composed of a smaller subunit (*2*).

Davison and his co-workers have characterized a presumptive neurofilament subunit, filarin, which has a molecular weight of 80,000 and which lacks the colchicine- and nucleotide-binding activities of the microtubule subunit (*3*, *4*). In the isolation method of Davison, axoplasm is extruded from the giant axon of the Chilean squid, *Docidicus gigas*, and fractionated to obtain the filaments. The major advantage of this method is the clear neuronal source of the filaments. The disadvantages are that this procedure cannot readily be applied to vertebrate brain, and that the difficulties in keeping a large squid such as *D. gigas* alive in the laboratory rule out many interesting metabolic studies on the neurofilament.

In order to circumvent these difficulties, we have attempted to isolate filaments from mammalian brain. In initial studies, a homogenate of a whole brain was fractionated in discontinuous sucrose density gradients. Fractions that were rich in filaments of 90-Å diameter were found at the interface between 1.5 and 2.0*M* sucrose. However, we could not determine whether these filaments were of neuronal, glial, or mixed origin. Since axoplasm is rich in filaments, the procedure was modified to use as an initial step the new procedure for isolation of axons (*5*, *6*), in which the myelin surrounding the axon functions as a "life vest" to float the axon away from other elements of nervous tissue. Examination with the electron microscope of axons prepared by this method confirmed their suitability as a starting material.

Because the structural integrity of the unmyelinated axons is not important in the filament purification, we have modified the method of DeVries and Norton (*6*) and incorporated it into the isolation scheme.

Myelinated axons were prepared from carefully dissected bovine white matter. White matter (25 g) was homogenized in 625 ml of 0.9*M* sucrose in 0.03*M* phosphate buffer containing 0.01*M* KCl at *p*H 6.5. Homogenzation was either at low speed in a blade-type homogenizer (Sorvall Omni-Mixer, setting 3) or in a glass Dounce homogenizer with a tight pestle. The suspension was then centrifuged in a Beckman L2-65B centrifuge in a Ti-14 rotor at 15,000 rev/min for 15 minutes or in an SW-27 rotor at 10,000 rev/min for 15 minutes. The pads of myelinated axons which had floated to the center or top, depending on the rotor used, were then harvested with care to keep them as free as possible from other materials in the rotor or tube. The pellet and sucrose solution, which consist of cell bodies, nuclei, capillaries, and debris, were discarded.

The pads were rehomogenized in 625 ml of the original homogenizing solution, and the flotation procedure was repeated three to four times. The pads from the final flotation were pooled, washed quickly by resuspension in 200 ml of 30 m*M* phosphate buffer at *p*H 6.5, and pelleted for 10 minutes at 20,000 rev/min. The pads at this stage were composed entirely of myelin and myelinated axons (*6*). The pellet was resuspended in 30 m*M* phosphate at *p*H 6.5 containing $10^{-2}M$ mercaptoethanol, homogenized vigorously in either a blade-type or Dounce homoge-