GSH is not. Meredith and Reed (*20*) also reported that depletion of mitochondrial GSH is accompanied by increased lipid peroxidation, indicating that the antioxidant function of GSH in mitochrondria may be its most critical role.

Entamoeba histolytica is also unusual in that it uses a more primitive form of cell division than higher eukaryotes and appears to lack microtubules (*10*, chapter 2). Thus, we cannot exclude the possibility that GSH plays a specialized role in microtubule function or mitosis in some higher eukaryotes (*22*). In many respects *E. histolytica* resembles anaerobic or microaerophilic bacteria more than it resembles most other eukaryotes. It does not tolerate normal oxygen tensions (*23*), has a high level of iron-sulfur protein (*14*), and does not produce glutathione. It meets many of the criteria outlined by Margulis (*24*) as characteristics of protoeukaryotes, the primitive anaerobes that served as cytoplasmic host in the symbiotic acquisition of mitochondria (*22*). Data from RNA and protein sequence studies may help us understand how *E. histolytica* is related to prokaryotes and to higher eukaryotes.

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Schwann Cell Galactocerebroside Induced by Derivatives of Adenosine 3′,5′-Monophosphate

Abstract. *Indirect immunofluorescence was used to show the presence of galactocerebroside (galC), a lipid found in myelin, on the surface of about half of the Schwann cells isolated from neonatal rat sciatic nerves and cultured for 1 day without neurons. By day 4 in vitro, the Schwann cells had all lost their surface galC. Three days after beginning treatment with 10^{-3} molar 8-bromo-adenosine 3′,5′-monophosphate (8-bromo cyclic AMP) or $N^6,O^{2'}$-dibutyryl adenosine 3′,5′-monophosphate (dibutyryl cyclic AMP), galC reappeared on the Schwann cells, and 2 days later 48 percent of the cultured Schwann cells showed surface galC. Tritium from tritiated D-galactose was incorporated into galC by the 8-bromo cyclic AMP– and dibutyryl cyclic AMP–treated cultures at a rate 15 times the control rate.*

Schwann cells synthesize myelin, a differentiated, galactolipid-rich form of plasma membrane, only when they are in contact with appropriate axons (*1*). The nature of the axonal signal that triggers Schwann cell myelin synthesis is not known.

Derivatives of adenosine 3′,5′-monophosphate (cyclic AMP) in millimolar concentrations stimulate biosynthesis of

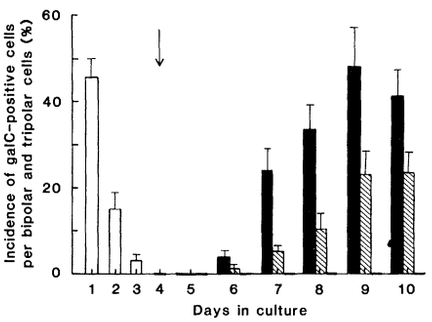

Fig. 1. Time course of disappearance of surface galC from cultured neonatal rat Schwann cells and of reappearance of this lipid upon treatment of the cultures with $10^{-3}M$ 8-bromo cyclic AMP (solid bars) or $10^{-3}M$ dibutyryl cyclic AMP (striped bars). The cyclic AMP derivatives were added to the medium on day 4 of culture (indicated by arrow). The incidence of galC-positive Schwann cells in control cultures without treatment is indicated by open bars. Results were expressed as the mean ± standard deviation of 5 to 10 determinations. Cover slips were processed at the times indicated. Each cover slip was rinsed with phosphate-buffered saline (PBS) ($1.4 \times 10^{-1}M$ NaCl, *p*H 7.4) then exposed to a 1:20 dilution of rabbit antiserum to galC for 20 minutes at 25°C. After being rinsed with PBS, the cover slips were exposed to a 1:50 dilution of rhodamine-conjugated goat antiserum to rabbit immunoglobulin G (Cappel) for 20 minutes at 25°C. The cover slips were exposed to 5 percent acetic acid in ethanol at 5°C for 5 minutes, then mounted in glycerol.

specialized plasma membrane components by human promyelocytic leukemia and F9 cells (*2*) and increase the specific activity of an enzyme characteristic of myelin—2′,3′-cyclic nucleotide 3′-phosphohydrolase—in both C6 glioma and cultured nonneoplastic oligodendroglia (*3*). We decided to test whether such cyclic AMP derivatives induce synthesis of myelin components by Schwann cells cultured in the absence of neurons.

Galactocerebroside (galC), a glycolipid found in myelin and myelin-forming cells, can be detected by indirect immunofluorescence on the surfaces of both newly cultured Schwann cells and newly cultured oligodendroglia (*4–6*). Whereas oligodendroglia continued to express surface galC for months in the absence of neurons, Schwann cells maintained without neurons uniformly lost their surface galC within 4 days in culture (*5*). We now report that such Schwann cells are induced to synthesize galC and to express this "myelin marker" on their surface by treatment with either 8-bromo cyclic AMP or N^6,$O^{2'}$-dibutyryl cyclic AMP.

Schwann cells were isolated from neonatal rat sciatic nerves by enzymatic dissociation followed by differential adhesion and were cultured on poly-D-lysine–coated glass cover slips in Eagle's minimum essential medium containing 10 percent calf serum (by volume) (*6*). More than 95 percent of the cells had the typical bipolar or tripolar morphology of of Schwann cells, and less than 5 percent appeared to be fibroblasts by morphological criteria and indirect immunofluorescence examination (*6*).

One day after the cultures were instituted, indirect immunofluorescence microscopy permitted detection of galC on the surface of almost half of the Schwann cells, but by day 4 in vitro, all of the

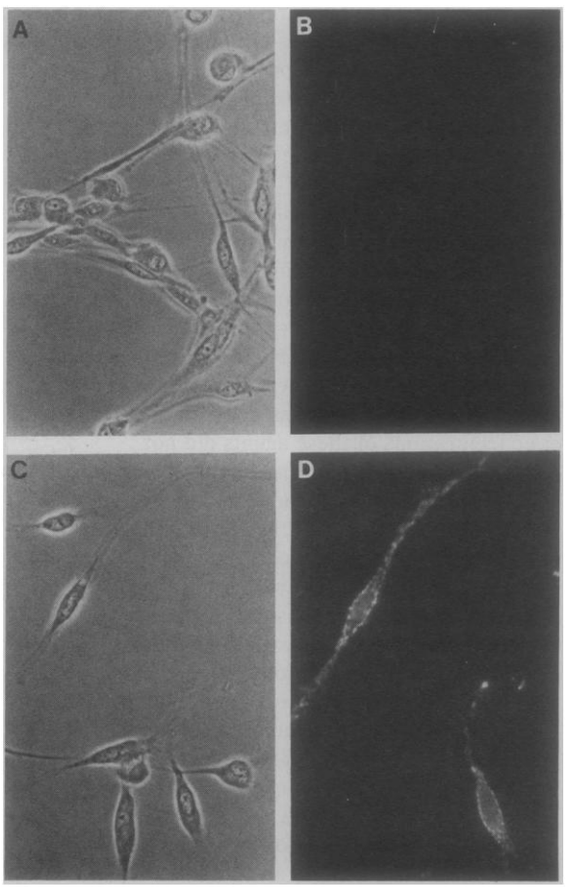

Fig. 2. Phase-contrast and indirect immunofluorescence microscopy with rabbit antiserum to galC for control cells [(A) and (B), respectively] and cells treated with dibutyryl cyclic AMP [(C) and (D), respectively]. The cells were maintained in culture for 9 days; the last 5 days were with or without $10^{-3}M$ dibutyryl cyclic AMP. Original magnification ×780.

Table 1. Induction of surface galC by cyclic AMP derivatives is concentration dependent, whereas butyrate has no effect. Schwann cells were applied to each polylysine-coated, round cover slip (diameter, 13 mm). The cyclic AMP derivatives were added on day 4 of culture, and the cells were examined by indirect immunofluorescence (as described in Fig. 1) on day 9 of culture. The cultures were inspected with a 50× fluorescence objective and a Leitz microscope. Since the cultures appeared to be radially symmetric, the total population of galC-positive cells per cover slip was estimated by counting galC-positive cells in an area measuring 0.2 by 13 mm along one diameter of the cover slip and correcting for total area. The percentage of Schwann cells with surface galC was calculated by dividing the number of galC-positive cells by the total number of bipolar and tripolar cells, and multiplying the result by 100. Results are means ± standard deviations, with the number of determinations indicated in parentheses.

Treatment	Cells with surface galC	
	Per cover slip (No.)	Total (%)
Control	0 (5)	0 (5)
8-Bromo cyclic AMP		
$10^{-5}M$	0 (5)	0 (5)
$10^{-4}M$	0 (5)	0 (5)
$5 \times 10^{-4}M$	485 ± 164 (5)	6.9 ± 4.2 (5)
$10^{-3}M$	4164 ± 802 (6)	48.4 ± 8.7 (10)
$10^{-2}M$	4418 ± 977 (4)	45.4 ± 7.4 (5)
Dibutyryl cyclic AMP		
$10^{-5}M$	0 (5)	0 (5)
$10^{-4}M$	0 (5)	0 (5)
$5 \times 10^{-4}M$	278 ± 145 (5)	4.4 ± 2.0 (5)
$10^{-3}M$	1286 ± 419 (5)	22.6 ± 5.8 (7)
$10^{-2}M$	<14 (3)	<1 (3)
Butyrate		
$10^{-3}M$	0 (3)	0 (3)
$5 \times 10^{-3}M$	0 (3)	0 (3)

Table 2. Incorporation of tritiated D-galactose into galactocerebroside. Radioactivity is expressed as disintegrations per minute per microgram of total cell protein. Schwann cells were cultured for 4 days in poly-D-lysine–coated wells (diameter, 16 mm). Then fresh medium, with or without the cyclic AMP derivative, was added. After 2 days, D-[1−^{3}H]galactose (3.9 Ci/mmole, New England Nuclear) was added at 15 μCi per milliliter of medium, and the cultures were washed and harvested 55 hours later. Lipids were extracted from the cell pellets with a 2:1 (by volume) mixture of chloroform and methanol, and the lipid extracts were washed by the Folch method. Cerebroside was isolated from the washed lipid extracts by thin-layer chromatography (TLC) on silica gel G plates, and galC was purified from total cerebroside by borate-TLC (*9*). Total cell protein (63 μg for the control, 48 μg for the cells treated with 8-bromo cyclic AMP, and 59.5 μg for the cells treated with dibutyryl cyclic AMP) was determined in parallel wells.

Treatment	Radioactivity recovered
None	4.4
8-Bromo cyclic AMP ($10^{-3}M$)	67.3
Dibutyryl cyclic AMP ($10^{-3}M$)	68.1

Schwann cells had lost their surface galC (Fig. 1). Addition of $10^{-3}M$ 8-bromo cyclic AMP or $10^{-3}M$ dibutyryl cyclic AMP on day 4 of culture resulted in the reappearance, within the next 3 days, of Schwann cells bearing surface galC. The proportion of galC-bearing Schwann cells peaked 5 days after addition of 8-bromo cyclic AMP or dibutyryl cyclic AMP (Fig. 1). Schwann cells positive for surface galC were not detected when concentrations of the cyclic AMP derivatives were less than $5 \times 10^{-4}M$. 8-Bromo cyclic AMP at $10^{-3}M$ was a more potent inducer of surface galC than was dibutyryl cyclic AMP at the same concentration. The high concentrations of the cyclic AMP analogs necessary to produce this effect may be attributable, at least in part, to relatively poor penetration of the analogs (*7*) into the Schwann cells. Larger concentrations of these agents did not increase the proportion of galC-positive cells (Table 1) and, in fact, treatment with $10^{-2}M$ dibutyryl cyclic AMP did not yield galC-positive Schwann cells. Because a maximum of 48 percent of the Schwann cells became positive for surface galC after treatment with the cyclic AMP derivatives, we cannot exclude the possibility that the cultures contained two populations of Schwann cells that appeared to be morphologically identical but that differed in their capacity to respond to such treatment.

To test whether induction of galC by dibutyryl cyclic AMP was attributable to butyrate, we cultured the cells in the presence of $10^{-3}M$ butyrate. No cells with surface galC appeared in such cultures during the ensuing week. Nor did treatment with $5 \times 10^{-3}M$ butyrate induce the appearance of galC-positive cells (Table 1).

Binding of antibodies to galC on the surface of cells treated with 8-bromo cyclic AMP and dibutyryl cyclic AMP showed a granulofloccular distribution, and the perinuclear region was more prominently fluorescent than the peripheral processes (Fig. 2). This distribution of binding of antibodies to galC resembled that observed in freshly isolated galC-positive Schwann cells.

In order to determine whether the appearance of galC on the surface of the treated Schwann cells was due solely to redistribution of this lipid from the interior of the cells or whether these cyclic AMP derivatives also stimulated Schwann cells to synthesize this myelin lipid, we incubated treated and control Schwann cells with D-[1-^{3}H]galactose. Incorporation of ^{3}H into galC by the 8-bromo cyclic AMP– and dibutyryl cyclic AMP–treated cultures was 15 times greater than that in the controls (Table 2).

These results indicate that derivatives of cyclic AMP can induce Schwann cells to synthesize galC and to express this myelin component on their surface; they also strengthen the possibility that cyclic AMP is a messenger involved in the process by which axons signal Schwann cells to synthesize myelin (*8*).

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8. Dibutyryl cyclic AMP has been reported to promote the proliferation of cultured rat Schwann cells [M. C. Raff, A. Hornby-Smith, J. P. Brockes, *Nature (London)* **273**, 672 (1978)]. We examined the effects of $10^{-7}M$ to $10^{-2}M$ 8-bromo cyclic AMP and dibutyryl cyclic AMP on incorporation of tritiated thymidine into the trichloroacetic acid–insoluble fraction of rat Schwann cell cultures. These cyclic AMP derivatives increased the uptake of tritiated thymidine by Schwann cells; maximum stimulation was observed at $10^{-4}M$ to $5 \times 10^{-4}M$ concentrations with either 8-bromo or dibutyryl cyclic AMP. However, at $10^{-3}M$ 8-bromo cyclic AMP or dibutyryl cyclic AMP, the optimum concentration for induction of surface galC, the incorporation of tritiated thymidine was slightly less than that in untreated control cultures.
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A New Charge-Mosaic Membrane from a Multiblock Copolymer

Abstract. *A charge-mosaic membrane was prepared from a pentablock copolymer of the BABCB type by selectively introducing anion and cation exchange groups into the microseparated phases. The three-layer lamellar structure of the starting pentablock copolymer film was not disturbed by the modifications. The membrane obtained was highly permeable only to sodium chloride in mixed aqueous solutions of sodium chloride and organic species of low molecular weight, such as sucrose. Marked* pH*-dependent permeabilities were also observed for amino acids.*

Charge-mosaic membranes, which are composed of cation- and anion-permeable domains, have been of continuing interest since being proposed by Sollner (*1*) in connection with biological phenomena. Theories concerning their structure have been presented (*2*); in practice, their transport properties, such as high permeability for salts, piezodialysis, or negative osmosis, have been observed in membranes prepared in various ways (*3, 4*). Early methods for preparing charge-mosaic membranes were reviewed by Leitz (*4*). However, those membranes were fragile and lacked well-defined domain structures.

If the microphase separation phenomenon of block copolymers (*5*) can be used to prepare charge-mosaic membranes, resulting membranes should have many pairs of anion and cation exchange microdomains. To prevent the formation of a poly-ion complex in such membranes, a triblock copolymer of the ABC type—composed of terminal polymer blocks A and C into which cation and anion exchange groups, respectively, are introducible and a middle polymer block B into which neither ion exchange group is introducible—should be used. This type of triblock copolymer forms a three-layer lamellar structure with the repeating unit -A-B-C-B-, where A, B, and C represent the domains consisting of their polymers (*6–8*). However, our early trial with triblock copolymers of the ABC type revealed that the lamellar structures do not withstand chemical treatments for introducing ion exchange groups, even if the neutral B domains are cross-linked.

Anionic polymerization allowed us to prepare a pentablock copolymer of the BABCB type that can generate the same three-layer lamellar structure as the ABC triblock copolymer (*8, 9*). But, in