lets indicates a hitherto unknown active participation of the insulin secretory vesicle and its membrane in the insulin release mechanism. While the role of binesis is still not clear, we suggest that it may represent a morphological concomitant of glucose-mediated activation of the insulin secretory vesicles occurring before emiocytosis of the vesicle contents. Similarly, the finding of binesis and vesicular fusion well within the interior of the beta cell supports the suggestion of Lacy (*6*) that insulin vesicles arrayed in tandem release their contents through a distal vesicle undergoing emiocytosis at the plasma membrane. Binesis may therefore be a useful phenomenon in the dissection of the insulin release sequence, especially since emiocytosis is a rapid process that cannot be adequately observed and quantified with ordinary transmission electron microscopy.

The observation of binesis and of its stimulation at elevated glucose concentration in islets does not appear to be influenced by the fixative employed. Rather, binesis appears to be more easily observed when the tissues are rapidly fixed by regional perfusion, or when isolated islets are used. Therefore, rapid and improved fixation may have allowed us to observe and quantitate this presumably fast and evanescent phenomenon. Indeed, inspection of published photomicrographs of rapidly fixed beta cells (fixation by regional perfusion and fixation of isolated islets) readily reveals examples of furrows and binesis by both transmission electron microscopy and by the freeze-fracture technique (*7*). Schramm *et al.* (*8*) showed that amylase granules from rat parotid gland form "pseudopods" during incubation of parotid gland slices, as well as in a cell-free preparation of secretory vesicles. The relative number of amylase granules bearing pseudopods increased during stimulus-induced secretion. Binesis may thus be of general significance in other secretory mechanisms as well.

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Adenosine 3′,5′-Monophosphate Analogs Promote a Circular Morphology of Cultured Schwannoma Cells

Abstract. *Dibutyryl adenosine 3′,5′-monophosphate (dibutyryl cyclic AMP) and 8-bromo cyclic AMP stimulated cells cultured from a rat Schwannoma to change their morphology from irregularly shaped to flattened circular and hollow circular forms within 30 minutes. The change in shape was specifically effected by analogs of cyclic AMP and cyclic AMP elevating agents, was reversible after removal of these additives, and was prevented by vinblastine and cytochalasin b, but was not affected by actinomycin D or cycloheximide.*

Schwann cells surround peripheral nerve cell processes, acting as an electrical insulator by synthesizing a multilayered myelin sheath (*1*). Once the nervous system has formed, Schwann cells do not normally divide but during the early stages of nervous system formation, when division does occur, they sometimes undergo a neoplastic transformation, developing into a malignant Schwannoma. These tumors have been used to generate established clonal lines of Schwannoma cells which are capable of performing functions unique to the nervous system and to myelin production (*2*).

Table 1. The effect of various chemicals and culture conditions on the morphology of Schwannoma clone RN22. Cells were plated at 2×10^3 cell/cm² and incubated for 24 hours. They were then exposed to the various agents at a final concentration of 1 m*M* (except where indicated) and any effect or morphology was observed after 2 hours of incubation at 37°C. Control cells and cells not affected by the additives had a predominance of the irregular or triangular shape, whereas cells treated with cyclic AMP analogs or prostaglandin E_1 had an increase in the number of hollow and flattened circular forms. At least 500 cells were counted in each instance; where differences were suggested by the data, at least 1000 cells were counted. The cells were not fixed. Comparisons were then made between the control group and various experimental groups.

Agent or condition	Percentage of cells exhibiting these forms after 2 to 4 hours of exposure		
	Flattened circular	Hollow circular	Irregular or triangular
No additives	9	10	81
Dibutyryl cyclic AMP (DBcAMP)	15*	34*	51*
8-bromo cyclic AMP (8-Br-cAMP)	14*	44*	42*
Cyclic AMP	7	8	85
Dibutyryl cyclic guanosine monophosphate	9	9	82
8-bromo cyclic guanosine monophosphate (8-Br-cGMP)	9	12	79
Cyclic GMP	10	7	83
Butyrate	9	8	83
5′-Adenosine monophosphate	9	5	86
Adenosine	12	5	83
Serum-free medium (24 hours)	9	8	83
Papaverine (0.1 mm)	8	10	82
Prostaglandin E_1 (25 μg/ml)	12	21*	67*
Prostaglandin $F_{2\alpha}$ (25 μg/ml)	8	10	82
DBcAMP and cytochalasin b (0.1 μg/ml)	12	12	76
DBcAMP and vinblastine (10 ng/ml)	3*	12	85
8-Br-cAMP and actinomycin D (5 μg/ml)	15*	36*	49*
8-Br-cAMP and cycloheximide (5 μg/ml)	11	36*	53*
8-Br-cAMP and 8-Br-cGMP	11	42*	47*
Isoproterenol ($10^{-5}M$)	12	11	77

* Statistically significant differences at a level of $P < .05$ as measured by Student's *t*-test.

Table 2. Cellular cyclic AMP levels after 4 hours' exposure to various biochemicals. Cells were grown to confluency in petri dishes 60 mm in diameter (approximately 2×10^6 cells per dish). The medium was not changed when the chemical was added. After 4 hours' exposure at 37°C, the medium was removed by aspiration and the cells were washed twice with 2.5 ml of Dulbecco's phosphate buffered saline. Two milliliters of 5 percent trichloroacetic acid at 0°C were then added to the plates, and subsequent cyclic AMP was analyzed by the method of Gilman (*20*). Duplicate samples treated with phosphodiesterase served as blanks. Protein was measured on duplicate plates by the method of Lowry *et al.* (*21*). These data represent five different experiments where assays were done in quadruplicate; N.D., not determined.

Additive	Cyclic AMP (pmole/mg of protein) in clonal cell line			
	RN1	RN2	RN22	RN3
None	5.4 ± 0.7	6.5 ± 0.6	9.2 ± 1.2	7.8 ± 1.0
Isoproterenol ($10^{-5}M$)	5.9 ± 0.6	6.4 ± 0.5	10.1 ± 1.7	8.4 ± 1.2
Butyrate (1 mM)	4.8 ± 0.7	5.7 ± 0.6	8.8 ± 0.9	7.2 ± 1.0
Prostaglandin E_1 (20 μg/ml)	5.3 ± 0.7	6.5 ± 0.9	16.0 ± 2.6*	N.D.
Prostaglandin $F_{2\alpha}$ (20 μg/ml)	5.7 ± 0.9	6.9 ± 1.0	8.3 ± 2.2	N.D.

* Only the RN22 cells treated with prostaglandin E_1 have a statistically significant difference from the cells with no additives, as analyzed by standard deviation and Student's t-test ($P < .05$).

Adenosine 3′,5′-monophosphate (cyclic AMP) has been shown to change the morphology of cultured animal cells of fibroblastic (*3, 4*), ovarian (*5*), hepatic (*6*), glial (*7*), and neural cell (*8*) origin. In the neuroblastoma system, the dibutyryl derivative of cyclic AMP induced the formation of nervelike axons; similar observations have been made when whole ganglion cultures were used (*9*) and in nerve regeneration studies (*10*). The Schwannoma cell culture provided another system in which to test a possible effect of cyclic AMP on cell shape.

Schwannoma cloned lines (RN1, RN2, RN22, and RN3) were derived from rat neurinoma by S. E. Pfeiffer (University of Connecticut), who sent them to us. The cells were routinely grown in Dulbecco's modified Eagle's medium supplemented with 10 percent calf serum (Colorado Serum Co.) and gentamycin (20 μg/ml). The medium was changed every 3 days and the cells were replated in dilutions of 1:10 after confluency (*11*). Specific methods used in these experiments are described in the legends to Tables 1 and 2.

The morphology of the cultured Schwannoma cell clone RN22 was changed perceptibly after exposure to dibutyryl cyclic AMP and 8-bromo cyclic AMP (Fig. 1). The effect was to change the irregularly shaped Schwannoma cells to a rounded, circular morphology. These circular forms were either hollow (ring-shaped) or flattened ("fried-egg" shaped), or a combination of these two forms. Prostaglandin E_1 (PGE_1) also had an effect on morphology after a longer exposure period (24 hours). Similar molecules, such as 5′-adenosine monophosphate, dibutyryl cyclic guanosine monophosphate, and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), had no significant effect on change in shape, nor did they counteract the effect of the cyclic AMP analogs under the conditions tested (Table 1). Basal levels of cyclic AMP were analyzed after exposure to several different agents to determine if any relation existed between morphological changes and the levels of cyclic AMP (Table 2). Only PGE_1 had a significant effect on the cyclic AMP level; it was also the only agent tested, aside from the cyclic AMP analogs, that had an effect on the shape of RN22 cells.

Only clone RN22, which is a subclone of RN2, showed a significant morphological effect after exposure to the cyclic AMP analogs; others tested were clones RN1, RN2, and RN3. Furthermore, only clone RN22 responded morphologically or biochemically (as shown by an elevated level of cyclic AMP) to the PGE_1. This effect—change in shape of cells of clone RN22 grown on plastic dishes—was obvious within 30 minutes after exposure to the cyclic AMP analogs and achieved maximum expression after 6 hours; the cells remained in their various forms as long as they were exposed to the additive (up to 72 hours). The rapidity of the change in shape is reminiscent of the change in Chinese hamster ovary cells (*5*) after exposure to dibutyryl cyclic AMP. Actinomycin D and cycloheximide did not inhibit the effect of the cyclic AMP analogs (Table 1). On the other hand, the change in shape was inhibitable by vinblastine and cytochalasin b, intimating a possible role for microtubules and microfilaments in the change-of-shape process. Similar effects by vinblastine and cytochalasin b on shape changes have been observed on other cell systems (*3, 5*). Other agents or conditions, such as papaverine (*12*) and serum-free medium (*13*) that have been shown to induce morphological changes in other cell systems, had little effect on the RN22 morphology (Table 1) or cyclic AMP levels (*14*).

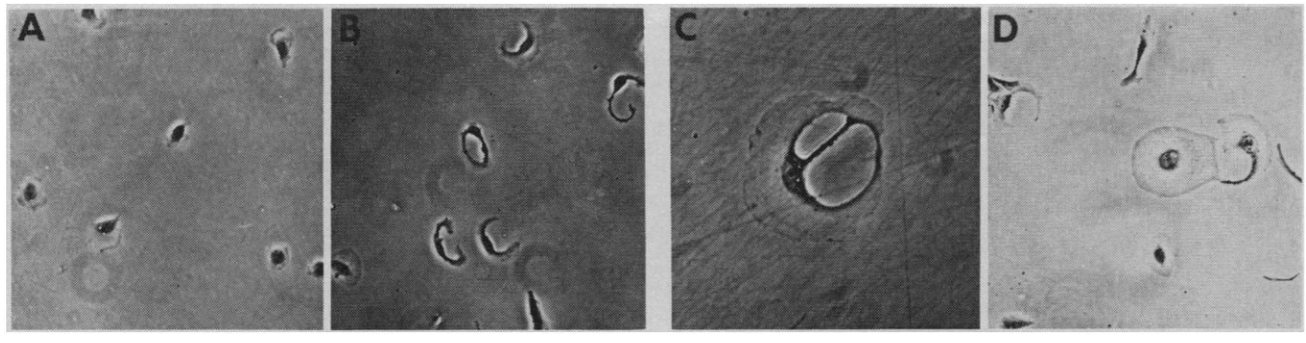

Fig. 1. RN22 Schwannoma cells were plated at 2×10^3 cell/cm^2 and incubated for 24 hours without any additives. The cells as they appear at this time are shown in (A). The cells were then exposed to 1 mM 8-bromo cyclic AMP. The ringlike or hollow circular form is illustrated in (B) and (C). The "fried-egg" or flattened circular form with an adjacent cell exhibiting a combination of the hollow and flattened forms is shown in (D). (C) A close-up of a completely hollow circular form. Note the process that spans the aperture; it may be actively extended by the cell and play a role in the generation of the ring or passively result from folded membrane. All the photomicrographs were made with a phase-contrast microscope; (A), (B), and (D) are magnified $\times$ 425 and (C) $\times$ 850.

The basis for the heterogeneous morphological response (hollow circular and flattened circular forms) to the cyclic AMP analogs and PGE_1 is not clear. It is possible that these Schwannoma clones are not genetically stable and recloning may provide lines with differing morphological effects. Another potential basis for the heterogeneity is that the open circular shape may be derived from the flat circular form. An invagination or membrane retraction may occur in the flattened circular, "fried-egg–like" form resulting in the open circular form. Formation of the myelin sheath in vivo is accomplished by movement of the nucleated portion of the Schwann cell around the nerve axon (*15*). In culture, the movement of the nucleus in the flattened circular form away from its initial central position could lead to retraction of the membrane, resulting in the hollow circular from (Fig. 1C may illustrate such an event). It should be emphasized that all of these various morphological forms exist in the untreated and control cultures but in much lower percentages than in the cyclic AMP analog–treated cultures. Furthermore, the effect is not a result of the enrichment of a given cell population through differential lethality. Cells exclude trypan blue after exposure to cyclic AMP analogs and cell number does not decrease. Actual numbers of circular cells, as well as percentages, increase as a result of the cyclic AMP analog treatment. Thus it appears that the major difference between control and cyclic AMP–treated cultures is quantitative, not qualitative.

Whether biochemical characteristics that distinguish Schwannoma cells in culture (*16*) are also affected by cyclic AMP must await further investigation. It is not yet clear whether cyclic AMP directly promotes the morphological changes accompanying cellular differentiation (*17, 18*) or effects the change by altering adhesiveness of the cells to substrate (*19*). Nevertheless, the cyclic AMP effect on these cultured Schwannoma cells may provide a model system useful in studying Schwann cell morphology, membrane synthesis, and myelination.

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When Left-Handed Mice Live in Right-Handed Worlds

Abstract. *Many C57BL/6J inbred mice were tested for paw preference. In unbiased worlds, half were left-handed, and females were more strongly lateralized. In biased worlds, approximately 10 percent exhibited lateral preferences inconsistent with the world bias, and males were more strongly lateralized. Influences of world bias appear to be superimposed upon an already laterally dichotomized population. Initial left-right sense, it is posited, arises as an outcome of a seemingly random process.*

Right- and left-handedness are not unique to humans. Mice also show a consistent hand preference. Experimental studies of laterality in inbred mice (*1*) and retrospective studies of handedness in man (*2*) have led me to conclude that lateral sense is not specified in any unambiguous manner by the individual's genome. If right- and left-handedness arise not from heritable genetic instructions, then how does lateral sense develop, and what influences affect its expression? The following studies of laterality in mice tested in unbiased and biased worlds may help in a solution to this question.

Mice of the highly inbred C57BL/6J strain (*3*), 709 animals 8 to 12 weeks of age, were first tested for paw preference in an unbiased world. Mice were deprived of food for 24 hours before testing, and were then placed into a cubicle in which sweetened rolled wheat (Maypo) was available in a feeding tube that was attached to the front wall equidistant from the right and left sides (*4*). This constitutes my definition of an unbiased or U world. Fifty reaches for food were observed for every mouse, and the number of right paw entries (RPE's) was used as one index of laterality. Each mouse was retested 1 week later. Figure 1a illustrates the RPE scores for 709 mice tested in the U world. Data were cast into 17 class intervals. Most mice were either strongly right-handed or strongly left-handed, whereas only a few were ambilateral. The distributions are strikingly "U"-shaped. In test 1, 41.8 percent of mice scored in the two most extreme class intervals, 0 to 2 and 48 to 50 RPE's; in test 2, 62.2 percent were in these intervals. If mice that scored exactly 25 RPE's are excluded, 334 of 702 or 47.6 percent of mice could be designated as dextral in test 1; and 337 of 707 or 47.7 percent, in test 2. The strength of lateralization was increased in the second testing. This difference is statistically significant (*5*).

Figure 1b illustrates the RPE scores for the same 341 female and 368 male mice. Individual test 1 and test 2 scores were averaged (*6*). Female mice were more strongly lateralized than males in the U world. Whereas 59.5 percent of female mice scored in the two extreme intervals, only 45.1 percent of male mice scored therein ($\chi^2 = 14.75$, $P < .001$). This result presents a clear example of a genetic effect on laterality.