the inhibitor (Table 1, experiment 4). The shift of the inducing activity from spinocaudal to deuterencephalic structures at 25 μg PVS (Table 1, experiment 5) can be explained by the activation in vitro of the mesodermalizing factor and the activation in vivo of the capacity of the ectoderm to differentiate into neural structures.

It is not unlikely that PVS competes with binding sites for a ribonucleoprotein or acidic glycoprotein in the complex of mesodermalizing factor and inhibitor complex (*11*). The action of PVS in ectoderm in vivo is more difficult to explain. The neuralizing action could be caused by interaction of PVS with a complex between the neuralizing factor and its inhibitor. The neuralizing factor is more easily activated than the mesodermalizing one. But the effect of PVS on ectoderm could also be caused by the interaction of PVS with DNA-histone bonds in chromatin or by interaction with cytoplasmic membranes. Polyethylene sulfate activates the aggregate RNA polymerase from rat liver nuclei (*12*).

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8. The polyvinylsulfate (Eastman Kodak) migrates in the analytical ultracentrifuge as a single peak with $s_{25°,w} \sim 7.4$ ($c \to 0$).
9. We thank the analytical laboratory of the Schering AG., Berlin, for performing the sulfur analyses.
10. Under these conditions the PVS which is implanted into a gastrula amounts to about 5×10^{-2} μg.
11. PVS has no effect on the inhibition of trypsin by soybean trypsin inhibitor.
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13. Other species are less suitable for these experiments. Gastrula ectoderm of *Ambystoma punctatum* and of *Triturus taeniatus* show a stronger neural reaction when mixed inducers like the phenol-extracted protein, which contain besides the mesodermalizing factor also some neuralizing factor, are tested.
14. Supported by the Deutsche Forschungsgemeinschaft.

3 January 1969; revised 14 March 1969

Colchicine-Inhibited Cilia Regeneration: Explanation for Lack of Effect in Tris Buffer Medium

Abstract. *In* Stentor coeruleus *growth of new daughter ciliates and experimentally induced regeneration of oral membranellar cilia are reversibly inhibited by low, nontoxic concentrations of colchicine. However, if the culture medium containing colchicine (or Colcemid) is made up in tris(hydroxymethyl)aminomethane buffer, growth of ciliated daughters and regeneration of oral cilia proceed normally. The evidence suggests that the mechanism of this reversal of the effects of colchicine (or Colcemid) is due to a chemical reaction between tris(hydroxymethyl)-aminomethane (or its hydrochloride, or both) and colchicine (or Colcemid), which reduces the effective concentration of these mitotic spindle inhibitors reaching the stentors.*

Stentor coeruleus can be experimentally induced to shed its oral membranellar cilia in response to toxic substances in the medium (*1*). If replaced into normal medium all shed cells regenerate their oral cilia within hours (*1*). Cilia and flagellar regeneration is sensitive to colchicine (*2*, *3*). Colchicine, approximately 10^{-6} to $10^{-8}M$, reversibly inhibits regrowth of the membranellar band in *Stentor* for up to 8 hours—as long as the cells remain in colchicine (*3*). The effect of colchicine and Colcemid in blocking cilia regeneration is entirely obliterated when Trizma® buffer (*4*) is used in the culture medium (Table 1). We now present evidence that colchicine and Colcemid react with tris(hydroxymethyl)aminomethane(tris) or with tris hydrochloride or with a combination (Trizma) to form new compounds.

In the course of testing the possible toxic effect of these drugs on normal stentors, we found that, although the cells survived for over 3 weeks, they seemed unable to produce normal daughters, presumably because formation of new cilia is inhibited and macronuclear abnormalities are produced (Fig. 1). Thus we studied the effect of colchicine and Colcemid (*5*) on the growth rate of stentors. Cell increase did not occur in the presence of either colchicine or Colcemid. This effect was also obliterated when the colchicine or Colcemid was made up in tris buffer (Fig. 2). The toxicity of Trizma alone was also investigated. Cells grew well in 10^{-5} to $10^{-2}M$ Trizma, but died in higher concentrations of this buffer. Unlike the usual dead stentors (*6*) car-

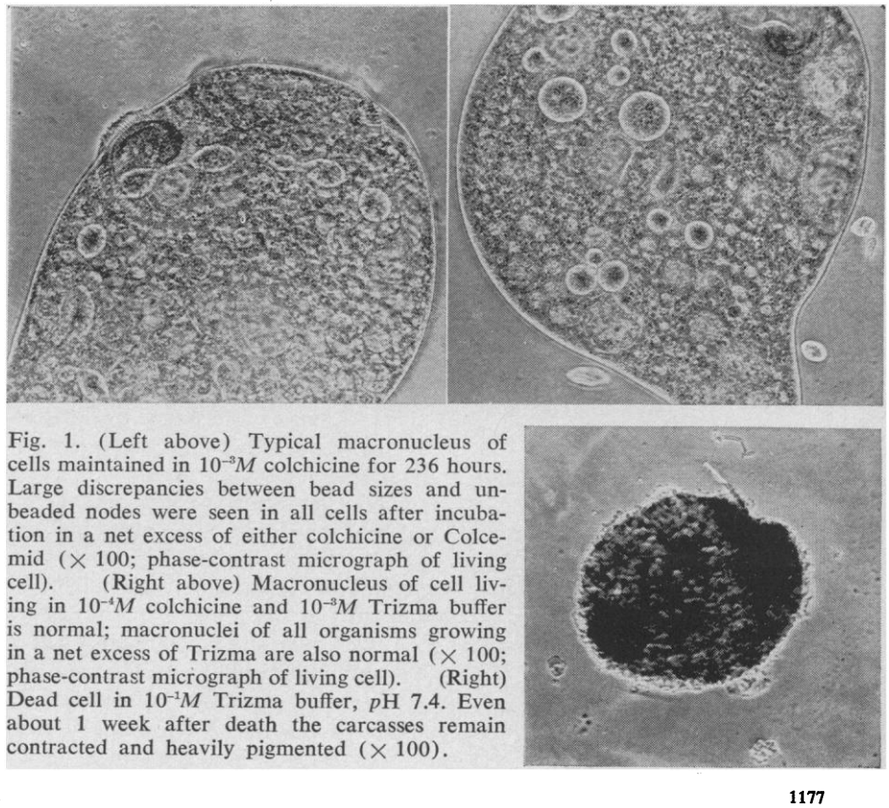

Fig. 1. (Left above) Typical macronucleus of cells maintained in $10^{-3}M$ colchicine for 236 hours. Large discrepancies between bead sizes and unbeaded nodes were seen in all cells after incubation in a net excess of either colchicine or Colcemid (× 100; phase-contrast micrograph of living cell). (Right above) Macronucleus of cell living in $10^{-4}M$ colchicine and $10^{-3}M$ Trizma buffer is normal; macronuclei of all organisms growing in a net excess of Trizma are also normal (× 100; phase-contrast micrograph of living cell). (Right) Dead cell in $10^{-1}M$ Trizma buffer, *p*H 7.4. Even about 1 week after death the carcasses remain contracted and heavily pigmented (× 100).

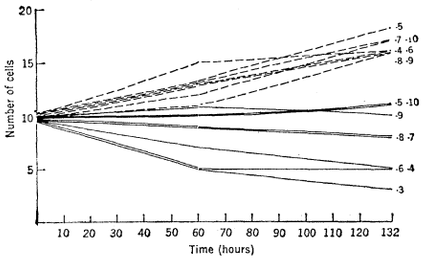

Fig. 2. The effect of colchicine and colchicine plus Trizma on the growth of *Stentor coeruleus*. (Solid lines) Colchicine (10^{-3} to $10^{-10}M$); (broken lines) colchicine (10^{-4} to $10^{-10}M$) plus Trizma ($10^{-5}M$); cells counted by hand; each entry represents an average of three cultures.

casses of those exposed to $10^{-1}M$ Trizma remained highly pigmented and continued to contract for more than a week after death (Fig. 1). The macronucleus was normal in cells living in Trizma or a net excess of Trizma, in contrast to cells surviving in net excesses of colchicine and Colcemid for long periods of time; the latter have aberrant macronuclei (Fig. 1). Flagellates and bacteria seemed unaffected by the $10^{-1}M$ Trizma; only stentors were killed. Cells dying from overdoses of colchicine cytolyze in previously described ways (*6*) and potentially then are distinguishable from cells killed by tris. In all experiments, the *p*H of the media remained between 5 and 7.5.

The consistent observation that sensitivity to colchicine (and Colcemid) was removed in cultures made up with tris buffer suggested that tris and colchicine (or Colcemid) were reacting in the medium so that the effective concentration of colchicine reaching the cells was lowered. When colchicine was in excess, cell division and cilia regeneration were arrested; when tris was in excess, these two processes were normal. Were new compounds derived from tris and colchicine (and tris and Colcemid)?

Table 1. Effect of medium on regeneration of shed *Stentor coeruleus*. Stentors were cultured in Long Island spring water taken directly from the spring at Cold Spring Harbor, Long Island, N.Y. Long Island spring water (LISW) alone or with additions as noted. An entry represents 50 to 200 stentors; in a given experiment all responded in the same way. Complete normal regeneration occurred in less than 24 hours; the inhibition was completely reversible.

Addition to LISW (mole/liter)		Result (*9*)
Trizma	Colchicine	
None	None	Regeneration
None	10^{-2}	Death
	10^{-3}	Death
	10^{-4}	Death
	10^{-5}	Death
	10^{-6}	Inhibition
	10^{-7}	Inhibition
	10^{-8}	Inhibition or regeneration
	$<10^{-9}$	Regeneration
10^{-5}	10^{-5}	Regeneration
10^{-5}	10^{-6}	Regeneration
10^{-5}	10^{-7}	Regeneration
10^{-5}	10^{-8}	Regeneration

We found a unique crystalline residue in dried out samples of Long Island culture medium taken from stock solutions used to grow cells and from concentrated equimolar aqueous solutions of colchicine and either tris, tris HCl, or Trizma. After 2 or 3 days under damp conditions, beautiful triclinic crystals came out of solution. The melting points of these crystals were 165° ± 1°C, colchicine-tris; 167° ± 1°C, colchicine-tris HCl; and 152° to 156°C, colchicine-Trizma. Comparable melting points (only poorer crystals) were obtained with Colcemid-tris (189° ± 1°C), Colcemid-tris HCl (188° ± 1°C), and Colcemid-Trizma (178° to 186°C). These differ from the physical mixtures of the parent compounds: colchicine and Trizma (134° to 150°C), Colcemid and Trizma (140° to 156°C). The melting points of the parent compounds recrystallized from Long Island spring water are 155° ± 1°C for colchicine, 164° ± 1°C for tris, 163° ± 1°C for tris HCl, 142° to 146°C for Trizma, and 185° ± 1°C for Colcemid. The new crystals also differed significantly in solubility. Colcemid-Trizma was more soluble in water than Colcemid and Trizma; colchicine-Trisma and Colcemid-Trizma (unlike Colcemid and Trizma) were extremely soluble in ethanol, and insoluble in benzene. Unlike colchicine and Colcemid, but like Trizma, colchicine-Trizma and Colcemid-Trizma were only sparingly soluble in chloroform. Although colchicine was slightly soluble in ethyl acetate (1 mg dissolved in less than 2 ml), the colchicine-tris HCl crystal was insoluble. The density of colchicine-Trizma was 1.32 ± .01 g/cm^3, as determined by flotation in methylene iodide and ethyl acetate. Preliminary crystallographic measurements of molecular weight, and chemical and mass spectrographic analyses are consistent with the hypothesis that single crystals contain both colchicine and tris.

Under very mild conditions, tris, a commonly used buffer in biological experiments to maintain a neutral *p*H seems to react with tropolone derivatives, colchicine and Colcemid, which inhibit cilia regeneration (*3*) and mitotic spindle microtubule formation, presumably by binding with protein (*7*, *8*). Regardless of any possible relation between the formation of these new colchicine-tris derivatives and the mechanism of action of colchicine and its analogs in vivo, caution must be exercised in the use of these substances together. This is especially pertinent because several investigators, concerned with the mechanism of action of colchicine on microtubules, have routinely used tris buffer in their procedures. Obviously the interaction of colchicine and tris and their derivatives may easily interfere with results of biological significance.

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23 January 1969

Rate of Intracellular Diffusion as Measured in Barnacle Muscle

Abstract. *The rate of intracellular diffusion of water, urea, and glycerol was measured in the giant barnacle* Balanus nubilus. *The calculated diffusion coefficients were not different from those values reported for dilute solutions.*

One of the basic assumptions for the measurement of cell membrane permeability is that diffusion in the cytoplasm proceeds at the same rate as in a dilute solution. Periodically, this assump-