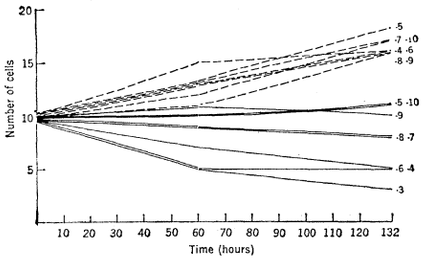

Fig. 2. The effect of colchicine and colchicine plus Trizma on the growth of *Stentor coeruleus*. (Solid lines) Colchicine (10^{-3} to $10^{-10}M$); (broken lines) colchicine (10^{-4} to $10^{-10}M$) plus Trizma ($10^{-5}M$); cells counted by hand; each entry represents an average of three cultures.

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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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-

Table 1. Diffusion coefficient values (D) measured intracellularly compared to values for dilute solution diffusion (25°C).

Solute	$D \times 10^{-5}$ (cm²/sec)		
	Intracellular	Dilute solutions	
Water	2.42 ± 0.28 [16]*	2.51 ± 0.01 (spin echo)	(4)
		2.28 (spin echo)	(5)
		2.25 ± 0.02 (^{3}H-tracer)	(6)
Urea	1.87 ± .15 [22]	1.36	(6)†
Glycerol	1.25 ± .25 [9]	0.95	(6)†

* Number in bracket indicates number of experiments. † Measured at 20°C and converted to 25°C by a factor 3 percent/°C = + 15 percent.

tion is questioned (*1*) and various authors have suggested a slower rate of intracellular diffusion, but few precise measurements have been attempted (*2*). We measured quantitatively the diffusion coefficient of radioactively labeled water, urea, and glycerol in the cytoplasm of the muscle fiber of the giant barnacle *Balanus nubilus* and then compared these results with the standard values measured in dilute solution.

The size of the muscle fiber (2 by 30 mm) allows dissection of individual fibers, therefore eliminating the potential difficulties of extracellular constituents and their contribution to diffusion. Each cell was tied by a fine thread at the tendonous end and hooked at the open end. It was then extended to normal resting length on a screw clamp, and placed vertically in a diffusion chamber with the open end immersed in the experimental solution. This apparatus allowed single dimensional diffusion from an essentially infinite volume of a dilute iso-osmotic solution containing labeled water, urea, or glycerol. The remaining part of the cell above the solution level was covered with a light oil to prevent drying and contamination via evaporation and condensation. After 90 minutes, the cell was removed, frozen, and sliced into 5-mm segments. Each segment was dissolved, its solute concentration was measured by liquid scintillation counting, and its diffusion coefficient was calculated.

The solution to Fick's law of diffusion for the nonsteady state of one dimensional diffusion from a source of constant concentration is given by the equation of Höber (*3*)

$$C_x = C_0\left(1-\frac{2}{\sqrt{\pi}}\int_0^y e^{-t^2}dt\right)$$

where $y^2 = x^2/4\,DT$; C_x is the concentration of the diffusion molecule at distance x from the source of concentration C_0 and at time T. C_x is assumed to be 0 at T_0; D is the diffusion coefficient expressed in square centimeters per second. Error function tables greatly simplify the calculations.

Our results, together with values obtained by others (*4–7*), are given in Table 1. The intracellular diffusion coefficient for water was $2.42 \pm .28 \times 10^{-5}$ cm²/sec, which is midway between the two values for the self-diffusion of water. Similarly, the experimental value for glycerol of $1.25 \pm .25 \times 10^{-5}$ cm²/sec does not differ from the measured value for diffusion in water. The value for urea of $1.87 \pm .15 \times 10^{-5}$ cm²/sec is somewhat higher than expected.

At least two possible complicating factors are present but, if significant, would tend to slow intracellular diffusion, not hasten it. Approximately 40 percent of the barnacle water is bound, and presumably binding could occur with the test molecules (*7*). The results suggest that the unbound water behaves, from a diffusional standpoint, as a dilute solution. Second, the concentration of barnacle cytoplasm is not dilute but is approximately 1*M*. However, the diffusion coefficient is proportional to the activity coefficient, and if activity coefficients change significantly with concentration they are lowered with increasing concentration.

The purpose of these experiments was to test the hypothesis that diffusion proceeds more slowly intracellularly than in dilute aqueous solution. This hypothesis was rejected for all three solutes. It would appear from calculation of permeability coefficients that one may reasonably assume that the rates of intracellular diffusion are equal to those in dilute solution.

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Diphtheria Toxin Subunit Active in vitro

Abstract. *Exposure of diphtheria toxin to dithiothreitol (and similar thiols) resulted in a subunit which was active in catalyzing the adenosine diphosphate–ribosylation of mammalian aminoacyl-transferase II in the presence of nicotinamide adenine dinucleotide. At the same time there was a marked increase in total ADP–ribosylation activity. A molecule which was apparently identical to the derived subunit in size and activity was detected in partially purified preparations of toxin.*

Diphtheria toxin has been crystallized in several laboratories. It is a simple protein of molecular weight about 65,000 (*1–3*). That the toxin molecule may be composed of more than one polypeptide chain is suggested by the observations that the sedimentation coefficient decreases from 4.2*S* to about 2*S* after treatment with sulfite (*3*) and that more than one equivalent of amino-terminal amino acid is present per 65,000 daltons of toxin (*4*). We now report the identification in supernatants from cultures of *Corynebacterium diphtheriae* of a molecular species that is approximately half the size of native toxin and exhibits the specific catalytic activity in vitro attributed to native toxin (*5, 6*). In addition we have derived a subunit of apparently identical size and high activity by exposure of native toxin to thiols such as dithiothreitol (DTT). Such treatment of native toxin also results in an increase in catalytic activity.

Diphtheria toxin inhibits protein synthesis in mammalian cells and cell-free systems derived therefrom (*7, 8*). This inhibition requires the presence of nicotinamide-adenine dinucleotide (NAD) and is effected through specific inactivation of aminoacyl-transferase