are altered by high concentrations of potassium ions and the removal of calcium ions.

Since both the excitability and the rate of incorporation of P^{32}-orthophosphate return to normal upon reimmersion of the nerves in ordinary Ringer's solution, it seems unlikely that any structural damage to the nerve fiber occurred during our experiments. A high external concentration of potassium ions does not significantly alter the internal K^+ concentration in the excitable tissues (*5*), so that the effect of K^+ probably occurs at the external surface. The surfaces most accessible to the exterior are the outer layer of the neurilemmal sheath and the axonal membrane at the region of the nodes of Ranvier. Since mitochondria are located in the axoplasm as well as in the neurilemmal sheath (*1*), and since the inhibitory action of potassium ions upon phosphorylation is almost complete, both regions must be affected, and a common inhibitory mechanism is suggested.

It has been postulated that a release of calcium ions from the excitable membrane occurs during depolarization and that this release is accompanied by an outflux of orthophosphate and ATP (*4*). The net flow of phosphates in the outward direction would indicate that phosphate adsorption at the interface is diminished by depolarization. It remains to be seen whether this outflux of phosphates and inhibition of phosphorylation accompanying depolarization is due to loss of Ca^{2+} or to the change in the K^+ concentration at the surface of the excitable membrane (*6*).

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Feedback Control of Purified Deoxycytidylate Deaminase

Abstract. *The activity of purified deoxycytidylate deaminase obtained from chick-embryo extracts is dependent upon the presence of deoxycytidine triphosphate and magnesium ions. The stability of the enzyme at 37°C is markedly enhanced by deoxycytidine triphosphate, and less so by the other deoxyribonucleotides. The inhibition by* p*-chloromercuribenzoate, urea, and deoxythymidine triphosphate, is reversed by deoxycytidine triphosphate. This reversal suggests that the regulation of enzyme activity is effected through configurational changes in the enzyme structure.*

We have recently described (*1*) the activating effect of dCTP (*2*) on deoxycytidylate deaminase, and the marked inhibition of the enzyme by dTTP. In view of the end-product regulatory influence of these nucleotides on the enzyme, and a similarity to the studies on aspartate transcarbamylase (*3*), a feedback mechanism was suggested which might possibly be invoked through conformational changes in the enzyme structure. These changes could explain the apparent increase in affinity of the enzyme for dCMP and its reversal by dTTP, and they suggest a control mechanism that may be important to numerous other enzymes. Subsequent to our original observations with extracts from chick and rat embryos, the enzyme has been highly purified from 6-day-old chick embryos and some of its properties have been described (*4*). The present report on studies with the purified enzyme supports the concept of a feedback control. Monod *et al.* (*5*) have recently reviewed a number of enzymes that appear to be activated or inhibited at a site (allosteric) other than the substrate site.

The activity of the purified enzyme is markedly dependent on the presence of dCTP and Mg^{++} when the substrate concentration was 2m*M* and less (Table 1). This observation would explain the activating effect of dCTP when added either to aged preparations or those incubated at 37°C (*1*), in which the endogenous dCTP had probably been depleted through enzymic hydrolysis. Similar results were recently reported (*6*) on a partially purified spleen preparation of deoxycytidylate deaminase. However, no apparent requirement for dCTP and Mg^{++} was described, which may be the result of differences in purity of the two preparations. The enzyme preparation was purified approximately 100-fold from the crude extract of 6-day-old chick embryos. The essential steps in the purification were calcium phosphate gel absorption of inert protein, precipitation of nucleic acids by protamine, saturation with ammonium sulfate, and fractionation on a cellulose phosphate column. A unit is defined as the amount of enzyme that will deaminate 1 μmole of dCMP in 10 minutes at 37°C. The initial specific activity was 0.50 unit per milligram of protein in a crude extract of chick embryos and the final purified enzyme preparation was capable of deaminating, in 10 minutes at 37°C, 50 to 60 μmoles of dCMP per milligram of protein when the concentration of dCMP in the substrate was 2m*M*.

When the substrate concentration of dCMP was 10m*M*, charcoal treatment of the purified enzyme preparation results in a partial requirement for dCTP, whereas the requirement was absolute at 2m*M* dCMP. There was a 30-percent decrease in activity at a concentration of 10m*M* dCMP, which could be partially restored by the addition of dCTP and Mg^{++}. It is not known whether this results from the stabilizing effect of dCTP (*7*) on the deaminase (Table 2), its role as a cofactor, or possibly an increase in affinity of the enzyme for its substrate. An increase in the affinity should be reflected in a decrease in the Michaelis-Menten constant (K_m) for the monophosphate in the presence of the triphosphate; however, as indicated in Table 1, little or no activity could be measured in the absence of the triphosphate and Mg^{++} at 2m*M* or less of dCMP, thus making it difficult to evaluate K_m accurately. Since this inability to detect significant activity may be due to the insensitivity of the assay, the question of whether a change in K_m is being effected by dCTP and Mg^{++} can possibly be resolved through the use of dCMP-2-C^{14} in the assay (*8*). It is also conceivable that two enzymes are present in the purified deaminase preparation, one with a low affinity for dCMP and the

Table 1. Requirement of deoxycytidylate deaminase for dCTP and Mg^{++}. In experiment 1, the reaction mixtures contained 1 μmole dCMP, 50 μmoles tris, *p*H 8.0, 1 μmole $MgCl_2$, Norite-treated enzyme (13 μg protein), and water to a final volume of 0.5 ml. They were incubated for 10 minutes at 37°C and assayed (*1*). In experiment 2, the conditions were the same as in experiment 1, except 0.04 μmole dCTP was added to each reaction and the enzyme was isolated from a Versene-washed phosphocellulose column.

Conditions	dCMP deaminated (μmole)
Experiment 1	
Minus dCTP	0.030
$+4 \times 10^{-6}$ *M* dCTP	.234
$+4 \times 10^{-5}$ *M* dCTP	.440
Experiment 2	
Minus Mg^{++}	.0
$+2 \times 10^{-5}$ *M* Mg^{++}	.142
$+2 \times 10^{-4}$ *M* Mg^{++}	.236

Table 2. Stability of deoxycytidylate deaminase at 37°C. Norite-treated enzyme (66 μg protein) was incubated at 37°C in the presence of 1 μmole $MgCl_2$, 15 μmoles phosphate (*p*H 7.5), and 0.2 μmole of the indicated nucleotides in a volume of 0.2 ml. After one hour, portions were removed from each tube and assayed for deoxycytidylate deaminase activity in the presence of 0.04 μmole added dCTP.

Compound added	dCMP deaminated (μmole)
None	0.0
dCTP	.284
dTTP	.210
dATP or dGTP	.0
dGMP	.202
Nonincubated control	.300

Table 3. Reversal of *p*-chloromercuribenzoate and urea inhibition by dCTP. Experimental conditions are the same as indicated in Table 1 with Mg^{++} present, and dCTP added to the following concentrations: *A*, 2×10^{-7} *M*; *B*, 4×10^{-5} *M*; *C*, 4×10^{-5} *M*. The compounds added are listed at the concentrations present in the reaction mixture.

Conditions	dCMP deaminated (μmole)
A	
Control	0.212
Control + 2×10^{-7} *M* *p*CMB	.080
Control + 6×10^{-7} *M* *p*CMB	.0
B	
Control	.356
Control + 2×10^{-7} *M* *p*CMB	.360
Control + 6×10^{-7} *M* *p*CMB	.046
C	
Control	.216
Control + 0.8*M* urea	.110
Control + 0.8*M* urea + 2.4×10^{-4} *M* dCTP	.201

other with a higher affinity in the presence of dCTP and Mg^{++}. That this possibility is not without precedent was indicated in a recent paper by Viñuela *et al.* (*9*) describing two apparently different hexokinases that act on glucose in the same liver preparation, one with a high affinity for glucose and the other with a lower one.

If the apparent activating effect of dCTP and Mg^{++} were related to a decrease in the K_m, the overall process could result from an attachment of the activators to a site removed from the substrate site, causing a more stable enzyme configuration. Support for this postulate may be reflected in the stability study of Table 2 and in the similarity of the effects observed with those of other enzymes (*5*). Whether dGMP acts in a similar manner to dCTP in stabilizing the enzyme is not known, but the fact that dGMP does not activate the enzyme suggests that this may not be the case.

The inhibition of the purified enzyme by dTTP has been found noncompetitive. This factor, coupled with the partial protective effect of dTTP upon the enzyme (Table 2), may indicate that dCTP and dTTP compete for the same site. In contrast to the studies with extract of chick embryo, dTDP was ineffective as an inhibitor and dCDP ineffective as an activator at concentrations comparable to the corresponding triphosphates, which could be explained by the presence of ATP in the crude extracts.

An interesting finding by Scarano *et al.* (*6*), which would support the concept of configurational change, is that dCTP reverses inhibitory action of *p*-chloromercuribenzoate on deoxycytidylate deaminase. We have made similar observations in which the amount of *p*-chloromercuribenzoate required for 50-percent inhibition was of the order of $10^{-7}M$ (Table 3) in the presence of dCTP, which, as indicated above (Table 1), is required for activity and also for the reversal of the inhibition (Table 3). Thus, it would appear that the binding equilibrium of *p*-chloromercuribenzoate is altered by dCTP, as a result of a configurational change in the enzyme structure. This change would have the effect of masking the sulfhydryl-sensitive site (or sites), causing a displacement of the indicated equilibrium to the left (*p*CMB + Enzyme$\rightleftarrows$*p*CMB·Enzyme). In support of this proposal is the reversal in urea inhibition by dCTP (Table 3) and a shift in the *p*H optimum from 7.6 for 10m*M* dCMP (minus dCTP) to 8.2 for 2m*M* dCMP (plus dCTP) (*10*).

In conclusion, the effective feedback inhibition of deoxycytidylate deaminase by dTTP and its equally effective reversal by dCTP may play a role in controlling the synthesis of dTTP (*11*).

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Photosensitizing Substance in Human Serum: Effect on HeLa Cells

Abstract. *The destructive effects of blue and white light on HeLa cells grown in monolayers is described. Apparently, there is a photosensitizer in the human-serum component of the growth medium. The photosensitizer may be a compound containing riboflavin.*

The photodynamic phenomenon originally described by Raab (*1*), which is also discussed by Blum (*2*), was a subject of great interest during the first 30 years of this century. The specific effects in mammalian tissue culture have been studied by several investigators. Earle (*3*) in 1928 showed that rabbit erythrocytes and leukocytes in protein-free, balanced saline were photosensitized by different