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*).

GLADYS F. MALEY
FRANK MALEY
Division of Laboratories and Research, New York State Department of Health, and Department of Biochemistry, Albany Medical College, Albany

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2. Abbreviations: dCMP, dCDP, dCTP, deoxycytidine 5′-mono-, di-, and triphosphate, respectively; dTMP, dTDP, dTTP, thymidine 5′-mono-, di-, and trisphosphate, respectively; dGMP, dGTP, deoxyguanosine 5′-mono-, and triphosphate; ATP, adenosine 5′-trisphosphate; *p*CMB, *p*-chloromercuribenzoate.
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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

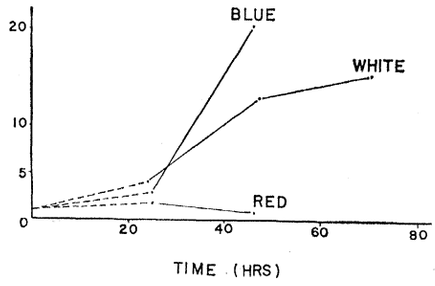

Fig. 1. The effects of red, blue, and white light on HeLa cells. Nonviability was defined by the ratio: (percentage of experimental cells that absorbed trypan blue)/(percentage of control cells that absorbed trypan blue). Ordinate, nonviability; abscissa, time (hours).

colors of light when erythrocytes were present. He described the cellular changes and suggested that the presence of red-cell pigment or its degradation products might be a possible requisite for the occurrence of the photodynamic phenomenon. Menke (*4*) described similar effects of light, in the presence of phloxine, on normal rat tissue and malignant rat sarcoma. Klein and Goodgal (*5*) described the photodynamic inactivation of monkey kidney cells grown in monolayers exposed to white light, or to light filtered through solutions of methylene blue and trypan blue.

We have observed the photodestruction of HeLa cells, grown in monolayers, exposed to white light and blue-filtered light (Fig. 1) and have searched for the cause of this photodynamic destruction.

Monolayers of HeLa cells were grown in Eagle's minimum essential medium (*6*) without phenol red, in Leighton tubes placed in a water bath (37°C) equipped with Corning glass filters, one of which passed red-orange light (maximum transmittance 672 mμ) and the other passed blue-violet light (maximum transmittance 458 mμ).

Cellular destruction was determined by viability tests with trypan blue, in which dye exclusion was the criterion of a viable cell.

Studies of the cells and components of the medium revealed that the human serum component of the medium was required for this phenomenon to occur. Cells maintained in direct light did not undergo degeneration if the medium used had no serum.

Examination of the literature indicates that a pigment is required for the photodynamic destruction described. Pigments present in human serum in relatively large quantities include bilirubin and carotenoids; riboflavin, which acts as a photosensitizer (*7*), is also present. If any of these substances were acting as photosensitizers, a more rapid and more pronounced destructive effect should occur if a higher than normal concentration were present. Thus, relatively high concentrations of bilirubin (from the serum of a hospital patient with 5 mg of bilirubin per 100 ml of serum), carotenoids (0.1 mg of vitamin A palmitate per milliliter), and riboflavin (2 μg/ml) were added separately to the growth medium of cells which were grown in blue light (Fig. 2).

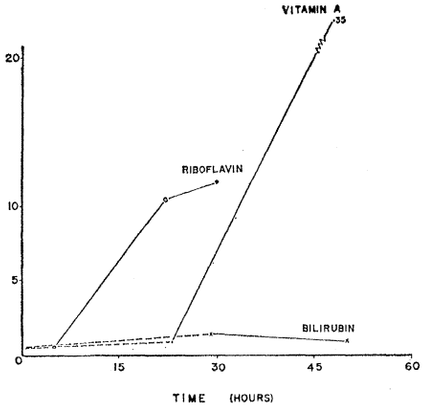

Fig. 2. The effects of blue light on HeLa cells grown in media enriched with riboflavin, vitamin A, and bilirubin. Ordinate, nonviability; abscissa, time (hours).

The increases in concentration of these substances were between 10 and 50 times the concentrations occurring in the normal medium. The curve representing the effect of high bilirubin concentration shows that there is no pronounced effect from exposure to blue light. Vitamin A enrichment produced a very pronounced destruction, but only after 48 hours of exposure to blue light. An increased concentration of riboflavin produced an early and definite cytopathogenic effect.

Therefore, it seems unlikely that bilirubin is the photosensitizer since it is unable to increase cellular destruction. Vitamin A satisfies one criterion, that of increased effect. However, this effect was not significantly rapid, and it might be somewhat different if it were acting as a sensitizer. Since vitamin A is very easily oxidized, it might be possible that the toxicity of a peroxide (*8*) would account for the greatly increased effect of exposure to blue light.

Riboflavin induced a very early destruction. After 30 hours of exposure to blue light, 100 percent of the riboflavin-treated cells were nonviable, whereas only 5 to 20 percent of the cells were nonviable in cultures treated with vitamin A enriched, elevated-bilirubin, and normal serum (*9*).

WILLIS B. WHEELER
ARIEL C. HOLLINSHEAD
Department of Medicine, Warwick Memorial Clinic, George Washington University School of Medicine, Washington, D.C.

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Thiamine: A Novel Conversion from Thiochrome

Abstract. *Reaction of thiochrome with α-chloropropionic acid yields thiamine and acetaldehyde. The reaction seems to proceed by way of a zwitterion intermediate that undergoes decarboxylation and hydrolysis. The biological significance of the reaction cannot yet be evaluated.*

Although the formation of thiochrome in the potassium ferricyanide oxidation of vitamin B_1 has been used for the quantitative assay (*1*) of the vitamin, the chemistry of the heterocycle has not been studied extensively (*2*). In the course of investigating the chemistry of thiochrome, we discovered an interesting decarboxylation. Under mild conditions, the reaction of thiochrome with α-chloropropionic acid yielded thiamine and acetaldehyde as the major products. The conditions for the reaction were as follows: thiochrome (250 mg, 0.9 mmole) and α-chloropropionic acid (98 mg, 0.9 mmole) were suspended in water (5 ml). Ethyl alcohol was then added to dissolve the mixture completely. Sodium hydroxide (40 mg in 2 ml of