counterpart, arc 2, precipitated by Pn2SS, which demonstrates that this doubly labeled rabbit antibody to pneumococcus retained its immunologic activity with the specific polysaccharide. Arc 2a is a mirror image of arc 2 formed by the same soluble substance in the middle trough. The long line of precipitation, arc 3, developed against RAF, is biphasic: the more rapid component has no counterpart in arc 1 or arc 2 and represents unconjugated ferritin, and the slower component exhibits the same mobility as arcs 1 and 2, and therefore contains ferritin in combination with immune rabbit globulin. The two or three sequential ultracentrifugations employed during the preparation of the double conjugates has removed any unconjugated rabbit globulin. The unconjugated ferritin can also be eliminated, if necessary, by starch-block or continuous-flow electrophoresis.

Experiments with fluorescein-ferritin–conjugated antisera described in this report indicate that by the tests employed there is no significant alteration in the specific activity of such doubly labeled antibody. It is anticipated that this technique will facilitate the task of the electron microscopist in the fine-structural study of antigen-antibody localization (*13*).

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Inhibition of Growth of Chick Embryo by Inhibition of Deoxycytidylate Deaminase

Abstract. *Deoxyguanylate when added to chick embryos grown in explant inhibited growth and development. Deoxycytidylate deaminase activity was inhibited both in the explants and in vitro; since the effect was quite specific, it is suggested that this may represent another control mechanism for deoxynucleotide synthesis.*

There appears to be a general correlation between the activity of dCMP (*1*) deaminase and the rate of cell proliferation or growth. Thus, high activities have been reported in embryonic tissues (*2*), in certain tumors (*3*, *4*), and in regenerating liver (*5*). This correlation is not surprising, since it has been shown that the product of dCMP deaminase action, deoxyuridylate, is utilized in the synthesis of thymidylate (dTMP), a DNA precursor.

Considering these observations, it is possible that the specific inhibition of dCMP deaminase in a rapidly growing system might inhibit growth.

In this report, some effects of deoxynucleotides on growth and dCMP deaminase activity of chick embryos grown in vitro and in the egg are presented.

The technique of explanting chick embryos of 11 to 13 somites with small extra-embryonic membranes has been described (*6*). Embryos were cultured on a whole-egg homogenate medium with a gas mixture consisting of 25 percent O_2 plus 75 percent air for 0 to 24 hours and 95 percent O_2 plus 5 percent CO_2 for 24 to 48 hours (*7*). After 48 hours of cultivation in vitro, between 9 and 15 embryos (or single embryos) from each group were homogenized in cold 0.25*M* sucrose solution in a 1-ml Ten Broeck homogenizer, and assays for total protein and dCMP deaminase (*4*, *8*) were carried out immediately.

Initial experiments with 2 μmole of dGMP (*9*) per milliliter of medium resulted in death of the embryos. The concentration was therefore reduced to a maximum of 0.1 μmole per milliliter (Table 1).

Embryos explanted in the presence of 0.05 μmole of dGMP per milliliter of medium were strikingly inhibited in growth and development, and the protein content of individual embryos was only one-half that of the controls (*10*). The specific activity of dCMP deaminase was only slightly reduced, however. Treatment with 0.10 μmole of dGMP led to a further reduction in protein content, and a marked reduction in dCMP deaminase specific activity. These effects were completely reversed by addition of dCMP to the medium. At a concentration of 0.10 μmole per milliliter, dAMP had no significant effect on the embryos, and caused no reversal of the inhibitory effects of dGMP when added to the reaction mixture with this latter nucleotide.

At a concentration of 0.10 μmole per milliliter, dG was also inhibitory to the growth of the embryos, giving about the same results as 0.05 μmole of dGMP.

These observations are in agreement with those of Karnovsky and Lacon (*11*) who reported a severe toxic effect when dG was injected into eggs. They also reported that this effect could be reversed by injection of dC.

Table 1. The effect of deoxynucleotides on the growth and enzyme activity of explanted chick embryos.

Total protein per embryo (mg)		dCMP deaminase specific activity (μmole/g prot/hr)	
Expt. 1	Expt. 2†	Expt. 1	Expt. 2†
	*None (control)**		
0.344	0.321	622	635
	dGMP (0.05 μmole)		
.142	.182	344	486
	dGMP (0.10 μmole)		
.104	.107	0	136
dGMP (0.05 μmole) + *dCMP (0.05 μmole)*			
.372		550	
dGMP (0.05 μmole) + *dCMP (0.10 μmole)*			
	.297		639
	dTTP (0.10 μmole)		
.280		1100	
	dG (0.10 μmole)		
	.184		261

*Total nucleotide in 1 ml of culture medium. Whole homogenate was used for assay of dCMP deaminase activity. †Average of two separate determinations in duplicate on single embryos performed by a micro modification of the Conway procedure.

Table 2. The effect of added deoxynucleotides on the deoxycytidylate deaminase activity of chick embryos grown in the egg. Approximately 24 to 36 embryos of the indicated age were homogenized in 0.25*M* sucrose solution and the homogenate was centrifuged at 92,000*g* (average) for 1 hr. Generally 0.4 ml of supernatant fraction, 0.5 ml of 0.1*M* tris buffer (*p*H 7.4), and nucleotides, in a total volume of 2.0 ml were incubated for 1 hour at 37°C. The incubation mixtures contained the quantities of added nucleotides indicated (in parentheses) in addition to 5 μmoles of dCMP as substrate. Chick embryo has no detectable dGMP or dG deaminase activity with the assay conditions used (*13*).

Addition (μmole)	dCMP deaminase specific activity (μmole/g prot/hr)
5-day embryos	
Control	1257
dGMP (2)	0
dG (2)	1210
dG (4)	1270
6-day embryos	
Control	1256
dGMP (0.1)	980
dGMP (0.2)	782
dGMP (0.4)	526
6-day embryos	
Control	1600
dTTP (0.063)	960
dTTP (0.25)	0

The results in Table 1, in conjunction with those in Table 2, suggest, however, that dGMP (rather than dG), by virtue of inhibition of dCMP deaminase activity, may be inhibitory to growth and development of the chick embryo. Further support for this view is given by the data in Table 2, which shows the effects of deoxynucleotides on dCMP deaminase activity in the supernatant fraction of chick embryos grown in the egg.

In these experiments, dGMP was strongly inhibitory to dCMP deaminase activity even at low concentrations, whereas dG had no effect. More detailed experiments in which the amounts of both dCMP and dGMP were varied have shown that the inhibition is competitive. Guanylate was also inhibitory to dCMP deaminase (not illustrated), and dTTP as reported by Maley and Maley (*12*) was strongly inhibitory. However, dA or dAMP in amounts up to 2 μmoles per milliliter had no effect on dCMP deaminase activity of the 5-day-old chick embryo, and this is in accord with experiments on explants.

In spite of the strong inhibitory action of dTTP, which on a molar basis is 10 to 20 times more effective than dGMP (*12*) as an inhibitor of dCMP deaminase (Table 2), it was predicted that this compound would have no effect on explanted embryos, since it is an end product of dCMP deaminase activity, and could be utilized as such by the embryos, as illustrated below.

$$dCMP \rightarrow dUMP \rightarrow dTMP \rightarrow dTTP \rightarrow DNA$$
$$\uparrow \quad \text{inhibition} \quad |$$

This turned out to be true (Table 1), and embryos explanted in the presence of 0.1 μmole per milliliter of dTTP grew and developed as well as the controls (Table 1).

The marked action of dGMP at what is probably a very low level of this compound in the embryo, and the appearance of dG and dGMP deaminase activity late in development (*13*), suggest that the deoxypurine nucleotide may play an important role as a regulator of development.

In a recent report Siedler and Holtz (*14*) showed that dCMP deaminase of *Lactobacillus acidophilus* was inhibited by dGMP, as well as by other nucleotides. Apparently dAMP was not tested. Although dGMP has a strong inhibitory action on dCMP deaminase of the chick embryo, it has also been reported that dGMP in low concentrations inhibits the conversion CDP → dCDP (*15*). This could be, therefore, an alternate or additional explanation of our results except for the fact that dAMP is also inhibitory to the reductive reaction but had no effect in the system that we studied (*16*).

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References and Notes

1. Abbreviations, dCMP, deoxycytidylate; dTMP, deoxythymidylate; dGMP, deoxyguanylate; dG, deoxyguanosine; dC, deoxycytosine; GMP, guanylate; dTTP, deoxythymidine triphosphate; dA, deoxyadenosine; dUMP deoxyuridylate; CDP, cytidine diphosphate; dCDP, deoxycytidine diphosphate.
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8. In addition to the method of Roth *et al.* (*4*) a micromodification, for 3- to 4-day-old embryos and 48-hr embryo explants was used. Details are in preparation for publication.
9. Nucleotides were obtained from Sigma, Schwarz BioResearch, or Calbiochem as free acids or sodium salts.
10. In general, the appearance of the embryo correlated well with the protein content. The embryos with lowered protein content were not only smaller, but showed considerably less development of hemoglobin, the circulatory system, and so forth. Only embryos with a pulsating heart were used.
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Poisoning by DDT: Relation between Clinical Signs and Concentration in Rat Brain

Abstract. *The severity of signs of poisoning in rats after a single dose of DDT is directly proportional to the concentration of the compound in their brains. The concentrations associated with death after one large dose are about the same as those after many smaller doses.*

The action of DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane] in animals is manifested almost entirely through the nervous system. The most prominent signs of poisoning from this compound are muscle tremor, incoordination, and convulsions. Studies of the effect of DDT on the nervous system have been reviewed elsewhere (*1*). Briefly, early studies in which measurements were made of DDT in brain and other tissues failed to relate the concentrations found to clinical signs of poisoning. In more recent work, Dale *et al.* (*2*) showed that the concentration of DDT in the brain of rats fed the *p,p'*-isomer of DDT, at a dietary level of 200 parts per million for 90 days, increased during a subsequent 10-day period of partial starvation. The increased concentration of DDT in the brain during starvation was correlated with the clinical signs of poisoning.

This report gives the concentration of DDT and DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethylene] in rats before, during, and after recovery from signs of illness following a single oral dose of DDT. The results show that clinical signs of poisoning are directly correlated with the concentration of DDT in the nervous system, as measured by the concentration in the brain. Special studies have shown that all parts of the nervous system are affected by DDT. However, a review (*1*) of published papers, and new research to be published elsewhere, indicates that