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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16. Supported by grants C–5264, AM–06408 and B–2238 from the National Institutes of Health and P–303 from the American Cancer Society. We thank Dr. H. Herrmann for the use of his facilities for the explant experiments.

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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

the brain is of major importance in this regard.

Forty male rats, 100 days old, of the Sherman strain, each weighing about 240 to 300 g, were kept in separate cages and given a single dose of 3-percent *p,p'*-DDT in peanut oil solution, by stomach tube, at a rate of 150 mg/kg of body weight. The rats had been reared on Purina laboratory chow with no DDT added, but were fasted overnight before being dosed with DDT.

Surviving rats were observed continuously for symptoms of poisoning during the first 12 hours after being dosed. They were killed according to the following schedule: group 1 (asymptomatic), 2 hours after dosing; group 2, at the onset of slight tremor; group 3, after 2 to 4 hours in severe tremor; group 4, at the onset of convulsions; and group 6, 26 hours after dosing, following observed severe tremor or convulsions, or both, with subsequent complete clinical recovery. Group 5 consisted of rats that died during or just after convulsions; samples were taken as in the other groups. The rats were assigned to their respective groups at the time of killing or death, and the brain, liver, kidneys, blood, and a fat specimen were taken for chemical analysis.

Since the rats in groups 1 to 4 were killed before or while they were showing signs of poisoning, there was no way to separate those that might have survived from those that might have died.

Blood was collected from living rats by cardiac puncture, while the rats were under ether anesthesia. After as much blood as possible was withdrawn, the rats were bled to death by severing the aorta. Blood for analysis was taken from the heart of dead rats within a few minutes after they died. Heparin was used in the syringe to prevent clotting.

The plasma was extracted by the method of Bloor (*3*). The DDT and DDE in the plasma extract were measured by the Schechter-Haller method, as modified by Schechter *et al.* (*4*). The brain, liver, kidneys, and fat tissues were analyzed for DDT and DDE by the Schechter-Haller method as modified by Mattson *et al.* (*5*).

No clinical signs of poisoning were observed in the rats during the first 2 hours after being given the single oral dose of DDT. Some of the rats began to show slight tremor after 3 hours, severe tremor after 4.5 hours, and convulsions after 5.4 hours of being dosed.

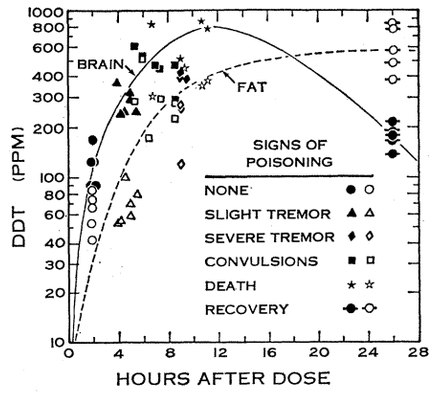

Fig. 1. Concentration of DDT in the lipids of brain (solid symbols) and fat (open symbols) of rats at different times after the administration of a single dose of the compound at the rate of 150 mg/kg. Different symbols indicate the clinical state of the animals when the samples were taken.

All of the rats that developed convulsions were in severe tremor when the convulsions began.

The concentrations of DDT and DDE found in the extractable lipids of the different tissues are shown in Table 1. It can be seen that there was a significant increase in the concentration of DDT in the brain of the poisoned rats which corresponded with the increase in the severity of the symptoms, but there was no significant difference between the concentrations of DDT in the brains of rats killed in severe tremor and those killed in convulsions. The concentration of DDT increased significantly in the plasma and kidneys until the onset of slight tremor, and in the liver until the time of severe tremor. There was no further increase in the concentration of DDT in the plasma and liver of the rats at the time of severe tremor, convulsions, or death. The sharp increase in the concentration of DDT in the kidneys of the rats that died as compared with the concentration in those killed after convulsions ($p < .001$) may have been due to renal failure.

Animals in group 6 were killed 26 hours after being dosed with DDT, when they were no longer showing signs of poisoning. The concentration of DDT in their brains had returned to levels below that of any rat showing toxic symptoms, and the concentrations in the plasma, liver, and kidneys had decreased also. However, the DDT in fat had continued to increase as it was mobilized from other tissues. The temporal relations of the changes in the brain and fat are shown in Fig. 1.

The major signs of poisoning by DDT concern the central nervous system, and the data presented here suggest that these signs are a direct response to the concentration of DDT found in the brain. In the rats that died (group 5), the concentration in the brain (524 to 848 ppm) was in good agreement with the range (205 to 985 ppm) reported elsewhere (*2*) for rats that died promptly when subjected to partial starvation after receiving small doses of DDT over long periods. This suggests that, regardless of how the DDT is administered, the probabil-

Table 1. The concentration of DDT and DDE in tissue lipids of male rats at various time intervals and in various clinical states after administration of a single oral dose of DDT at the rate of 150 mg/kg. The results are presented as the range, mean, and standard error.

No. of rats	Time between administration of DDT and death (hr)	DDT (ppm)					DDE (ppm)
		Brain*	Plasma	Liver	Kidney	Fat	Plasma
		Group 1: no clinical signs of poisoning					
6	2	90 – 168†	178 – 417	737 – 1074	286 – 1002	29 – 84	<0.5‡
	2	119 ± 14	268 ± 40	947 ± 58	597 ± 126	58 ± 8	
		Group 2: slight tremor					
6	4.00 – 5.58	242 – 371	178 – 1190	1706 – 4626	1029 – 1894	54 – 99	<0.5‡
	4.75 ± 0.23	287 ± 21	833 ± 147	2839 ± 470	1495 ± 130	68 ± 7	
		Group 3: severe tremor					
3	9.00 – 9.50	386 – 433	215 – 1086	4602 – 6657	1030 – 1211	118 – 266	99 – 193
	9.17 ± 0.17	404 ± 15	645 ± 251	5818 ± 622	1100 ± 56	213 ± 47	129 ± 32
		Group 4: convulsions					
6	5.50 – 8.50	289 – 606	643 – 1348	2420 – 7609	1341 – 1597†	173 – 512	<0.5 – 145
	7.05 ± 0.55	468 ± 43	1007 ± 114	4149 ± 734	1443 ± 45	293 ± 47	98 ± 21‡
		Group 5: convulsions and death					
4	6.80 – 11.25	524 – 848	417 – 893	4270 – 6602	2885 – 3994†	309 – 424	298 – 476
	9.06 ± 1.12	737 ± 73	685 ± 107	5698 ± 500	3315 ± 343	361 ± 24	372 ± 45
		Group 6: recovery after severe tremor or convulsions, or both					
5	26	138 – 213	119 – 178	755 – 1162	591 – 871	377 – 810	298 – 476
	26	176 ± 12	131 ± 12	958 ± 82	720 ± 51	598 ± 82	405 ± 34

* *p*-Values: group 1 versus 2, < .001; 2 versus 3, < .01; 3 versus 4, > .2; 4 versus 5, < .01; 2 versus 6, < .005. † One sample lost. ‡ In calculating the mean and standard error, values below the detectable limits (0.5 ppm) were counted as zero.

ity that death will occur increases if the concentration of DDT in the brain exceeds 500 ppm in otherwise healthy rats, or exceeds 200 ppm in debilitated rats.

These data confirm the known fact that the concentration of DDT in the fat is not related to acute toxicity.

The appearance of measurable amounts of DDE in the plasma before other tissues parallels the results of Rothe *et al.* (*6*) who found that DDE appeared in chyle collected from the ductus lymphaticus during the absorption of DDT from the intestine. Values for DDE in brain, liver, kidneys, and fat are not shown in the table, because the concentrations were all below the experimental limits of the method.

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Actinomycin D: Its Effect on Antibody Formation in vitro

Abstract. *The formation of antibodies to bacteriophage T_2 in vitro was inhibited by 5×10^{-8}M actinomycin D. This result is consistent with the concept that antibody formation depends upon DNA-dependent RNA synthesis.*

The polypeptide antibiotic actinomycin D, at low concentrations, has been shown to inhibit cellular RNA synthesis while having little or no effect on DNA synthesis (*1*). It was therefore of considerable interest to test the effect of this antibiotic on antibody formation. In the studies reported here, it has been shown that $5 \times 10^{-8}M$ actinomycin D can inhibit completely both the continuation of primary antibody formation and the secondary antibody response induced in vitro to T_2 bacteriophage.

Lymph nodes were obtained from rabbits and cultivated in vitro according to the technique described by Michaelides and Coons (*2*). The rabbits were immunized twice, at a 2-week interval, with 2×10^{11} T_2 phage in saline distributed in the four footpads. After 6 to 34 weeks, both popliteal lymph nodes were removed and were cut into 1-mm^2 fragments. About 15 fragments were placed in each tube. The fragments were "fixed" to the glass by addition of one drop of heparinized plasma, and 1 ml of medium 1066 (*3*) containing 20 percent rabbit serum was added, followed by T_2 phage (2×10^{10}/ml) or actinomycin D (*4*), or both, as indicated. The tubes were placed in an incubator at 37°C with 5 percent CO_2. After 24 hours and, subsequently, every 3 to 4 days, the medium was replaced with fresh medium with or without actinomycin D. Antibody determinations were made by the standard titration for phage antibody (5). (Results are expressed as k, the inactivation constant of the rate of neutralization of phage by a given antiserum.)

The results of a representative experiment are depicted in Figs. 1 and 2. The antibody concentration (k) of each tube is plotted on a logarithmic ordinate against time on the abscissa. As can be seen in Fig. 1, the maintenance of antibody concentration in tubes to which T_2 had not been added, was prevented by $3 \times 10^{-7}M$ actinomycin D (0.3 μg/ml). The evidence that antibody is synthesized in vitro and is not formed in vivo and merely released during incubation is: (i) before incubation, only small amounts of antibody could be demonstrated in washed lymph node cells after repeated freeze-thawing; and (ii) the actinomycin D effect as described here. Figure 2 shows that larger amounts of antibody were usually formed in tubes to which T_2 had been added, indicating that a secondary response was stimulated in vitro; this response was also prevented by actinomycin D.

Thus actinomycin D can prevent the secondary response induced in vitro and the continuation of primary antibody formation in vitro. Additional experiments have revealed the following. (i) At a concentration of $5 \times 10^{-8}M$, actinomycin D completely inhibited both types of antibody formation; the effect on continuation of primary synthesis was detected as early as 72 hours after addition of actinomycin D to the medium. (ii) Antibody formation during the second week of culture was also

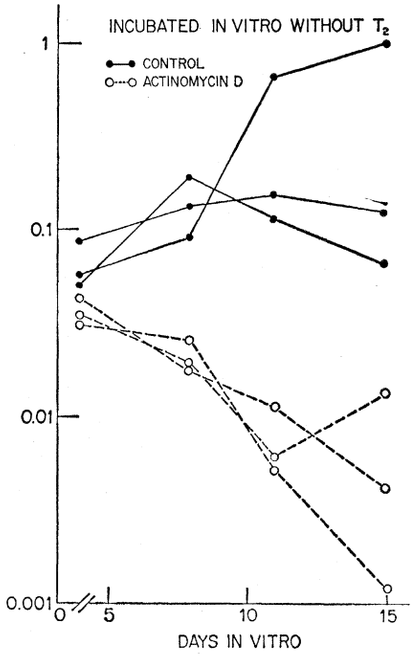

Fig. 1. The concentration of antibody to bacteriophage T_2 in each of six tubes. The culture medium was changed every 3 to 4 days; actinomycin D ($3 \times 10^{-7}M$) was added to three of the tubes.

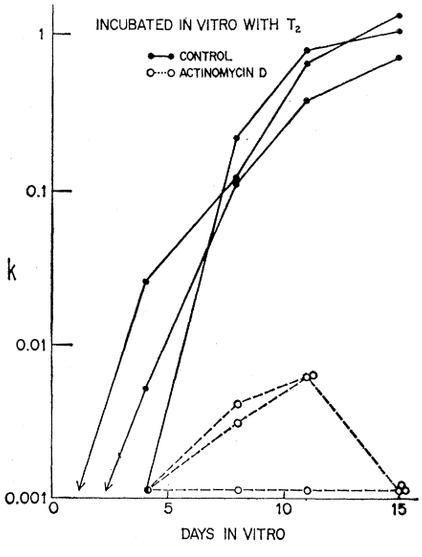

Fig. 2. The concentration of antibody to bacteriophage T_2 in each of six tubes. Actinomycin D ($3 \times 10^{-7}M$) was added to three of the tubes. Bacteriophage T_2 (10^{10}/ml) was added at the start of the experiment; the medium was changed on day 1 and every 3 to 4 days thereafter.