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Effect of Chlorpromazine on DNA Synthesis in a Cell Free System.* (30518)

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Chlorpromazine (CPZ) is known to suppress a wide variety of enzyme activities(1-8) including DNA synthesis(9-11) in intact cells and animals. Rosenberg(9) observed that administration of CPZ to rats with ascites hepatoma results in diminished incorporation of P^{32} into the nuclei of tumor cells. Also, radioautography demonstrated that CPZ inhibits *in vitro* incorporation of H^3 thymidine into human marrow cells engaged in DNA synthesis, followed by delay of mitosis(11,12).

Since CPZ affects cell permeability(13,14) as well as many other biologic activities not necessarily involved with enzymes(15-17) its effect on DNA synthesis in a cell free system was studied. It is felt that a cell free system avoids some of the variables imposed by a cell membrane and the numerous chemical reactions that simultaneously occur in an intact cell or organism. Our approach uses a multi-enzyme source from regenerating rat liver which can promote DNA synthesis.

Material and methods. Table I lists the components of the incubation mixture employed in these experiments; methods were adopted from those recommended by Bollum and Potter(18) and by Main and Walwick (19-21). Primer DNA‡ was obtained from calf thymus.

TABLE I. DNA Synthesis in a Cell Free System(20).

Tris-HCl, pH 7.7	20	μmoles
$MgCl_2$	3	mμmoles
Glucose	25	μmoles
ATP (K salt)*	.5	"
DPN*	1.25	"
dAMP*	25	mμmoles
dGMP*	25	"
dCMP*	25	"
Tritiated thymidine* (Sp. act. = 3 c/mM)	33	"
Protein enzyme†	1-2	mg
DNA	200	"
Test drug (Added either to incubation mix less enzyme or to enzyme)	.2	μmole

(Total volume 0.5 ml) Incubate 1-4 hr at 37°C.

* Source of reagents: ATP (K salt) Pabst Laboratories, Trace ADP; DPN, Pabst; H^3 thymidine, Schwarz, radiochemical purity 99%; dAMP, Calbiochem; dGMP, Calbiochem; dCMP, Calbiochem; all other reagents, chromatographically homogenous.

† Protein enzyme derived from regenerating rat liver homogenate, prepared 24 hr after partial hepatectomy.

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‡ Worthington Biochemical Corp., Freehold, N. J.

The DNA synthesizing system was prepared from Holtzman strain rats weighing 125-160 g. Seventy-five per cent of the liver was removed under ether anesthesia and the

TABLE II. Effect of Test Drugs on Incorporation of H^3 Thymidine into DNA in a Cell Free System. 2-Hr incubation

Drug:*	No drug	CPZ	PMZ	TDZ	CPZ-sulfoxide	Aspirin	Diphenhydramine	Amidopyrine	Diphenylhydantoin
cpm/mg protein:	7,888	10,762	9,728	3,443	not done	8,713	7,031	8,327	6,740
	8,533	10,559	8,292	3,867	—	7,830	7,328	7,621	6,342
	8,158	10,353	9,962	3,623	—	7,980	7,627	6,478	6,868
Mean:	8,190	10,558	9,327	3,644	—	8,174	7,329	7,475	6,650
				Drug added to substrate					
cpm/mg protein:	9,844	6,329	16,520	1,426	11,220	11,351	14,872	10,584	12,465
	11,024	6,180	13,146	1,799	9,130	12,896	10,992	11,465	13,027
	11,418	6,516	16,761	1,781	—	13,829	12,340	11,376	10,622
Mean:	10,762	6,342	15,456	1,669	10,175	12,643	12,735	11,142	12,038
				Drug added to enzyme					

* Source of reagents: CPZ, Smith, Kline & French; PMZ, Wyeth; TDZ, Sandoz; CPZ sulfoxide, Smith, Kline & French; diphenhydramine, HCl, Parke-Davis; amidopyrine, Matheson, Coleman & Bell; diphenylhydantoin, Parke-Davis.

residual regenerating liver was removed following decapitation 24 hours later. The residual liver was homogenized in an all glass Potter-Elvehjem homogenizer in an ice bath, into 0.25 M sucrose and then centrifuged at 107,000 $\times$ g in a Spinco ultracentrifuge, model L, for one hour. The protein content of the supernate was determined by the method of Lowry(22). The incubation mixture was prepared and kept in individual test tubes at —20°C until used. To the thawed incubation mixture in an ice bath, the required amount of H^3 thymidine was added and then the measured amount (usually about 1.5 mg of protein) of regenerating rat liver extract.

All test drug solutions were freshly prepared in a concentration to provide 0.2 μmole per incubation mix. Two sets of experiments were performed; to one set, the test drug was added directly to the incubation mixture minus the protein-enzyme (hereafter called the substrate). In the other group, the test drug was added one hour before the enzyme protein. In each experiment, the enzyme alone or the drug enzyme mixture was added at time zero. The reported results represent the mean of 3 determinations.

The incubation mixture was incubated with shaking at 37°C in a reciprocal action water bath for 2 hours and then chilled in ice. DNA was isolated, hydrolyzed and radioactivity determined in a liquid scintillation counter according to the procedures of Main and Walwick(19-21). The results are expressed as counts per minute per mg of protein incubated.

In some experiments, the incorporation of H^3 thymidine into DNA was determined at hourly intervals for 4 hours.

Variation in enzymatic activity was observed with different liver homogenates. Repeated determinations, using the same liver homogenate, showed satisfactory agreement in enzymatic activity so that each simultaneous set of determinations provided its own control.

Results. Table II shows the effect of various test drugs on incorporation of H^3 thymidine into DNA in a cell free system after a 2-hour incubation. The parallel lines of data illustrate the conditions where the test drug is added to the incubation mixture (substrate) less liver-enzyme (top), or to the enzyme (bottom). The results, expressed as cpm per mg of protein, disclosed that CPZ, promethazine, acetylsalicylic acid, diphenhydramine, amidopyrine and diphenylhydantoin did not affect the incorporation of H^3 thymidine in 2 hours. Thioridazine (TDZ) showed a moderate suppression under the conditions of this experiment. When the drugs were added first to the enzyme, CPZ inhibited the incorporation of H^3 thymidine by about 50%; TDZ inhibited to an even greater degree. The other test drugs, including promethazine and CPZ sulfoxide did not inhibit.

The effect of CPZ on the rate of incorpora-

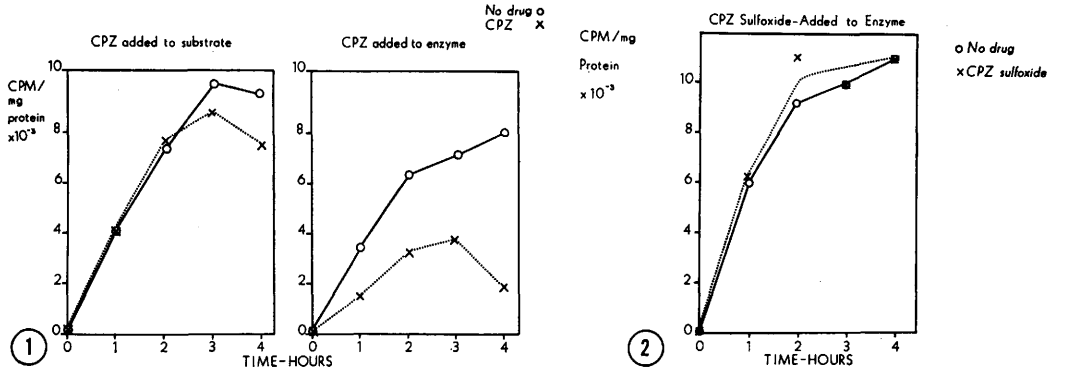

FIG. 1. Effect of chlorpromazine on rate of incorporation of H^3 thymidine into DNA. Left panel, CPZ added to substrate; right panel, CPZ added to enzyme.

FIG. 2. Effect of CPZ-sulfoxide on rate of incorporation of H^3 thymidine into DNA. Drug added to enzyme-protein.

tion of H^3 thymidine is illustrated in Fig. 1 and 2. When added to the substrate, CPZ did not alter the rate of incorporation on the 4-hour observation period (Fig. 1). However, when CPZ was added to enzyme, suppression of incorporation of H^3 thymidine was observed during the entire period of observation (Fig. 1). Addition of CPZ sulfoxide to the protein did not affect rate of incorporation of H^3 thymidine (Fig. 2). Each set of determinations was performed at a different time using a different enzyme preparation which showed slight variation in activity in the controls.

Other experiments confirmed further that TDZ suppressed the rate of incorporation of H^3 thymidine to an even greater degree. On the other hand, the promethazine treated preparations exhibited greater incorporation of H^3 thymidine into DNA than did the controls regardless of whether the drug was added to substrate or enzyme.

Discussion. It is well established that CPZ suppresses a wide variety of enzymes. Our experiments further suggest that it suppresses the DNA synthesizing enzymes. To show this effect, it is necessary to add the drug directly to the enzyme protein.

Suppression of DNA synthesis by CPZ may explain many of its biologic and toxic effects. Large concentrations of CPZ have suppressed embryonic development in many models including fertilized axolotl(23) and chicken eggs(24,25). Smaller litters and decreased mean litter weights have been observed when CPZ was given to pregnant mice(26). In man, treatment of certain susceptible individuals with large doses of CPZ may result in suppression of bone marrow function with depletion of circulating leukocytes(27). Of some interest in this connection is the observation that promethazine, a closely related phenothiazine, has never been clinically implicated as a bone marrow suppressant, nor does it inhibit DNA synthesis *in vitro*.

Summary. Addition of an extract of regenerating rat liver to a mixture of monophosphate nucleotides, in the presence of an energy generating system, was found to promote the incorporation of H^3 thymidine into DNA. Addition of chlorpromazine and thioridazine to the enzyme protein suppressed the rate of incorporation of H^3 thymidine. Other test drugs such as CPZ sulfoxide, promethazine, aspirin, amidopyrine, and diphenylhydantoin, did not affect this parameter. Addition of CPZ and other test drugs directly to the substrate did not affect incorporation of H^3 thymidine. Thioridazine did so, to a moderate degree. Some biologic toxicity reactions of CPZ may be related to its anti-enzyme effect, possibly to its anti-DNA enzyme activity.

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Effect of Interferon on Vaccinia Virus Yield and Plaque Formation.* (30519)

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The effects of interferon on virus yield and on plaque formation have been widely used to measure the antiviral action of interferon. The two procedures have not been compared often although dosages of interferon obtained in plaque inhibition assays are employed in other types of studies. The present study relates the effect of various plaque-depressing units of interferon upon vaccinia virus yield and plaque formation in chick embryo fibroblast cultures.

Materials and methods. Interferon. Interferon was produced by inoculation of chick embryo tissue cultures with Chikungunya virus(1). Cultures were incubated at 37° for 24 hours and the supernatant fluids removed, centrifuged to remove cell debris, and treated with 0.02 M zinc acetate at pH 6.0 according to the method of Lampson *et al*(4). The precipitated interferon was collected by centrifugation and solubilized by adding 0.01 M HCl to pH 2.5. The preparations were dialyzed against 20 volumes of 0.9% sodium chloride at pH 6.0 and finally with Gey's balanced salt solution (BSS) at pH 7.2. After dialysis the interferon-containing fluids were heated at 65° for 30 minutes to inactivate any residual virus.

Media and tissue cultures. Growth medium for the establishment of chick cell monolayers consisted of Gey's BSS with 0.0025 M Tris, 5% calf serum, 0.25% lactalbumin hydrolysate and 0.1% proteose peptone. Mainte-

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