lated intraperitoneally with 0.05 ml amounts, and 2 were inoculated subcutaneously with 0.5 ml amounts. None of the mice appeared ill or died during a 14-day observation period except for 2 mice which were found missing within 24 hours of the time they were inoculated.

Discussion and summary. The data presented indicate that Toluca-3 virus has properties characteristic of the enteroviruses but that it is antigenically distinct from all previously described serotypes. Despite the absence of generally accepted definitions of the polioviruses, Coxsackie viruses, and ECHO viruses(4) this agent has been designated tentatively as the prototype strain of ECHO type 33 by the Panel for Picornaviruses of the National Institute of Allergy and Infectious Diseases.

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Effect of o,p'-DDD on Hepatic Metabolism of Pentobarbital in Rats.* (29853)

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The experiments described here began with the initial observation that both the depth and duration of pentobarbital anesthesia were reduced in rats treated with the compound 2,2 bis (2-chlorophenyl, 4-chlorophenyl) 1,1-dichloroethane (o,p'-DDD).† This observation suggested that o,p'-DDD might stimulate hepatic metabolism of pentobarbital since Hart and Fouts(1) reported that administration to rats of DDT (a structural analog of DDD) is capable of inducing the hepatic microsomal enzymes which metabolize hexobarbital.

Experiments were performed to test the effects of administration of o,p'-DDD on pentobarbital metabolism in the rat. Sleeping time following injection of pentobarbital was used as an indirect measure of *in vivo* metabolism while *in vitro* metabolism was determined chemically in liver slices. In an attempt to demonstrate enzyme induction by o,p'-DDD, ethionine, an inhibitor of protein synthesis(2), was given with o,p'-DDD. Inhibition of the effect of an enzyme inducer by ethionine has been put forward as evidence for an induction of *de novo* enzyme synthesis by the inducer rather than activation of existing enzymes(3).

Methods. Male rats of the Holtzman strain were maintained on a diet of Purina Laboratory Chow and water *ad libitum*. A preliminary experiment utilized 10 rats weighing 225 to 250 g, 5 of which served as controls while 5 received 300 mg/kg o,p'-DDD by the oral route. In subsequent studies 34 rats weighing 100 to 150 g were divided into the following treatment groups: (a) control, (b) o,p'-DDD 100 mg/kg in peanut oil subcutaneously, (c) o,p'-DDD 300 mg/kg orally, (d) d,l-ethionine 150 mg/kg intraperitoneally (i.p.), and (e) d,l-ethionine with o,p'-DDD.‡ The number of rats per treatment group is given in Table I. All doses quoted in both

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† Aldrich Chemical Co., Milwaukee, Wisconsin.

‡ Results were identical with a combination of ethionine and either 100 or 300 mg/kg o,p'-DDD; therefore, the data were pooled and are presented as one group.

TABLE I. Sleeping Times and Hepatic Metabolism of Pentobarbital in Rats Pre-Treated with o,p'-DDD and o,p'-DDD + dl-Ethionine.

Treatment	No. of rats	Sleeping time (min)*	Hepatic metabolism		Liver/body wt ratio (g/100 g)
			Unit activity†	Hepatic capacity‡	
Control	14	86 ± 9	146 ± 6	6.6 ± .4	4.6 ± .1
o,p'-DDD (100 mg/kg)	6	29 ± 3	166 ± 8	9.4 ± .6	5.6 ± .2
o,p'-DDD (300 mg/kg)	8	20 ± 2	242 ± 9	12.4 ± .6	5.1 ± .1
dl-ethionine (150 mg/kg)	8	137 ± 4	108 ± 6	4.7 ± .5	4.3 ± .2
dl-ethionine + o,p'-DDD	8	78 ± 6	131 ± 8	5.7 ± .3	4.4 ± .2
		Probability Values			
Control vs o,p'-DDD		<.005	<.005	<.005	<.005
100 mg vs 300 mg o,p'-DDD		>.25	<.005	<.005	<.05
Control vs ethionine		<.005	<.005	<.005	>.25
Control vs ethionine + o,p'-DDD		>.25	>.1	>.1	>.1

* Pentobarbital sodium 40 mg/kg IP. † mg × 10^{-3}/g hr. ‡ mg/kg BW hr.

preliminary and subsequent experiments are daily doses and treatment was continued for a period of 3 days. Data from the total 44 rats were pooled and analyzed by analysis of variance(4).

Sleeping time in response to i.p. injection of pentobarbital (40 mg/kg) was determined during the third day of treatment. Sleeping time was taken as the elapsed time between injection of pentobarbital and regaining of righting reflex.

On the fourth day following initiation of treatment the rats were killed by decapitation and the livers rapidly excised and rinsed in cold saline. Slices were prepared using a Stadie-Riggs hand microtome and 500 ± 5 mg portions of slices were weighed on a torsion balance. The slices were placed in 50 ml beakers containing 6 ml of Krebs-Ringer-bicarbonate glucose medium to which had been added 1 μM of sodium pentobarbital. The beakers were incubated for 1 hour at 37.5°C, under an atmosphere of 5% CO_2 and 95% O_2, in a Dubnoff-type incubator. Duplicate control beakers received a like amount of liver slices and medium with sodium pentobarbital and were kept on ice for 1 hour. At the end of the incubation period, aliquots of the incubation medium were removed and analyzed for pentobarbital by the method of Brodie(5). The amount of pentobarbital metabolized was determined as the difference between the amount remaining in control and incubated beakers.

Results. Administration of o,p'-DDD in doses of either 100 or 300 mg/kg substantially increased the ability of animals so treated to metabolize pentobarbital. This change in the metabolic handling of the drug is first suggested by shortened sleeping times of the animals receiving the drug (Table I). In contrast to the control group which slept 86 ± 9 minutes, the groups of animals receiving the drug at 100 mg/kg and 300 mg/kg slept 29 ± 3 and 20 ± 2 minutes, respectively. When ethionine was given with both dose levels of o,p'-DDD, the abrupt decrease in sleeping times seen in the two above groups was abolished and the mean time for the experimental animals (78 ± 6 min) was not statistically different from mean control time ($P>.25$). When ethionine alone was given, sleeping times were lengthened such that the mean approached 150% of control time.

The increase in rate of metabolism of pentobarbital suggested by the shortened sleeping times in the animals receiving o,p'-DDD was confirmed by the *in vitro* studies. Liver slices from both groups of animals receiving o,p'-DDD alone were capable of degrading pentobarbital much more rapidly than comparable slices from control animals. These values are reported in Table I and are shown both as unit activity (mg metabolized/g liver · hr) and hepatic capacity (mg metabolized/kg BW · hr). Hepatic capacity was calculated as the product of unit activity and the ratio of liver weight to body weight (g/100 g) and is thought to be a better expression of *in vivo* metabolism since the relative mass of the metabolizing tissue is

taken into consideration. A statistical comparison of either unit activity or hepatic capacity of the two treated groups with the control group shows the differences to be highly significant ($P<.005$), furthermore, the rates of metabolism of pentobarbital by the 2 treated groups are different between themselves ($P<.005$), with the group receiving the highest dose of o,p'-DDD metabolizing pentobarbital at the greatest rate. Slices taken from the livers of animals treated with ethionine alone show a distinctly retarded capability to metabolize pentobarbital. Table I shows that slices from these animals degraded pentobarbital at a mean hepatic capacity of 4.7 $\pm$ 0.5 mg/kg · hr in contrast to control values of 6.6 $\pm$ 0.5 mg/kg · hr. This represents a diminution in activity of approximately 30% from control values ($P<.005$).

When given in combination with o,p'-DDD, ethionine effectively prevented the increase in *in vitro* metabolic activity seen when o,p'-DDD was given alone. The hepatic capacity of the animals so treated was not statistically different from control values.

Liver weight to body weight ratios in both groups of animals given o,p'-DDD alone were significantly larger than control ratios, but not in direct proportion to the dose of the drug given. The liver weight ratios of the other 2 groups of animals (ethionine and ethionine + DDD) were not different from control ratios.

Discussion. A number of chemically unrelated compounds have been shown to induce the microsomal enzymes which metabolize drugs and other foreign compounds(1,6). Most of these inducers have been found to be non-specific in that they induce microsomal enzymes capable of metabolizing a variety of apparently unrelated chemical substances. Hart and Fouts(1) reported that administration of DDT to rats results in an increase in the enzyme systems which metabolize not only hexobarbital, but aminopyrine and p-nitrobenzoic acid as well. Thus, one might expect o,p'-DDD to induce enzymes which would degrade compounds other than pentobarbital. Toward this end an investigation was made into the possibility that o,p'-DDD might alter the rate of inactivation of adrenocortical steroids. A comparison of rates of delta-4 reduction of Ring A of desoxycorticosterone by liver slices from control rats and rats fed o,p'-DDD revealed that this pathway was not influenced by the drug. Thus, the non-specificity of induction does not extend to this system.

The mechanism by which enzyme inducers exert their effect is not known; however, some inducers have been shown to have an initial inhibitory effect which is possibly related to the subsequent induction(7). In an attempt to determine if o,p'-DDD exhibited such an inhibitory effect, the compound was added to an *in vitro* system of rat liver slices in approximately the same concentration as the substrate (pentobarbital). The slices to which o,p'-DDD was added failed to metabolize pentobarbital either slower or faster than did controls. These preliminary studies suggest that o,p'-DDD does not belong to that group of inducers characterized by a biphasic effect on drug metabolizing enzymes. We recognize, however, that problems of solubility or entrance of the drug into the tissue may be limiting when it is added to the incubation medium. This possibility is suggested by the studies recently reported by Cazorla and Moncloa(8) who found that addition of o,p'-DDD to incubating slices of dog adrenal failed to produce the characteristic decrease in response to ACTH seen when the drug is administered to the animal 2 hours prior to the *in vitro* tests.

Nichols(9) has reported that dogs treated with technical grade DDD (200 mg/kg) exhibited extreme prolongation of pentobarbital sleeping time whereas rats given the same drug (200 mg/kg) showed no changes in sleeping time. These data are in direct contrast to the shortened sleeping times of the rats reported here. It might, therefore, be suspected that the technical grade DDD used by Nichols and his group lacked enough o,p'-DDD to induce enzymes, but in our hands all 3 of the isomers found in technical grade DDD are equally efficient in shortening the sleeping times of both males and females of 2 different strains of rats (unpublished observations). While the different effects of o,p'-DDD in the dog and rat could be explained

on the basis of dissimilar species, the differences between rats remain unexplained.

From examination of Table I it is seen that the increase in hepatic capacity accompanying administration of o,p'-DDD is the result of both an increase in unit activity and the ratio of liver weight to body weight. The 300 mg dose of o,p'-DDD appears to have a smaller effect on liver size than does the 100 mg dose, but this apparent difference is due to the presence of older rats in the group receiving the highest dose level of the drug. Our data and those of others(10) suggest that the ratio of liver weight to body weight decreases with age.

Contrary to the findings of others(3,6), ethionine alone increased sleeping time and caused a significant decrease in hepatic metabolism of pentobarbital. These findings are in agreement with those reported for carbon tetrachloride and other liver poisons but not for ethionine, which is said to be a specific agent for inhibiting the induction of drug metabolizing enzymes(6). Both Conney(3) and Kato *et al*(6) used a single injection of ethionine (150 to 250 mg/kg) given 24 to 48 hours before determination of either sleeping time or enzyme assays and found no effect of ethionine alone. In the studies reported here, the rats were given daily injections of ethionine (150 mg/kg) for 2 days prior to determination of sleeping time and for 3 days prior to *in vitro* enzyme assays and it seems likely that this longer period of treatment with ethionine is responsible for the contradictory findings. This does not necessarily refute the claim that prevention of enzyme induction by ethionine is evidence for *de novo* synthesis but it does suggest that ethionine may possess a hepatotoxicity similar to carbon tetrachloride.

Summary. Both subcutaneous (100 mg/kg day) and oral (300 mg/kg day) treatment of rats for 2 days with the compound, o,p'-DDD, shortened pentobarbital sleeping time. Hepatic metabolism of pentobarbital was measured in liver slices taken from these animals after 3 days of treatment and found to be significantly increased. Concomitant treatment with ethionine (150 mg/kg), an inhibitor of protein synthesis, blocked the effects of o,p'-DDD on both sleeping time and *in vitro* hepatic metabolism of pentobarbital. On the basis of these criteria it is suggested that o,p'-DDD induces hepatic enzymes which metabolize pentobarbital.

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Further Studies of Drug Combinations for Complete Cure of DNA Virus Infection in Cultured Cells.* (29854)

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In previous papers(1,2) it was shown that 5-methyltryptophan (5-MTP) and noformicin (NOF), out of 60 selected compounds,

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