Barbiturate Potentiating Action of DDD and Perthane.* (24002)

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DDD (2,2-bis(parachlorophenyl)-1,1-dichloroethane) has been reported to cause adrenal cortical atrophy(1) species specific for the dog(1,2). In experiments involving surgical procedures on dogs previously fed technical grade DDD and anesthetized with barbiturates it has been noted that a high mortality results, regardless of the nature and extent of the surgery. Despite reports that production of adrenal cortical atrophy by DDD is species specific for the dog, attempts have been made to employ it(3-5) and Perthane(6), the ethyl derivative, (2,2-bis(para-ethylphenyl)-1,1-dichloroethane) as an adrenal inhibiting agent in the human. Because of the possibility of a therapeutic compound related to DDD becoming available in the future it is deemed advisable to record this barbiturate hypnotic potentiating action of DDD.

Methods. Eighteen mongrel dogs of both sexes weighing 5-8.8 kg were arbitrarily divided into 4 groups, 2 groups of 5 and 2 groups of 4. During a control period all dogs were anesthetized by intravenous administration of sodium pentobarbital 40 mg/kg. Blood samples were drawn at times indicated in Table and barbiturate was determined by the method of Goldbaum(7). The endpoint of barbiturate action was taken as when the dogs were able to walk after being placed in an erect posture. Five dogs were given Tech. DDD 200 mg/kg/day. Because it has been shown that the most active principle(s) of Tech. DDD are contained in petroleum ether extractable mother liquors(8), a second group of 5 dogs received DDD recrystalized 8 times from petroleum ether. The dose was the same as in the first group. This recrystalized material consists almost entirely of p, p'-DDD. A third group of 4 dogs received the combined supernatant mother liquors from the preceding extractions, following evaporation of the extraction solvent, in daily doses of 50 mg/kg. This preparation contains, among possible other active principle(s) o,p'-DDD (9) which has previously been shown to cause adrenal atrophy(8). Perthane has also been reported to cause adrenal atrophy in the dog(10) so the fourth group of 4 dogs were given Tech. Perthane 200 mg/kg. All compounds were given orally in gelatin capsules once daily. Dogs in Groups I and II were anesthetized after 1, 10, and 14 days of feeding while dogs in Groups III and IV were anesthetized only after 14 days feeding. The results, excepting those at one day which showed no change from the control, are shown in Table I. An additional 2 dogs were dosed with Tech. DDD (200 mg/kg/day) for 14 days. Pentobarbital anesthesia was induced at the start and end of this period and blood samples drawn at 0, 15 minutes and 6 hours were analyzed for sugar and potassium. Since DDD does not cause histological changes in the rat adrenal it was deemed advisable to repeat portions of the experiment with this species. Thirty male Wistar rats weighing 200-230 g were used. Ten rats were given sodium pentobarbital 40 mg/kg intraperitoneally and the sleeping time noted. The rats were then fed 200 mg/kg of Tech. DDD dissolved in corn oil by stomach tube once daily for 2 weeks. Then the rats were again anesthetized and sleeping time noted. Another group of 10 rats were subjected to a different stress, namely anoxia. After being fed Tech. DDD in the same dose schedule as the preceding group of rats, these rats plus 10 controls were, over 4 minutes, taken to a simulated altitude of 25,000 feet and maintained there for an hour. A vacuum desiccator was used which permitted a continuous sweep of air through the chamber. Again on the following day the DDD fed animals and controls were subjected to a simulated alti-

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TABLE I. Effect of DDD and Perthane on Duration of Anesthesia from Pentobarbital in Dogs and Rats and Blood Barbiturate Levels in Dogs.

		Control dogs, blood barbiturates in mg/100 ml blood					10 days feeding, blood barbiturates					14 days feeding, blood barbiturates					
Material	Wt (start)	15 min.	3 hr	6 hr	24 hr	Sleeping time (hr)	15 min.	3 hr	6 hr	24 hr	Sleeping time (hr)	15 min.	3 hr	6 hr	24 hr	Sleeping time (hr)	Wt (finish)
Tech. DDD	8.8	4.6	3.4	2.9	Nil	7	4.5	3.8	3.2	Nil	10	3.9	3.2	2.8	Nil	18	8.5
	6.7	4.3	3.7	3.0	"	7	3.7	3.4	2.5	"	26	4.1	3.7	3.3	"	36*	6.0
	5.5	4.1	2.5	2.2	"	8	3.9	3.4	2.9	"	12	3.8	2.9	2.6	"	36*	5.8
	8.5	4.1	3.6	3.4	"	7	4.2	3.8	3.3	"	10	Died†					8.0
	6.5	4.4	3.4	2.8	"	8	4.0	3.6	2.6	"	26	4.5	3.3	2.8	"	30	6.0
Recrystalized DDD	6.4	4.4	3.4	2.8	"	6	3.9	3.2	2.3	"	8	3.8	2.7	2.1	"	14	6.2
	8.5	3.4	2.4	2.0	"	8	4.1	3.5	2.3	"	10	3.8	3.4	2.5	"	16	8.0
	6.5	4.1	3.7	3.1	"	7	4.1	3.8	2.9	"	10	3.7	3.1	2.5	"	15	6.3
	6.0	3.4	2.5	1.9	"	6	3.9	2.5	1.9	"	10½	3.8	2.7	2.1	"	14	6.5
	5.7	2.5	2.0	1.7	"	6½	3.9	2.5	1.9	"	10	3.8	2.7	2.1	"	15	5.5
Mother liquor	8.0	3.7	3.3	2.4	"	6½						3.6	2.3	1.6	"	12	8.2
	8.0	4.7	3.2	2.4	"	6½						4.3	3.6	2.6	"	14	7.5
	5.0	3.9	2.6	1.5	"	7						3.7	3.4	2.7	"	24	5.2
	6.0	4.2	2.9	2.5	"	7						3.3	2.2	1.8	"	18	5.8
Tech. Perthane	7.5	4.1	2.3	2.0	"	6½						3.7	3.2	1.7	"	12	7.5
	6.5	3.4	2.4	1.7	"	6						3.8	3.2	1.9	"	12	6.2
	7.5	3.8	2.9	1.7	"	7						Clotted				14	7.5
	7.0	3.6	2.6	2.4	"	7						3.3	2.9	1.7	"	14	6.5
Tech. DDD	Rats (10)	218 ± 5 (g)				4.0 ± .1										4.2 ± .2	232 ± 8

* Sacrificed at 36 hr.
† Died immediately after inj. of anesthetic.

tude of 28,000 feet for 30 minutes. Both groups were immediately decompressed and sacrificed. The adrenals, pituitaries, livers, hypothalami of all dogs and rats were fixed in formalin, sectioned, and stained with hematoxylin and eosin.

Results. From the Table it can be seen that dogs fed Tech. DDD, ether extractable mother liquor from Tech. DDD, recrystalized DDD, and Tech. Perthane sleep longer after the same dose of barbiturates than before feeding with these materials. This effect is not apparent after the first day of feeding but is pronounced after 2 weeks of feeding. It appears that Tech. DDD is more potent in this respect than recrystalized DDD and Tech. Perthane. The effects of supernatant mother liquor are not strictly comparable because the dose used was one-fourth that of other compounds.

Microscopic examination of pituitaries and hypothalami of all dogs showed no unusual features, this is in agreement with early reports(1). Sections of adrenals from animals treated with Tech. DDD, mother liquor, and Tech. Perthane revealed usual atrophy which has been repeatedly described(1,10) and to about equal degree despite the fact that the dose of mother liquor was about one-fourth that of the other 2 compounds. Sections of adrenals from animals treated with recrystalized DDD showed no evidence of atrophy, in keeping with a previous paper(8). Sections of livers from all dogs revealed in each dog very minimal or no fatty change as contrasted with the more extensive fatty changes previously reported(1).

The fact that dogs receiving recrystalized DDD did not have atrophic adrenals but yet exhibited prolonged sleeping after barbiturate administration indicates that prolonged sleeping may not be mediated through the adrenals. It must, of course, be borne in mind that physiological functioning of the adrenal cannot be judged by histological appearance. The cause of this increased sleeping time is not apparent from these experiments. From the early report of liver damage by DDD(1), it was logical to presume that there may be delayed metabolism of administered barbiturates. However, this is not the case as is attested by the fact (Table I) that barbiturates are cleared from the blood stream with equal rapidity before and after feeding DDD. Finnegan *et al.*(11) have reported that DDD accumulates in fatty depots of the body, including the brain. Perhaps local changes in cellular metabolism of the brain not reflected in histological appearances may be the cause.

Lamson *et al.*(12,13) have shown that intravenous glucose and its metabolites such as lactate, among other substances, can prolong barbiturate anesthesia in some dogs. This action does not occur with lactate in the adrenalectomized guinea pig. Lasagna(14) has shown that K^+ also possesses this capacity. Despite the uncertainty in mode of action of the foregoing phenomenon it was decided to ascertain if blood sugar and K^+ were significantly affected by barbiturate anesthesia in DDD fed dogs. In 2 dogs fed DDD for 2 weeks and anesthetized with pentobarbital blood sugar and K^+ showed no significant deviations from similar analyses made prior to DDD feeding.

Administration of Tech. DDD to rats in same dosage as to dogs did not significantly prolong sleeping time under sodium pentobarbital (Table I). Since DDD does not produce adrenal cortical atrophy in the rat (1), this emphasizes another difference in the effects of DDD on the two species. In the anoxia tests applied to DDD-treated rats, a simulated altitude of 25,000 feet maintained for one hour had no untoward effect. However, a succeeding exposure to 28,000 feet for 30 minutes caused death of 5 of 10 DDD-treated rats but of none of 10 controls. It appears, therefore, that DDD in the dose used, in some fashion *slightly* lowers the threshold of the rat to anoxia. Sections of the organs from the rats showed no histological changes.

Conclusions. Tech. DDD, recrystalized DDD, supernatant mother liquor of Tech. DDD, and Tech. Perthane cause prolonged sleeping time in dogs given sodium pentobarbital. This increased sleeping time is not seemingly related to atrophy of adrenal cortex, and the very slight amount of fatty

change in the liver does not cause prolonged clearance of barbiturates from the blood. This prolonged sleeping time is not obtained with similar doses of Tech. DDD in the rat although a *slight* lowering of tolerance to anoxia may result.

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Learning Deficit in Mature Rats Recovered from Early Post-Natal Infection With West Nile Virus. (24003)

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It has been established that certain viruses, which cause disease of the central nervous system (CNS) of man, can also cause a fatal CNS infection in rats, provided young enough animals are tested. A uniformly fatal encephalitis occurs in 7-day-old rats following either intracerebral injection or intranasal instillation of either St. Louis encephalitis virus (SLE) or Japanese B encephalitis virus (JBE). However, at 12 days of age rats manifest a high degree of resistance to both viruses and at 21 days of age are completely resistant, as far as clinically apparent infection is concerned(1,2). The resistance of older rats is not absolute, however, since, as has been demonstrated in the case of SLE, an inapparent infection occurs in 21-day-old rats (unpublished data). Furthermore, it has been observed that an occasional 12-day-old rat may develop a clinically apparent infection with JBE and then recover(2). When our studies were undertaken, the age-susceptibility pattern of the rat to West Nile virus (WN), a virus closely related to both SLE and JBE was being studied. It was found that the rat reacts to intracerebrally injected WN in the same manner that it does to JBE in that 7-day-old rats develop a uniformly fatal encephalitis while, in 12-day-old rats, some may succumb but most survive without evidence of CNS involvement although an occasional animal manifests signs indicating CNS dysfunction with subsequent recovery. On the other hand, rats 3 to 4 weeks of age never develop a clinically apparent infection. Although rats which have resisted or recovered from infection with above viruses do not subsequently show obvious abnormalities it seemed reasonable to assume that the viruses may have caused some damage to the CNS which could be reflected in subsequent behavior of the animal, especially behavior near the upper limit of complexity. To test this hypothesis one behavioral pattern, the learning of a moderately complex maze, was investigated. Rats which had received WN intracerebrally and rats which had received no virus, but whose brains had been similarly traumatized, were studied in a 14 choice-point modified Stone Multiple T-maze and the re-