together with the infectious virus. The small-particle antigen elutes after the infectious virus and is not sedimented under conditions of centrifugation which sediment the infectious virus. Sephadex gel filtration was shown to be more effective than direct centrifugation of rubella-infected fluids in separating the two different antigens.

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Received July 18, 1966. P.S.E.B.M., 1966, v123.

Some Central Nervous System Actions of Beta-Mercaptoethylamine. (31597)

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The cardiovascular actions of β-mercaptoethylamine (MEA), a radio-protectant compound, have received considerable attention (1-4). However, little is known concerning its action on the central nervous system (CNS), although investigators have noted convulsions and tremors in animals treated with it. Recently Benigo and Palazzoadrino have reported that MEA synergizes with barbiturates in a specific manner to prolong their central action without depressing vital centers (5). On the other hand, Yam and co-workers have reported that pentobarbital is an excellent antidote against the early, excitant toxicity of MEA(6).

The purpose of this preliminary report is to explore the pattern of CNS activity of MEA by combining a CNS stimulant or a depressant with it. Specifically, interactions of MEA with pentylenetetrazole and pentobarbital are reported.

Methods. Walter Reed, Bagg-Swiss, male mice† weighing between 21-30 g were used. Animals had access to food and water at all times prior to the experiment. Ten mice were selected randomly from colony cages for each experiment group. Each group was maintained in a separate cage during the experiment.

β-mercaptoethylamine HCl was dissolved in distilled water (23.4 mg/ml) and injected intraperitoneally (150 mg/kg of the base). Pentylenetetrazole solution (60 mg/kg) and pentobarbital sodium solution (130 mg/kg) were prepared in a dilution such that the volume for each injection was 9.6 ml/kg, and injected intraperitoneally. Pentylenetetrazole was injected at 60, 30, and 15 minutes before MEA, simultaneously with MEA, and at 15, 30, and 60 minutes after MEA.‡ Pentobarbital was injected according to the same schedule. In control experiments 0.9% sodium chloride solution was substituted for MEA. Mortality was recorded at 24 hours after the injections.

Results. Table I shows results obtained with combinations of pentylenetetrazole and MEA and each agent with saline. The MEA and pentylenetetrazole doses were non-lethal. When the pentylenetetrazole was adminis-

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† The mice were supplied by Walter Reed Army Inst. of Research, shipped by air express to our laboratory and allowed to become accustomed to their new surroundings 5-7 days before use in an experiment.

‡ Additional observations made on pentylenetetrazole administered 120 min after MEA.

TABLE I. Lethality of MEA-Pentylenetetrazole Combinations.

Drug or combination	Time of injection—pentylenetetrazole or 0.9% sodium chloride solution —60	—30	—15	0	+15	+30	+60	+120
MEA, sodium chloride	—	—	—	0	0	0	0	0
Pentylenetetrazole, sodium chloride	—	—	—	0	0	0	0	0
MEA, pentylenetetrazole	0	0	0	70	90	100	90	50

Figures recorded as percent mortality—group of 10 animals. Doses: MEA 150 mg/kg (base), pentylenetetrazole 60 mg/kg. Volume of all injections was 9.6 ml/kg. Zero time indicates simultaneous injection. MEA, or saline in place of MEA, always injected at zero time.

TABLE II. Lethality of MEA-Pentobarbital Combinations.

Drug or combination	Time of injection—pentobarbital or saline —60	—30	—15	0	+15	+30	+60
Pentobarbital, saline	—	—	—	80	50	70	70
MEA, saline	—	—	—	0	0	0	0
Pentobarbital, MEA	60	70	50	90	100	90	100

Figures recorded as percent mortality—group of 10 animals. Doses: Pentobarbital 130 mg/kg, MEA 150 mg/kg (base). Volume of all injections was 9.6 ml/kg. Zero time indicates simultaneous injection. MEA, or saline in place of MEA, always injected at zero time.

tered as much as 15 minutes before MEA there was no lethal synergism. When administered simultaneously synergism was obtained, which quickly reached a maximum intensity and endured for at least 2 hours. The animals died in tonic convulsions. Nonlethal convulsions and tremors were observed in the animals treated with pentylenetetrazole or MEA and saline.

Table II shows results with combinations of MEA with pentobarbital and of each agent with saline. Pentobarbital killed 67.5% of the mice when combined with saline. Pentobarbital killed 60.0% of mice when injected prior to MEA. The same dose of pentobarbital killed 95.0% of mice when injected simultaneously with and after MEA. Chi Square analysis revealed no difference in number of mice killed by pentobarbital and saline and pentobarbital preceding MEA. When pentobarbital was injected simultaneously with or after MEA, there was an increase in mortality (Chi Square 11.7, 1 d.f.).

Discussion. MEA acts synergistically with pentylenetetrazole to produce deaths in mice. The compound must be injected simultaneously with or following MEA for synergism to be manifested. The synergistic action of MEA establishes itself promptly after an intraperitoneal injection and lasts for at least 2 hours. Mechanism of additive effect of these chemicals cannot be deduced from the data presented. Assuming that both agents are CNS stimulants, lack of synergism of pentylenetetrazole with MEA when injected before MEA may be due to short duration of action of pentylenetetrazole; however, multiple possibilities exist, such as altered distribution or metabolism of either agent. Some other lethal factor not related to the central effect of MEA or pentylenetetrazole might have been present. The animals died in convulsions and this supports the theory of additive central effects.

Lack of additive toxicity when pentobarbital was injected prior to MEA might be due to slow absorption of MEA in the deeply anesthetized mouse. However, Benigo obtained additive depression when non-anesthetizing doses of phenobarbital were injected prior to MEA and YaM was able to control lethal convulsions in rats by prior injection of pentobarbital (30 mg/kg)(6,7). These results may not be contradictory if it is assumed that microsomal enzymes which metabolize pentobarbital were saturated with it, non-competitively, before MEA was injected and that the initial detoxification period (0-15 minutes) is critical in the lethality of pentobarbital. When MEA was injected simultaneously with or before pentobarbital the same enzymes were available for blockade by MEA, leading to decreased barbiturate degradation during the critical 0-15-minute period. It is known that high concentrations of MEA inhibit hexobarbital metabolism by rat liver enzymes(7,8).

The fact remains that a more direct, slowly developing, depression of the CNS, in correct time relationship with barbiturate depression, might have accounted for the additive effect. This explanation would be inconsistent with the lack of additive toxicity when pentobarbital was injected prior to MEA.

Summary. 1. MEA synergizes with pentylenetetrazole to produce death in mice when it is injected simultaneously with it, or at 15, 30, 60, or 120 minutes preceding it. Thus, MEA has a stimulant action which comes on rapidly after intraperitoneal injection and endures for at least 2 hours. 2. A central depressant, pentobarbital, does not interact lethally with MEA when injected prior to MEA. When MEA was injected simultaneously with or 15, 30, or 60 minutes prior to pentobarbital, it increased pentobarbital lethality. These results are most logically explained by assuming competitive interference with barbiturate metabolizing enzymes by the agents.

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Received July 21, 1966. P.S.E.B.M., 1966, v123.

Renal Changes in Dietary Hepatic Injury in Rats.* (31598)

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In previous studies(1) renal changes have been observed in rats fed cirrhosis producing rations. These changes were concentrated mainly in the convoluted tubules of the cortex, showing in the acute stage an alteration which was interpreted as "acute necrotizing nephrosis." There was in many instances distinct correlation between changes of the liver and of the kidneys, especially in the experiments using methionine. Supplement of methionine prevented both the hepatic and renal changes. This parallelism extended to some extent also to observations with basal ration in these experiments(1) which contained vitamin-free casein in low proportion (8-10%) as sole source of protein. Vitamins A and D were given mostly as cod liver oil mixed with the diet (2%) or 3 drops of percomorph oil per week. Thiamine HCl 20 μg, riboflavin 20 μg, pyridoxine-HCl 20 μg, Capantothenate 100 μg, and menadione 20 μg all dissolved in 1 ml were given 3 times weekly (twice 2 ml and once 3 ml) in small dishes separately from the diet. The fat content was rather high (22-40%): lard or Crisco (hydrogenated cottonseed oil). In general more severe hepatic and renal changes were found when lard and cod liver oil were given compared with Crisco. This was related to the vitamin E content of Crisco. Supplements of tocopherol have also reduced the incidence and severity of the renal changes. However, this protective effect of Crisco or of α-tocopherol was never complete and in many experiments (even 30 μg of α-tocopherol in daily supplement) statistically not significant.

* Supported in part by Grants A-5921 and T1-HD-17 of Nat. Inst. Health.

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