Coramine (Nikethamide) as Adjuvant to Atropine in Treatment of Poisoning by EPN (ethyl p-nitrophenyl thionobenzenephosphonate) (19191)

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There exists a number of organic phosphates (DFP, TEPP, parathion, EPN, *et al.*) having considerable economic and possible military importance. Since these compounds inhibit cholinesterase(1-3), they are extremely hazardous; the search for efficacious antidotes is important. At present, atropine because of its powerful parasympatholytic activity is the drug of choice in the treatment of poisoning by these agents(4).

It is believed that death following organophosphate poisoning results from respiratory failure(2). The administration of a respiratory stimulant, then, would seem to be advantageous. The purpose of this paper is to report the beneficial effect of a respiratory stimulant, coramine (nikethamide), when used as an antidote in poisoning by EPN.

Methods. Male and female albino rats were given graded doses of EPN in corn oil solution by stomach tube. Since the absorption of EPN may depend in part on the volume of oil given, approximately equal volumes were given each sex. All rats used were 2 to 3 months old; female rats weighed between 140-180 g and males between 200-240 g. To compensate for differences in body weights and in LD50's (Table I), the males received a 5% solution, the females a 1% solution. Atropine (90 mg/kg) and coramine (120 mg/kg) were given intraperitoneally (Table I) immediately after the stomach tubing procedure. The data represent twenty-four hour mortalities and were treated according to the method of Litchfield and Wilcoxen(5).

Results. It is evident from Table I that the simultaneous administration of coramine and atropine to *female* rats raised the LD50 ten times over that of EPN alone. Atropine when used by itself was capable of producing only a twofold increase in the LD50 of EPN.

The antidotal properties of the atropine-coramine combination were not so striking in EPN poisoned *males*. The LD50 in this instance was raised four times over that of EPN, whereas a 2-fold increase was evident when atropine was the sole antidote. Coramine alone was not an effective antidote and did not change the LD50 of EPN in either males or females.

The mechanism of action of the atropine-coramine combination is obscure. That it does not protect red blood cell cholinesterase levels was borne out by an experiment in which adult female rats were given a certainly lethal dose of EPN (60 mg/kg). Some were given atropine-coramine, some coramine alone, some no further treatment. All were sacrificed when the last-named group exhibited severe symptoms; blood was withdrawn by cardiac puncture. Individual samples tested by the method of Michel(6) showed no differences in the reduction of red blood cell cholinesterase activity regardless of treatment.

A number of the commonly used analeptics have also been given to EPN poisoned rats. These included metrazol, benzedrine (amphetamine), picrotoxin and caffeine; in each case the effect was *increased mortality*. There-

TABLE I. Dose-Effect Relationships Comparing the Antidotal Properties of Atropine and of Atropine-Coramine.

	Females			Males		
	LD_{50}, mg/kg	19/20 limits, mg/kg	No. of rats	LD_{50}, mg/kg	19/20 limits, mg/kg	No. of rats
EPN	6.7	7.1–6	71	33	39–28	95
EPN atropine	11.6	12.2–11	50	74	104–53	46
EPN atropine-coramine	71	86–59	80	120	216–67	53

fore, a word of warning is indicated: it is probable that these analeptics should *not* be given a desperately poisoned human patient.

Since all the observations reported herewith have been on rats, it is not implied that the findings can be directly applied to the treatment of human poisoning. In the rat tests, the antidotes were given simultaneously with the EPN, such conditions would rarely be possible in industrial or accidental human exposures.

Summary. The administration of coramine and atropine together was considerably more effective in the treatment of EPN poisoned rats (both male and female) than was atropine alone.

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A Simple Method for the Determination of Urinary Amino Nitrogen.* (19192)

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Russell(1,2) adapted Folin's colorimetric technic(3) for the determination of blood amino nitrogen. According to Russell, this technic could not be used for urine because of interference by ammonia and uric acid. Therefore, it was the purpose of this investigation to adapt her procedure to the quantitative determination of urinary amino nitrogen.

Procedure. The reagents required, color reaction and technic were those described by Russell with the exception of Norit-A Decolorizing Carbon.

Removal of Ammonia: Volumes of urine ranging from 0.5 to 2.0 ml were pipetted into 200 ml Erlenmeyer flasks etched at the 100 ml mark. Approximately 75 ml of distilled water was added followed by 4 drops of 0.25% phenolphthalein in 95% ethanol. 0.1 N sodium hydroxide was added dropwise until the flask contents retained the pink color. This condition was reached at a pH between 9.0 and 9.5. The solution was boiled gently for 25 minutes. Base was added during the boiling process until the pink color no longer faded.

Removal of Uric Acid: After boiling off ammonia, the flask and its contents were cooled in an ice bath, and the solution acidified dropwise with 0.2 N hydrochloric acid until the pink color disappeared. Then one drop in excess was added. The solution was diluted to the 100 ml mark with distilled water and chilled in an ice bath for 10 minutes. Fifty mg of Norit-A Decolorizing Carbon was then added. The use of charcoal as an adsorptive agent for uric acid in urine was adapted from a procedure suggested by Bodansky(5).

After the addition of charcoal, the flask was stoppered, vigorously swirled for about a minute, and then allowed to stand at 5°C for a 2-hour period. At the end of this time, about 10 ml of the charcoal treated solution was transferred to a test tube and centri-

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