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Effects of Oral Doses of Carbaryl on Man

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INTRODUCTION

Best and Murray [2] studied a group of workers engaged in synthesizing, handling, and shipping carbaryl (1-naphthyl-N-methylcarbamate) for inhibition of cholinesterase and toxic actions. The most heavily exposed group of people (those making the compound) excreted relatively large amounts of 1-naphthol in their urines, occasionally had slightly subnormal levels of whole blood cholinesterase, and experienced no signs or symptoms of poisoning. Miosis, profuse salivation, and muscular incoordination induced in a 19-month-old child by carbaryl have been reported [3].

Carbaryl is not particularly toxic to the rat [4,5], the guinea pig [5], the rabbit [5], the cat [5], or the dog [5] when given orally. Carbaryl is a fairly potent inhibitor of cholinesterases in vitro, but its most striking effect in vivo, in the mouse, is to inhibit an enzyme that hydrolyzes triacetin; hydrolysis of acetylcholine may be enhanced [6].

After preliminary studies of our own in animals [7], we felt justified in proceeding to studies of the effects of ingestion of carbaryl on man. The

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animal studies had shown that carbaryl is much less toxic to the monkey than to the rat and the dog. They had shown, however, that carbaryl produces vacuolization of the epithelial cells of the proximal tubules of the kidneys in both the rat and the monkey, although no interference with the formation of urine by the kidney was detected in those experiments. The apparent greater safety of carbaryl for primates than for other animal species encouraged us to proceed with the studies in volunteers; the effect on tubular epithelium suggested that a measure of the capacity of the proximal tubular epithelium to transfer material should be used in the human experiments.

MATERIALS AND METHODS

The maximal daily intake of carbaryl in a normal human diet under existing tolerances for this compound was calculated to be 2.5 mg. A 6-week study was undertaken to ascertain whether daily ingestion of carbaryl in dosages of 0.06 mg/kg (corresponding to a dose of from 2.4 mg for a 40-kg subject to one of 5.4 mg for a 90-kg subject) and 0.12 mg/kg (corresponding to daily doses of from 4.8 mg for a 40-kg subject to a 10.8 mg for a 90-kg subject) would be truly safe for man.

Two sets of hard gelatin capsules, red and green, were prepared for us by the Sterling-Winthrop Research Institute. Each set consisted of one subset containing an inert placebo and of another subset containing carbaryl. The green carbaryl-containing capsules were made to contain 2.5 mg of the carbamate; the red ones were made to contain 10 mg.

These capsules were analyzed by Dr. Henry Fishback of the Food and Drug Administration. The red and the green placebo capsules contained no carbaryl; the green carbaryl-containing capsules actually held 2.5 mg of the carbamate (mean of 6 capsules; range, 1.93 to 2.94 mg), whereas the red ones yielded 11.2 mg (mean of 6 capsules; range, 10.5 to 13.4 mg).

The volunteer subjects were male prisoners at Clinton Prison in Dannemora, New York, of ages ranging between 25 and 57 years, with a mean of 36 years. They were informed carefully by the prison physician (Dr. Jameson) of the known and possible dangers in taking part in the experiment. Those who elected to continue as subjects were given thorough physical examinations by the prison physician and an initial set of laboratory examinations, including removal of Bromsulfophthalein (BSP) from the blood and recording of the EEG pattern from scalp electrodes.

A preliminary experiment consisted of giving to different pairs of men single oral doses of 0.5, 1.0, or 2.0 mg/kg of carbaryl. The men were observed carefully for objective signs of intoxication, including inhibition of cholinesterases in blood plasma and in whole blood, and were questioned about subjective effects. Neither objective nor subjective effects were noted. The pair who took 2.0 mg/kg of carbaryl collected urine fractionally, as voided, during the first day of the experiment and for each whole day thereafter through the fourth day. These urine specimens were frozen immediately and sent to Dr. J. B. Knaak at the Mellon Institute for examination for metabolites of carbaryl. An abstract of that work has been published [8]; a full report will be published separately.

The main experiment was run in two sections. Groups of 5 men began taking either the green placebo capsules or those containing carbaryl (in a dosage of 0.06 mg/kg/day) on August 30, 1966. Up to September 12, there was no evidence of deleterious action within either of these groups, which were continued on their same regimens for an additional 4 weeks. At that time two new groups of 6 men each started taking either red placebo or carbaryl-containing capsules (nominal dose, 0.12 mg/kg; dose from Dr. Fishback's analysis, 0.13 mg/kg). These two groups also took their daily doses for 6 weeks.

The capsules were distributed to the volunteers by the prison pharmacist, who observed the men while they took them and examined their mouths after the capsules had been swallowed.

Blood, urine, and stool specimens were collected just before the initial dose of carbaryl and at weekly intervals during the experiment. At the end of the experiment, the physical examination, BSP removal from blood, and recording of the EEG were repeated. Blood samples were examined for hematocrit; hemoglobin; erythrocyte and leucocyte counts; differential count; urea nitrogen (BUN); glucose; plasma and whole blood cholinesterases; cholesterol; prothrombin time; serum glutamic oxalacetic transaminase (SGOT); sodium; and potassium. Urine specimens were examined for turbidity, pH, protein, glucose, erythrocytes, squamous epithelial cells, crystals, and the ratio of amino acid nitrogen to creatinine. Stool specimens were examined for occult blood.

Most chemical examinations of biological samples were performed with the Technicon Autoanalyzer, using "N" series methodologies. Estimation of total amino acid nitrogen in urine was carried out by the method of Schroeder et al. [9]. Plasma and whole blood cholinesterases were estimated by the pH-stat method [10]. Erythrocyte cholinesterase was approximated from the plasma and whole blood cholinesterase values and the hematocrit.

RESULTS

The removals of BSP from the plasma before and after the course of doses of carbaryl were not different. The final EEG recordings for both the control and the treated groups were somewhat more synchronized than the initial ones. The spiking reported to appear in EEG's of subjects exposed to organophosphorus inhibitors of cholinesterase appeared in none of these subjects. Neither the plasma nor the calculated erythrocyte cholinesterase levels of the subjects underwent significant alteration during the experiment.

Table 1 summarizes the symptoms and signs that appeared in the experimental subjects, none of which is unequivocally referable to carbaryl. The low-dose placebo group experienced somewhat more effects than the low-dose carbaryl group. The high-dose carbaryl group had a number of complaints that were not matched in the corresponding placebo group. About 60% of these complaints occurred on the day after ingestion of carbaryl had ended, so that they may represent a sort of withdrawal syndrome. The remaining three complaints include two, difficulty in sleeping and abdominal cramps, that would be recognized as more or less typical effects of cholinesterase inhibitors. They can also be caused by other influences, however. The third of the re-

Table 1
Symptoms and Signs during 6-Week Period

Group	Symptoms	Signs
Control 1 (5)	2: headache (12th day)	1: slight decrease in plasma ChE (3rd day)
	1: headaches (5th week)	1: elevated SGOT (1st and 2nd week)
		1: transient decreases in RBC, HCT, and Hb (2nd week)
		1: elevated SGOT (6th week)
0.06 mg/kg (5)	1: abdominal cramps (15th day)	1: slight decrease in plasma ChE 3rd day)
	1: stiff thumbs (29th day)	
	1: pain in neck (42nd day)	
Control 2 (6)		
0.12 mg/kg (6)	2: epigastric cramps (43rd day)	
	2: hot and cold flashes (43rd day)	
	1: paroxysmal tachycardia (43rd day)	
	1: difficulty in sleeping	
	1: pupillary dilatation (2nd day)	
	1: abdominal cramps (4th week)	

maining complaints, pupillary dilatation, is not a standard effect of an anticholinesterase chemical. Because it occurred in only one subject on one occasion, we doubt that it has any real significance.

The hematic, blood chemistry, and urine examinations reveal in general no significant effects that can be attributed to the administration of carbaryl. The one variable that seems to show an effect of carbaryl is the ratio of the concentration in urine of amino acid nitrogen to that of creatinine. Figure 1 is a graph of the values of this ratio at intervals during the experiment with the daily dose of 0.06 mg/kg of carbaryl. The value of the ratio for this experimental group never rose above that for the placebo group.

Figure 2 is a similar graph of the data derived from the experiment with the higher dose of carbaryl. The placebo and the carbaryl groups started at about the same mean level of this ratio, but the ratio for the subjects receiving carbaryl rose definitely above the placebo line during the second week of administration of the insecticide and remained there until

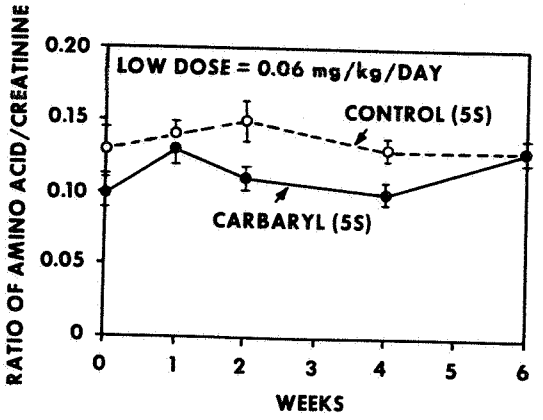

Fig. 1. Mean values for the ratio of the concentration in urine of amino acid nitrogen to that of creatinine for men given daily doses of placebo or of 0.06 mg/kg of carbaryl by mouth during a 6-week period.

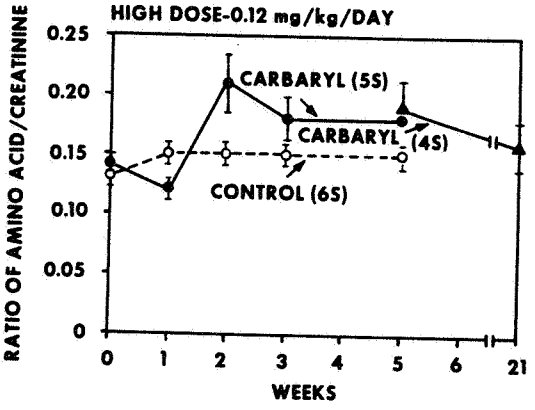

Fig. 2. Mean values for the ratio of the concentration in urine of amino acid nitrogen to that of creatinine for men given orally daily doses of placebo or of 0.13 mg/kg of carbaryl during a 6-week period. The two points marked by triangles are from 4 men of the carbaryl-ingesting group from whom urine samples were obtained 15 weeks after the end of the experiment.

ingestion of carbaryl was discontinued. Only 5 subjects are represented by the points for the carbaryl-receiving subjects in this figure because one urine sample from one subject was lost; this subject was excluded from the graph. Urine samples from 4 of the subjects were obtained 15 weeks after the discontinuance of carbaryl ingestion. The values of the ratio for these 4 men both near the end of the experiment and 15 weeks after its termination are plotted at the extreme right of Fig. 2. The final point is below that near

the end of the experiment and in the same range as those at the start of the experiment.

DISCUSSION

The increased synchronization seen in the second EEG's of all our subjects is a well-known change when subjects submit to repeated recordings of their EEG patterns. With the single exception of the ratio of the concentration in urine of amino acid nitrogen to that of creatinine, all the subjective and objective observations during this experiment indicate that daily oral doses of carbaryl up to 0.13 mg/kg produce no harmful effects in human subjects. The ratio of the urinary concentration of amino acid nitrogen to that of creatinine is a rough measure of the reabsorptive capacity of the proximal tubule. We conclude, therefore, that the only evidence of a deleterious effect produced by the higher dose of carbaryl was a slight decrease in the ability of the proximal convoluted tubule to reabsorb amino acids and possibly other substances. Figure 2 shows that this change is a reversible one, at least under the conditions of this experiment. Because these experiments lasted for only 6 weeks, we cannot say whether the decrease in the ability of the proximal convoluted tubule to reabsorb amino acids might not have become more serious or more persistent with more prolonged exposure to carbaryl. We cannot conclude, therefore, that the higher dose of carbaryl is a safe one.

CONCLUSIONS

1. Daily administration for 6 weeks of 0.06 mg/kg of carbaryl produced no objective or subjective evidence of deleterious action in human subjects.
2. Daily administration for 6 weeks of 0.13 mg/kg of carbaryl produced no changes clearly attributable to the insecticide other than a slight, reversible decrease in the ability of the proximal convoluted tubule to reabsorb amino acids.

SUMMARY

Groups of male volunteers who took carbaryl by mouth in daily doses of 0.06 or 0.13 mg/kg for 6 weeks suffered no subjective or objective changes clearly attributable to carbaryl other than a slight, reversible decrease in the ability of the tubule of the kidney to reabsorb amino acids in the group on the higher dose.

REFERENCES

[1] J. H. Wills, E. Jameson, A. Stein, D. Serrone, and F. Coulston, *Toxicol. Appl. Pharmacol.*, **10**, 390 (1967).
[2] E. M. Best, Jr., and B. L. Murray, *J. Occupational Med.*, **4**, 507 (1962).

[3] E. V. Henson, quoted by Best and Murray, Ref. [2].
[4] T. B. Gaines, *Toxicol. Appl. Pharmacol.*, **2**, 88 (1960).
[5] C. P. Carpenter, C. S. Weil, P. E. Palm, M. W. Woodside, J. H. Nair, III, and H. F. Smyth, Jr., *J. Agr. Food Chem.*, **9**, 30 (1961).
[6] R. L. Barron, J. L. Casterline, Jr., and O. G. Fitzhugh, *Toxicol. Appl. Pharmacol.*, **6**, 402 (1964).
[7] D. M. Serrone, A. A. Stein, and F. Coulston, *Toxicol. Appl. Pharmacol.*, **8**, 353 (1966).
[8] J. B. Knaak, L. J. Sullivan, and J. H. Wills, *Toxicol. Appl. Pharmacol.*, **10**, 390 (1967).
[9] W. A. Shroeder, L. M. Kay, and R. D. Mills, *Anal. Chem.*, **22**, 760 (1950).
[10] J. Jensen-Holm, H. H. Lausen, K. Milthers, and K. O. Moller, *Acta Pharmacol. Toxicol.*, **16**, 384 (1959).