Effect of Banol and Parathion on Operant Learning Behavior of Rats Fed Adequate and Inadequate Casein Diets

by James L. Casterline, Jr., Robert E. Brodie, and Thomas J. Sobotka
Division of Pesticide Chemistry and Toxicology
Bureau of Foods
Food and Drug Administration
Washington, D. C.

The nature of neuronal interactions in affecting behavior is not yet fully understood, but it is generally believed that biochemical mechanisms are involved, one of which involves acetylcholine (ACh) and cholinesterase (ChE). Recent studies have shown that a low protein diet did not retard the development of brain ChE in rats, but did increase the inhibitory action of a carbamate and an organophosphate upon brain ChE activity (1). Rosencrans et al. (2) found that behavioral effects were related to brain ChE inhibition and ACh elevation, and other investigators have reported that nutritional deficiencies altered performance in pyschomotor and intelligence tests (3, 4). It was, therefore, considered important to study the effects of low protein intake and the combined action of low protein intake and a low level of pesticide administration on the acquisition of an operant learning procedure and the activity of ChE and monoamine oxidase (MAO) in the brain.

Materials and Methods

Male Osborne-Mendel rats bred in FDA, caged individually and fed ad libitum, were used. The composition of the synthetic diet, as percent by weight, was: vitamin-free casein, 5; sucrose, 39; corn starch, 39; corn oil, 10; mineral salts, 2; vitamin mixture, 3; and Alphacel, 2. When casein was increased from 5 to 18%, a corresponding amount of the carbohydrates (1 sucrose:1 corn starch) was reduced so that all diets were approximately isocaloric. The protein content of normal rat chow is about 22% protein, but synthetic diets with casein content of 15 to 18% were shown to produce normal growth (5). The 5 and 18% casein diets were each divided into three portions; one portion was kept for control diets, 1000 ppm Banol[1] was added to the second, and 4 ppm parathion[2] was added to the third. These doses were selected since they slightly inhibit ChE and other enzymes (6). A group of 7 rats was given either the basal 5 or 18% casein diet and another was given a diet of the same composition but with added pesticide; a total of 56 rats were used, including a group of control rats for each pesticide. All animals were maintained on their respective diets for 11 weeks.

---

[1] Banol is a trademark of the Upjohn Co., Kalamazoo, Mich., for 6-chloro-3,4-xylyl methylcarbamate.

[2] Parathion (common name for O,O-diethyl-O-p-nitrophenyl thiophosphate) was obtained from the American Cyanamid Co., Princeton, N.J.

Bulletin of Environmental Contamination & Toxicology,
Vol. 6, No. 4, 1971, published by Springer-Verlag New York Inc.

Behavioral procedures. Fourteen or 18 days after rats were weaned, the synthetic diets were begun and the rats were subjected to daily 1-hour training sessions, 5 days a week, in a standard operant conditioning chamber. Each trial consisted of 5 seconds of conditioned stimuli (light and sound) (CS) alone, followed immediately by 5 seconds of an unconditioned stimulus (shock from electrified grids) (UCS) and the CS, at the end of which time both UCS and CS were terminated. The intertrial interval was 10 seconds. A lever press during the CS alone was recorded as an avoidance; a lever press during the UCS plus CS period was recorded as an escape. No lever press during either period was recorded as "No Escape". Responses are expressed as the average percent of the total number of trials per week. This experimental procedure was continued for 10 weeks.

These operant data were analyzed statistically by the Mann-Whitney U test.

Brain enzyme assays. At the end of 9 weeks in the parathion experiment and 10 weeks in the Banol experiment, the rats were killed by decapitation. The brains were quickly removed and the cerebellum and cerebrum were separated and cooled to 4° C. Each part of the brain was homogenized in cold saline and assayed for enzyme activity immediately.

Cholinesterase activity in either the cerebellar or the cerebral homogenate was measured by utilizing a radiometer apparatus and acetylcholine chloride as substrate, as previously described (1). Monoamine oxidase was assayed according to a method based upon the formation of a fluorescent product from homovanillic acid and the hydrogen peroxide released during assay (7); fluorescence was measured with an Aminco-Bowman spectrophotofluorometer at an activation wavelength of 315 λ and fluorescence wavelength of 425 λ. It was necessary to increase the amount of substrate slightly to obtain a satisfactory reaction. The results were analyzed statistically by the Student's "t" test.

Results

Food consumption and growth. Food consumption of the rats fed the 5% casein diets containing either Banol or parathion was lower than that of the rats fed the 18% casein diet (Table 1); therefore the pesticide intake was also lower. The decreased caloric intake was reflected by the smaller average changes in body weight.

Enzyme activities. The activities of cholinesterase and monoamine oxidase in the cerebellum and the cerebrum were not significantly affected by either the low casein diet or the presence of pesticide in the diet at the concentrations used in this study.

TABLE 1

Food Consumption and Growth of Rats Fed Two Levels of Casein in Diets With or Without Pesticides

Pesticide	% Casein in Diet	Total Food Consumed (g/week)	Body Wt. Changes (g/week)[a],[b]
Control	5	72.1	+ 1.5
	18	114.9	+ 19.4
Banol	5	55.3	- 4.0
	18	98.5	+ 11.3
Control	5	75.8	+ 2.1
	18	107.4	+ 23.3
Parathion	5	50.7	- 2.5
	18	106.0	+ 19.5

[a] Initial weights were 107 grams for Banol-fed rats and 95 grams for parathion-fed rats.

[b] Six or seven rats per casein level.

Operant conditioning. None of the diet-pesticide combinations used resulted in significant changes in avoidance responding of the experimental animals. There were, however, marked changes in the "No Escape" scores (Tables 2 and 3).

Rats fed the 5% casein, Banol-free diet had temporary but significant increases in "No Escape" scores at weeks 2 and 3 of training, as compared to the scores of the rats fed the 18% casein, Banol-free diet (Table 2). The presence of Banol at a dose of 1000 ppm in the 5% casein diet resulted in even more marked and prolonged increases in "No Escape" scores than those observed with 5% casein diet alone; these increases were significantly different from the scores of the 18% control group from week 2 throughout the 10 weeks of training. When this same dose of Banol was fed to rats in the 18% casein diet, no observable effects on "No Escape" scores were found.

TABLE 2

Effect of Dietary Banol (1000 ppm) on Behavior of Rats Fed Adequate and Inadequate Protein Diets

Week of Training	% No Escape[a]			
	5% Casein	5% Casein + Banol	18% Casein	18% Casein + Banol
1	36	49	32	37
2	33[b]	42[b]	16	28
3	46[b]	62[b]	8	19
4	36	75[b]	10	9
5	31	78[b]	9	6
6	34	78[b]	11	4
7	35	79[b]	8	4
8	35	84[b]	10	3
9	25	81[b]	9	1
10	23	84[b]	8	1

[a] Averages of the percentages of times the rats (6-7 per group) failed to press the lever as a response to conditioned and unconditioned stimuli during 1 hour of testing 5 days a week.

[b] Significantly different ($P<0.01$) as compared to values at the 18% casein level.

The effect of 4 ppm parathion in a 5% casein diet on "No Escape" scores (Table 3) was qualitatively comparable to but quantitatively less pronounced than that of Banol in a 5% casein diet. Rats fed parathion in a 5% casein diet had significantly greater "No Escape" scores at weeks 2, 3, 4, 5, 6, 7, and 9 of training than the control rats given the normal 18% casein diet (training was terminated in this group after 9 weeks). The group of rats fed the parathion-free, 5% casein diet exhibited "No Escape" scores greater than those of controls at weeks 2, 3, 4, and 8. Parathion in a normal 18% casein diet had no effect on "No Escape" scores.

TABLE 3

Effects of Dietary Parathion (4 ppm) on Behavior of Rats Fed Adequate or Inadequate Protein Diets

Week of Training	% No Escape[a]			
	5% Casein	5% Casein + Parathion	18% Casein	18% Casein + Parathion
1	71	90	76	75
2	70[b]	69[b]	40	38
3	71[b]	63[b]	26	20
4	58[b]	55[b]	13	8
5	35	53[b]	11	6
6	28	51[b]	5	5
7	11	34[b]	2	5
8	16[b]	41	1	5
9	20	51[b]	1	3

[a] Averages of the percentages of times the rats (6-7 per group) failed to press the lever as a response to conditioned and unconditioned stimuli during 1 hour of testing 5 days a week.

[b] Significantly different ($P<0.05$) as compared to values at the 18% casein level.

As can be seen in Tables 2 and 3, the general "No Escape" scores at the onset of training for the animals in the Banol experiment were lower than the scores of the animals in the parathion experiment. This difference was entirely unexpected and may have been due to a number of variables including the different litters or the different times of the experiments.

Discussion

The present experiment has shown that feeding Banol in a protein-deficient diet at a dietary concentration which has no demonstrable effect when given in a normal diet has markedly detrimental effects on the performance of rats in an operant

conditioning procedure. This was also true of parathion but to a lesser extent. The effects were primarily restricted to a reduction in lever-press performance of the animals; the treated animals demonstrated a reduced ability to associate lever-pressing with termination of the noxious stimulus (shock). Although the low protein diet itself did contribute to the reduced performance of the rats, the addition of the pesticide caused a potentiation of this retarded performance.

The retarded behavioral performance of the rats was not accompanied by changes in cerebral or cerebellar ChE or MAO activities at the termination of the training period; in fact none of the pesticide-diet combinations employed had altered the activities of these enzymes. However, this does not preclude the possibility that enzymatic changes had taken place in the earlier phases of training but the rats may then have adapted to the continual presence of the pesticide or low protein diet. A recent study has shown that brain ChE activity in rats fed an 8% casein diet for 28 days was slightly inhibited by either 3.75 ppm parathion or 1000 ppm Banol (6). Adaptation to ChE inhibition has also been previously shown to occur (8). Since brain function can be affected in a short time by anticholinesterase agents (9) it seems possible that significant alterations of the ChE or MAO enzyme systems may have occurred during the early critical early critical phases of conditioning, resulting in a lasting effect on the performance during the later phases of training. However, alternative possibilities do present themselves such as subliminal but effective alterations of these enzymes, changes in these enzyme systems at locations other than cerebrum or cerebellum, alterations in the activities of any of a multitude of different enzyme systems that are necessary for brain function, interference with protein synthesis, changes in endocrine systems related to emotional drives of the animal in the stressful situation of aversive conditioning, select effects on the sensorium of the rats which may alter the threshold to the pain of shock, or some peripheral changes which secondarily influence central nervous function. A general debility due to low body weight may be discounted as an explanation for the neurotoxic effect seen with the pesticide-containing, low protein diet, since the low body weight was related to the low protein diet rather than to the presence of pesticide.

The present experiment does show that dietary sufficiency does interact in a subtle way with compounds that may be environmental contaminants to produce behavioral changes. Further investigation to elucidate the nature of the interaction of dietary deficits and pesticidal contaminants would be most valuable.

Summary

A study eas made of the effects of prolonged pesticide feeding on operant conditioning and brain ChE and MAO activities of rats fed normal and low protein diets. Four ppm of parathion in a 5% casein diet retarded the lever press performance and 1000 ppm Banol almost completely inhibited such responding in rats. These pesticides had no effect on the performance of the rats fed 18% casein diet. Neither ChE nor MAO of the rat brain were altered at the end of the training procedure. Other possible explanations for the changes in operant conditioning are discussed.

References

1. CASTERLINE, J.L., JR., and WILLIAMS, C.H., Toxicol. Appl. Pharmacol. 14, 266 (1969).

2. ROSENCRANS, J.A., DREN, A.T., and DOMINO, E.F., Int. J. Neuropharmacol. 7, 127 (1968).

3. CRAVIOTO, J., DELICARDIE, E.R., and BIRCH, H.G., Pediatrics 38, 319 (1966).

4. STOCH, M.B., and SMYTHE, P.M., S. Afr. Med. J. 41, 1027 (1967).

5. FRIEDMAN, L., Fed. Proc. 25, part 1, 137 (1966).

6. CASTERLINE, J.L., JR., and WILLIAMS, C.H., Toxicol. Appl. Pharmacol., in press (1971).

7. SNYDER, S.H., and HENDLEY, E.D., J. Pharmacol. Exp. Ther. 163, 386 (1968).

8. STAVINOHA, W.B., RYAN, L.C., and SMITH, P.W., Ann. N. Y. Acad. Sci. 160, Art. 1, 378 (1969).

9. GOLDBERG, M.E., JOHNSON, H.E., and KNAAK, J.B., Psychopharmacologia 7, 72 (1963).