soluble collagen from the tissue. Speculatively to separate the catabolic mechanism of insoluble collagen from the anabolic process does not appear to be inconsistent with what is currently known of collagen metabolism *in vivo*(12,13) and might be consistent with the cyclic rate of isotope incorporation into newly synthesized collagen recently described by Bentley and Jackson(14). That is, the independent disappearance of a mature form of collagen from a biological "compartment" results in a marked increase in rate of synthesis of the precursors of this component. Whether this disappearance of a more mature form of collagen from the biological "compartment" refers to its leaving the cells as tropocollagen or to its being precipitated as insoluble collagen or to its loss from the tissue entirely, the net result of its loss would be an increased rate of synthesis of its appropriate precursors.

These speculations are not inconsistent with what is currently known and they do to a degree partially explain the data described here as well as the effect of stress (8), cortisol(9) and Dilantin(10) upon the chemistry of the connective tissue described elsewhere.

Summary and conclusions. The dermal chemical response to atropine administration involved primarily losses of salt soluble, insoluble and total collagens and increased amounts of acid soluble collagen effecting the hexosamine content of the tissue. The increased dermal concentration of acid soluble collagen and the decreased dermal concentration of salt soluble collagen were maintained during the entire course of atropine administration, whereas the decreased concentration of both total and insoluble collagen was no longer significant by the last day of drug administration. To explain these changes in the dermal concentrations of the various collagens the possibility was suggested that atropine stimulated specifically the catabolism of insoluble collagen, and the changes in the dermal concentrations of the soluble collagens represented an anabolic response to this loss.

1. Houck, J. C., *J. Invest. Dermatol.*, 1962, v39, 241.

2. Busch, H., Allen, H., Anderson, D., *J. Pharm. Exp. Ther.*, 1959, v127, 200.

3. Houck, J. C., Jacob, R. A., PROC. SOC. EXP. BIOL. AND MED., 1960, v105, 324.

4. Dische, Z., Shettles, L., *J. Biol. Chem.*, 1951, v192, 579.

5. Warren, L., *ibid.*, 1959, v234, 1971.

6. Neuman, R., Logan, M., *ibid.*, 1950, v186, 549.

7. Houck, J. C., Jacob, R. A., Vickers, K., *Am. J. Path.*, 1962, v40, 431.

8. Houck, J. C., *Surgery*, 1962, v51, 770.

9. ———, *Am. J. Pathol.*, 1962, v41, 365.

10. Houck, J. C., Jacob, R. A., Maengwyn-Davies, G. D., *J. Clin. Invest.*, 1960, v39, 1758.

11. Shafer, W. G., PROC. SOC. EXP. BIOL. AND MED., 1961, v106, 205.

12. Harrington, W., VonHippel, P., *Adv. Prot. Chem.*, 1961, v16, 1.

13. Jackson, D. S., *New Eng. J. Med.*, 1958, v259, 814.

14. Bentley, J. P., Jackson, D. S., *Biochem. Biophys. Res. Commun.*, 1963, v10, 271.

Received November 22, 1963. P.S.E.B.M., 1964, v115.

Effect of Amphetamine on the Learning and Performance of Mice in a Swimming Maze.* (29021)

MARY ELLEN KOSMAN (Introduced by L. G. Abood)

Research Laboratories, Department of Psychiatry, University of Illinois College of Medicine, Chicago

The ability of the amphetamines to enhance performance has been studied extensively. In a recent review Weiss and Laties (1) concluded that these drugs improve performance on a wide variety of tasks. In some

* This study was supported by a contract between Edgewood Arsenal, U. S. Army Chemical Research and Development, and University of Illinois.

recent studies of the effect of amphetamine on swimming ability, it was found that the drug improves swimming speed and endurance(2,3,4,5). In contrast to performance studies, there is little information on the effects of amphetamine on learning. Several studies have shown that the amphetamines facilitate the acquisition of a variety of responses(6,7,8,9). No attempt was made, however, to determine whether this enhanced performance during training was a relatively permanent effect which would carry over into the undrugged state. The literature contains one experiment in which amphetamine-injected rats were trained to run a maze for a food reward, and were later tested without the drug(10). The drug impaired performance, but an immediate improvement occurred when injections were discontinued, indicating that the effect was on performance and not on learning.

The present experiment was designed to obtain more conclusive evidence concerning the effect of amphetamine on both learning and performance in a situation which was not influenced by the effects of the drug on appetite. The task employed was a simple swimming maze, and the experiment was designed to show both learning and performance effects.

Methods. The subjects for these experiments were 160 two-month-old male albino mice of the Swiss-Webster strain. The animals were housed in groups of 5 or 6 in cages measuring 8 × 10 × 5 inches. Food and water were present in the home cages at all times.

The apparatus was a simple galvanized iron swimming maze illustrated in Fig. 1. The maze was 10 inches high and was filled with water to a depth of 4 inches. The water was maintained at a temperature of 27°C. The subject was placed in the water at the "start" area and was required to swim to the ramp. After it climbed onto the ramp it was lifted from the maze, placed in a sawdust-filled box, and dried by an infra-red heat lamp located 3 feet above the box.

The procedure for training and testing was the same. Training consisted of 4 trials on one day; 6 test trials were given one week later. The mice were injected intraperitoneally with either 5 mg/kg body weight amphetamine sulfate in a 2.5 mg/cc solution, or with an equivalent volume of saline. Training or testing trials were begun one-half hour after injection. The animals were injected in groups of 5 or 6, and the group was kept together in a box both before and between maze trials. Each subject was run through the maze individually. Swimming time and errors were recorded; an error consisted of the entering of a blind alley. Five minutes was the maximum amount of time allowed to swim the maze. If a mouse had not reached the ramp within this time, it was gently prodded to the end and given a time score of 300 seconds. The interval between trials for each subject was approximately 10 minutes.

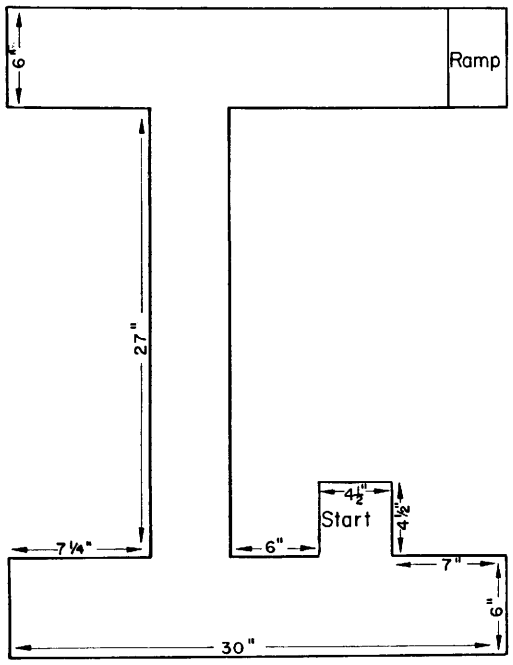

FIG. 1. Swimming maze.

The 160 subjects were divided into 5 groups. The first group was trained on saline and tested on saline (S-S). The second group was trained on saline and tested on amphetamine (S-A). Group 3 was both trained and tested on amphetamine (A-A), and group 4 was trained on amphetamine and tested on saline (A-S). Group 5 was in-

TABLE I. Log Time on Test Trials 1 and 6.

Group	Trial 1	Trial 6
S-S	1.38	1.64
S-A	1.36	1.07
A-A	1.38	1.09
A-S	1.32	1.19
A after-S	1.33	1.20

TABLE II. $\sqrt{X} + \sqrt{X+1}$ Errors on Test Trials 1 and 6.

Group	Trial 1	Trial 6
S-S	3.9	4.4
S-A	3.6	2.6
A-A	3.8	2.5
A-S	3.7	2.5
A after-S	3.6	2.4

jected with amphetamine one hour *after* training and was tested on saline (A after -S). The *test* data were then analyzed to determine whether the presence of the drug during training had a residual effect (learning effect) and/or if the presence of the drug during testing produced significant differences (performance effect). Group 5 was included in this experiment to control for possible long-term drug effects.

Results. The data from test trials 1 and 6 were analyzed. The time scores were transformed into log time and the error scores to $\sqrt{X} + \sqrt{X+1}$(11). Table I contains the mean time scores and Table II, the error scores. Analysis of variance showed no significant differences among the groups in either time or errors on test trial 1 ($F = < 1$ in both cases). On test trial 6, however, there was a highly significant difference in both time and errors ($F = 10.69$ for time and 6.78 for errors; $P = .001$ in both cases). The individual means were then compared by Tukey's(12) procedure. These tests separated the S-S group from the other 4 groups (Sig. gap = .20 for time and .92 for errors). No straggler group and no excessive variability were found among the remaining 4 means.

Discussion. On the first test trial there were no significant differences among the groups, but on the last trial, the mice which received amphetamine at *any* time were highly superior to those which had never received the drug. Since the subjects which were injected with amphetamine after training (A after -S) did as well on Trial 6 as those which were trained on the drug (A-S), there is no evidence that the presence of the drug during training had a residual effect (*i.e.*, no "learning" effects were demonstrated).

Amphetamine definitely improved performance on the last test trial. These results are consistent with some human studies which have shown that amphetamine improves the performance of fatigued, bored, or sleepy subjects, but has no effect on rested subjects (9,13,14,15) and that the drug will prevent the usual decrement in performance that occurs with time(16). In the present experiment, the test performance of the A after -S group indicates that this particular effect of amphetamine is still evident a week after injection. Studies on the disposition of radioactive amphetamine in rat brain have shown that the drug disappears from the brain within 4 hours after injection(17); therefore, it is unlikely that these results are due to a long-term drug action.

This prolonged effect could be caused by some structural or metabolic change which was produced in the animals by the drug. There is no information in the literature concerning persistent bodily changes resulting from single injections of amphetamine, but Ehrich and Krumbhaar(18) found no detectable lesions in rats which were sacrificed after repeated injection of a wide variety of sublethal doses. Biochemical changes have been found in rat brain after injection of varying doses of d-amphetamine. The drug lowered brain noradrenaline and increased brain seroronin, and, in a group of animals given repeated injections of a very high dose (17 mg/kg), the effect persisted for at least 5 days(19). However, in the present study, prolonged behavioral effects were obtained with only one injection of a moderate dose of the racemic mixture.

This study has shown that a single dose of amphetamine can influence behavior at a time when the drug is no longer present in the central nervous system. Since nothing is known about the long-term effects of a single injection, no conclusions may be drawn con-

cerning the specific mechanisms involved. However, it appears that amphetamine must have produced some long-lasting physiological change in the animals which alleviated fatigue, sustained motivation, or enhanced their ability to withstand stress.

Summary. Five groups of mice were trained to swim a maze in 4 trials and were given 6 test trials one week later. Group I was trained on saline and tested on saline (S-S); Group II was trained on saline and tested on amphetamine (S-A); Group III was both trained and tested on amphetamine (A-A); Group IV was trained on amphetamine and tested on saline (A-S), and Group V was injected with amphetamine one hour *after* training and was tested on saline (A after -S). The *test* data for trials 1 and 6 were then analyzed to determine whether the presence of the drug during training had a residual effect (learning effect) and/or if the presence of the drug during testing produced significant differences (performance effects). No learning effects were found on either trial, but, on the last trial, amphetamine significantly improved performance, and this effect outlasted the drug's presence in the central nervous system. It was hypothesized that amphetamine produced some persistent physiological change in the animals which maintained their performance.

The assistance of Genevieve Lowden in carrying out these experiments is gratefully acknowledged. Thanks are also due to Smith, Kline and French for generous provision of amphetamine.

1. Weiss, B., Laties, V. G., *Pharmacol. Rev.,* 1962, v14, 1.
2. Battig, K., *Psychopharmacologia,* 1963, v4, 15.
3. Smith, G. M., Beecher, H. K., *J. Am. Med. Assn.,* 1959, v170, 542.
4. ———, *ibid.,* 1960, v172, 1623.
5. Smith, G. M., Weitzner, M., Beecher, H. K., *J. Pharmacol. Exp. Therap.,* 1963, v139, 114.
6. Aceto, M. D., Lynch, V. D., Thoms, R. K., *J. Pharmaceut. Sci.,* 1961, v50, 823.
7. Eysenck, H. J., Casey, S., Trouton, D. S., *J. Ment. Sci.,* 1957, v103, 645.
8. Franks, C. M., Trouton, D. S., *J. Comp. Physiol. Psychol.,* 1958, v51, 220.
9. Kornetsky, C., Mirsky, A. F., Kessler, E. K., Dorff, J. E., *J. Pharmacol. Exp. Therap.,* 1959, v127, 46.
10. Minkowsky, W. L., *J. Comp. Psychol.,* 1939, v28, 349.
11. Winer, B. J., *Statistical Principles in Experimental Design,* New York, McGraw-Hill, 1962.
12. Tukey, J. W., *Biometrics,* 1949, v5, 99.
13. Barmack, J. E., *J. Psychol.,* 1938, v5, 125.
14. ———, *J. Exp. Psychol.,* 1939, v25, 494.
15. Kornetsky, C., *J. Pharmacol. Exp. Therap.,* 1958, v123, 216.
16. Payne, R. B., Hauty, G. T., *J. Exp. Psychol.,* 1954, v47, 267.
17. Young, R. L., Gordon, M. W., *J. Neurochem.,* 1962, v9, 161.
18. Ehrich, W. E., Krumbhaar, E. B., *Ann. Int. Med.,* 1937, v10, 1874.
19. McLean, J. R., McCartney, M., PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 77.

Received November 26, 1963. P.S.E.B.M., 1964, v115.

Stem Cell Replacement in Normal Thymus Grafts.* (29022)

DONALD METCALF AND RESA WAKONIG-VAARTAJA (Introduced by H. Koprowski)
Cancer Research Laboratory, Walter and Eliza Hall Institute, Royal Melbourne Hospital, Victoria, Australia, and Queen Elizabeth Hospital, Woodville, S. A., Australia

It has been postulated by some that thymic lymphocytes may be a special class of cell, distinct from other lymphocytes in the body. This implies the existence in the thymus of a permanent population of special thymic lymphoid stem cells, which by division could maintain the supply of thymic lymphocytes. However, evidence from the repopulation of the thymus in irradiated, bone marrow-injected animals(1,2) and of thymus grafts in thymectomized recipients(3) sug-

* This investigation was supported by the Anti-Cancer Council of Victoria and the Anti-Cancer Foundation, University of Adelaide.