central nervous system, it seems more reasonable to assume tentatively, on the basis of existing data, that the method or timing of PKU induction is a more important factor.

It is indeed likely that administration of excessive phenylalanine during fetal or neonatal life, or during both, would have a greater and perhaps a permanent behavioral effect. The results of such experiments are contradictory, however. Woolley and van der Hoeven (*3*) have reported that mice administered DL-phenylalanine and L-tyrosine from birth until 7 or 8 weeks of age showed "subnormal learning ability as measured in the maze test" after being fed normal diet for at least 3 days. On the other hand, three other groups of investigators (*3*) have failed to detect an impairment in complex behavior of rats administered phenylalanine under an impressive variety of prenatal and neonatal treatments. Therefore, although the balance of evidence seems to point to the absence of permanent behavioral effects of fetal or neonatal treatment, the question is by no means settled. Nevertheless, the most important implication of the results of the present experiment is that the weanling or older rat is not the appropriate experimental animal preparation for further research on this problem.

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References and Notes

1. R. Karrer and G. Cahilly, *Psychol. Bull.* **64**, 52 (1965) provide a recent review which refers to most of the research relevant to this report.
2. E. S. Kang, J. L. Kennedy, Jr., L. Gates, I. Burwash, A. McKinnon, *Pediatrics* **35**, 932 (1965); D. S. Kleinman, *ibid.* **33**, 123 (1964).
3. V. J. Perez, *Amer. Psychol.* **19**, 498 (1964) abstr.; T. L. Perry, G. M. Ling, S. Hansen, L. MacDougall, *Proc. Soc. Exp. Biol. Med.* **119**, 282 (1965); W. R. Thompson and K. Kano, *J. Psychiat. Res.* **3**, 91 (1965); D. W. Woolley and Th. van der Hoeven, *Science* **144**, 883 (1964); ———, *ibid.* **144**, 1593 (1964).
4. V. J. Polidora, D. E. Boggs, H. A. Waisman, *Proc. Soc. Exp. Biol. Med.* **113**, 817 (1963).
5. V. J. Polidora, R. F. Cunningham, H. A. Waisman, *J. Comp. Physiol. Psychol.*, in press; prepublication copies available from V.J.P.
6. Five percent P-diet, prepared by adding 5 g of NRC (National Academy of Sciences–National Research Council) grade L-phenylalanine to every 95 g of ground Rockland rat diet (C-diet), was selected as an optimal concentration on the basis of our previous findings (see *5*).
7. Mean phenylalanine dosage (grams per 100 g of body weight per day) actually administered during the three 10-day intervals (in the order 20-29, 30-39, 40-49, respectively) for the seven P-groups were: I—9.1, 8.5, 6.6; II—8.3, 7.8; III—8.3; IV—6.5; V—7.4, 6.2; VI—7.1; VII—8.5, 6.2.
8. Swimming speeds on the first test day were comparable for all groups. Swimming speeds through the maze on test days 2 to 6 showed a pattern of differences between groups identical to that indicated by the error data reported (compare *5*).
9. Analyses of variance of unweighted means were performed on the data on total errors in the 50-day test (Fig. 2*A*) and the 75-day test (Fig. 2*B*). For the 50-day test, the effects of diets (P- versus C-groups), groups (I through VII), and their interactions were statistically significant (diets $F = 94.13$, $df = 1/200$, $p < .001$; groups $F = 6.17$, $df = 6/200$, $\mathrm{p} < .001$; diets by groups interaction $F = 2.97$, $df = 6/200$, $p < .01$). Multiple *t*-tests subsequent to the analysis of variance revealed that each P-group made significantly more errors than did the corresponding C-group ($p < .001$ in each case). Duncan's new multiple-range tests established that: the seven C-groups did not differ significantly from one another ($p > .05$); the three, 10-day P-groups did not differ ($p > .05$), nor did the three, 20-day P-groups differ from each other or from the 30-day group ($p > .05$); each of the 20- or 30-day groups made significantly ($p < .01$) more errors than did any of the 10-day groups. For the 75-day test, no main effect or interaction was significant. Statistical tests were taken from B. J. Winer, *Statistical Principles in Experimental Design* (McGraw-Hill, New York, 1962).
10. Approximately the same number of errors *per day* were made by C-groups in the 50- and 75-day tests; the apparent lack of comparability (compare Fig. 2*A* and 2*B*) stems from the fact that the data of the 50-day test were based on 5 days and the 75-day test, on 2 days.
11. Selection of any single "valid" behavioral testing procedure for animals must, of course, be questioned (*1, 12*) and cross-validating results must be sought. Our justification for using the described water-maze test in this series of experiments is threefold: (i) Maze-learning in rats has been extensively studied and shown to be a sensitive measure of a wide range of experimental treatments upon behavior. (ii) A test based upon the rats' obvious and persistent motivation to escape from cold water as quickly and efficiently as possible is a naturally occurring tendency which avoids most of the confounding problems associated with behavior motivated by electric shock, or by deprivation of food or water (which affects phenylalanine intake). (iii) The present and previous experiments have demonstrated that this test is a reliable index of relatively complex behavior in normal rats, and it also detects replicable and dose-dependent behavioral deficits associated with PKU. Thus we have to a certain extent avoided a judgment of test validity; we have sought instead to base the firmness of our conclusions upon the degree to which related experiments, each using the same behavioral test, produce internally consistent and replicable results.
12. D. E. McMillian, *Science* **145**, 952 (1964).
13. J. C. Voss and H. A. Waisman, *Comp. Biochem. Physiol.*, in press; H. A. Waisman and H. F. Harlow, in *Proceedings of the 2nd International Congress on Mental Retardation* (Karger, New York, 1963), p. 54; M. N. Woods and D. B. McCormick, *Proc. Soc. Exp. Biol. Med.* **116**, 427 (1964).

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Anticholinesterase-Induced Amnesia and Its Temporal Aspects

Abstract. *Injection of the anticholinesterase drug diisopropyl fluorophosphate into the hippocampi of rats, 30 minutes after escape learning, produces partial amnesia with full recovery 5 days after injection. No such amnesia is produced if the injection takes place 3 days after learning. However, with injections 5 days after learning there is again an effect, and at 14 days amnesia is complete though no normal forgetting occurs within this period.*

Experimentally induced amnesia has been widely used as a tool in the investigation of the physiological basis of memory (*1*). Our study suggests that amnesia can be produced by interference with at least two processes which differ in their temporal characteristics and that such amnesia may be temporary.

Amnesia was induced in rats by the injection of the anticholinesterase drug diisopropyl fluorophosphate (DFP) into the hippocampi after learning. Flexner, Flexner, and Stellar (*2*) have recently produced amnesia by the intracerebral injection of puromycin, a protein synthesis inhibitor which produces an effect on cholinesterase activity (*3*). Cholinesterase may play an important role in learning (*4*).

Male rats (Sprague-Dawley, Holtzman strain, 350 g) were trained in a Y-maze to run into the illuminated arm to escape from shock (0.75 ma) applied to the grid floor. The position of the safe arm was varied randomly from trial to trial. A series of ten consecutive correct choices was the criterion of learning both during initial learning and retraining. The rats were divided into nine groups, six experimental and three control. The groups were run in small sections randomly interspersed with others, so that possible variations in procedure and drug sample could be counterbalanced. The differences between the experimental and control groups were large, and the variance within these groups was small, an indication of consistency of the effect that was independent of the time when a section of a group was tested. There were no significant differences in initial learning scores between the groups.

The first three experimental groups were used to determine if DFP pro-

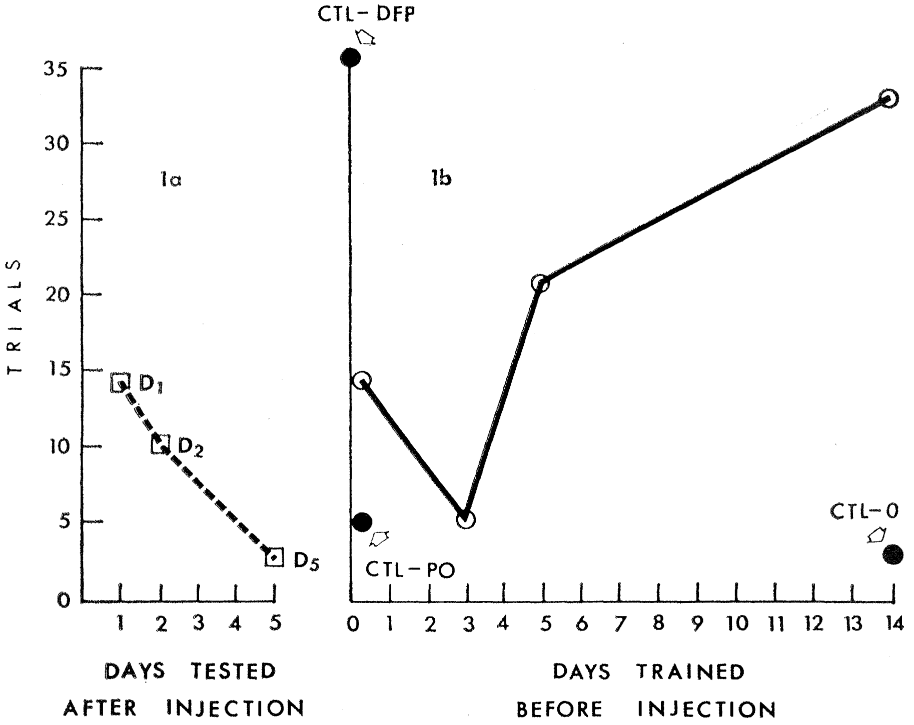

Fig. 1 (*a*) The effect of varying injection-retest interval with original training-injection interval kept constant. The squares indicate the relearning scores of groups D-1, D-2, and D-5. Initial training always occurred 30 minutes before injection. (*b*) The effect on retest scores of varying original learning-injection interval with injection-retest interval kept constant. The open circles indicate the number of trials to criterion, at retest 24 hours after injection of groups trained various times before injection. Solid circles indicate controls; *CTL-O* indicates animals trained and retested without intervening treatment; *CTL-DFP* indicates animals injected with DFP without original training; *CTL-PO* indicates animals trained and injected with peanut oil 30 minutes after training.

duced amnesia and to see if the lost memory would return spontaneously after a lapse of time. Such a return is normal with human retrograde amnesia of traumatic origin (*5*), though it has not been reported for animals (*1*). Approximately 30 minutes after training the rats in these groups were injected with DFP under nembutal anesthesia in a stereotaxic instrument. The drug was injected in an anhydrous solution of peanut oil containing 1 mg of DFP per ml (Floropryl, isoflurophate USP). The placement of the bilateral injection was anterior 3, lateral 3, vertical +2; and anterior 3, lateral 4.75, vertical −2, according to the atlas of DeGroot (*6*). Each rat received four injections of 0.01 ml of solution. Ten brains were examined histologically, and the correctness of placement of injection was confirmed. The relearning test took place 1 day after injection in group D-1 ($N = 13$), after 2 days in group D-2 ($N = 5$), and after 5 days in group D-5 ($N = 10$). One control group ($N = 10$) was injected with peanut oil 30 minutes after training and tested 24 hours later to provide a base line of retention when no drug was administered. Animals in group D-1 ($N = 13$) took 14.5 trials to relearn, whereas animals in the peanut oil control group took 5 trials ($t = 5.20$, $P < .001$) (Fig. 1*a*). This difference reflected mainly a memory loss. If injection of DFP was made 3 days after training (group 3-D) and the rats were tested 24 hours after injection, five trials were required for relearning. This number was the same as the score of the control group on peanut oil alone and rules out confusion as an explanation for the retention loss shown by group D-1. There was a gradual and nearly complete recovery of memory. The rats in group D-5 (tested 5 days after injection) took an average of 2.5 trials to relearn. The difference between group D-5 and the control group on peanut oil is not significant ($t = 1.66$, $P < .10$).

We also investigated the susceptibility of memory to disruption at various times after learning. Rats in three groups were trained at various times before injection. However the time from injection to relearning was 24 hours for all these groups. Differences in retention between these groups must have been due to the only difference in treatment, which was in time of training before injection. Animals in group D-1 were anesthetized as soon as they had been trained and were then injected. Rats in group 3-D ($N = 9$) were injected 3 days after training, those in group 5-D ($N = 9$) 5 days after, and those in group 14-D ($N = 6$) 14 days after. They were retrained 24 hours after injection. Two control groups were also used. The purpose of the first control group ($N = 13$) was to establish how fast an untrained animal could be trained 24 hours after an injection of DFP. This measure would then serve as a base line to assess the amount of amnesia in other groups. Another control ($N = 7$) was used to determine the amount of normal forgetting over a 14-day period. Animals were trained and tested 15 days later without any intervening treatment.

Injection of DFP obliterates a 2-week-old memory completely (Fig. 1*b*). Rats trained 14 days before injection showed no significant difference in trials to relearn the habit from those in the group which had never learned before injection and so had no memory of the habit. Further, the score of the group trained 14 days before injection is different from that of the control group which received peanut oil ($t = 13.03$, $P < .001$). That such a result is not due to a normal forgetting is shown by rats in the group trained and tested 15 days later without intervening injection. Such rats took only three trials to relearn. After 5 days of storage the memory is more resistant to the drug than after 14 days, whereas at 3 days it seems completely immune to the effects of the drug. The group with 3-day delay between training and injection was retrained in 5.0 trials, which is the same as for the peanut oil control group. The score reflects the effects, 24 hours later, of the operative procedure. In spite of this lack of effect on a 3-day-old memory, there is a definite effect on memory if the drug is injected nearer the time of learning. This effect is shown by the difference in the scores of group D-1 (14.5) and group 3-D (injected 3 days after training) ($t = 4.61$, $P < .001$).

The results of the second experiment reveal two components of the physical basis of the memory process. The first phase shows the familiar picture of retrograde amnesia where older memories are less affected by treatment or, as in humans, by trauma (*5*). The second phase, beginning only after 3 days shows the reverse: the older memories are more susceptible to effects of the drug. The memory process may have two biochemical phases which are differentially sensitive to disruption.

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References and Notes

1. J. A. Deutsch, *Ann. Rev. Physiol.* **24**, 259 (1962).
2. J. B. Flexner, L. B. Flexner, E. Stellar, *Science* **141**, 57 (1963).
3. A. Burkhalter, *Nature* **199**, 598 (1963).
4. M. R. Rosenzweig, D. Krech, E. L. Bennett, *Psychol. Bull.* **57**, 476 (1960).
5. R. W. Russell and P. W. Nathan, *Brain* **69**, 280 (1946).
6. J. DeGroot, *Verhandel. Koninkl. Ned. Akad. Wetenschap. Afdel. Natuurk. Sect. II*, **52**, 1 (1957).
7. We thank Dr. A. Marcus of Merck, Sharp and Dohme for peanut oil. Supported by NIH grant No. MH-10997-02 and NSF grant No. NSF-GB-2882 to J.A.D. and NIH fellowship 1F3MH-23,108-01 to H.D.

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Extraterrestrial Dust as a Source of Atmospheric Argon

Tilles (*1*) points out that solar-wind bombardment may emplace small amounts of noble gases on the surfaces of extraterrestrial dust particles. Heating of the particles during entry into the earth's atmosphere would release the volatiles. Tilles suggests, on the basis of an estimate of the influx of black spherules to the earth (*2*), that as much as 20 percent of the argon-36 and argon-38 in the atmosphere could be contributed by this mechanism. The strength of this source thus depends directly on the influx of extraterrestrial material to the earth.

The influx rate assumed by Tilles (Table 1) was based on black spherules collected from snows of the Greenland ice cap. Probably not all the spherules recovered from polar snows are of extraterrestrial origin. Giovinetto and Schmidt (*3*) found black spherules to be concentrated in snow layers deposited at the South Pole during years when paroxysmal volcanic eruptions took place. The spherule concentration was especially great in the snow layer representing 1883, when the famous eruption of Krakatoa occurred. The earlier suggestion by Schmidt (*4*) that volcanic dust could not be transported great distances to the polar regions is not supported by the newer data. Occurrence of volcanic dust in polar snows is consistent with the work of Flowers and Viebrock (*5*), who attributed a decrease in solar radiation at the South Pole to dust carried to Antarctica from the eruption of Mount Agung in Bali. It therefore appears likely that estimates of "cosmic" spherule flux, based on particle collections from either Greenland or Antarctic snows, are too great by some unknown amount. Even if it is assumed that all the spherules collected from polar snows are of extraterrestrial origin, the Greenland value is not characteristic of the spherule influx at other polar sites. It is about an order of magnitude greater than the mean influx rate determined for black spherules occurring in the Antarctic ice cap (Table 1). If the Antarctic influx value is used in the equations, only about 2 percent of atmospheric argon-36 and -38 could be accounted for by the mechanism proposed by Tilles.

Spherules of probable meteoritic composition were recovered from deep-sea sediments by Pettersson and Fredriksson (*6*). Assuming for the moment that these spherules entered the atmosphere as discrete particles and thus satisfied the criteria of Tilles' mechanism, the influx rate yields a negligible amount of argon-36 and argon-38 (Table 1). However, it is more likely that the value derived from Pettersson and Fredriksson's influx rate represents an upper limit to the amount of noble gases contributed by the spherules, which appear to be droplets resulting from atmospheric ablation of larger meteorites.

While the influx data for probable extraterrestrial spherules indicate only a negligible contribution of noble gases, other varieties of extraterrestrial dust may be present in sufficient quantity to fulfill the requirements of Tilles' ingenious mechanism. Investigation of this possibility should be provided for in future collections of dust particles of extraterrestrial origin.

Table 1. Comparison of amounts of argon introduced into the atmosphere under different assumed rates of influx of extraterrestrial spherules. The amount of argon-36 and argon-38 is based on the calculation presented by Tilles. The only parameter changed is the influx of extraterrestrial spherules.

Influx rate of extraterrestrial dust (g cm^{-2} year^{-1})	Volume of argon per square centimeter over Earth's surface (cm^3)	Percentage of total Ar^{36} and Ar^{38} due to extraterrestrial dust
	Greenland snow (2)	
2×10^{-7}	4.6	~20.0
	Antarctic snow (4)	
2×10^{-8}	0.5	~2.0
	Deep-sea sediments (6)	
1×10^{-9}	0.03	~0.002

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References

1. D. Tilles, *Science* **148**, 1085 (1965).
2. C. C. Langway, Jr., *Proc. Intern. Assoc. Sci. Hydrol. Publ. No. 61* (Berkeley, 1963), p. 191.
3. M. B. Giovinetto and R. A. Schmidt, *Trans. Amer. Geophys. Union* **46**, 116 (1965).
4. R. A. Schmidt, *Ann. N.Y. Acad. Sci.* **119** (1), 186 (1964).
5. E. C. Flowers and H. J. Vierbrock, *Science* **148**, 493 (1965).
6. H. Pettersson and K. Fredriksson, *Pacific Sci.* **12**, 71 (1958).

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Old Faithful

I find myself concerned with J. S. Rinehart's hypothesis [*Science* **150**, 494 (1965)] of a dual cavity to explain the bimodal nature of Old Faithful's period. It would seem wise to search for a simpler model first, and indeed the data seem to suggest one. If, in the previous eruption, the cavity was incompletely emptied, then one might expect new activity to build up more quickly and the period to be briefer. The evidence that during short periods seismic activity starts immediately, whereas during long periods there is a 20- to 30-minute quiet, supports this alternative hypothesis. It might be tested by comparing the duration, or better the volume, of eruption with the time interval preceding the next eruption.

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