force us to reconsider our notions of the origins of intelligence (particularly with respect to sex differences), and to look more closely to the genetic structure of the child and the ecology provided by the parents for traces of these origins.

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References and Notes

1. For example, the correlation between total development test scores at 8 months and I.Q. at 4 years is .02 [N. Bayley, *J. Genet. Psychol.* **75**, 165 (1949)]. A correlation of .32 between 6 months and 5 years is reported by C. B. Hindley [*J. Child Psychol. Psychiat.* **6**, 85 (1965)]. However, one study on *preverbal vocalizations*, not overall intelligence, between 6 and 18 months reports *r*'s of about .45 with I.Q. at 3 years [F. L. Catalano and D. McCarthy, *J. Psychol.* **38**, 203 (1954)].
2. Comparisons of the relations between children's I.Q.'s and abilities of true and of adoptive parents have been made by M. P. Honzik [*Child Develop.* **28**, 215 (1957)] and by N. Bayley [*Monographs Soc. Res. Child Develop.* **29** (6, whole No. 97) (1964)]. See also G. M. Whipple, Ed., *The Thirty-Ninth Yearbook of the National Society for the Study of Education. Intelligence: Its Nature and Nurture* (Public School Publishing Co., Bloomington, Ind., 1940).
3. A full description of the longitudinal study can be found in H. E. Jones and N. Bayley, *Child Develop.* **12**, 167 (1941). The Tryon method of cluster analysis is described in R. C. Tryon, *Educational Psychol. Meas.* **18**, 3 (1958) and in R. C. Tryon, *Cluster Analysis* (Edwards, Ann Arbor, Mich., 1939). A full report of the cluster analysis of these infant developmental test data is in preparation as a monograph. A factor with slightly different item content, derived from scores for sexes combined, was reported by N. Bayley in *Analyses of Concept Learning*, H. J. Klausmeier and C. W. Harris, Eds. (Academic Press, New York, 1966), p. 117.
4. Correlations of .42 between 4 and 18 years, for 211 subjects, and .61 between 6 and 18 years, for 214 subjects, are reported by M. P. Honzik, J. W. Macfarlane, and L. Allen, *J. Exp. Educ.* **17**, 309 (1948). N. Bayley [*J. Genet. Psychol.* **75**, 165 (1949)], using an average of three consecutive tests to obtain more reliable scores, obtained *r*'s of .62 between 4 and 18 years and .86 between 6 and 18 years for 40 subjects.
5. Domain validity, as defined in the Tryon system, is equivalent to the correlation between subject's actual score in a particular domain of ability and a hypothetical score which measured *without error* his standing in that domain or area. See R. C. Tryon [*Educational Psychol. Meas.* **18**, 3 (1958)] for computational formula.
6. At 6 and 7 years of age the Berkeley Growth Study subjects were given the 1916 Stanford-Binet, for which no methods are available for determining separate verbal and performance scores. At 13 and again at 15 years they received the Terman-McNemar test, which also has no separate verbal and performance scores. At ages 8 through 12 and again at 14 and 17 the revised Stanford-Binet intelligence tests (L and M) were administered, for which verbal and nonverbal factor score formulas are available [Q. McNemar, *The Revision of the Stanford-Binet Scale: An Analysis of the Standardization Data* (Houghton Mifflin, Boston, 1942)]. At the remaining ages (16, 18, 21, and 26 years) the Wechsler-Bellevue test, which is divided into verbal and performance sections, was used.
7. Supported, in part, by grant MHO8135 from the National Institute of Mental Health. Marjorie P. Honzik contributed to earlier phases of work on this study.

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Puromycin Effect on Memory May Be Due to Occult Seizures

Abstract. *Intracerebral injections of puromycin, which have been shown to impair memory 3 hours after training, increase the susceptibility of mice to seizures after administration of normally subconvulsive doses of pentylenetetrazol. Cycloheximide, which antagonizes the puromycin-induced amnesia 3 hours after training, also antagonizes the puromycin effect on susceptibility to seizure. The anticonvulsant diphenylhydantoin antagonizes the puromycin effect on memory. The puromycin effect on memory may be due to occult seizures.*

We have reported that mice that are injected intracerebrally with puromycin 5 hours before training can learn to escape shock in a one-choice maze but have markedly impaired retention when tested 3 hours later or thereafter (*1*). Since puromycin inhibited cerebral protein synthesis very extensively, it seemed possible that it impaired memory by interfering with the synthesis of protein required for memory storage. However, injections of cycloheximide, which inhibited cerebral protein synthesis more extensively than puromycin, did not interfere with memory 3 hours after training and antagonized the effects of puromycin (*2*). The studies with cycloheximide suggested that the puromycin effect on memory 3 hours after training might not be due to interference with the synthesis of a protein required for memory storage but rather to some other action. This impression was supported by our finding that recordings from the hippocampal region of the brain made 5 hours after intracerebral injection showed markedly diminished and irregular activity in puromycin-injected mice but not in mice which had been injected with cycloheximide or saline (*3*). The present studies provide further evidence that the puromycin effect on memory 3 hours after training may be related to its production of abnormalities in cerebral electrical activity.

To extend our observations of the effects of intracerebral injections of drugs on electrical activity of the brain, recordings were made at a number of times after injection. We found that, within the first few hours after intracerebral injection of puromycin, recordings from the hippocampal region of the brain showed frequent seizure activity which was not apparent from observation of the animal's behavior. Recordings from cycloheximide- or saline-injected mice showed far less frequent seizure activity. It seemed, therefore, that the marked decrease in electrical activity observed in the puromycin-injected mice 5 hours after administration of the drug probably represented a post-ictal phenomenon after repetitive occult convulsive discharges. Since cycloheximide was found to antagonize the amnesic effect of puromycin 3 hours after training, the effect of a combination of cycloheximide and puromycin on cerebral electrical activity was determined. Cycloheximide was found to antagonize the electrical abnormalities produced by puromycin, but the finding was difficult to qualify. Quantification of data on large numbers of mice was considerably simplified by studying the effects of normally subconvulsive doses of pentylenetetrazol on overt seizure activity in mice which had been injected intracerebrally with puromycin or other drugs. Since the recordings that we made suggested that the primary abnormality that puromycin produces is an increase in seizure activity, we reasoned that subconvulsive doses of pentylenetetrazol might produce overt seizure activity in puromycin-injected mice, and that the cycloheximide antagonism to puromycin might be expressed in a reduction in susceptibility to pentylenetetrazol-induced seizures.

The details of the procedure that we used and the results we found are shown in Table 1. Puromycin-injected mice were far more prone to develop seizures than were cycloheximide-, acetoxycycloheximide-, or saline-injected mice when pentylenetetrazol was administered subcutaneously either 1 or 5 hours after intracerebral injections. Addition of cycloheximide to the puromycin solution markedly decreased the effect of puromycin on susceptibility to seizure. Hydrolysis of the puromycin also diminished its effect.

Studies were also made of susceptibility to pentylenetetrazol administered 5 minutes after intracerebral puromycin. Such treatment produced no increase in seizure activity. This suggests that it may be necessary for puromycin to diffuse more extensively than it has at 5 minutes, or to be chemically altered, before it can act to increase brain irritability. The product of such a chemical alteration might be peptidyl-puromycin, which is formed by incorporation of puromy-

Table 1. Average scores of mice injected subcutaneously with pentylenetetrazol (50 mg/kg) at various time intervals after intracerebral injections. Mice were observed for 5 minutes after injection of pentylenetetrazol and scored for overt seizure activity by an observer who had no knowledge of the nature of the material that was injected intracerebrally. The scoring system used was: 0, no seizure activity; 1, occasional twitching of body or jerky elevation of tail; 2, frequent twitching of body or jerky elevation of tail; 3, prominent seizure which involved only a portion of the body; 4, generalized major seizure. Numbers in parentheses refer to the number of mice in each group. Statistical comparisons were made with the Mann-Whitney U test. The mice injected with puromycin differed significantly from every other group at the .02 level of significance or better.

Substance injected intracerebrally	Total dose (μg)	Time after injections		
		1 hour	5 hours	11 hours
Puromycin	200	2.6 (19)	2.5 (22)	
Saline	0	0.4 (15)	0.9 (10)	
Acetoxycycloheximide	20	0.4 (15)	0.6 (10)	0.9 (11)
Puromycin and cycloheximide	200 of each	0.7 (15)	1.6 (19)	
Hydrolyzed puromycin	200		1.5 (20)	

cin into a growing polypeptide chain (*4*). Since cycloheximide might block the formation of peptidyl-puromycin (*5*), this may be the mechanism by which it antagonizes the effect of puromycin on seizure susceptibility and memory.

When pentylenetetrazol was administered 24 hours after intracerebral injection, all mice were extremely prone to develop seizures, and there was no significant difference found between puromycin-, acetoxycycloheximide-, or saline-injected animals. This delayed change in irritability could be due to the beginning of scar formation in the injection tract.

Since it seemed likely that the puromycin effect on memory 3 hours after training was related to the abnormalities of electrical activity which it produced, the effect of the anticonvulsant agent diphenylhydantoin on memory in puromycin-injected animals was studied. Mice were injected intracerebrally with a total of 200 μg of puromycin. Immediately thereafter they were injected intraperitoneally with diphenylhydantoin (35 mg/kg) or with saline. Five hours after injection they were trained to choose the correct limb of a T-maze to a criterion of three out of four consecutive correct responses (*6*). Three hours after training they were tested for retention (*6*). Thirty-one mice injected with diphenylhydantoin had an average of 57 percent savings, whereas 19 puromycin-injected mice that did not receive the anticonvulsant had an average of 24 percent savings. Diphenylhydantoin treatment significantly improved retention ($P < .02$, Mann-Whitney U test). This confirms our impression that the amnesia noted in puromycin-injected animals 3 hours after training may be related to seizure activity.

We have recently found that mice injected intracerebrally with acetoxycycloheximide learned a one-choice maze normally, to a criterion of three out of four consecutive correct responses, and remembered normally 3 hours after injection, but had markedly impaired savings 6 hours after injection and thereafter (*6*). Because of the finding that the puromycin effect on memory was related to abnormalities in electrical activity we have evaluated the possibility that acetoxycycloheximide might also be acting in this manner. As shown in Table 1, acetoxycycloheximide-injected mice are no more susceptible to pentylenetetrazol-induced seizures than are saline-injected mice. This was true not only 5 hours after intracerebral injection, the time when the mice were usually trained, but also 11 hours after injection, a time when acetoxycycloheximide-injected mice have forgotten what they learned 6 hours previously (*6*). The effect of diphenylhydantoin on acetoxycycloheximide-induced amnesia was also determined. Eleven mice were injected with diphenylhydantoin immediately after intracerebral injection of 20 μg of acetoxycycloheximide. They were trained 5 hours later and tested 6 hours after training. They had an average of 27 percent savings 6 hours after training. Therefore, diphenylhydantoin does not apparently prevent the acetoxycycloheximide-induced amnesia.

We conclude from these experiments that, because of its effect on cerebral electrical activity, puromycin is not a useful drug for studying the hypothesis that memory storage is based on the synthesis of protein which facilitates synaptic connections, and that the mechanism of the amnesic effect of puromycin in our experiments (*1, 2*) and those of others (*7*) is uncertain. However, on the basis of the present evidence, acetoxycycloheximide does not produce general cerebral abnormalities, and it remains possible that it exerts its effect on memory (*6*) by preventing the synthesis of protein which facilitates synaptic connections.

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References and Notes

1. S. H. Barondes and H. D. Cohen, *Science* **151**, 594 (1966).
2. ———, *Brain Res.* **4**, 44 (1967).
3. H. D. Cohen, F. Ervin, S. H. Barondes, *Science* **154**, 1557 (1966).
4. D. Nathans, *Proc. Nat. Acad. Sci. U.S.* **51**, 585 (1964).
5. A. R. Williamson and R. Schweet, *J. Mol. Biol.* **11**, 358 (1965).
6. S. H. Barondes and H. D. Cohen, *Proc. Nat. Acad. Sci. U.S.*, in press; H. D. Cohen and S. H. Barondes, *Proc. Amer. Psychol. Ass.*, in press.
7. J. B. Flexner, L. B. Flexner, E. Stellar, *Science* **141**, 57 (1963); R. E. Davis, P. J. Bright, B. W. Agranoff, *J. Comp. Physiol. Psychol.* **60**, 162 (1965).
8. Supported by grant No. MH-12773 from the PHS. Acetoxycycloheximide was kindly supplied by Dr. T. J. McBride, John L. Smith Memorial for Cancer Research, Charles Pfizer and Co., Maywood, N.J. (supported by NIH contract PH 43-64-50). H.D.C. is an interdisciplinary fellow supported by grant No. ID MH-6418 from PHS, and S.H.B. is supported by career development award No. K3 MH 18232 from PHS.

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Amnesic Effects of Small Bilateral Brain Puncture in the Mouse

Abstract. *A small acute brain puncture produced retrograde amnesia in a passive avoidance learning situation in mice. If injury to the hippocampus was inflicted either immediately, 1 hour after the learning, or 1 hour before the learning, the animals showed a retention deficit; the degree of this deficit was related to the time interval. No effect of this injury was observed on retest performance when the animals were treated as long as 6 hours before or after the learning trial.*

Retrograde amnesia has been noted in animals after treatments such as electroconvulsive shock (ECS), anesthesia, spreading cortical depression (*1*), and intracerebral injection of certain antibiotics (*2*). However, brain injury or ablation alone has never hitherto been shown to produce a memory loss that depended on the retention interval. Simply inserting a needle or just touching the brain may sometimes produce profound changes in function (*3*). In view of the fact that many investigators