Infantile Experience and Resistance to Physiological Stress

It has been reported (*1*) that rats which had been "gentled" (that is, picked up and stroked once daily for 21 days postweaning) were heavier and reacted with less cardiovascular and gastrointestinal damage under conditions of immobilization for 48 hours than did the "nongentled" controls. In addition, adrenal glands of the nongentled were heavier than those of the gentled animals. Subsequent research (*2*) has revealed that rats handled prior to weaning showed significantly less mortality following 5 days of total food and water deprivation than did nonhandled rats or rats handled after weaning. The present experiment (*3*) was designed to investigate the response of rats, handled and nonhandled, in infancy (days 1 through 20) to physiological stress.

Twenty-seven male Sprague Dawley-Holtzman albino rats were handled from day 1 through day 20. Handling consisted of removing the pup from the nest, placing it in a 2.5- by 3.5- by 6-in. compartment, and then returning it to the nest. This procedure was followed once daily until weaning, on day 21. Twenty-nine rats were not handled in any manner through the first 20 days of life and then were handled only once, at weaning. The experimental treatments of handling and nonhandling were randomly assigned to complete litters. All rats within each litter received the same treatment.

All animals received no further handling until 70 days of age. At this time 20 rats from the handled group and 20 rats from the nonhandled group were given a 20-percent solution of glucose, injected intraperitoneally at a dosage of 7.5 ml/100 g of body weight, according to the method described by Brogi (*4*). A 21.4-percent mortality has been reported with this dosage for normal animals. Following the administration of the glucose, the animals were placed in individual cages without food or water. Twenty-four hours after the glucose injection the surviving animals were permitted to drink for 1 hour. The amount of water consumed was recorded. They were then sacrificed, and the left adrenal was removed and weighed. The remaining seven handled and nine nonhandled rats were not given the glucose injection but were sacrificed, and the adrenal weights were determined.

The mean body weight for the groups at weaning (21 days of age) was 47.74 g for the handled group and 44.34 g for the nonhandled group. This difference was significant beyond the 0.05 level. The mean weight for the groups at 70 days was 248.78 g for the handled group and 230.28 g for the nonhandled group. This difference was significant beyond the 0.01 level. These results are consistent with those previously reported (*5*) under identical conditions.

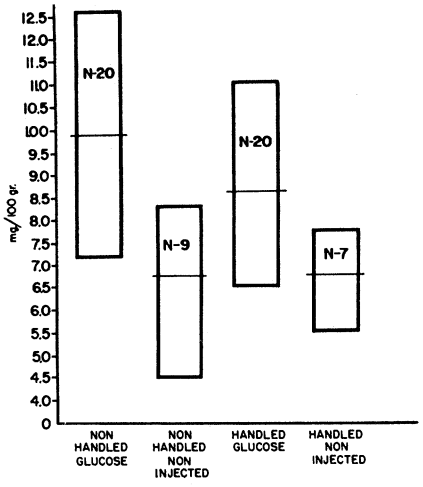

Fig. 1. Comparison of weights of adrenals of the various groups. The lengths of the bars indicate the range of individual values; the light lines, the means; and the numbers, the subjects within each group.

Although there was no significant difference in mortality between the groups following the glucose injection (three handled and two nonhandled rats died), there was a significant difference at the 0.02 level in the adrenal weights following the glucose injection. The adrenal weights for all the groups are presented in Fig. 1. It is important to note that, although the weights of the adrenals of the nonhandled animals were greater than the weights of those of the handled, both groups receiving the glucose injection had significantly greater adrenal weights than had their nonstressed controls. The noninjected groups had almost identical mean adrenal weights. Finally, the handled group consumed 4.29 g of water following glucose injection, whereas the nonhandled group consumed 2.11 g. This difference is significant beyond the 0.01 level. The difference between the handling procedure used in this experiment and the gentling procedure used by Wieninger should be recognized. Whereas Wieninger stroked and fondled his rats, the rats in this study were merely transported from the nest to a compartment and after 3 minutes were returned to the cage. That this procedure produces results similar, if not more profound, than those produced by the gentling procedure argues against the concept of "gentling" and the surplus meaning associated with this term as the major variable in the effects of early experience on behavior under stress in adulthood. It has been proposed (*6*) that handling constitutes a stressful situation for the infant organism and that early experience with stress results in a greater ability of the organism to adapt to psychological and physiological stress in adulthood.

Bovard (*7*) has suggested that, as a result of early handling, the threshold for emotional reactivity is raised. The present data suggest a more generalized hypothesis—namely, that the nonhandled animals are more profoundly affected by stress, both psychological and physiological. The greater adrenal weights of the nonhandled animals following glucose injection suggests a greater output of ACTH as a result of stress. The differences in the water intake may be a result of greater ADH production by the nonhandled rats following stress. Insofar as both ACTH (*8*) and ADH (*9*) have been shown to increase markedly following noxious stimulation, the hypothesis that handling in infancy reduces the physiological response to stress is supported.

Since ACTH (*10*) and ADH (*11*), and in general the stress reaction (*12*), appear to be mediated through the central nervous system, it is my belief that the effects of early experience modify later reactivity of the central nervous system under stress conditions. The precise nature of this modification is still unknown.

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References and Notes
1. O. Wieninger, *Science* 119, 285 (1954).
2. S. Levine and L. Otis, unpublished data.
3. This investigation was supported by research grant PHS M-1630 from the National Institute of Mental Health of the National Institutes of Health, U.S. Public Health Service.
4. M. P. Brogi, *J. Endocrinol.* 11, 344 (1954).
5. S. Levine, *J. Comp. Physiol. Psychol.*, in press.
6. ———, *J. Personality* 25, 70 (1956).
7. E. W. Bovard, *Science* 120, 187 (1954).
8. H. Selye, *Annual Report on Stress* (Acta, Montreal, 1951).
9. I. A. Mirsky, M. Stein, G. Paulisch, *Endocrinology* 54, 491 (1954).
10. G. W. Harris, in *Hypothalamic-Hypophysical Inter-relationships*, W. S. Fields *et al.*, Eds. (Thomas, Springfield, Ill., 1956).
11. M. Stein and I. A. Mirsky, *ibid.*
12. M. S. Goldstein and E. R. Ramey, *Federation Proc.* 16, 48 (1957).

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Inapparent Lymphocytic Choriomeningitis Infection in Folic Acid-Deficient Mice

Administration of amethopterin, or maintenance on folic acid-deficient diets, frequently causes mice to survive usually fatal lymphocytic choriomeningitis (LCM) infections, the virus being recoverable for long periods (*1, 2*). The experiments reported in this article deal with persistence of virus in treated mice having mild or inapparent infections.

Male mice from NIH stock were used, either Swiss "general purpose" or strain C57B1. The former, weighing 16 to 18 g when inoculated, were given bread-and-milk diets; the latter, weighing 7 to 9 g,

Table 1. Influence of amethopterin and of folic acid deficiency on inapparent LCM infection in mice. Data were compiled from four independent experiments. Virus was injected subcutaneously (s.c.) or intraperitoneally (i.p.) (column 2). Diet C2F was deficient in folic acid and contained 0.5 percent DL-methionine in addition to the published ingredients (*3*); the mean weight of these mice was 15 g. Diet C2 was the same as diet C2F, except that it contained 3 mg/kg of folic acid; the mean weight of the mice was 26 g. The mice in groups E and F were C57; the other mice were Swiss, which received bread-and-milk diets (*2*).

Group	Injection	Treatment or diet	Virus recoveries/No. of trials at postinoculation intervals						Last recovery (last test) (day)	
			< 10 days		10 to 20 days		> 20 days		Blood	Pool*
			Blood	Pool*	Blood	Pool*	Blood	Pool*		
A	s.c.	Amethopterin	4/6	3/3	4/4	2/2	3/7	4/5	24(38)	28(38)
B	s.c.	No treatment	1/6	2/3	0/4	0/2	0/7	0/5	6(38)	6(38)
C	i.p.	Amethopterin	6/6	3/3	3/5	3/3	6/7	5/6	50(50)	35(35)
D	i.p.	No treatment	3/6	3/3	0/5	1/3	0/6	0/6	6(36)	17(35)
E	s.c.	Diet C2F	2/6		1/1		3/3		36(36)	
F	s.c.	Diet C2	1/2		0/1		0/4		9(36)	

* Pool of liver, spleen, and kidney.

were given synthetic diets (*3*) starting 6 weeks before inoculation. One basal synthetic diet, designated C2F, was deficient in folic acid; the other, designated C2, contained 3 mg of folic acid (pteroylglutamic acid) per kilogram. Lymphocytic choriomeningitis virus was an intracerebrally passaged strain (*3*), given subcutaneously or intraperitoneally as infected brain emulsion, 0.15 ml of 10^{-3} dilution, or intracerebrally in 0.03-ml doses of 10^{-5} dilution. Viremia was detected by intracerebral injection of heart blood diluted 1/10; five mice were used for each test. For virus demonstration in other tissues, the dilution was 10^{-3}, given intracerebrally to each of five mice; emulsions were made by hand grinding in mortars; dilutions were made with buffered saline. Amethopterin, 0.3 mg/ml in 2-percent $NaHCO_3$, was given intraperitoneally on the third, fifth, seventh, and ninth days after virus injection.

In four experiments, inapparent infection was produced by giving LCM virus subcutaneously or intraperitoneally (*4*). Data are presented in Table 1. Virus was not recovered from control, nondeficient mice after the ninth day, with a single exception on the seventeenth. In amethopterin-treated mice, and in those on folic acid-deficient diet, virus was regularly demonstrated for 4 weeks or longer.

In another experiment, mice given LCM virus subcutaneously were challenged 2 weeks later (after immunity had developed) by homologous virus inoculated intracerebrally; some were treated with amethopterin, and others were left untreated. No virus was recovered from blood or brains of mice in either group in eight attempts between the sixth and fifteenth days after intracerebral challenge; in parallel tests on nonimmune mice, virus was recovered from treated mice as reported previously (*2*).

The significant effect common to these and previous (*1, 2*) experiments is the prolongation of infection by interference with folic acid activity. The contrast between this persistent infection and the normally self-limited one is illustrated by the following fortuitous observation. Two C57 mice of a group of five on a nondeficient diet (*5*) happened to survive an intracerebral injection of virus, although they became extremely ill. After their recovery, attempts to demonstrate viremia on the seventeenth and twenty-fourth postinoculation days were unsuccessful. Two other groups of C57 mice (four in one, five in the other) maintained on a folic acid-deficient diet (*5*) had viremia on each of these days, although none had appeared ill at any time.

One interpretation of these results is that mice with defective folic acid activity fail to develop immunity in the normal manner. Consequently, termination of virus replication is delayed. (As is indicated in a preceding paragraph, when immunity was well established before folic acid metabolism was disturbed, virus replication was not detectable.) In the inapparent infections reported here, there is a basic alteration of the mouse's reaction to initial contact with LCM virus, manifested by the development of a carrier state. In the normally fatal infections reported previously (*1, 2*) the creation of the carrier state was possible because, along with virus persistence, there was sparing of the host. This condition of equilibrium between mouse and LCM virus, with both surviving, is known to occur in two other circumstances—namely, when mice are infected *in utero* (*6*), and when they are exposed to x-rays before injection of virus (*7*).

At some stage in the evolving LCM infection in the mouse, there appear to be potentialities for different pathways. One leads to rapid virus replication and death of the mouse, another to host immunity and virus suppression, and a third to an intermediate situation characterized by survival of mouse and virus. Folic acid is one factor, although not the only one, in determining which route the infection will take.

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References and Notes

1. V. H. Haas and S. E. Stewart, *Virology* **2**, 511 (1956).
2. V. H. Haas, S. E. Stewart, G. M. Briggs, *ibid.* **3**, 15 (1957).
3. G. M. Briggs *et al.*, *Proc. Soc. Exptl. Biol. Med.* **8**, 113 (1952).
4. V. H. Haas, *J. Infectious Diseases* **94**, 187 (1954).
5. Nondeficient diet was C2 plus 0.5 percent DL-methionine and 7 percent gelatine; mice weighed 24 g at time of virus injection. Deficient diet was same, minus folic acid; mice weighed 16 g at time of virus injection.
6. E. Traub, *J. Exptl. Med.* **69**, 801 (1939).
7. W. P. Rowe, *Proc. Soc. Exptl. Biol. Med.* **92**, 194 (1956).

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