National Institutes of Health, U.S. Public Health Service.

3. The stimuli were single square wave pulses of 20 μsec duration. Reflexes were recorded monophasically from cut ventral roots, usually the seventh lumbar or the first sacral. D-Tubocurarine chloride was administered to prevent reflex movements.
4. In recordings from peripheral nerves, this prolonged firing can only be found in those supplying physiological flexor muscles. Hence it is undoubtedly the late phase of the well-known flexor reflex.
5. H. Gasser, *Ohio J. Sci.* 41, 145 (1941). In fine nerve filaments the position of the fibers with respect to the recording electrodes is relatively unimportant in determining the amplitude of the recorded impulses. This can be corroborated by separating a filament into several finer strands and rearranging these on the recording electrodes.

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Infantile Experience and the Maturation of the Pituitary Adrenal Axis

It has previously been proposed (*1*) that repeated handling of the infant animal constitutes a stress situation and that experience in infancy with stress results in a greater ability of the organism to adapt to stress in adulthood. Although there have been numerous experiments which have shown that infantile experience affects adult behavior, there has been little work on the effects of infantile experience on developmental processes in the infant organism.

Jailer (*2*) reported that infant rats, when they were subjected to cold stress, failed to show adrenal ascorbic acid depletion prior to 16 days of age. At 16 days of age, a 19 percent depletion in adrenal ascorbic acid was found. Jailer (*3*) postulated that the pituitary of rats younger than 16 days released ACTH as rapidly as it was synthesized, so that, prior to the 16th day, there was no intracellular storage of ACTH to be liberated under stress. As a result, before 16 days of age, the animals were not able to respond to an acute stress with increased release of ACTH. Jailer further reasoned that chronic stress prior to 16 days of age should lead to facilitation of ACTH production and intracellular storage, resulting in increased liberation of the hormone in response to later acute stress. Since it has been postulated that handling in infancy is a stress, it would be expected, on the basis of Jailer's hypothesis, that rats that had been handled up to 16 days of age would liberate more ACTH than nonhandled rats when subjected to cold and would show greater adrenal ascorbic acid depletion. The experiment described in this report (*4*) was designed to investigate the effects of handling during infancy on ACTH release as measured by adrenal ascorbic acid depletion (*5*) in the 16-day-old rat.

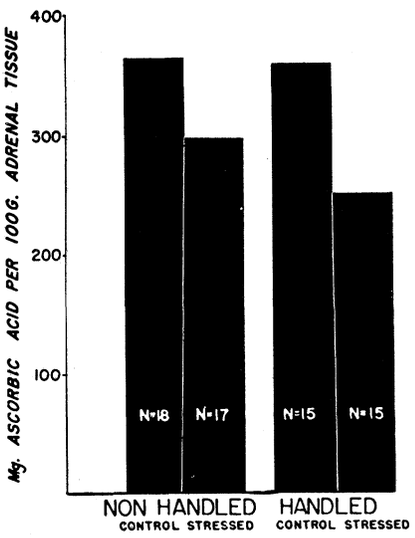

Fig. 1. Comparison of adrenal ascorbic acid concentration in the various groups of infant albino rats.

Sixty-five infant Sprague-Dawley-Holtzman albino rats were used as subjects. Thirty pups were handled from day 1 through day 15, and 35 were not handled in any manner during the first 15 days of life. The handling procedure was the same as that previously described (*6*). At 16 days of age the pups were randomly assigned to either the stress or control condition. There were thus four groups: (i) handled, nonstressed ($N = 15$); (ii) handled, stressed ($N = 15$); (iii) nonhandled, nonstressed ($N = 17$); and (iv) nonhandled, stressed ($N = 18$). The nonstressed animals were removed from their cages, killed by cervical spinal separation, and weighed. Their adrenals were removed, weighed on a 25-mg Roller-Smith balance, and analyzed for ascorbic acid content. The stressed animals were removed from their cages, placed in small individual compartments, and subjected to a cold stress of 5°C for 90 minutes. They were then killed, and their adrenals were removed, weighed, and assayed.

Adrenal ascorbic acid was assayed by a modification of the micro technique of Glick *et al.* (*7*). After being weighed, the adrenals were placed in a 15-ml ground-glass stoppered centrifuge tube and were thoroughly ground in 2 ml of 0.5 percent oxalic acid. Five milliliters of *N*-amyl alcohol were added to the tube; next were added 3 ml of a 4 mg percent aqueous solution of sodium 2,6-dichlorophenol indophenol dye. The tubes were then thoroughly shaken and centrifuged, the colored alcohol layer was removed, and its optical density was determined on the Beckman DU spectrophotometer at a wavelength of 546 mμ.

The handled and nonhandled groups showed essentially the same body weights and adrenal-weight/body-weight ratios at 16 days of age. In addition, the mean adrenal ascorbic content of the handled and nonhandled control groups was almost identical (nonhandled, nonstressed = 359 mg percent; handled, nonstressed = 365 mg percent). The handled, stressed animals showed a mean depletion of 109.73 mg percent and the nonhandled-stressed group, a mean depletion of 73.05 mg percent (Fig. 1). Thus, the handled animals showed 36.68 mg percent greater depletion, a difference significant beyond the 0.025 level of confidence ($t = 2.42$, $P < 0.025$). In terms of percentage depletion, the nonhandled animals showed 20 percent depletion, corresponding to the 19 percent obtained by Jailer, whereas the handled animals had a depletion of 30 percent.

The results of this experiment tend to support both (i) the hypothesis that infantile handling constitutes stress and (ii) Jailer's hypothesis concerning the effects of chronic stress on the facilitation of ACTH production in the infant animal. It should be noted that whereas Jailer's 16-day animals and our nonhandled animals showed only a 20 percent depletion, the 30 percent depletion exhibited by the handled animals in this study closely resembles the adult response to stress, which has been reported to be between 30 percent and 60 percent depletion (*8*). The question remains whether these results indicate greater intracellular storage, which results in more ACTH liberation at 16 days of age, or more rapid maturation of the pituitary adrenal axis. Preliminary results obtained in our laboratory indicate that handled infant rats respond to cold stress with increased ACTH release earlier than 16 days; this indicates that handling leads to more rapid maturation of the pituitary adrenal axis.

SEYMOUR LEVINE
MORTON ALPERT*
GEORGE W. LEWIS
Columbus Psychiatric Institute and Hospital, Ohio State University Health Center, Columbus

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* Present address: Department of Anatomy, Ohio State University, Columbus.

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