proven, a statistical comparison of condition 1B with condition 2 provided a *t*-ratio for correlated means less than unity. Note the performance in condition 3. For each rat the effect of free ESB during the 22 seconds of lever withdrawal served to increase the number of extinction responses occurring during the 10 minutes following the reinsertion of the lever, as compared with condition 2 where no free ESB was administered. A statistical comparison between conditions 3 and 2 yielded a *t*-ratio for correlated means of 3.93 (3 degrees of freedom), allowing the rejection of the null hypothesis at better than the .05 level of confidence. We may conclude on the basis of this experiment that (i) the Howarth and Deutsch findings are essentially replicable, and (ii) free ESB during the period of lever withdrawal serves to increase the number of unreinforced responses emitted during the immediately succeeding extinction phase. The latter finding is in conformity with expectations from Deutsch's drive-decay hypothesis.

The second experiment, with four fresh rats, was a repetition of the experiment just described in all details except one. During the several days of lever-press training preceding the extinction sessions, the lever was withdrawn from the box every 5 minutes for a period of 22 seconds each time. The lever was then returned to the box and *rewarded* training continued. At the end of "lever-out, lever-in" training, all four rats in the second experiment displayed a latency between lever insertion and the first rewarded response of less than 1 second. The three types of extinction procedure were run as before and the results are indicated in the lower panel of Fig. 1. A consideration of those data reveal, first, that the neat data relationships among the several extinction procedures in the first experiment have been dramatically changed. In two cases, rats 3 and 4, condition 1B produced more, rather than fewer, responses during extinction. In two cases, rats 1 and 2, free ESB during lever withdrawal served to reduce rather than increase the number of responses emitted during extinction. A second finding from those data is that, in general, overall extinction output and data variability for the four rats is greater than in the first experiment. One statistical comparison was made. The total response output over the three extinction procedures was computed for each of the eight rats. The mean output for the first experiment was compared with the mean output for the second. The *t*-ratio for uncorrelated means of 4.29 (6 degrees of freedom) is significant at the .01 level. The training procedure, therefore, was a powerful variable in determining extinction performance.

If extinction after ESB reward is understandable solely in terms of a drive-decay process akin to that suggested by Deutsch (*1*), then the outcome of our second experiment should have been similar to the first. The fact that striking differences were found attests to the theory's limited predictive value. The data presented by Howarth and Deutsch (*2*) and in the first experiment described above certainly point to some role played by a time-dependent process. Its exact role, generality, and importance remain to be determined (*5*).

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2. C. I. Howarth and J. A. Deutsch, *Science* **137**, 35 (1962).
3. The lever employed in these experiments was a solonoid-operated, retractable lever manufactured by Foringer and Co., Rockville, Md.
4. The actual sequence of the three extinction procedures was varied for the several animals. The results of the first experiment indicated that order effects were unimportant.
5. The experiments reported here were performed under contract No. DA 49-193-2288 between the Office of the Surgeon General, U.S. Army, and the University of Maryland.

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Early Developmental Stress and Later Behavior

Abstract. *The effects of behavioral stress on mice during pregnancy on the behavior of offspring are mimicked by epinephrine injection of mice during pregnancy; hydrocortisone and norepinephrine injection also produce behavioral changes in the offspring. Similar results were obtained in chicks hatched from injected eggs.*

Work by Thompson, Watson, and Charlesworth and by Keeley (*1*) indicates that severe behavioral and physiological stress to rats and mice during pregnancy (conditioned anxiety, crowding, epinephrine injection) produces permanent changes in the behavior of offspring (changes in open-field activity, defecation, and maze-learning). I here report some preliminary work on the mechanism by which such changes occur. Injection of stress-syndrome hormones (*2*) into pregnant mice and into chicken eggs produces changes similar to those produced by subjecting pregnant mice to a behavioral stressor.

In the first experiment, pregnant mice (C57BL/6 strain) (pregnancy determined by the plug method) were divided into five groups: a saline-injected control; an epinephrine-injected group; a norepinephrine-injected group; a hydrocortisone-injected group; a group stressed behaviorally [crowding of females in an 8- by 11- by 5-inch (20.3- by 27.9- by 12.7-cm) cage with ten aggressive males (aggressiveness shown by frequent fights and even killing among the males)]. Treatment was administered during the second trimester of pregnancy; injected groups received four subcutaneous injections on days 8, 10, 12, and 14 of pregnancy. Animals weighed about 21 g, and each injection contained 0.25 μmole of epinephrine, 0.25 μmole of norepinephrine, or 2.5 μmole of hydrocortisone in 0.10 ml of physiological saline solution. Mothers gave birth in individual cages and were not disturbed until 18 days after parturition, at which time cages were cleaned; young were weaned at 30 days. The small numbers of animals prevented cross-fostering.

At 35 days of age, offspring were given individual 10-minute trials in an open-field apparatus [a 20- by 20- by 5-inch (50.8- by 50.8- by 12.7-cm) box ruled off in 2-inch (5.08-cm) squares and illuminated by a 60-watt bulb]. Measurements of locomotion (lines crossed per unit time), defecation, escape jumps (attempts to jump out of the apparatus), and self-grooming activity (a nonnumerical estimate) were made. At 120 days, animals were killed and measurements were taken of brain weight, body weight, and gross brain serotonin and norepinephrine. While the delay is long, measurement at this time is justified by previous experimental findings (*1*) that the behavioral effects persist to this age.

A summary of the behavioral testing with the appropriate *F*- and *t*-tests is seen in Table 1. The results indicate increased activity and decreased defecation in the offspring of crowded and epinephrine-injected groups when compared to offspring of the saline control, and decreased activity and increased defecation in offspring of the hydrocortisone- and norepinephrine-injected

groups when compared to the offspring of the controls. Of the 12 *P* values presented, four are significant at the .05 level or less, and a fifth value is significant at the .06 level. Five of the seven nonsignificant differences are in directions consistent with the significant ones. Neither comparison of the offspring of the crowded group with offspring of the epinephrine-injected group nor comparison of offspring of the hydrocortisone-injected group with offspring of the norepinephrine-injected group yielded any significant differences. If *P*'s are computed for a comparison of offspring of either the hydrocortisone- or the norepinephrine-injected group with offspring of either the crowded or the epinephrine-injected group, five of the 12 possible comparisons are significant at less than the .01 level, and the other seven comparisons are in directions consistent with these.

Estimates of grooming activity agree with the movement and defecation findings. Offspring of epinephrine-injected and crowded animals groomed more than offspring of the controls, while offspring of hydrocortisone- and norepinephrine-injected animals groomed less.

No significant relationships were found between these behavioral differences and brain or body weight or neurohumoral level.

Differences in open-field activity and defecation have long been considered measures of fear and emotionality (*3*). With such an interpretation, offspring of the crowded and epinephrine-injected groups can be considered less emotional and fearful than offspring of the saline control, and offspring of the hydrocortisone- and norepinephrine-injected groups more emotional and fearful.

These results confirm previous findings that behavioral stress in pregnant rodents alters the behavior of the offspring. Further, they indicate that hormones involved in the stress syndrome can also alter behavior in the offspring. Epinephrine is found to mimic the behavioral stressor used and so is perhaps involved in transmission of stress to the offspring in this particular case; hydrocortisone and norepinephrine also change performance of the offspring on the measures used, but in the opposite direction.

Since hormones have been implicated in the transfer of behavioral stress to the offspring and many have been demonstrated to cross the placental barrier (*4*), the question of how hormones alter behavior is raised: do they further alter the maternal or the placental exchange systems, thus producing secondary effects which alter the embryo, or do they themselves cross the placenta and affect the developing system directly? One way to attack this problem is to inject chicken eggs and test the hatched animals.

Table 1. Effects of behavioral stress or chemical injection of pregnant mice on the behavior of offspring. *P* values shown in body of the table are based on a two-tailed *t*-test comparison of the experimental groups with the control group. NS = not significant. Scores shown are mean scores per 10-minute trial.

Parental group	Offspring				
	No. of animals	No. of litters	Locomotions*	Escape jumps †	Defecations ‡
Saline control	18	4	431	.50	.83
Crowded	13	3	528 ($P = .001$)	.32 (NS)	.92 (NS)
Epinephrine-injected	12	4	508 ($P = .025$)	.75 (NS)	.75 (NS)
Norepinephrine-injected	17	4	379 ($P = .06$)	.06 ($P = .08$)	1.23 (NS)
Hydrocortisone-injected	12	3	392 ($P = .10$)	.00 ($P = .05$)	1.50 ($P = .025$)

* $P < .001$ based on *F*-test comparison of five groups on locomotions. † $P < .01$ based on *F*-test comparison of five groups on escape jumps. ‡ $P < .1$ based on *F*-test comparison of five groups on defecations.

Three dozen eggs (Hall "sex linked," Hall Brothers, Wallingford, Conn.) were divided into three groups (a saline control; an epinephrine group; a hydrocortisone group) and given injections on days 12 and 14 of development; injections were made into the egg (weight = 55 g), not the embryo, and were made up as described above. Birds were hatched in the dark (hatching time, 21 days) and at 12 hours were imprinted for 10 minutes to a bobbing, red triangle placed in a lighted, 24- by 36- by 18-inch (61- by 91.4- by 45.7-cm) box. They were then returned to the dark and at 36 hours were tested with the imprinted object. The test situation consisted of placing the chick 3 ft (0.91 m) from the bobbing triangle and timing the latency of social response (the time required to approach the object and peck, scratch, or seek ventral contact).

Six of the 12 epinephrine-injected eggs and four of the 12 saline-injected eggs hatched; hydrocortisone, however, in the dosage given seems to be lethal. When tested, the birds from epinephrine-injected eggs responded to the object after an average of 12.5 seconds, while the birds from saline-injected eggs required 68.5 seconds on the average. A two-tailed *t*-test yields a *P* significant at less than .01. Moreover, the epinephrine group's social responses were more vigorous and intense; such observations, however, are difficult to quantify.

The results suggest that epinephrine can act directly on the developing embryo to produce changes in behavior. Since the epinephrine group performed more vigorously than the saline group, it is unlikely that the hormone acts by affecting the general vigor of the organism. While more complex maternal and placental changes may be involved in producing behavioral changes in the mammal, at least part of the effect is probably due to direct hormonal action.

Whether or not such mechanisms operate in humans is unknown, but the possibility exists that severe emotional stress during pregnancy leaves its mark on the unborn through sympathetic activation of the stress syndrome and migration of hormones across the placental barrier (*5*).

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References and Notes

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