band (*23*). The muscles were cut into three pieces (one containing the stained end plates), and the pieces were weighed and assayed for radioactivity in a gamma counter. Specific end-plate radioactivity (*SC*) was determined for each muscle by subtracting the pooled radioactivity bound to the pieces without end plates from the piece containing the end plates on a per weight basis. A ratio of the specific counts for denervated to innervated junctions was determined for each animal; the average at each time is presented in Fig. 1. We have determined that there is no significant difference between the amount of radioactivity bound to the left and right muscles under control conditions. Furthermore, it has been established (*13*) that the turnover rate for junctional ACh receptors determined by the gamma counting method is very close to that determined by EM autoradiography for the radioactivity at the postjunctional membrane.

Figure 1 shows that after denervation the radioactivity progressively decays more rapidly at denervated junctions than at innervated junctions. Eight days after denervation the half-time for turnover of denervated junctional receptors was 2 ± 0.4 days, which is consistent with the value derived from EM autoradiography (approximately 2.5 days) (*13*).

An independent comparison of the turnover rates of denervated extrajunctional and junctional receptors with those of normal junctional receptors was also made. Fourteen animals were injected with ^{125}I-labeled α-BGT 11 days after denervation. Four, seven, and three mice were killed 8 hours, 2 days, and 3 days after the injections, respectively, and the muscles were removed. The specific end-plate counts for denervated and innervated muscles and the extrajunctional radioactivity were each normalized to 100 percent at time zero and plotted semilogarithmically (Fig. 2). These data show the exponential nature of the decay for the denervated junctions over this 3-day period.

Eleven days after denervation the half-time for denervated junctional receptors was 34 ± 5 hours (Fig. 1), and the independently derived value was 33 ± 8 hours (Fig. 2). Fifteen days after denervation, the half-time for turnover at denervated junctions reached 30 ± 5 hours. By that time, the turnover rate for junctional ACh receptors was well within the range of values reported (*7, 8*) for extrajunctional receptors in noninnervated muscle and approaching the value of 17.5 hours given in Fig. 2 (*24*).

It has been reported that a maturation period is required between the time that clusters of ACh receptors first appear at the forming NMJ's and the time that the turnover rate of these junctional receptors decreases (*4-6, 25*). The present data suggest that there is a finite period after denervation during which the neuronal influence on turnover of junctional ACh receptors fades, even though the dense clustering remains unchanged (*13*). Thus the acceleration of turnover may reflect a reversal of the process of maturation of junctional receptors seen during development.

Note added in proof: Since this report was accepted for publication, we have obtained evidence that Fig. 1 can be modeled by assuming that the junction contains a dual population of receptors: the original receptors, present at the time of denervation, with a slowly increasing turnover rate, and new ones, with a turnover rate equivalent to that of extrajunctional receptors (*26*).

TAMI A. LEVITT
RALPH H. LORING*
MIRIAM M. SALPETER
Section of Neurobiology and Behavior and *School of Applied and Engineering Physics, Cornell University, Ithaca, New York 14853*

References and Notes

1. D. M. Fambrough, *Physiol. Rev.* **59**, 165 (1979).
2. D. Purves, in *International Review of Physiology*, R. Porter, Ed. (University Park Press, Baltimore, 1976), p. 125.
3. E. Gutman, *Annu. Rev. Physiol.* **38**, 177 (1976).
4. S. J. Burden, *Dev. Biol.* **61**, 79 (1977).
5. C. G. Reiness and Z. W. Hall, *Neurosci. Abstr.* **5**, 1654 (1979).
6. J. H. Steinbach, J. Merlie, S. Heinnemann, R. Bloch, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3547 (1979).
7. D. K. Berg and Z. W. Hall, *J. Physiol. (London)* **252**, 771 (1975).
8. S. Burden, *Dev. Biol.* **57**, 317 (1977).
9. C. C. Chang and M. C. Huang, *Nature (London)* **253**, 643 (1975).
10. P. N. Devreotes and D. M. Fambrough, *J. Cell Biol.* **65**, 335 (1975).
11. A. Shainberg, S. A. Cohen, P. G. Nelson, *Pfluegers Arch.* **361**, 255 (1976).
12. T. S. Brett and S. G. Younkin, *Neurosci. Abstr.* **5**, 2568 (1979).
13. R. Loring and M. M. Salpeter, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 2293 (1980).
14. S. Heinneman, J. Merlie, J. Lindstrom, *Nature (London)* **274**, 65 (1978).
15. C. G. Reiness, C. B. Weinberg, Z. W. Hall, *ibid.*, p. 68.
16. C. D. Linden and D. M. Fambrough, *Neurosci. Abstr.* **4**, 527 (1978).
17. C. C. Chang and C. Y. Lee, *Arch. Int. Pharmacodyn. Ther.* **144**, 241 (1963); C. Y. Lee, L. F. Tseng, T. H. Chiu, *Nature (London)* **215**, 1177 (1967).
18. J. M. Gardner and D. M. Fambrough, *Cell* **16**, 661 (1979).
19. J. P. Merlie, J. P. Changeux, F. Gros, *Nature (London)* **264**, 74 (1976).
20. H. C. Fertuck and M. M. Salpeter, *J. Cell Biol.* **69**, 144 (1976).
21. H. C. Fertuck, W. Woodward, M. M. Salpeter, *ibid.* **66**, 209 (1975).
22. The specific activity of the labeled toxin was 93 to 200 Ci/mmole and the median lethal dose was about 4.5 μg per 100 g of body weight.
23. M. J. Karnovksy and L. Roots, *J. Histochem. Cytochem.* **12**, 219 (1964).
24. The measured turnover rate of extrajunctional receptors is uncertain, since there is an increase in the number of non-plasma membrane α-BGT binding sites after denervation [R. Loring and M. M. Salpeter, *Neurosci. Abstr.* **4**, 1930 (1978)]. The turnover rate of these sites may deviate from that of the extrajunctional receptors in the plasma membrane. However, preliminary data suggest that after the initial rapid loss of nonspecific radioactivity, the labeled toxin bound to non-plasma membrane sites turns over at about the same rate as does the toxin bound to the membrane.
25. G. D. Fischbach, E. Frank, T. M. Jessel, L. L. Rubin, S. M. Scheutze, *Pharmacol. Rev.* **30**, 411 (1979).
26. T. A. Levitt and M. M. Salpeter, in preparation.
27. We thank T. Podleski and S. Jones for their helpful comments. Supported by NIH grant NS 09315-10.
* Present address: Department of Pharmacology, Harvard Medical School, Boston, Mass. 02115.

14 April 1980

Maternal Glucocorticoid Hormones Influence Neurotransmitter Phenotypic Expression in Embryos

Abstract. *Treatment of pregnant rats with reserpine prevented the normal disappearance of catecholamine fluorescence in presumptive neuroblasts of the embryonic gut. These cells normally express the noradrenergic phenotype transiently during embryonic development. The effect of reserpine was reproduced by treating mothers with hydrocortisone acetate. Moreover, the reserpine effect was blocked by treatment with dexamethasone, which inhibits the stress-induced increase in plasma glucocorticoids, and by mitotone, which causes adrenocortical cytolysis. It is concluded that reserpine, through the mediation of maternal glucocorticoid hormones, alters the phenotypic expression of these embryonic neuroblasts.*

Although it is recognized that maternal-fetal relations critically influence the developing nervous system, the processes by which maternal experience affects embryonic development are ill-defined. Exposure of pregnant or nursing animals to antipsychotic agents (*1*), opiates (*2*), or reserpine (*3*) is associated with neurological and behavioral abnormalities in the offspring. Moreover, maternal conditions such as toxemia, advanced age (*4*), and stress (*5*) also contribute to neurological defects.

The current study was undertaken to define the effects of neurologically active drugs on the embryonic nervous system. Specifically, we sought to characterize the influence of pharmacologic agents on

0036-8075/80/1031-0551$00.50/0

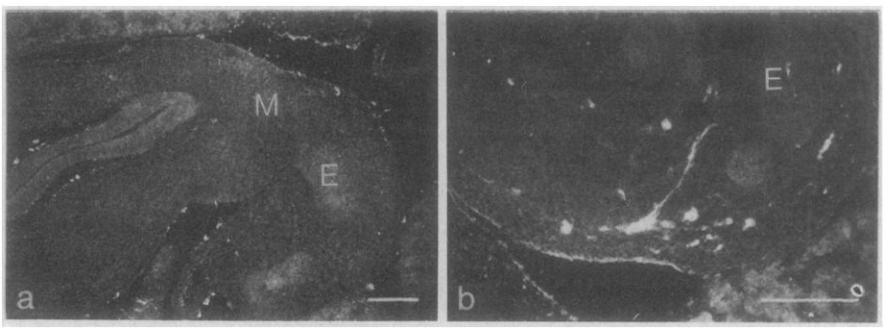

Fig. 1. Effects of reserpine on noradrenergic gut cells. Pregnant rats injected intraperitoneally with reserpine (10 mg/kg) on day 11.5 of gestation were killed 2 days later and the embryos were examined for catecholamine FIF. Saline-injected mothers served as controls. (a) Cross section of embryonic gut from control animal at E13.5. (b) Gut from embryo whose mother was treated with reserpine. *E*, Lumenal epithelium; *M*, mesenchyme. Scale bar, 50 μm.

a recently described population of presumptive neuroblasts in embryonic rat gut (*6*). We report that the administration of reserpine to pregnant rats alters neurotransmitter phenotypic expression in the embryonic neuroblasts through the mediation of maternal glucocorticoid hormones. Furthermore, the effects of reserpine administration can be mimicked by the administration of hydrocortisone to pregnant rats.

We have been studying a population of presumptive neuroblasts in the embryonic rat intestine which transiently expresses the noradrenergic phenotype (*6*). At 11.5 days of gestation (E11.5; full term in the rat is 22 days) these cells simultaneously express several noradrenergic characters, including immunoreactivity to biosynthetic enzymes, and endogenous catecholamine fluoresence (*6*). Over the next 24 hours, this population increases in number, but by E13.5 loses these noradrenergic characters. Since cells exhibiting specific, high-affinity uptake for norepinephrine persist in the gut beyond E13.5, this population probably does not die, but remains in the intestine in an altered phenotypic state [discussed in (*7*)].

To characterize these remarkably plastic cells pharmacologically, we treated pregnant rats with resperine on E11.5, when the noradrenergic gut cells first appear (*6*). Reserpine depletes vesicular stores of monoamine transmitter in mature neurons (*8*) and also crosses the placenta (*9*). We therefore expected that if vesicular storage of catecholamines were a property of this population, reserpine administration would cause premature loss of catecholamine fluorescence through depletion. Guts examined for formaldehyde-induced fluorescence (FIF) (*10*) after 24 hours were not depleted, but instead contained cells with unusual fluorescence intensity (data not shown). We therefore examined guts with FIF 2 days after reserpine administration, when noradrenergic characters have normally disappeared. As previously reported, embryos from control mothers contained few, if any, fluorescent cells in the developing gut mesenchyme at this time (*6*, *7*). In marked contrast, bright catecholamine fluorescence was still present at E13.5 in embryonic guts from reserpine-treated mothers (Fig. 1). This paradoxical result, obtained with a drug expected to deplete catecholamines, prompted us to seek other explanations for the reserpine effect.

In the adult, reserpine administration induces catecholamine-synthesizing enzymes in sympathetic ganglia and adrenal medulla through reflex activitation of transsynaptic mechanisms (*11*). To determine whether a similar mechanism was involved in this instance, we treated mothers with phenoxybenzamine, another agent that induces noradrenergic enzymes through transsynaptic mechanisms (*12*). Administration of phenoxybenzamine (20 mg/kg, intravenously) on E11.5 and E12.5 did not cause persistence of fluorescence in the embryonic gut cells on E13.5. Consequently, the action of reserpine was apparently not attributable to reflex activation of the sympathetic system in the mother.

Since glucocorticoid hormones mediate (*13*) and even mimic (*14*) the induction of noradrenergic enzymes in adults, and since reserpine administration results in a transient increase in plasma glucocorticoid concentrations (*15*), we examined the effects of increased maternal glucocorticoids on the embryonic gut cells. Pregnant rats received subcutaneous implants of hydrocortisone acetate pellets on E11 (*16*). Embryos were examined 2 days later. Hydrocortisone reproduced the effects of reserpine administration: catecholamines persisted in the embryonic gut cells (Fig. 2a).

To determine whether the reserpine effect was, in fact, due to increased maternal glucocorticoids, pregnant rats were treated with dexamethasone (1 mg/kg), which inhibits the stress-induced increase in plasma glucocorticoids (*17*). The dexamethasone was injected subcutaneously 1 day before reserpine administration. This treatment prevented the reserpine-induced persistence of catecholamine fluorescence, suggesting that the maternal pituitary-adrenal axis mediated the reserpine response.

Consistent with the above observations was our finding that the integrity of the maternal adrenal cortex was also necessary for the full reserpine effect. Administration of mitotane (dichlorodiphenyldichloroethane, *o*,*p*′-DDD), a drug cytotoxic to the adrenal cortex (*18*), severely diminished the reserpine effect (Fig. 2b).

Our observations suggest that maternal reserpine administration prolonged

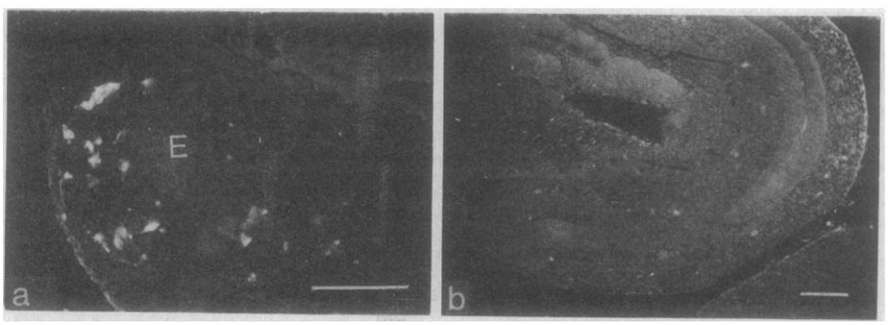

Fig. 2. (a) Effects of hydrocortisone on noradrenergic gut cells. Pellets of hydrocortisone acetate were made by melting and resolidifying the drug (*16*). On E10.5 or E11.0 mothers were anesthetized with halothane and pellets (175 to 200 mg) were implanted subcutaneously in the cervical area. Mothers with sham operations served as controls. Embryos were taken at E13.5 and examined for catecholamines by FIF. Scale bar, 50 μm. (b) Effects of mitotane on reserpine-induced fluorescence. Mothers received subcutaneous injections of mitotane twice daily (75 mg/kg per injection) beginning on E5.5. Reserpine was administered on day 11.5, and embryos were examined at E13.5 for FIF. Mitotane inhibited the effect of reserpine. (Compare with Fig. 1.) Scale bar, 50 μm.

the expression of the noradrenergic phenotype in these embryonic neuroblasts. The reserpine effect appeared to be mediated by the maternal pituitary-adrenal axis and release of maternal glucocorticoids since it was (i) mimicked by hydrocortisone administration; (ii) blocked by dexamethasone, which inhibits the stress-induced increase in plasma glucocorticoids; and (iii) diminished by mitotane. It is likely that maternal steroids directly affected the embryos, since steroid hormones can cross the placenta (*19*).

The precise mechanisms responsible for the persistence of catecholamines in these embryos remain to be defined. Glucocorticoid hormones exert a complex variety of actions on developing neuronal tissue, including prolonged survival of degenerating extra-adrenal chromaffin tissue in vivo (*20*), preferential selection of noradrenergic traits in cell populations expressing more than one neurotransmitter phenotype in vitro (*21*), and effects on the cell cycle that alter the sequence of neuronal genesis during development of the central nervous system (*22*). Although it is unclear which of these actions is relevant to the noradrenergic gut cells, several tentative conclusions may be warranted. Glucocorticoids probably do not simply enhance survival of catecholaminergic cells in the gut, since previous work has suggested that the cells normally persist even after losing most noradrenergic characters (*6, 7*). Moreover, reserpine probably causes other noradrenergic characters (such as the catecholamine biosynthetic enzymes) to persist, since these traits appear and disappear simultaneously under normal circumstances (*6*).

Regardless of the intracellular mechanisms involved, it is clear that maternal experience can affect phenotypic expression in developing embryonic neurons. Specifically, our studies indicate that maternal hormones, such as glucocorticoids, may significantly influence the developing nervous system. In addition to regulating normal neurologic development, it is entirely possible that maternal steroids, directly or indirectly, contribute to abnormal development and birth defects when they are present in increased concentration. The presumptive neuroblasts in the embryonic gut may be a valuable system for studying these issues.

G. MILLER JONAKAIT
MARTHA C. BOHN
IRA B. BLACK
Division of Developmental Neurology, Cornell University Medical College, New York 10021

References and Notes

1. S. R. Tonge, *Br. J. Pharmacol.* **47**, 425 (1973); J. W. Dailey, *Neuropharmacology* **17**, 583 (1978).
2. C. Lau, M. Bartolomé, T. A. Slotkin, *Neuropharmacology* **16**, 473 (1977); T. A. Slotkin and T. R. Anderson, *Addict. Dis.* **2**, 293 (1975).
3. J. Bartolomé, F. J. Seidler, T. R. Anderson, T. A. Slotkin, *J. Pharmacol. Exp. Ther.* **197**, 293 (1976).
4. S. Hay and H. Barbano, *Teratology* **6**, 271 (1972).
5. I. L. Ward and J. Weisz, *Science* **207**, 328 (1980); J. A. Moyer, L. R. Herrenkohl, D. M. Jacobowitz, *Brain Res.* **144**, 173 (1978).
6. P. Cochard, M. Goldstein, I. B. Black, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2986 (1978); G. Teitelman, T. Joh, D. J. Reis, *Brain Res.* **158**, 229 (1978).
7. G. M. Jonakait, J. Wolf, P. Cochard, M. Goldstein, I. B. Black, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4683 (1979).
8. A. Carlsson, in *Handbook of Experimental Pharmacology*, V. Erspamer, Ed. (Springer-Verlag, New York, 1965), vol. 19.
9. B. Kovacic and R. L. Robinson, *J. Pharmacol. Exp. Ther.* **152**, 37 (1966).
10. B. Falck and C. Owman, *Acta Univ. Lund Sect. 2* (No. 7) (1965).
11. R. A. Mueller, H. Thoenen, J. Axelrod, *J. Pharmacol. Exp. Ther.* **169**, 74 (1969); *Eur. J. Pharmacol.* **10**, 51 (1970).
12. H. Thoenen, R. A. Mueller, J. Axelrod, *Nature (London)* **221**, 1264 (1969).
13. U. Otten and H. Thoenen, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1415 (1975).
14. I. Hanbauer, W. Lovenberg, A. Guidotti, E. Costa, *Brain Res.* **96**, 197 (1975).
15. L. A. Carr and K. E. Moore, *Neuroendocrinology* **3**, 285 (1968); R. P. Maickel, E. O. Westermann, B. B. Brodie, *J. Pharmacol. Exp. Ther.* **134**, 167 (1961).
16. J. S. Meyer, D. J. Micco, B. S. Stephenson, L. C. Krey, B. S. McEwen, *Physiol. Behav.* **22**, 867 (1979).
17. E. Zimmerman and V. Critchlow, *Am. J. Physiol.* **216**, 148 (1969).
18. A. A. Nelson and G. Woodward, *Arch. Pathol.* **48**, 387 (1949); O. Vilar and W. W. Tullner, *Endocrinology* **65**, 80 (1959).
19. M. Dalle and P. Delost, *J. Endocrinol.* **82**, 43 (1979); K. Milkovic, C. A. Winget, T. Deguchi, J. D. Barchas, S. Levine, R. Ciaranello, *Am. J. Physiol.* **226**, 864 (1974); G. Carson, J. D. Bolla, J. R. G. Challis, *Endocrinology* **104**, 1053 (1979).
20. O. Erankö and L. Erankö, *Acta Physiol. Scand.* **84**, 125 (1972); M. Lempinen, *Acta Physiol. Scand. Suppl.* **62**, 231 (1964).
21. I. S. McLennan, C. E. Hill, I. A. Hendry, *Nature (London)* **283**, 206 (1980); D. Sandquist, A. C. Black, Jr., S. K. Sahu, L. Williams, T. H. Williams, *Exp. Cell Res.* **123**, 417 (1979); D. H. Edgar and H. Thoenen, *Brain Res.* **154**, 186 (1978).
22. M. C. Bohn and J. M. Lauder, *Dev. Neurosci.* **1**, 250 (1978).
23. This work was supported by NIH grants NS 10259 and HD 12108 and aided by a grant from the Dysautonomia Foundation Inc. G.M.J. is a recipient of NIH fellowship NS 06142. I.B.B. is a recipient of the Irma T. Hirschl Career Scientist Award. M.C.B. is a recipient of NIH fellowship NS 06400. We thank Jody Cohen and Elise Grossman for excellent technical assistance.

28 July 1980

Differential Development of Brainstem Potentials in Healthy and High-Risk Infants

Abstract. *Maturation along the brainstem acoustic pathway, as well as the integrity of these structures, has been shown to be reflected in brainstem evoked potential recordings. Trajectories formed from repeated sequential measurements of several brainstem response variables reveal distinct developmental curves for healthy and high-risk infants. Longitudinal analysis offers a means of determining temporary or permanent maturational effects on the central nervous system in early life.*

The infant born at "high risk" for central nervous system (CNS) damage is more likely to manifest neurological and intellectual deficits in early childhood (*1*). Certain brainstem structures along the auditory pathway are preferentially vulnerable to many of the perinatal conditions that compromise the infant (*2*). Subtle impairments to these subcortical regions may go unnoticed until blatant behavioral defects become apparent in later life. In this regard the brainstem auditory evoked potential (BAEP) technique offers a sensitive index of brainstem maturation and function (*3*). A strong relationship between altered BAEP's and specific or generalized sensory and neurological disorders has been documented in adults (*4*). The diagnostic value of BAEP recordings has recently been extended to newborns (*5*). Yet aberrant BAEP's at one point may or may not imply clinical significance for a later stage of development (*6*). By establishing longitudinal trajectories based on multiple observations of both healthy and sick infants, we show that the maturational curve not only differs for selected measures of the BAEP between the two groups but continues to distinguish the risk population at 1 year of age. In addition, we demonstrate the utility of the longitudinal approach in determining exactly when an individual begins to depart from the expected developmental pattern.

A total of 342 infants participated in this study. All subjects were seen at least twice, but most were seen on three or more occasions from birth to 1 year of age (corrected for prematurity). Of these, 245 were full-term normal babies born without incident. The remaining 97 subjects had been kept in the intensive care nursery (*7*). These infants sustained various complications of birth or pregnancy and were judged to be at risk for CNS involvement. Most were born prematurely and had suffered respiratory distress, some form of oxygen deprivation (asphyxia, hypoxia, and so forth), or both.

All normal babies were tested in open cribs, and most high-risk infants in iso-

0036-8075/80/1031-0553$00.50/0