References and Notes

1. The available terminology was criticized by G. H. Dury, *Earth Sci. J.* **3**, 77 (1969).
2. G. H. Dury, in *Studies in Australian Geography*, G. H. Dury and M. I. Logan, Eds. (Heinemann, Melbourne, Australia, 1968), pp. 16–20.
3. ———, *Encyclopaedia Britannica*: "Duricrusts," in press.
4. ——— and T. Langford-Smith, *Aust. J. Sci.* **27**, 171 (1964); T. Langford-Smith and G. H. Dury, *Amer. J. Sci.* **263**, 170 (1965); R. B. McConnell, *Geogr. J.* **134**, 506 (1968).
5. G. H. Dury, *Geogr. J.*, in press.
6. W. E. Parham, *Clay Mineralogy and Geology of Minnesota's Kaolin Clays* (Minnesota Geological Survey, Minneapolis, 1970). We thank W. E. Parham for drawing our attention to so he'pful a work.

11 June 1971; revised 17 August 1971

Norepinephrine Stimulated Increase of Cyclic AMP Levels in Developing Mouse Brain Cell Cultures

Abstract. *Norepinephrine causes a four- to sixfold increase in the intracellular level of cyclic AMP (adenosine 3′,5′-monophosphate) reaggregated brain cell cultures derived from embryonic mouse brain. The cyclic AMP level of adult brain is increased by norepinephrine; however, embryonic mouse brain does not show a cyclic AMP response. The aggregate cultures thus demonstrate an event of differentiation very similar to that seen in vivo.*

Norepinephrine produces a fivefold increase in the level of cyclic AMP (adenosine 3′,5′-monophosphate) in slices of cerebral cortex of both rat and mouse (*1*). This stimulation of cyclic AMP levels is not observed in brains of newborn rats and appears only after 4 days of age (*2*). Thus, the response to norepinephrine can be considered to be a developmental event occurring during brain maturation.

Recently a brain cell culture system has been developed which shows patterns of biochemical differentiation similar to that of maturing mouse brain in vivo (*3*). This cell culture system is founded on the ability of dissociated cells to reassociate and to form aggregates during rotation culture (*4*). The brain cell aggregates are organized structures that often possess a cellular architecture resembling normal brain tissue (*5*). In addition, electron microscopic observations show the presence of highly developed synaptic regions in the aggregates after several weeks in culture (*6*).

Since the brain cell aggregates undergo several events of differentiation during culture, we determined their ability to develop a cyclic AMP response to norepinephrine. Isoproterenol was also used to test the β-adrenergic nature of the effector mechanism and to eliminate the possibility of inhibitory effects of α-adrenergic receptor stimulation on accumulation of cyclic AMP. The results are shown in Table 1. As expected, the fetal tissue does not respond to norepinephrine or isoproterenol. Although aggregate formation is essentially complete by 15 hours of culture, the catecholamines again fail to elicit an increase in levels of cyclic AMP in the cells. However, after 9 days of culture the aggregates show a four- to sixfold increase in cyclic AMP levels in response to both norepinephrine and isoproterenol—an effect of similar magnitude to that seen in adult mouse brain.

Other experiments (not shown) have indicated that the magnitude of the response does not change with longer culture times. In addition, when incubations were done in the absence of theophylline, the catecholamine-stimulated level of the cyclic nucleotide was not appreciably different from the levels presented in Table 1. In this respect the aggregate cell cultures also resemble slices of guinea pig and rabbit brain (*1*).

Clonal lines of rat glial tumors show large (> 200-fold) stimulatory effects of catecholamines on intracellular levels of cyclic AMP (*7*), suggesting that such response in normal brain and in the cultures described above may be due primarily to responding glial elements. Furthermore, primary monolayer cultures derived from embryonic rat brain show similar 50- to 100-fold stimulatory effects of catecholamines (*8*). Since considerable cell division takes place in the monolayer cultures, there is undoubtedly enrichment for glial cells, supporting the hypothesis that predominant cell type responding to norepinephrine in rat brain is glial.

The more "normal" magnitude of the response seen in the aggregates may be reflection of more natural proportions of neurons and glia present in these cultures. Presumably this is a consequence of the restriction of cellular division in a system where cell-cell interactions are prominent (*3*). The value of aggregate cell cultures is indicated by the fact that the development of norepinephrine responsiveness in these aggregates is the fifth phenomenon they have shown that is characteristic of the program of differentiation of the developing mouse brain. Others include increased specific activities of choline acetyltransferase, acetylcholinesterase, glutamate decarboxylase (*3*), and the formation of synapses (*6*).

Table 1. Effect of catecholamines on cyclic AMP levels of fetal mouse brain slices and brain cell aggregates. Brain cell aggregates and fetal tissue were preincubated for 30 minutes in Eagle's basal medium plus 0.4 percent glucose and $10^{-3}M$ theophylline at 37°C in an atmosphere of 5 percent CO_2 and 95 percent air. Norepinephrine and isoproterenol were added at a concentration of $10^{-4}M$ each and incubation was continued for 15 minutes. The medium was rapidly removed and the aggregates or tissue were homogenized in ice-cold 5 percent trichloroacetic acid. After centrifugation at 15,000*g* for 5 minutes, the supernatants were assayed for cyclic AMP content (*9*). The trichloroacetic acid precipitate was resuspended in 0.1*N* NaOH and assayed for protein (*10*).

Additions	Cyclic AMP (pmole/mg of protein)		
	Fetal tissue	Cell culture	
		15 hours	9 days
None	11	12	8.5
Norepinephrine	11	10	38
Isoproterenol	14	14	45

NICHOLAS W. SEEDS
Departments of Biophysics and Genetics and Psychiatry, University of Colorado Medical Center Denver 80220
ALFRED G. GILMAN*
Laboratory of Biochemical Genetics, National Heart and Lung Institute, Bethesda, Maryland 20014

References and Notes

1. T. W. Rall and A. Sattin, in *Role of Cyclic AMP in Cell Function*, P. Greengard and E. Costa, Eds. (Raven, New York, 1970), p. 113.
2. M. J. Schmidt, E. C. Palmer, W-D. Dettbarn, G. A. Robison, *Develop. Psychobiol.* **3**, 53 (1970).
3. N. W. Seeds, *Proc. Nat. Acad. Sci. U.S.* **68**, 1858 (1971).
4. A. A. Moscona, in *Cells and Tissues in Culture*, B. M. Willmer, Ed. (Academic Press, New York, 1965), p. 483.
5. G. R. DeLong, *Develop. Biol.* **22**, 563 (1970); R. L. Sidman, in *The Neurosciences Second Study Program*, F. O. Schmidt, Ed. (Rockefeller Univ. Press, New York, 1970), p. 100.
6. N. W. Seeds and A. E. Vatter, *Proc. Nat. Acad. Sci. U.S.*, in press.
7. A. G. Gilman and M. W. Nirenberg, *ibid.* **68**, 2165 (1971).
8. A. G. Gilman and B. K. Schrier, in preparation.
9. A. G. Gilman, *Proc. Nat. Acad. Sci. U.S.* **67**, 305 (1970).
10. O. Lowry, N. Rosebrough, A. Farr, J. Randall, *J. Biol. Chem.* **193**, 265 (1951).

11. Supported in part by PHS grant (NS-09818). This paper is No. 479 from the Eleanor Roosevelt Institute for Cancer Research and the Department of Biophysics and Genetics, University of Colorado Medical Center.

* Present address: Department of Pharmacology, University of Virginia School of Medicine, Charlottesville, 22903.

6 July 1971