transcriptase activity (*10–12*), transforming ability in vitro (*14*), and poly(A) content and size.

D. GILLESPIE
Litton Bionetics, Inc.,
Bethesda, Maryland 20014
K. TAKEMOTO
Laboratory of Viral Disease,
National Institute of Allergy and
Infectious Diseases, Bethesda 20014
M. ROBERT, R. C. GALLO
Laboratory of Tumor Cell Biology,
National Cancer Institute,
Bethesda 20014

References and Notes

1. D. Gillespie, S. Marshall, R. C. Gallo, *Nature New Biol.* **69**, 227 (1972).
2. M. M. C. Lai and P. H. Duesberg, *Nature* **235**, 383 (1972); M. Green and M. Cartas, *Proc. Nat. Acad. Sci. U.S.A.* **69**, 791 (1972).
3. Y. Yoga and E. Wimmer, *Proc. Nat. Acad. Sci. U.S.A.* **69**, 1877 (1972).
4. L. Lim and E. S. Canellakis, *Nature* **227**, 710 (1970).
5. J. E. Darnell, personal communication.
6. ———, L. Philpson, R. Wall, M. Adesnik, *Science* **174**, 507 (1971).
7. H. Thormar, *Nat. Inst. Neurol. Dis. Blindness Monogr. 2* (1965), p. 335.
8. K. K. Takemoto, C. F. T. Mattern, L. B. Stone, J. E. Coe, G. Lavelle, *J. Virol.* **7**, 301 (1971); J. E. Coward, D. H. Harter, C. Morgan, *Virology* **40**, 1031 (1970).
9. D. H. Harter, J. Schlom, S. Spiegelman, *Biochim. Biophys. Acta* **240**, 435 (1971).
10. L. B. Stone, E. A. Scolnick, K. K. Takemoto, S. A. Aaronson, *Nature* **229**, 257 (1970).
11. F. H. Lin and H. J. Thormar, *Virology* **6**, 702 (1970).
12. J. Schlom, D. H. Harter, A. Burny, S. Spiegelman, *Proc. Nat. Acad. Sci. U.S.A.* **68**, 182 (1971).
13. L. B. Stone, K. K. Takemoto, M. A. Martin, *J. Virol.* **8**, 573 (1971).
14. K. K. Takemoto and L. B. Stone, *ibid.* **7**, 770 (1971); E. H. MacIntyre, C. J. Wintersgill, H. Thormar, *Nature New Biol.* **237**, 111 (1972).
15. M. Robert, D. Gillespie, R. Gallo, unpublished observations.
16. D. L. Fine, E. W. Kingsbury, M. G. Valerio, M. T. Kubicek, J. C. Landon, H. C. Chopra, *Nature New Biol.* **238**, 191 (1972).
17. D. Slater, I. Slater, D. Gillespie, *Nature*, in press.
18. D. Slater and D. Gillespie, unpublished results.
19. J. A. Armstrong, M. Edmonds, H. Nakazato, B. A. Phillips, M. H. Vaughan, *Science* **176**, 526 (1972).
20. R. E. Pemberton and C. Baglioni, *J. Mol. Biol.* **65**, 531 (1972).
21. In collaboration with S. Spiegelman and coworkers and W. Feller and co-workers.
22. We thank S. Gillespie and C. Davis for assistance in preparing and analyzing the visna RNA; to A. Shatkin, D. Housman, J. Schlom, and S. Marshall for preparations of EMC virus RNA, rabbit reticulocyte hemoglobin mRNA, MP-MV RNA, and mouse liver total RNA, respectively. M.R. holds a fellowship from the Leukemia Society of America, Inc.

14 September 1972; revised 25 October 1972

Isolated Adrenal Cortex Cells: Hypersensitivity to Adrenocorticotropic Hormone after Hypophysectomy

Abstract. *Cells from the adrenals of hypophysectomized rats (up to 28 days after operation) require less adrenocorticotropic hormone to induce one-half maximal rate of production of 3′,5′-adenosine monophosphate than do cells from the adrenals of intact rats. A corresponding increase in sensitivity is reflected in the steroidogenic response to adrenocorticotropic hormone up to 2 days after hypophysectomy.*

In the absence of pituitary tropic hormones, target endocrine glands develop a depression of secretory capacity. For example, in vivo studies have demonstrated that hypophysectomy is followed by a reduction in the rate of secretion of corticosteroids in response to a given dose of adrenocorticotropic hormone (ACTH) (*1*). On the other hand, rate of accumulation of the "second messenger," 3′,5′-adenosine monophosphate (cyclic AMP) is not diminished in response to the administration of ACTH (*2*). This interesting difference prompted us to examine the two responses in vitro; both ACTH-induced accumulation of cyclic AMP and steroidogenesis have been determined in suspensions of isolated cells prepared from adrenals removed at various times after hypophysectomy.

Suspensions of cells were prepared from the fasciculata-reticularis region of 32 to 40 rat adrenals according to the method of Sayers *et al.* (*3*). Thirty microcuries of [8-^{14}C]adenine were added to the dispersion medium for the labeling of adenosine triphosphate, the precursor of cyclic AMP. After dispersion with trypsin, the cell suspensions were centrifuged, and the pellet was resuspended in a volume of buffer medium so that each 0.9-ml portion taken for incubation contained approximately 350,000 cells. Cortrosyn ($ACTH_{1-24}$) (Organon Inc.) was added in a volume of 0.1 ml of buffer medium. This medium only, in a volume of 0.1 ml, was added to blanks. After 60 minutes of incubation, cells plus medium were extracted with methylene chloride. In samples of the aqueous layer cyclic AMP labeled with ^{14}C was determined by the method of Kuo and De Renzo (*4*), as modified for use in isolated adrenal cortex cells by Beall and Sayers (*5*). Corticosterone in the methylene chloride extracts was determined by the method of Silber *et al.* (*6*).

Logarithmic dose response curves for production of cyclic AMP and of corticosterone by isolated adrenal cortex cells (350,000 cells per milliliter of incubate) in response to various doses of $ACTH_{1-24}$ are displayed in Figs. 1 and 2, respectively. The isolated cells are characterized by the following parameters: (i) $cAMP_{max}$ and B_{max}, the maximum rate of production of cyclic AMP and the maximum rate of production of corticosterone, respectively, and (ii) A_{50C} and A_{50B}, the doses of $ACTH_{1-24}$ that induce one-half $cAMP_{max}$ and B_{max}, respectively. Computer estimates of these parameters with standard errors represent least square fits by a nonlinear method (*7*). The data presented in Fig. 1 show that $cAMP_{max}$ for cells prepared from the adrenals of hypophysectomized rats was greater than $cAMP_{max}$ for cells from intact rats. The increases for rats hypophysectomized for 2, 14, and 28 days were 33 ± 2.6, 15 ± 2.5, and 25 ± 3.4 percent, respectively, over values for intact rats. The A_{50C} was estimated to be 760 ± 150 pg for cells from intact rats and 160 ± 20, 240 ± 30, and 160 ± 30 pg for cells from rats hypophysectomized for 2, 14, and 28 days, respectively. The data indicate that the cells from hypophysectomized rats are about five times more sensitive to ACTH than cells from intact rats.

As expected, B_{max} progressively decreased with time after hypophysec-

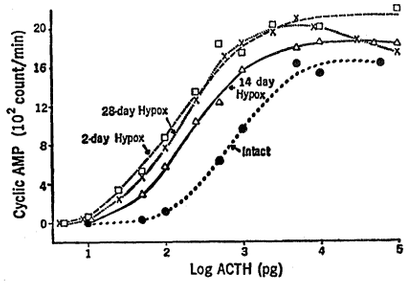

Fig. 1. Logarithmic dose response curves for cyclic AMP production by samples of adrenal cortex cells in suspension prepared from the fasciculata-reticularis region of adrenals of intact rats and of rats killed 2, 14, and 28 days after hypophysectomy. Net cyclic AMP production, as counts per minute of cyclic [8-^{14}C]AMP per 350,000 cells per 60 minutes of incubation, is plotted on the ordinate; dose of $ACTH_{1-24}$ in picograms on the abscissa. The points are the means of analyses on three samples of the cell suspension; *Hypox*, hypophysectomized.

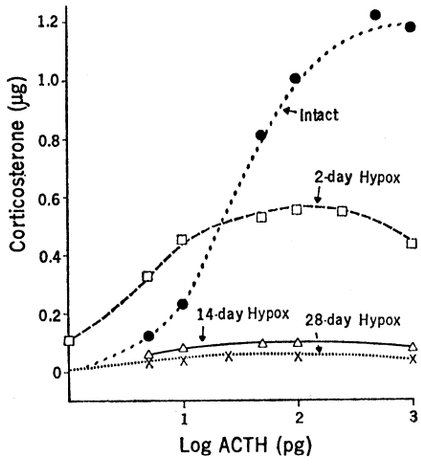

Fig. 2. Logarithmic dose response curves for corticosterone production by samples of adrenal cortex cells in suspension prepared from the fasciculata-reticularis region of adrenals of intact rats and of rats killed 2, 14, and 28 days after hypophysectomy. Net corticosterone production (ACTH stimulated minus blanks) per 350,000 cells per 60 minutes of incubation is plotted on the ordinate; dose of $ACTH_{1-24}$ in picograms on the abscissa. The points are the means of analyses on three samples of the cell suspension. Insulin or glucagon in a dose of 100 μg did not stimulate steroidogenesis when added to suspensions of cells from the adrenals of intact rats or of hypophysectomized rats; *Hypox*, hypophysectomized.

tomy (Fig. 2). After 14 and after 28 days, corticosterone production is markedly reduced. However, after 2 days, at which time B_{max} for cells from hypophysectomized rats is reduced by only 50 percent, sensitivity to ACTH is increased, as shown by the crossing-over of the curves for cells from rats hypophysectomized for 2 days and for cells from intact rats. The A_{50B} for cells from hypophysectomized rats was estimated to be 3.34 ± 0.34 pg, while that for cells from intact rats was 27.3 ± 2.2 pg—a highly significant difference ($P < .001$). At a dose of 1.0 pg, ACTH induced no response in cells from intact rats and 0.12 μg of corticosterone in cells from rats hypophysectomized for 2 days; at 5 pg, 0.11 μg of corticosterone was produced in intact rats and 0.33 μg in those hypophysectomized for 2 days; and at 10 pg, the values were 0.22 and 0.45 μg, respectively. In each of ten additional experiments, cells from rats hypophysectomized for 2 days have exhibited the same order of hypersensitivity to ACTH in terms of production of corticosterone.

Since after hypophysectomy the capacity to produce cyclic AMP is slightly enhanced, we may conclude that the receptor–adenylate cyclase complex of the plasma membrane retains its functional integrity after hypophysectomy. Of considerable interest is the hypersensitivity to ACTH as reflected in marked decreases in the quantity of the polypeptide hormone required to induce A_{50C}. Hypersensitivity is also reflected in the quantity of ACTH required to induce A_{50B}, provided the cells are examined before drastic reductions in steroidogenic capacity have developed.

The changes observed with time after hypophysectomy cannot be ascribed to alteration of cells of the glomerulosa; this region of the gland was removed by decapsulation. It seems unlikely, but we cannot rule out the possibility, that the differences in sensitivity are a result of differences in the effect of the trypsinized buffer medium on adrenal tissue from hypophysectomized and from intact rats. Also unlikely is the possibility that cells from intact rats in contrast to cells from hypophysectomized rats have a fraction of their receptors occupied by endogenous ACTH at the time of incubation with added ACTH. In the first place, the cells are exposed to trypsin during the dispersion process and ACTH is an excellent substrate for this enzyme. In the second place, cells from intact rats, incubated 60 minutes in the absence of added ACTH, produce no significant quantity of corticosterone. Among possibilities to be explored we include (i) increased number of receptors per cell, (ii) more efficient coupling of the signal generated by ACTH-receptor interaction and activation of adenylate cyclase, (iii) alterations in affinity of ACTH for receptors, and (iv) decrease in phosphodiesterase.

Classic studies in hypophysectomized animals clearly indicate that, with time after removal of the pituitary, a given dose of ACTH induces progressively less of an increase in corticosteroids secreted into the adrenal vein (*1*). Reduction in the capacity of the cells of the adrenal cortex to produce corticosterone after hypophysectomy appears to be a result of functional atrophy of the cellular processes involved in steroid biosynthesis. For example, the contents of P-450 in the mitochondria and the microsomes of the adrenal cortex undergo progressive decreases with time after hypophysectomy with an estimated half-life of 3 to 4 days (*8*). In the absence of ACTH, we presume that lack of cyclic AMP is at least partly responsible for the atrophy, as suggested by the observations of Ney (*9*) to the effect that administration of dibutyryl cyclic AMP to hypophysectomized rats results in partial maintenance of adrenal weight and content of DNA, RNA, and protein. Dibutyryl cyclic AMP also partially maintains corticosterone production in response to the administration of ACTH. In the hypophysectomized animal, rate of secretion of corticosteroids into the adrenal vein in response to ACTH is determined to an important degree by the functional integrity of the steroid biosynthetic machinery. The isolated adrenal cortex cell system reveals that hypersensitivity to ACTH does exist in the brief period following removal of the pituitary before atrophy becomes marked.

The phenomenon described here for the adrenal cortex has certain similarities to denervation hypersensitivity of skeletal muscle. Following denervation, skeletal muscle is hypersensitive to acetylcholine (*10*), and contractile force progressively decreases (*11*). In analogy to denervation hypersensitivity we suggest "detropication hypersensitivity" for the changes that occur in cells of the adrenal cortex after loss of its tropic hormone.

GEORGE SAYERS, ROBERT J. BEALL
Department of Physiology, School of Medicine, Case Western Reserve University, Cleveland, Ohio 44106

References and Notes

1. W. F. Ganong, in *Review of Medical Physiology*, W. F. Ganong, Ed. (Lange Medical, Los Altos, Calif., ed. 5, 1971), p. 279; G. W. Liddle, D. Island, C. K. Meador, *Rec. Progr. Horm. Res.* **18**, 125 (1962).
2. D. B. Grahame-Smith, R. W. Butcher, R. L. Ney, E. W. Sutherland, *J. Biol. Chem.* **242**, 5535 (1967).
3. G. Sayers, R. L. Swallow, N. D. Giordano, *Endocrinology* **88**, 1063 (1971).
4. J. F. Kuo and E. D. De Renzo, *J. Biol. Chem.* **244**, 2252 (1969).
5. R. J. Beall and G. Sayers, *Arch. Biochem. Biophys.* **148**, 70 (1972).
6. R. H. Silber, R. O. Busch, R. Oslapas, *Clin. Chem.* **4**, 278 (1958).
7. W. W. Cleland, *Advan. Enzymol.* **29**, 1 (1967).
8. J. L. Purvis, J. A. Canick, J. I. Mason, J. L. McCarthy, R. W. Estabrook, *Proceedings of the Fourth International Congress on Endocrinology, Washington* (1972), abstr., p. 40; D. R. Pfeiffer, J. W. Chu, T. H. Kuo, S. W. Chan, T. Kimura, T. T. Tchen, *Biochem. Biophys. Res. Commun.* **48**, 486 (1972).
9. R. L. Ney, *Endocrinology* **84**, 168 (1969).
10. R. Miledi, *J. Physiol.* **151**, 1 (1960).
11. T. Kiku-Iri, *Jap. J. Physiol.* **14**, 400 (1964).
12. Supported by NSF grant GB-27426. We thank Theodore W. Rall for his comments on the manuscripts and appreciate the skilled technical assistance of Mary Vegh and Barbara Brady; Kevin Cummins helped in certain phases of the experiments. We are grateful to H. Strade of Organon Inc. for supplies of Cortrosyn. S. Seelig estimated parameters and standard errors by computer.

22 November 1972