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 24. Assuming a cell volume of 30 pl, a Ca^{2+} current of 0.5 nA would correspond to an increase in $[\text{Ca}^{2+}]_i$ of 0.08 $\mu\text{M}/\text{msec}$. The fura-2 Ca^{2+} transient suggests that $[\text{Ca}^{2+}]_i$ is changing at not less than 0.03 $\mu\text{M}/\text{msec}$ at 5 msec after depolarization. Assuming that the free Ca^{2+} represents 2% of the total Ca^{2+} released (the remainder being rapidly bound), then intracellular calcium must be rising at about 1.5 $\mu\text{M}/\text{msec}$. Thus the rate of Ca^{2+} release by the SR is at least an order of magnitude greater than the flux arising from the Ca^{2+} current.
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 26. There is increasing evidence for more than one Ca^{2+} channel type in various tissues. In atrial cells, B. P. Bean [*J. Gen. Physiol.* **86**, 1 (1985)] showed a low threshold Ca^{2+} channel that activated at about -40 mV. B. Nilius, P. Hess, J. B. Lansman, and R. W. Tsien [*Nature (London)* **316**, 443 (1985)] described a T-type Ca^{2+} channel in guinea pig ventricular muscle. Although our Ca^{2+} currents do not exhibit current components that might be ascribed to T-type Ca^{2+} channels, it is possible that they were undetected because we measured the D-600-sensitive current. It is not known whether D-600 blocks T-type Ca^{2+} channels. If this type of channel were present in rat ventricular cells, it might provide the Ca^{2+} needed to activate the CICR mechanism in the voltage range where we first detect changes in $[\text{Ca}^{2+}]_i$.
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 31. Supported by NIH grant HL25675 (W.J.L.) and by grants from the American Heart Association and the Maryland Heart Association to M.B.C., J.R.B., and W.J.L. W.J.L. is an Established Investigator of the American Heart Association and its Maryland Affiliate. M.B.C. was supported by a Young Investigatorship of the Maryland Affiliate of the American Heart Association. We thank K. MacEachern for assistance in manuscript preparation.

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Technical Comments

Virus-Induced Increases in Plasma Corticosterone

E. M. Smith *et al.* (1) reported results consistent with the hypothesis that cells of the immune system are able to initiate an adrenocortical stress response by releasing adrenocorticotropin (ACTH), indicating a "lymphoid-adrenal axis." The basis for their hypothesis was the observation that injection of hypophysectomized mice with Newcastle disease virus (NDV) elevated plasma concentrations of corticosterone, together with their earlier observation that lymphocytes in vitro responded to NDV exposure by releasing ACTH (2).

Smith *et al.* report testing the completeness of hypophysectomy by visual inspection of the sella tursica; functional testing by the stress of cold-water immersion was performed in a separate group of mice that did not receive NDV. This is important because Moldow and Yalow (3) have shown that, unless all corticotrophic tissue is removed, cells may survive, multiply, and eventually restore full corticotrophic function. We have repeated the experiments of Smith *et al.* and obtained somewhat different results in hypophysectomized mice in which the completeness of hypophysectomy was verified by prior restraint or CRF administration.

In our experiments (4), each mouse was tested for the completeness of hypophysectomy (with restraint) and for adequate adrenocortical function (by injecting ACTH) before we administered NDV. Three days after hypophysectomy, each mouse was placed in a restraining device for 60 minutes, after which approximately 150 μl of blood was collected from the tail vein (in 5

to 15 minutes). On the next day, each mouse was injected subcutaneously with ACTH₁₋₂₄ (Organon, 1 $\mu\text{g}/\text{g}$), and tail blood was again sampled 30 minutes later. On the fifth day after hypophysectomy, mice were intraperitoneally injected with NDV (0.3 ml) or control allantoic fluid (5) and were decapitated 8 hours after injection to collect trunk blood. Plasma corticosterone was assayed by radioimmunoassay (6).

We present in Table 1 the combined results of two separate experiments with the same design (7). Hypophysectomized mice

did not show elevated plasma corticosterone concentrations after restraint. ACTH-induced concentrations of plasma corticosterone were slightly lower in hypophysectomized mice than in sham-operated controls, consistent with a small loss of adrenocortical sensitivity. As would be expected after removal of the pituitary, the plasma of hypophysectomized mice contained lower concentrations of corticosterone than did sham-operated controls, whether or not they were injected with NDV. NDV-injected sham-operated mice showed statistically significant elevations of plasma corticosterone relative to vehicle-injected controls. In these two experiments, there was a small increase in the plasma corticosterone concentrations of hypophysectomized mice injected with NDV as compared with vehicle, but this effect was not statistically significant. Thus hypophysectomy prevented the

Table 1. Plasma corticosterone concentrations (nanograms per milliliter) after NDV administration to hypophysectomized or sham-operated mice. Three days after hypophysectomy mice were tested for completeness of hypophysectomy by restraining them for 60 minutes and collecting a sample of blood from the tail vein. Adrenocortical responsiveness was tested the next day 30 minutes after subcutaneous injection of ACTH₁₋₂₄ (1 $\mu\text{g}/\text{g}$). On the fifth day after hypophysectomy, mice were injected intraperitoneally with 0.3 ml of NDV (750 hemagglutination units) or with control allantoic fluid 8 hours before trunk blood was collected for assay of corticosterone by radioimmunoassay. Three hypophysectomized mice displaying plasma corticosterone concentrations greater than 50 ng/ml after restraint and two exhibiting corticosterone concentrations less than 100 ng/ml after ACTH were excluded from the hypophysectomized group. The results after NDV injection were similar whether or not any mice were excluded.

Treatment-injection	Plasma corticosterone concentrations (ng/ml)			
	N	Prior restraint	ACTH injection	NDV injection
Sham-operated				
Vehicle	17	284 ± 19	409 ± 25	85 ± 13
NDV	17	284 ± 23	448 ± 34	167 ± 21*
Hypophysectomized				
Vehicle	17	19 ± 3	327 ± 38†	21 ± 5
NDV	16	24 ± 4	336 ± 39†	37 ± 9

*NDV injection caused a statistically significant elevation of plasma corticosterone compared with that in the vehicle-injected group in sham-operated mice [$t(df = 32) = 3.29, P < 0.005$], but not in hypophysectomized mice. †The plasma concentrations of corticosterone after ACTH injection were significantly lower in the hypophysectomized than in the sham-operated mice [$t(df = 65) = 2.86, P < 0.01$].

normal increase in plasma corticosterone after NDV injection.

The range of values for plasma corticosterone concentrations Smith *et al.* reported for unstressed hypophysectomized mice is similar to what we observed. We do not know whether the NDV-induced increases in plasma corticosterone they observed in hypophysectomized mice were comparable with those observed in intact or sham-operated mice, because they did not present data from the latter. Nevertheless, the values they reported for plasma corticosterone after NDV injection in hypophysectomized mice are similar to those we found in intact or sham-operated mice. The major difference between the results of our respective studies is that we did not find an increase in the plasma corticosterone concentrations of hypophysectomized mice after injection with NDV under conditions where such an increase was observed in sham-operated mice. A small proportion of verified hypophysectomized CD-1 mice (4 of 47) did show relatively high plasma corticosterone concentrations after NDV administration, and it is these few mice that accounted for the small (not statistically significant) increases observed in this group (in two of the five experiments). Thus it is possible that a small proportion of mice can initiate an adrenocortical response by an extra-pituitary mechanism. However, because we injected NDV two or more days after the restraint test, it is just as likely that some recovery of pituitary corticotroph function had occurred in these few animals. Nevertheless, it is clear that an extra-pituitary mechanism cannot account quantitatively for the increase in plasma corticosterone normally observed after NDV injection in intact mice.

The major respect in which our experiments differ from that reported by Smith *et al.* is that we used male CD-1 mice from Charles River, whereas they used female Swiss Webster mice from Taconic Farms (8). In separate experiments on three batches of hypophysectomized female Swiss Webster mice from Taconic Farms, we found that a high proportion of the hypophysectomies appeared to be incomplete (25 of 57 mice did not pass our restraint test, as compared with 11 of 91 from Charles River). In these three batches some of the mice were not completely healthy; nevertheless the results after NDV injection were similar to those after injection of CD-1 mice.

We found that almost all of the hypophysectomized mice that we excluded because they showed increases in plasma corticosterone after restraint also responded to NDV (five of seven CD-1 mice, 13 of 16 Swiss Webster mice). Our observation that a high proportion of incompletely hypophysecto-

mized mice were among those supplied by Taconic Farms suggests that the results of Smith *et al.* may have been due to inclusion of such mice in their experiments. We note that results from only five mice tested with cold water immersion were included in their report.

Our results call into question the proposed role of lymphocytes in initiating a pituitary-adrenal response after an immune challenge and suggest rather that the pituitary is indeed normally involved in the increase in plasma corticosterone that occurs after such a challenge. We believe the response to NDV more likely involves indirect activation of pituitary ACTH release by interleukin-1, as suggested by Besedovsky *et al.* (9), and may be secondary to the fever.

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4. Hypophysectomized CD-1 male mice or sham-operated controls were obtained from Charles River (Wilmington, MA). They were housed in individual cages on a 12:12 lighting cycle (lights on at 7 a.m.).
5. NDV of the mesogenic [N. J. Roakin-1946 (Daubney)] strain was grown in chick embryos according to the procedure of Henle and Hilleman [*Diagnostic Procedures for Viral and Rickettsial Infections*, E. H. Lennette and N. J. Schmidt, Eds. (American Public Health Association, New York, 1969), pp. 483-490]. A vehicle control was obtained from uninoculated embryos. The sample of virus used was infectious in BHK cells, but no live virus was obtained from the lungs or spleens of mice 8 hours after injection. We found it necessary to use 0.3 ml of NDV inoculum to obtain 750 hemagglutination units (close to the 800 hemagglutination units in 0.2 ml described by Smith *et al.*). In pilot experiments with intact mice we verified that peak concentrations of plasma corticosterone occurred approximately 8 hours after NDV injection.
6. Corticosterone concentrations in plasma were determined by radioimmunoassay of methylene chloride-extracted plasma. The antibody and procedure of A. Gwosdow-Cohen, C. L. Chen, and E. L. Besch [*Proc. Soc. Exptl. Biol. Med.* **170**, 29 (1982)] were used.
7. We have performed five experiments in CD-1 mice involving a total of 172 mice (91 hypophysectomized). In one of these the verification of hypophysectomy was performed by injecting CRF; in this and in two other experiments, adrenocortical function was not verified, and NDV was injected 12 to 19 days after hypophysectomy. In no experiment did we observe a statistically significant increase in plasma corticosterone in hypophysectomized mice after NDV administration.
8. We have attempted to replicate the experiments of

Smith *et al.* as closely as possible. Because Smith *et al.* performed the NDV injections 5 days after hypophysectomy, this time schedule was used in our last two experiments (Table 1).

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Response: It is now well established that leukocytes produce and secrete adrenocorticotropin (ACTH) either spontaneously or in response to immunostimulants, such as Newcastle disease virus (NDV) or bacterial endotoxin (1). Furthermore, they harbor the messenger RNA for proopiomelanocortin (POMC) (2) and have a POMC response to corticotropin-releasing factor (CRF) (3). We have previously shown that sufficient ACTH was produced in NDV-infected hypophysectomized mice to elicit a corticosterone response (4). When these findings are considered collectively, the reason for the inability of A. J. Dunn *et al.* to reproduce our in vivo results is especially puzzling. These authors suggest that this is due to incomplete hypophysectomy of the animals we employed. While this is a possibility, we continue to believe this is not the case, since our plasma corticosterone concentrations for unstressed hypophysectomized mice were similar to those observed by Dunn *et al.* Furthermore, we verified the completeness of the hypophysectomy by visual inspection of the sella tursica under a dissecting microscope and by functional testing the stress of cold-water immersion. Dunn *et al.* are correct that functional testing was performed on a separate group of mice. However, it would seem unlikely that complete hypophysectomies would have segregated to this group. One important difference between the studies which could account for the discrepancy is the omission of a crucial control in the present study. We showed that the spleens of NDV-infected animals in our study actually produced ACTH. In contrast, Dunn *et al.* have not verified the production of splenocyte ACTH under their experimental conditions. Perhaps the number of verified hypophysectomized CD-1 mice (4 of 47) that did show relatively high plasma corticosterone concentrations after NDV administration was small because those mice were the only ones that produced splenocyte ACTH under the experimental conditions of Dunn *et al.* This is a particularly important control, since they employed a mesogenic strain of virus in their studies, while we used a lentogenic strain. We do not know whether leukocytes consistently produce ACTH in response to a mesogenic strain of NDV, and Dunn *et al.* have not tested this idea.

Virus-induced increases in plasma corticosterone

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