nary artery wedge method in the bovine is increased at high altitude in brisket disease. Aviado (9) showed an increase in pulmonary artery wedge pressure in hypoxia in dogs. Gilbert *et al.* (10) have demonstrated that epinephrine, norepinephrine, histamine, and 5-hydroxytryptamine at appropriate dose levels cause pulmonary venoconstriction in isolated perfused lungs and could therefore under some circumstances induce pulmonary edema.

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Prevention of Drop in Adrenocortical Activity in the 7-Day-Old Rat by Pretreatment with ACTH* (33797)

J. E. Philpott,[1] M. X. Zarrow, and V. H. Denenberg
Department of Biological Sciences and Psychology, Purdue University, Lafayette, Indiana 47907

It has been recently established that the neonatal rat responds to certain stressors with increased plasma corticosterone (1–3). The response is high at birth, diminishes to a low value by day 7, and then progressively increases, reaching the adult-type response by day 21. This biphasic pattern of corticosterone production has also been demonstrated after treatment with ACTH (4, 5).

An important question that can be raised is why the adrenal cortex fails to respond in the 7-day-old rat? Since hypophysectomy leads to a decreased sensitivity of the adrenal cortex to ACTH (6), we argued that a relative lack of ACTH might be the responsible factor and could lead to the decreased steroidogenic response to ACTH (7). On the basis of such reasoning, the following experiment was designed to test this hypothesis: neonatal rata were primed with ACTH in beeswax-oil on day 5 of age and tested with ACTH in 1% albumin on day 7.

Materials and Methods. In all experiments female Purdue-Wistar rats and their litters were maintained under normal colony conditions: a light schedule of 13 hr light and 11 hr dark (lights went on at 7:00 a.m.), a temperature range of 72–74°F, and a relative humidity of 45–55%. Food and water were given *ad libitum*. The morning on which the litters were found was designated as day 1. On this day all litters were reduced to 6 female pups and were not disturbed until day 5.

At 3:30 p.m. on day 5 the litters were removed, weighed, and marked, and given a single (subcutaneous) injection of either 2, 5, or 10 IU of ACTH[2] suspended in 0.1 ml of beeswax-oil[3] (8–10) or the vehicle alone.

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[1] Trainee in Neurobiology, 5-T01-MH-10267, NIMH.

[2] The ACTH was obtained through the courtesy of Dr. James W. Bastian, Armour Pharmaceutical Co., Kankakee, Ill.

[3] The beeswax-oil vehicle was obtained through the courtesy of Dr. Robert Kroc, Warner-Chilcott Laboratories, Morristown, N. J.

Those animals which received ACTH were killed by decapitation 1 or 2 days following treatment (i.e., at 6 or 7 days of age) and plasma samples were taken. Similarly, beeswax oil-injected controls were killed on days 5, 6, and 7. Plasma samples were stored at —15° until assayed for corticosterone by the procedure of Glick *et al.* (11).

In the second experiment animals were "primed" at 5 days of age with 2 IU of ACTH as above or given beeswax-oil (non-primed group). At 3:30 p.m. on day 7 the control rats were killed immediately. The rats treated with ACTH in 1% albumin or albumin alone were first anesthesized with 30 mg/kg of sodium pentobarbital. This was done to facilitate injection and to immobilize the animals. All injections were given intraperitoneally and plasma was collected 40 min after injection.

Results. The results of the first experiment are presented in Fig. 1. Treatment with 2, 5, and 10 IU of ACTH suspended in beeswax-

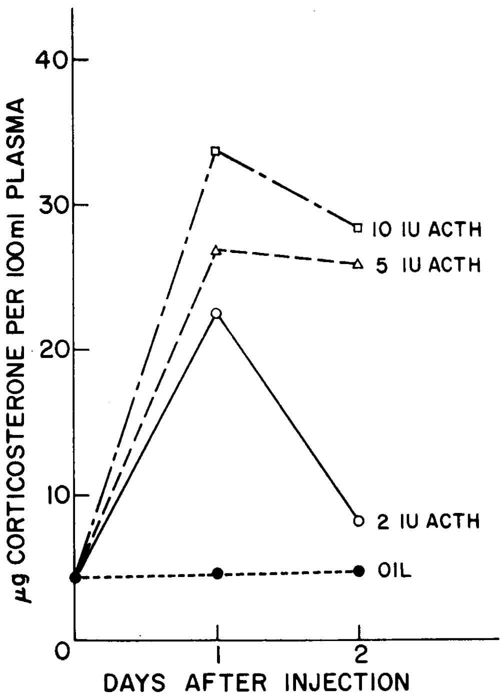

FIG. 1. Effect of priming doses of ACTH on plasma corticosterone levels at 24 and 48 hr after treatment: each point represents the average of 10–24 rats.

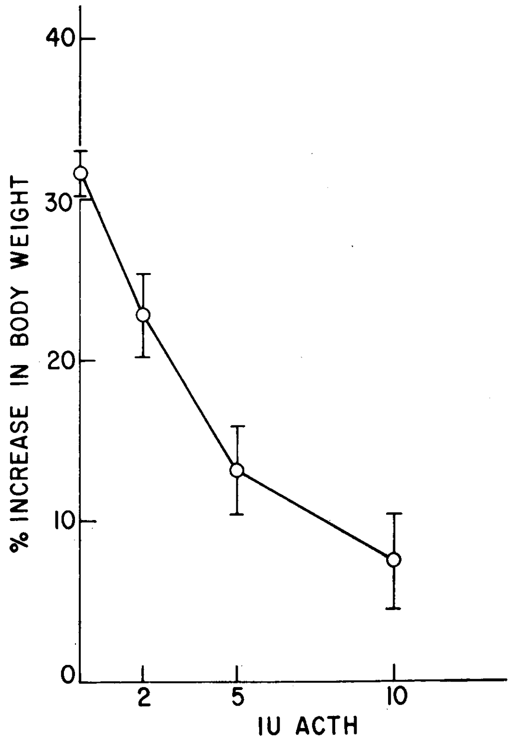

FIG. 2. Inhibition of body weight increase following a single injection of ACTH in beeswax oil: the rats were injected at 5 days of age and the weight change over a 48 hr period was obtained.

oil caused a significant elevation in plasma corticosterone levels 24 hr later. At this time plasma corticosterone levels rose from a pretreatment level of 4.34 ± 0.45 μg/100 ml to levels of 22.5 ± 1.8, 26.9 ± 2.9, and 33.7 ± 3.2 μg/100 ml in the rats treated with 2, 5, and 10 IU respectively. Oil-injected controls showed levels of 4.45 ± 0.65 μg/100 ml. By 48 hr after the injection the corticosterone fell to 8.2 ± 0.9 μg/100 ml for the groups treated with 2 IU of ACTH. This was a significant drop ($p < .01$) The corticosterone values for the 2 groups treated with 5 and 10 IU of ACTH remained high at 48 hr (25.9 ± 2.3 and 28.3 ± 3.5, respectively). Controls had levels of 4.51 ± 0.39 μg/100 ml.

In addition to affecting corticosterone, the above treatment also caused an inhibition in rate of increase of body weight. While the control rats showed a body weight increase of 31.6 ± 1.4%, the rats treated with 2, 5, and

10 IU of ACTH showed increased of 22.7 ± 2.6, 13.1 ± 2.8, and 7.4 ± 3.0%, respectively, (Fig. 2).

The above experiment indicated that the group injected with 2 IU of ACTH was a baseline value 2 days after injection. Thus, this particular combination was chosen as the priming condition for the next experiment. Rats were primed with 2 IU of ACTH suspended in beeswax-oil on day 5 of age and plasma corticosterone values obtained following a single injection of ACTH in 1% albumin on day 7 of age (plasma was collected 40 min after the injection of ACTH). The data indicate a significant rise in plasma corticosterone levels in the nonprimed rats from a level of 5.4 ± 0.5 μg/100 ml after albumin alone to 7.9 ± 0.6 ($p<0.01$) after 2 mU of ACTH (Fig. 3). However, a further increase in plasma corticosterone levels was not noted with increasing dosages of ACTH. The primed rats, however, showed a highly significant, linear log-dose response curve over the entire dosage range of 2–8 mU of ACTH ($\lambda = 0.15$). Plasma corticosterone values of 19.7 = 0.9 μg/100 ml were obtained in the primed animals followig 8 mU of ACTH whereas the nonprimed rats showed levels of 7.2 ± 1.3 μg/100 ml.

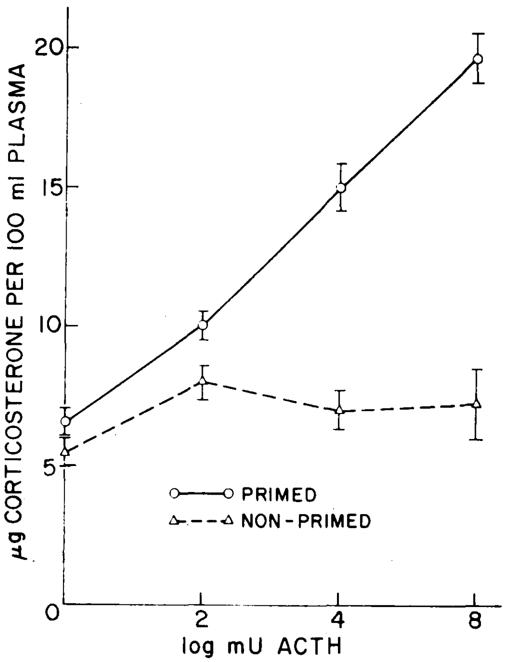

FIG. 3. Response of primed vs nonprimed 7-day-old rats to ACTH in 1% albumin: rats were primed with 2 IU of ACTH in beeswax-oil on day 5 of age, injected with test doses of ACTH in 1% albumin on day 7 of age, and blood was collected for corticosterone levels 40 min later.

Discussion. The biphasic response of the hypothalamic–hypophyseal adrenal axis in the neonatal gland is characterized by an ability of the adrenal gland of the 2- and 21-day old rat to respond to ACTH or a stressor (1–3, 4, 7, 9). The response in the 7-day-old rat, however, is markedly reduced. In an attempt to explain this finding, we have postulated that the adrenal cortex of the 7-day-old rat is temporarily deprived of ACTH, although such is not the case in the 2-day-old rat. Thus the ability of the adrenal gland of the 7-day-old rat to respond to ACTH is depressed, a situation comparable to the decreased sensitivity to ACTH seen with time following hypophysectomy (6). The current results are consistent with this hypothesis since priming the rats with ACTH results in an adrenal gland able to respond in a manner comparable to that seen in both the 2- and 21-day old.

This raises the question of what the circumstances are that might deprive the 7-day-old rat of circulating ACTH, since the hormone is present in adequate amounts in the 2-day old. One possible explanation may be found in the reports of Glydon (12) and Campbell (13). These investigators report that the hypothalamic portal system is not complete until 10–15 days of age. If this is the case one might then assume that CRF could reach the pituitary gland of the late fetus or the 2-day-old rat by diffusion [cf. Dicker, (14)] which becomes more difficult with increased growth of the rat. A point in time might be reached, as seen in the 7-day-old rat, when diffusion is inadequate and the hypothalamic-portal system is not yet complete. However, Florsheim and Rudko (15) reported that the hypothalamic–pituitary portal system is functional in the rat by the fourth postnatal day.

The inhibition of body growth obtained in the priming experiment which pharmacologic doses of ACTH confirms the well-known findings that ACTH (16–18) or glucocorticoids (19–21) inhibit body growth. Our results also indicate that the adrenal cortex of the 5–7-day-old rat may be induced to release sufficient amounts of corticosterone to produce these profound disturbances.

The decreased adrenal function in the 7-day-old rat is not due to the presence of an inhibitory level of ascorbic acid (22, 23) as adrenal levels of this vitamin also follow the biphasic pattern (24). More likely explanations lie either at the level of ACTH-dependent energy sources [Staehelin *et al.* (6)] or in the levels of the precursor, cholesterol, which Dexter *et al.* [25] have shown to be dependent upon ACTH secretion. A fourth possibility is that activity and/or levels of the enzymes necessary for steroidogenesis, themselves, may be ACTH dependent.

Summary. It was demonstrated that the plasma corticosterone response to ACTH is extremely weak in the 7-day-old rat. The present results indicate that pretreatment with 2 IU of ACTH in a beeswax-oil suspension on day 5 of age resulted in a 7-day-old rat that is capable of responding to ACTH in a manner comparable to that seen in younger and older rats. It is concluded from these studies that the "biphasic pattern" seen in the neonatal rat (characterized by a depressed adrenal response at day 7) is due to a depressed ability of the adrenal cortex to respond to ACTH. The hypothesis is presented that the depressed ability of the adrenal cortex to respond is the result of a relative lack in circulating ACTH. It is suggested that decreased ACTH secretion is the result of a failure of CRF to get to the pituitary because the hypothalamic–pituitary system is not complete in the 7-day-old rat.

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