Summary. A method is described which permits both the ascorbic acid and corticosterone content of the same adrenal gland to be determined. Adrenal response to the stress of unilateral adrenalectomy was investigated in normal and hydrocortisone-inhibited rats. Hydrocortisone administration prevented the ascorbic acid depletion which occurred in untreated animals in response to stress. The steroid-inhibited animals, however, exhibited an increase in adrenal corticosterone concentrations equal to that of the saline treated controls, although the basal level from which this increase occurred was markedly lower. It is suggested that pretreatment with hydrocortisone may alter the physiological "set" at which the corticoid component of the stress response is operating, but does not inhibit the response itself.

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Neonatal Adrenal Cortical Response to Stress and Vasopressin.* (27384)

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The neuroendocrine response to noxious stimuli is one of the basic adaptive reactions of animals. This vital homeostatic mechanism does not function at birth, at least in the rat, and apparently develops slowly during the postnatal period. Criterion for activation of the stress mechanism has generally been ascorbic acid depletion from the neonatal adrenal gland, and a wide range of physiological and pharmacological stressor agents have been found to be ineffective in this regard(1-5). We propose the term "stress-non-responsive period" (S-N-R period) to indicate that stage in an animal's early life when pituitary-adrenal activation is not evoked by stressor agents. The S-N-R period is apparently not due to inability of the adrenal gland to respond to ACTH(1,2, 5), and it has been suggested that the block is in the central nervous system(2). In the present report we demonstrate that during the S-N-R period there is no evidence of adrenal cortical activation in response to stress when both ascorbic acid and adrenal corticosterone concentrations are determined. Our data also indicate that commercial and synthetic vasopressin will regularly cause corticoidogenesis during the S-N-R period, and less regularly will cause ascorbic acid depletion. Endocrinologists have been made increasingly aware that different parameters of adrenal cortical activation may be dependent upon the integrity of different central nervous system regions. For this reason, we were also interested in determining whether the time of maturation of the corticoidogenic response to stress would coincide with the appearance of the ascorbic acid response.

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Methods. A breeding colony of Long-Evans rats was established in our laboratory and records kept of time of mating, number of litters, parentage, etc. Females were not allowed to produce more than 6 litters. Time of parturition was determined with a maximal error of 12 hours. Standard Purina laboratory pellets were supplied *ad libitum.*

Stress procedure. On the day of experiment the litter from any one female was divided into 2 groups: Group I received subconvulsive electric shock by means of a charged grid, for a period of 5 minutes, made up of successive periods of 15 seconds on, 15 seconds off. The severity of this stress was indicated by the very active struggling and squealing of the rats in even the youngest age groups. Group II rats (controls) were left with their mothers during this period. Sixty minutes later all rats were sacrificed by decapitation, the adrenal glands were dissected out with their capsules, weighed to the nearest 0.1 mg and placed in 2.5 ml of 20% ethanol. The adrenal glands from each group for any particular experiment were pooled. *Chemical determinations.* Adrenal ascorbic acid(6) and corticosterone(7) were determined as previously described(8) on aliquots of the ethanol extract. *Drug administration. Vasopressin.* Previous pilot experiments had suggested that 10 milliunits of vasopressin was the minimal effective dose as to adrenal activation, while 50 milliunits caused a maximal response. The young from any one litter were divided into 3 equal groups, consisting of ratlets from several mothers which had delivered within 12 hours of each other. Group I received 0.05 ml saline IP by means of a 30-gauge needle. Groups II and III received 0.05 ml saline containing, respectively, 50 milliunits pitressin (Parke Davis), or synthetic lysine vasopressin.‡ *ACTH.* The young from any one litter were divided into 2 equal groups. 50 milliunits ACTH in 0.05 ml saline was administered IP to one group while the other received 0.05 ml saline.

Sixty minutes after either drug or saline administration the rats were decapitated and the adrenals from each group pooled and treated as previously described(8).

Results. Fig. 1 summarizes the response to electric shock stress at different ages as indicated by both ascorbic acid (AAA) and corticosterone (ACS) changes measured in the same adrenal glands. Before day 8 there were insignificant changes in either of these substances, and both these indices of adrenal cortical activation seemed to mature at approximately the same time. The markedly increased corticosterone responses at day 21 and 25 were based upon 2 separate sets of animals for each age group. Day 21 consisted of 2 experimental groups of 10 and 7 animals with an equal number of controls; day 25 consisted also of 2 experimental groups of 5 and 10 animals with their respective controls. The results in each case were in general agreement, giving greater assurance to the unexpected increased response found at these time periods.

Response to ACTH and vasopressin during the S-N-R period. Fig. 1 shows that 50 milliunits of ACTH given to 1-3-day-old rats provoked a decrease in AAA and a concomitant increase in ACS. Table I illustrates the effect of both synthetic and commercial vasopressin upon AAA and ACS when administered during the S-N-R period. In all experiments both compounds increased adrenal gland corticosterone. Ascorbic acid values for the saline treated controls were rather irregular. Nevertheless, Table I indicates that neither vasopressin regularly influenced ascorbic acid as consistently as it did corticosterone.

Discussion. Our data demonstrate that during the early postnatal period in rats the severe stress of electric shock is ineffective in provoking either ascorbic acid depletion or corticoidogenesis. Time of onset of both these responses seems to be about the same; thus our attempt to show that these 2 adrenal cortical responses may represent the maturation of different mechanisms was unsuccessful. The greater corticoid response observed at day 21 and 25, with a subsequent lowered and more stabilized response from day 30, may reflect the growing functional importance, beyond day 25, of the steroid feedback mechanism acting to brake or moderate

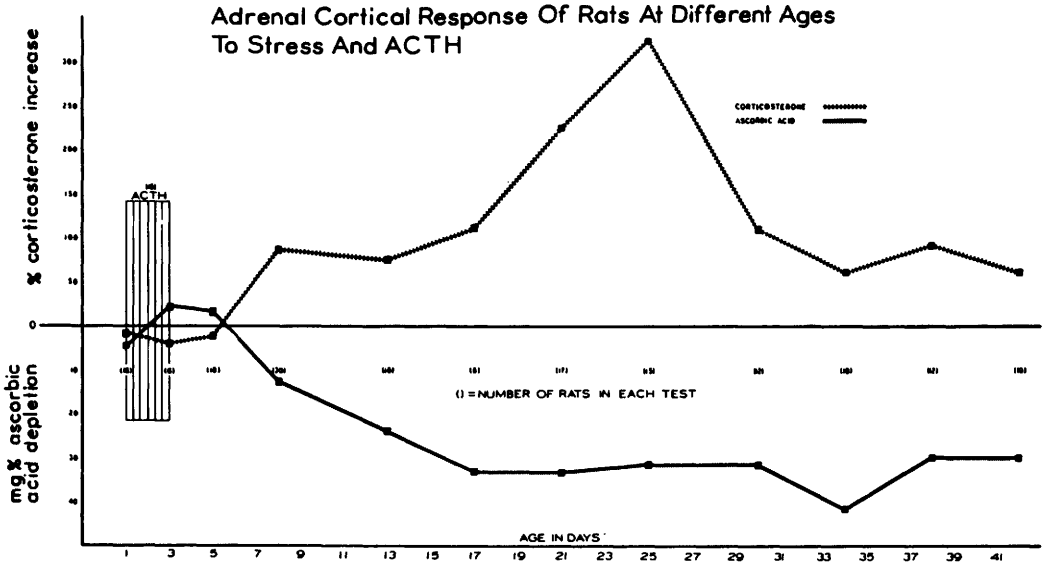
‡ We are pleased to acknowledge the generous gift of this material from Prof. V. DuVigneaud.

ACTH outflow in response to stress. The corticosterone changes probably do not indicate the maximum response, as the time curves for ascorbic acid and corticosterone changes following stress are probably not the same. It is indeed possible that the apparently diminished response beyond day 29 actually represents a partial restoration of corticoid values to their pre-stress levels.

The effects of vasopressin on adrenocortical

FIGURE 1

Adrenal Cortical Response Of Rats At Different Ages To Stress And ACTH



Each point represents values for % change in adrenal gland ascorbic acid and corticosterone as determined on pooled groups of animals of indicated ages 60 min. after electric shock. Both control groups (non-shocked) and experimental groups (shocked) comprised the designated number of animals; *i.e.*, day 8, 30 animals were shocked, while an additional 30 animals served as controls. ACTH (50 milliunits) or saline was administered respectively to 2 groups of sixteen 1-3-day-old rats (32 animals in all) and 60 min. later they were sacrificed. In all cases chemical determinations were made on the pooled glands from within each group.

TABLE I. Effect of Saline, 50 Milliunits of Commercial Vasopressin or Synthetic Lysine Vasopressin on Content of Ascorbic Acid and Corticosterone in Adrenal Glands of Litter Mate Infant Rats During S-N-R Period.

No. rats*	Age (days)	Saline AAA mg %	Saline ACS μg/g	Commercial vasopressin AAA mg %	Commercial vasopressin ACS μg/g	Synthetic lysine vasopressin AAA mg %	Synthetic lysine vasopressin ACS μg/g
		(a)	(b)	(c)	(d)	(e)	(f)
30	1	451	6.6	339	57.8	443	28.3
21	1	324	28.0	363	75.8	383	101.9
30	2	382	30.0	380	101.4	363	70.9
30	2	339	16.1	265	68.7	314	57.8
24	2	396	3.2	318	5.4	339	6.2
24	3	468	17.3	330	45.1	308	36.0
21	3	325	33.4	234	66.2	288	51.7
21	3	486	4.0	350	24.4	357	11.9
15	3	274	12.6	187	23.2	252	28.0
15	3	470	26.7	212	43.6	217	44.2
15	3	312	12.9	238	29.8	254	22.0
24	4	359	14.8	379	41.0	304	32.4
Avg with S.E.		382±19	17±3	299±19	48±7	318±18	41±7
Tests of significance		a-c	a-e	c-e	b-d	b-f	d-f
% difference		−22	−17	+3	+180	+140	−15
P values		<.01	<.05	NS	<.01	<.01	NS

* Number of animals was divided equally among the 3 sub-groups.

activity during the S-N-R period are of especial interest. Both synthetic and commercial preparations consistently produced an approximately equivalent increase in adrenal gland corticosterone concentration. The ascorbic acid results were not as uniform or reproducible as the corticosterone changes. In several batches of ratlets negligible or no response of AAA occurred to either or both vasopressins, suggesting that the ascorbic acid component of the stress mechanism, at least during the S-N-R period, is less responsive to vasopressin than the corticoidogenic component. It may also indicate differential maturation rates of vasopressin sensitive steps involved in the stress reaction. The qualitative similarity in our results with both synthetic and commercial vasopressin is compatible with the hypothesis, reviewed by Nichols(9), that vasopressin can serve as the hypothalamic neurohumoral mediator of the stress response, and suggests that during the S-N-R period the neurohumoral link which presumably relays the stress response from the central nervous system to the anterior pituitary is non-functional. However, under some circumstances vasopressin may act directly upon the adrenal gland(10); therefore, in order more definitively to establish the above hypothesis this possibility must be excluded. (It is also possible that vasopressin may exert both an indirect and a direct effect upon the adrenal cortex.)

Several observations in the literature considerably complicate conclusions as to the precise state of the stress mechanism during the S-N-R period. It has not been definitively established whether the pituitary gland of such animals contains(5,11) or does not contain(12) ACTH. As Hellerstein *et al.* (13) observed eosinopenia in very young rats following administration of hypothalamic extracts, and as we have frequently obtained ascorbic acid depletion with vasopressin, any effects obtained using whole pituitary gland extracts may be caused by material present in the posterior part of the gland. Roffi(14) and Heller and Lederis(15) found that vasopressin appears in the pituitary even before birth and increases thereafter. Another complicating factor is the report by Kitchell and Wells(16) that unilateral adrenalectomy of fetal rats caused compensatory adrenal hypertrophy. This hypertrophy could be prevented by subcutaneous implants of cortisone into the fetus. Unless maternal influences§ were not completely excluded, these results as well as those of Schmidt and Hoffman(18) suggest that the steroid feedback mechanism may be operative in the fetus. If it is functioning at this time, why is there no evidence of adrenal cortical activation in response to stress during the S-N-R period shortly after birth?¶

Though the role of the hypothalamus in neuroendocrine control and autonomic coordination is well known, there have been few reports of the biochemical and physiological maturation of this region. According to Buchanan and Hill(20), there is a relationship between density of myelin in the hypothalamus and ability of the young rat to regulate its body temperature, and this capacity matures at approximately the same time that the stress mechanism responds to temperature extremes. Cohn and Richter(21) found that phosphomono-esterases and acetyl-cholinesterase in the rat hypothalamus increase rapidly from day 1-15 and thereafter tend to decline. Adolph(4) observed an impaired water diuresis in infant rats in response to a water load and interpreted this as indicating lack of sensitivity of hypothalamic osmoreceptors to hemodilution with an associated impaired adjustment of antidiuretic hormone secretion. The response to stress in the adult is accompanied by an increased secretion of this hormone(22) and our results suggest that the S-N-R period may be related to the immaturity of the hypothalamic mechanism which underlies the regulation of vasopressin secretion.

Summary. The adrenal cortical response to electric shock stress was studied in rats of various ages. Measurements of both adrenal

§ For example, it has been shown that placental tissue contains ACTH, and the functional importance this may have for the fetus is not clear(17).

¶ A comprehensive review on the interaction between fetal and maternal endocrine systems in development of birds and mammals has recently been made available(19).

corticosterone and ascorbic acid changes in the same gland indicated that no response occurred until the animals were approximately 8 days old, at which time stress caused changes in both these substances. The early postnatal period in the rats' life when stress does not result in pituitary-adrenal activation we propose to call the stress-non-responsive period (S-N-R period). Both synthetic and commercial vasopressin administered to rats during the S-N-R period caused an increase in corticosterone content of the adrenal gland, and less consistently a decrease in ascorbic acid. It is suggested that the S-N-R period may be related to immaturity of the hypothalamic osmoreceptor mechanism which underlies adjustments in vasopressin secretion.

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Effect of Oxygen at High Pressure (OHP) on Asphyxial Survival Time of Rats.* (27385)

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Hypothermia is well known to be effective in increasing the tolerance of animals to the deleterious effects of anoxia and asphyxia(1). Theoretically, another means whereby the oxygen demands of tissue metabolism can be met, even in absence of effective O_2 transport by hemoglobin, is to increase the amount of physically dissolved O_2 in blood and tissue fluids by exposure to O_2 at high pressure(OHP). The possibility that combining OHP and hypothermia might significantly increase the tolerance of animals to the effects of anoxia and asphyxia above that seen with either treatment alone thus appeared worthy of investigation.

This report presents the results of experiments designed to test whether or not moderate OHP (*i.e.,* 3 atmospheres absolute) has any effect on asphyxial survival time of normothermic and hypothermic rats. Experiments are also presented in which the effects

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