treated animals. Data from the 2 different ashing temperatures showed changes in the same direction, though not always of equivalent magnitude.

The uptake of Ca^{45} measured as cpm per gram of ash (Table III) was about equal for the 2 groups at 5 days. However, as the AAN-treated group showed much greater total mineral content of the callus than the normal (Table II), total Ca^{45} of the AAN calluses was much greater than that of the normal. At 10 days, uptake of Ca^{45} by the AAN callus was considerably higher per gram of ash than by the normal callus. Since at 10 days mineralization of the AAN callus still exceeded that of the normal, total Ca^{45} uptake of the AAN callus sharply exceeded that of the normal. As was noted with the total calcium content of ash and of callus, these differences were lost by the 20th day. In fact, Ca^{45} data exhibited a tendency toward reversal of the earlier position.

Summary. Calcium content and Ca^{45} uptake have been measured in the fracture callus of normal and AAN-treated rats. It appears that total calcium deposition and Ca^{45} uptake are both higher in the young callus, 5 and 10 days after fracture, of the AAN-treated animals. By the 20th day, mineralization of the callus in both groups is similar.

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Corticoid Response to Stress in the Steroid-Inhibited Rat. (27383)

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It is generally accepted that high circulating levels of certain hormones inhibit the secretion of their respective tropic hormones. As regards pituitary-adrenal function, this relationship has been repeatedly confirmed when ascorbic acid depletion or adrenal gland weight is used as a measure of adrenal activity. However, since proper analytical technics were not available until quite recently, there are only a few reports in the literature concerning direct measurements of adrenal corticoid activity in the steroid-inhibited animal. Peron and Dorfman(1) have recently shown a decrease in rat adrenal gland corticosterone following chronic treatment with cortisone. The work of Holzbauer(2) indicated that, at least in the rat, adrenal corticoid concentration is an accurate reflection of adrenal secretory activity.

Endocrinologists are increasingly aware that different parameters of adrenal function may be influenced by different adrenocorticotropic substances or by central nervous system mechanisms. For example, certain hypothalamic lesions(3) which block the stress response, as measured by ascorbic acid depletion, apparently do not interfere with the capacity to mobilize corticoids.

The present experiments were designed to compare changes occurring in both adrenal ascorbic acid and corticosterone content of the same adrenal gland of the untreated rat and the steroid-inhibited rat in response to stress of unilateral adrenalectomy.

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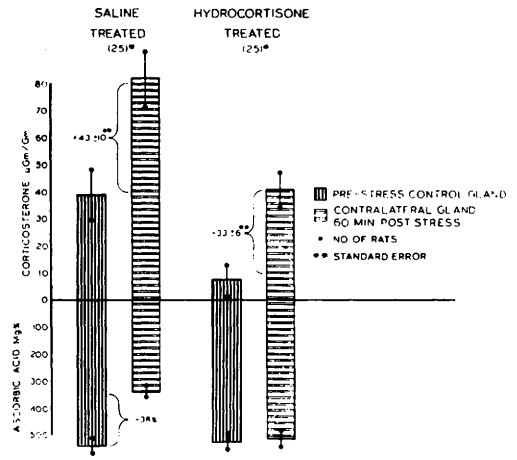

FIG. 1. Adrenal gland ascorbic acid and corticosterone concentration of saline treated or hydrocortisone treated rats before and after the stress of unilateral adrenalectomy.

Methods. Male Wistar rats housed in individual cages and weighing between 200-250 g were handled for several days to accustom them to the necessary manipulations. The day previous to experiment they were weighed. The following morning hydrocortisone acetate (6 mg/100 g body weight) or saline (.2-.5 ml) were injected intraperitoneally into experimental and control groups, respectively. Four hours later the animals were anesthetized with sodium pentobarbital (3 mg/100 g intraperitoneally); 15 minutes later a unilateral adrenalectomy was performed. Each adrenal was weighed and placed immediately in 5 ml of alcoholic saline (4 ml saline + 1 ml ethanol). Sixty minutes later the other adrenal was removed and treated as described above. The glands were homogenized individually by means of a motor driven glass homogenizer, centrifuged, and 3 ml of the supernatant were used for corticosterone analysis by the method of Guillemin, *et al.*(4). To 1 ml of the remaining supernatant, 2 ml of 10% trichloroacetic acid were added and ascorbic acid was determined by the Munson modification(5) of the method of Roe and Kuether(6). The above procedure enables one to measure both ascorbic acid and corticosterone content of the same adrenal gland.

Results and discussion. In these experiments exogenous hydrocortisone was used to interfere with the response to stress. Using the criterion of ascorbic acid depletion, this blockade was indeed effective (Fig. 1) as has been demonstrated by many investigators. Despite this ascorbic acid blockade, however, there was a clear demonstration that the stress employed caused an increase in corticosterone content of these same adrenal glands (Fig. 1). Hydrocortisone administration resulted in a decreased pre-stress concentration of adrenal corticosterone, as observed by Peron and Dorfman to accompany chronic hydrocortisone treatment(1). The site of action of the steroid in decreasing basal adrenal gland corticosterone content could be at the pituitary gland, upon hypothalamus or other central nervous system structure(7), or even by a direct action upon the adrenal gland itself(8,9). Regardless of the site of action, our results suggest that the acute administration of hydrocortisone, while effectively preventing the ascorbic acid depletion response to stress, acts merely to depress the basal level, or physiological "set" from which the corticoid component of the stress mechanism is operating.† This interpretation is further supported by the absolute increase in corticosterone concentration which occurred following stress in both groups. Thus, following the stress of adrenalectomy, the control group had an increase of 43 µg/g while in the hydrocortisone-inhibited group there was an increase of 33 µg/g. There was no significant difference between these 2 values.

Hydrocortisone pretreatment may also prevent an initial elevation in corticoids due to handling, laboratory disturbances and other non-surgical manipulations as has been shown by Fortier(10), and the initial higher level found in the non-treated animals could have been caused by these "psychic stresses". The additional surgical stress of adrenalectomy may then have resulted in the similar increase in adrenal corticosterone concentration actually observed.

† After completion of this study an analogous concept was presented by Yates *et al.*, (Yates, F. E., Leeman, S. E., Glenister, D. W., Dallman, M. F., *Endocrinology,* 1961, v69, 67). These authors, however, confined their observations to changes in plasma corticosterone concentration.

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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