Functional Response of Regenerating Adrenal Glands to Stress.* (26264)

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Although enucleation of the adrenal gland in the rat is followed by rapid regeneration, the mass of regenerated cortical tissue never comes to equal that of both glands in normal animals of the same size, age and sex(1). Previous reports have shown that regenerating adrenal cortical tissue can respond to nonspecific stress(1) but, whether or not normal functional capacity is ever regained by such glands has not been clearly established. Development of micro-methods for determination of corticosterone in plasma has made possible the *in vivo* study of adrenal cortical functional capacity under many experimental conditions. Guillemin *et al.*(2) have shown, for example, that the level of systemic plasma corticosterone accurately reflects the secretory activity of the adrenal cortex. Utilizing such methods, Fortier and De Groot(3) showed that rats bearing a single regenerating adrenal had a normal resting plasma corticosterone level as early as 8-16 days after enucleation. The object of the present study was to determine *in vivo* the functional response of regenerating adrenal glands to nonspecific stress throughout the period of adrenal regeneration as reflected by the corticosterone level of systemic plasma.

Methods. Three groups each consisting of 128 female Holtzman rats weighing 50-60 g were (A) unoperated, (B) bilaterally adrenal enucleated, and (C) unilaterally adrenalectomized and unilaterally adrenal enucleated, respectively. At 2, 3, 6 and 12 weeks, 24 animals from groups A, B, and C were stressed by bilateral fracture of the tibia and fibula under ether anesthesia. Thirty, 60 and 120 minutes after stress the rats were decapitated, blood was collected from the severed neck in heparinized beakers and plasma obtained as rapidly as possible following centrifugation in the cold. Plasma from non-stressed animals was obtained from 8 rats of groups A, B and C after 12 hours isolation in a dark, quiet environment. Adrenals were removed, freed of adherent fat and weighed to nearest tenth of a milligram.

Corticosterone was determined in plasma by the fluorometric method of Zenker and Bernstein(4). In agreement with others, (2,3) a relatively constant residual fluorescence was found in the plasma of rats 48 hours after bilateral adrenalectomy which cannot be ascribed to corticosterone. Aliquots of plasma pools from stressed rats were analyzed for corticosterone by this fluorometric procedure and by an isotopic dilution technic using corticosterone-4-C^{14}† similar to that described by Peterson(5). Corticosterone accounted for 90% of the fluorescence in plasma from stressed rats after the value for constant residual fluorescence had been subtracted from total fluorescence determined by the Zenker-Bernstein method. This agrees well with previously published data on the validity of other fluorometric procedures for corticosterone determination in rat plasma (2).

Results. Results are illustrated in Fig. 1-4. In all instances plasma corticosterone of control rats with intact adrenals increased approximately 8 to 10 fold following initiation of stress. At 2 weeks plasma corticosterone of animals bearing a single regenerating gland increased less than 2 fold and those bearing 2 regenerating glands increased less than 4 fold in response to stress. At 3, 6 and 12 weeks a progressively greater rise of plasma corticosterone occurred in response to stress in rats with one or 2 regenerating adrenals, but in neither circumstance was a normal response attained. The rise in plasma corticosterone following stress was greater in animals with 2 than with 1 regen-

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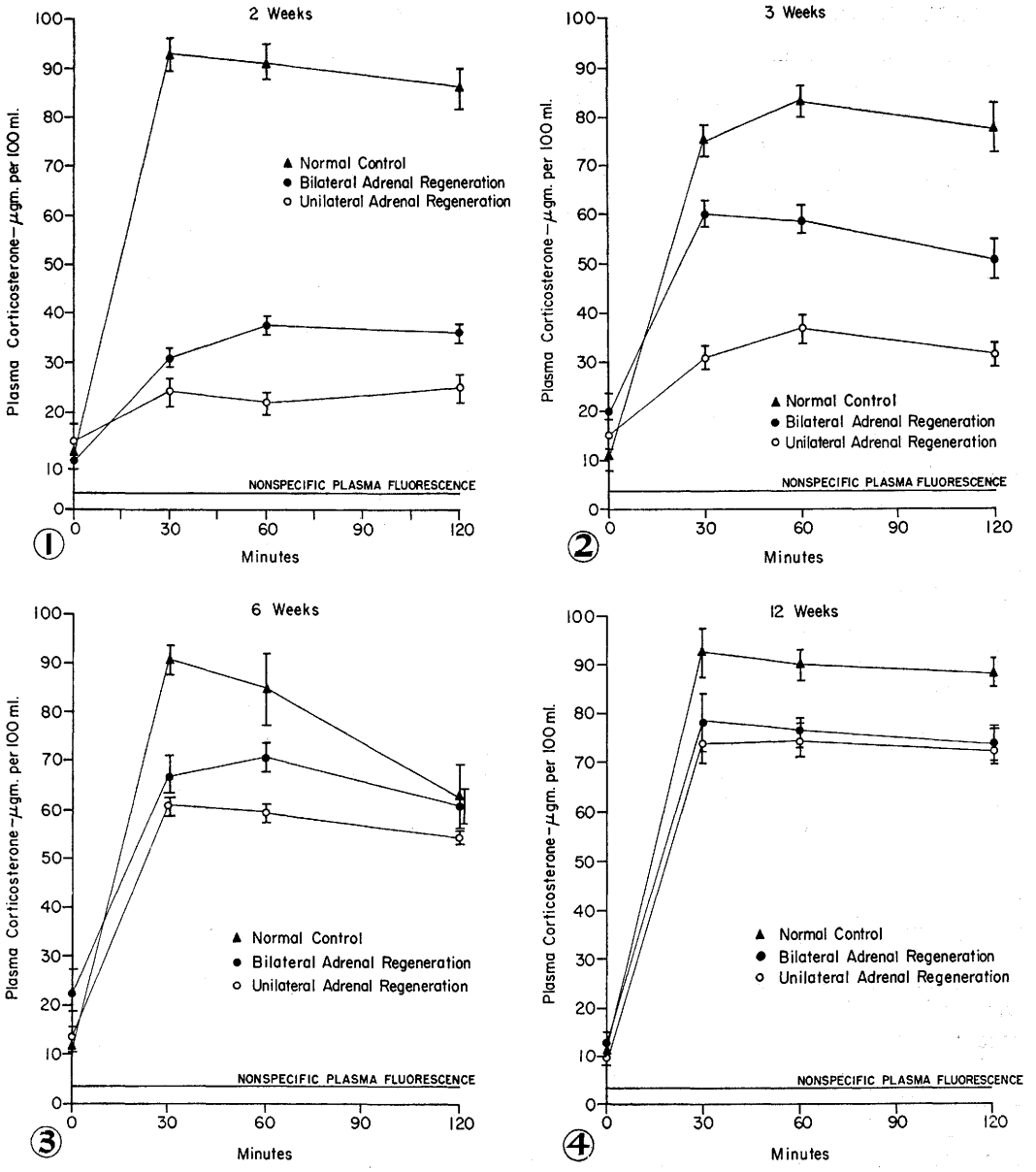

FIG. 1-4. Response of normal and regenerated adrenal cortex to stress as reflected by changes in plasma corticosterone levels. Vertical bars represent stand. error of the mean.

erating gland at 2, 3 and 6 weeks, but at 12 weeks no difference was apparent. When calculated as a percentage of the response in control animals (Table I), rats with 2 regenerating glands showed a more rapid return toward normal than those with one regenerating gland. Maximum response occurred after 12 weeks regeneration when the mean 2 hour response of rats with 2 regenerated glands and one regenerated gland was 84% and 82% of control values, respectively (Table I). Calculation of regenerated cortical mass according to data reported by De Groot and Fortier(3) (Table I) shows that the magnitude of plasma corticosterone increase following stress was greater than the increase in weight of the regenerated cortex, suggesting that response was not solely de-

TABLE I. Response of Normal and Regenerated Adrenals to Stress as Reflected by Plasma Corticosterone Levels.

Weeks	Plasma corticosterone, μg/100 ml, mean 2 hr response*			% response		
	Control	Bilat. regen.	Unilat. regen.	Bilat.: Control	Unilat.: Control	Unilat.: Bilat.
2	89.8 ± 2.3†	34.8 ± 1.6	23.4 ± 1.6	39	26	67
3	77.6 ± 5.1	56.7 ± 2.1	32.9 ± 1.5	73	42	58
6	79.4 ± 4.1	66.1 ± 2.3	58.7 ± 1.2	83	74	89
12	90.1 ± 2.3	76.4 ± 2.5	73.8 ± 2.2	84	82	97
	Calculated cortical wt, mg			% ratio of calculated cortical wt		
2	43.9 ± .7	18.2 ± .5	11.9 ± .5	41	27	65
3	38.0 ± .7	22.4 ± .5	15.8 ± .4	59	42	70
6	41.9 ± .7	27.8 ± .6	21.7 ± .5	66	52	78
12	44.4 ± .8	34.6 ± .6	25.0 ± .7	78	56	72

* Avg of uncorrected values obtained at 30, 60 and 120 min. after stress.
† Stand. error of mean.

pendent upon mass of regenerated adrenal cortical tissue.

Discussion. Decrease in adrenal concentration of ascorbic acid, lymphoid atrophy, fall in circulating eosinophils and survival in the face of exposure to various noxious stimuli has indicated that regenerated adrenals were capable of functional response to nonspecific stress, but whether or not a normal response in terms of hormone production was ever attained has remained in doubt. *In vitro* incubation studies of Masson, Koritz and Peron(6) demonstrated that regenerating adrenals (21 and 30 days after enucleation) secreted corticosterone into the incubation medium but the amount secreted by regenerated adrenal tissue was less than that of normal adrenal tissue, especially when ACTH was added. Corticosterone secretion into adrenal vein blood also has been reported to be less from regenerating glands than from intact adrenals(6). Although these observations probably reflect decreased functional response of such glands to ACTH, both methods have made it difficult to quantitate the magnitude of the functional response of regenerating adrenal tissue to stress since *in vitro* procedures are unphysiologic and adrenal vein cannulation is itself a stress. The experiments reported here obviated these difficulties inasmuch as corticosterone levels were determined in systemic blood plasma under physiologic conditions of stress and non-stress. Results confirm the marked functional deficiency of regenerating adrenal tissue at 2 weeks and, although functional capacity increased with time such glands failed to regain the ability of normal adrenals to elevate systemic blood levels of corticosterone in response to stress. The greater increase in functional capacity than in mass of regenerated tissue also lends support to the suggestion that regenerated tissue is more active per unit of weight than normal adrenal cortical tissue.

Summary. Plasma corticosterone changes were studied in response to stress in normal rats and in animals bearing 2 regenerating adrenals and 1 regenerating adrenal at intervals of 2, 3, 6 and 12 weeks after enucleation. Such glands were capable of elevating systemic blood corticosterone levels in response to stress by 2 weeks, the increase being greater in those with 2 regenerating glands than in those with one. The functional capacity of regenerating adrenals as determined by the elevation in plasma corticosterone following stress progressively increased from minimal ability at 2 weeks to maximum capability at 12 weeks, but the functional capacity of normal adrenals was never regained.

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Connective Tissue. II. Distant Dermal Collagen Response to Local Inflammation.* (26265)

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It has been demonstrated that there is a significant decrease in total dermal concentration of hydroxyproline or collagen in skin distant from the site of local croton oil induced inflammation(1,2,3). This loss of dermal collagen from uninjured tissue is not associated with a change in either total dermal nitrogen or hexosamine(3).

Recently, we have developed a technic for extractive disection of rat skin into ground substance, neutral soluble collagen, procollagen and insoluble collagen(4,5). Employing this procedure, we have investigated the nature of the "distant dermal collagen response to local inflammation."

Material and methods. Twenty-four Sprague-Dawley rats, averaging 270 g in weight were divided into 3 equal groups of 8 each, 2 of which were injected intradermally with 0.4 ml of a 75% solution of croton oil in peanut oil, the third group with a similar volume of the carrier oil alone. This latter group was sacrificed by etherization within a few minutes after injection, and 5 g of shaved abdominal skin distant from site of injection (left abdominal area) were removed. The chemical analysis of skin from animals injected with carrier alone did not differ significantly from normal 0, 4 or 14 days after injection(3). The eschar formed 4 days after injection of the irritant, and one group of animals was sacrificed at this time. Similarly, the third group was killed after 14 days when the eschar had sloughed, and the apparently uninjured skin collected. All tissues were shaven, dissected clean of adhering fat, fascia and muscle, and finely minced. Triplicate samples of pooled tissue from 8 animals were extracted sequentially in 0.15 and 0.5M NaCl and citrate buffer as described previously(5). The hexosamine(6), hydroxyproline(7) and nitrogen content of the extracts, as well as that of the whole skin of each animal separately, was determined in triplicate after acid hydrolysis(5). These extracts have been shown to remove quantitatively the ground substance, neutral soluble collagen and "procollagen" respectively(5). The difference between total tissue content of each component and the sum of the soluble material in μmoles/g of starting tissue defined the composition of the insoluble residue.

All results were expressed in μmoles/g of skin as the mean of triplicate analyses performed upon triplicate tissue extracts obtained from the pooled tissue of each group of 8 animals. The analytical variation between the triplicate extracts obtained from each group of animals was less than 10%. Mean values of 9 determinations for each fraction are presented along with the appropriate standard deviation below. The tissue samples were stored for a few weeks in the frozen state after cleaning but prior to either hydrolysis or extraction. Water loss *via* sublimation was essentially constant for all tissues at 30% w/w as has been described previously(4).

Results and discussion. The pathology of this type of injury, a non-suppurative ulcerous defect lightly outlined by erythema, has been described(2). No significant changes in dermal hexosamine or nitrogen were found in the skin distant from site of local croton oil induced inflammation. Changes in hy-

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