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Status of the Pituitary-Adrenocortical-Liver Axis following Partial Hepatectomy[1] (41048)

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Abstract. Since the liver is the primary site of steroid degradation and plasma binding protein synthesis, studies were undertaken to evaluate adrenocortical activity in partially hepatectomized (H) male rats. Two days after either 10, 30, 40, or 70% H, significant decreases in liver mass were observed, while elevations in total and free plasma corticosterone levels, adrenal corticosterone content, and adrenal weights were noted only in the 70% H group. Hepatic Δ^4-steroid hydrogenase activity was significantly depressed in both whole and microsomal, but not in the supernatant, fractions of the regenerating liver of the 70% H group. Although plasma ACTH levels were unchanged in these animals, the *in vitro* adrenocortical responsiveness to ACTH was increased. These data suggest that a portion of the increase in total plasma corticosterone levels post-70% H was due to a reduction in the hepatic capacity to inactivate corticosterone, while the remainder may be due to an increase in adrenocortical secretion as a result of an enhanced responsiveness of the adrenal gland to ACTH.

Since the liver is the main site of corticosteroid degradation and the source of corticosteroid plasma binding proteins, changes in liver function would be expected to alter hypothalamic-pituitary-adrenocortical (HPA) activity. Recently Marotta *et al.* (1) described the adrenocortical status of rats from 1 to 14 days following 70% hepatectomy (H). Based upon plasma and adrenal corticosterone concentrations, *in vitro* adrenal corticosterone production, and adrenal gland weights, these investigators demonstrated increased adrenocortical activity for at least the first 3 days post-70% H, which gradually subsided following a course inversely related to that of hepatic restoration. Using histologic techniques, others (2, 3) have also shown enhanced adrenocortical activity following partial H. These findings are not in accord with the accepted feedback control of endocrine gland activity; that is, one would expect a decrease in adrenal gland weight and/or secretion in the presence of elevated steroid levels resulting from a reduced hepatic capacity to metabolize corticosteroids (4). Therefore, the adrenocortical response to partial H presents an intriguing question which requires further elucidation.

Using the partially H rat, the present investigation was designed to determine: (a) the minimal amount of liver mass that must be removed to produce an imbalance in the HPA system; (b) the concentrations of free and bound plasma corticosterone, since it is well known that partial H decreases plasma corticosteroid binding proteins (5, 6); (c) hepatic Δ^4-steroid hydrogenase activity, the most important enzyme system involved in the degradation of corticosteroids (7); and (d) plasma and pituitary adrenocorticotrophic hormone (ACTH) levels and the *in vitro* responsiveness of the adrenal cortex to ACTH.

Materials and methods. Male Holtzman rats (180–200 g), which were housed in a constant temperature (22°) room with a lighting schedule of 12L:12D and fed a diet of Purina Rat Chow and water *ad libitum*, were used in all experiments. Rats were divided into six groups in the partial hepatectomy experiment: intact (I), sham operated (S), and 10, 30, 40, and 70% H. Under ether anesthesia, various degrees of H were obtained by removing after ligation the fol-

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lowing different combinations of liver lobes (8, 9): caudate lobe (10%), left lateral lobe (30%), left lateral and caudate lobes (40%), and left lateral and median lobes (70%). Sham operation consisted of a midline abdominal incision, delivering the respective lobes of the liver from the abdomen, replacing them, and closing the incision. Based on the adrenocortical response observed during hepatic restoration (1), all nonfasted animals were decapitated 2 days after surgery, between 0800 and 1000 hr, and trunk blood was collected in heparinized tubes. Body, adrenal gland, and liver (wet and dry) weights were measured. Plasma and adrenal corticosterone concentrations were determined fluorometrically (10). Protein bound corticosterone was measured in pooled (two to four animals) plasma samples after equilibrium dialysis (11). Plasma samples (2.0 ml) were placed in dialysis tubing (Union Carbide, Chicago, Ill.) and incubated with gentle shaking at 4° for 48 hr in flasks containing 8.0 ml Krebs–Eggleston phosphate saline buffer (pH 7.4). Total plasma corticosterone levels before dialysis, as well as the protein bound corticosterone remaining in the retentate, were measured.

Hepatic Δ^4-steroid hydrogenase activity was assayed in whole homogenates, as well as in the microsomal and supernatant fractions, of each liver from S (11 rats) and 70% H (10 rats) groups 2 days postoperative. The various fractions were prepared according to the method of Kato *et al.* (12) and duplicate samples, using corticosterone (0.5 μmole) as the substrate and all the necessary factors (i.e., 12.5 μmole $MgCl_2$, 140 μmole phosphate buffer at pH 7.4, 20 μmole glucose 6-phosphate, 1 unit glucose 6-phosphatase, 1.5 μmole reduced nicotinamide adenine dinucleotide phosphate and H_2O to a final volume of 2.5 ml), were assayed for Δ^4-steroid hydrogenase activity (13) and protein concentration (14).

The *in vitro* responsiveness of the adrenal cortex to ACTH was determined using the incubation procedure of the Kitay *et al.* (15). The adrenal glands from S and 70% H animals (2 days postoperative) were weighed, quartered, placed in Teflon beakers, and preincubated for 30 min with gentle shaking at 37° in Krebs–Ringer bicarbonate buffer (pH 7.4) gassed with 95% O_2 and 5% CO_2. The buffer was then removed and replaced with 2.0 ml fresh buffer containing either 0, 5, 25, or 125 mU cortrosyn ($ACTH_{1-24}$; Organon, West Orange, N.J.). After 60-min incubation, the buffer was collected and analyzed for corticosterone.

Plasma and pituitary ACTH were measured in S and 70% H rats on Day 2 postoperative. Pituitaries were removed in the cold and homogenized in 2.0 ml 0.1 *N* HCl. ACTH was assayed in pooled plasma samples (seven pools for the S group; nine pools for the 70% H group) and individual pituitary homogenates (six for the S group; eight for the 70% H group) using a radioimmunoassay kit from Amersham/Searle (Arlington Heights, Ill.) containing ^{125}I-ACTH. The anti-ACTH serum in the kit was developed with $ACTH_{1-24}$ and has maximum reactivity with the 11–24 polypeptide which is common to both rat and human ACTH. Furthermore, Matsuyama *et al.* (16) have shown excellent cross-reactivity of rat plasma ACTH with antiserum to human ACTH. The free ^{125}I was counted in a gamma counter (Packard, Downers Grove, Ill.) and the interassay variation was 11 pg/ml.

Significance for all data was determined by analyses of variance and nonpaired *t* tests with $P < 0.05$ being accepted as significant.

Results. Two days following 10, 30, 40, and 70% H approximately 94, 87, 75, and 55%, respectively, of wet liver mass and 88, 75, 65, and 43%, respectively, of dry liver mass were present in the H groups when the latter groups were compared to similar parameters in the S groups (Fig. 1). The dry liver weights observed on Day 2 postoperative showed significant ($P < 0.05$) reductions in liver mass in all percentage H groups. Significant elevations ($P < 0.05$) in total plasma corticosterone levels and adrenal gland weights 2 days following H were observed only in the 70% H group (Fig. 2). These animals had mean plasma corticosterone levels of 9.7 ± 1.0 μg/100 ml and adrenal gland weights of 50.0 ± 1.9 mg, while these parameters in the S group were 5.9 ± 1.1 μg/100 ml and 43.3 ± 1.2 mg,

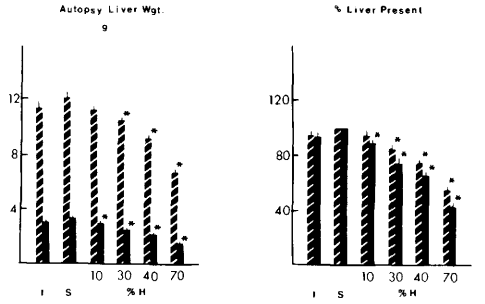

FIG. 1. Wet (slashed bars) and dry (solid bars) liver weights and percentages (% of S group) of liver present in intact (I), sham (S), 10, 30, 40, and 70% hepatectomized (H) rats 2 days postoperative. Bars represent the means of not less than 8–10 rats/group with SEM above. Asterisk denotes $P < 0.05$ from the S group.

respectively. Similarly, the percentages of protein bound plasma corticosterone (Table I) were not markedly different in the 10, 30, and 40% groups from the I or S groups; however, the 70% H rats had significantly ($P < 0.05$) lower protein bound and higher free plasma corticosterone levels. On the other hand, the adrenal corticosterone concentrations were significantly ($P < 0.05$) greater in both the 40 and 70% H animals than in the S group (Fig. 2).

Since the previous experiments revealed that adrenocortical activity was not altered until approximately 70% of the liver mass was removed, the remaining studies focused on the 70% H animals. The hepatic Δ^4-steroid hydrogenase activities in the whole, microsomal, and supernatant fractions of these animals were less than in the

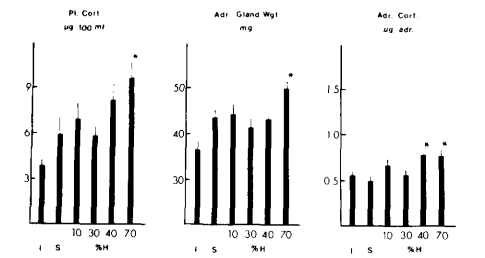

FIG. 2. Plasma corticosterone levels, adrenal gland weights, and adrenal corticosterone concentrations of intact (I), sham (S), and hepatectomized (H) rats 2 days postoperative. Bars represent the means of 8–10 rats/group with SEM above. Asterisk denotes $P < 0.05$ from the S group.

S animals with the differences being significant ($P < 0.05$) for the whole and microsomal fractions (Table II). In the latter fraction, this enzymatic activity metabolized 42.9 ± 4.8 µmole corticosterone/100 mg protein/hr for the 70% H rats, compared to 95.6 ± 9.6 µmole corticosterone/100 mg protein/hr in the S group. This represented a 55% decrease in Δ^4-steroid hydrogenase activity 2 days after 70% H. Based on the amount of wet liver mass present, the concentration of liver protein and the enzymatic activity in the whole homogenate, the total hepatic capacity to metabolize corticosterone was approximately 17 and 44 µmole/hr in the 70% H and S groups, respectively.

In the absence of ACTH the *in vitro* secretion of corticosterone (Fig. 3) by the adrenal glands of 70% H animals (0.0151 ± .002 µg/mg/hr) was significantly ($P < 0.05$) greater than that of the S animals (0.0095 ± 0.001 µg/mg/hr). In addition, the responses of the adrenal glands to ACTH were consistently greater at each dose in the 70% H animals than those of the S groups. These differences were significant ($P < 0.05$) at 25 and 125 mU ACTH. Furthermore, the response of the adrenal glands from the H group continued to increase as the dosage of ACTH increased, while that of the control group did not increase above the response noted with 5 mU ACTH.

The plasma ACTH levels of the S (77 ± 12 pg/ml) and 70% H (79 ± 7 pg/ml) groups 2 days postoperative were not significantly different ($P > 0.05$). On the other hand, the pituitary ACTH content of the 70% H group (362 ± 7 ng/pituitary) was slightly, but significantly ($P < 0.05$), greater than in the S group (321 ± 17 ng/pituitary).

Discussion. The present study demonstrates that increased HPA activity, as indicated by elevations in total plasma and adrenal corticosterone concentrations and adrenal gland weights, was noted only when 70% of the liver was removed. Similarly, the 70% H group showed a substantial decrease in protein bound plasma corticosterone levels. The latter is in accord with reports that 70% H in rats decreases corticosteroid binding globulins (5) and albumin (6). Thus, the data obtained in the 10, 30,

TABLE I. PLASMA PROTEIN BOUND AND FREE CORTICOSTERONE 2 DAYS POSTOPERATIVE

Groups[a]	No. of pools	Total cort. (μg/100 ml)	Protein bound (%)
I	3	3.8 ± 0.5[b]	85.7 ± 1.8
S	12	5.9 ± 1.1	83.1 ± 1.4
10% H	4	6.9 ± 1.1	79.8 ± 3.4
30% H	4	5.8 ± 0.6	80.2 ± 1.9
40% H	6	8.2 ± 1.1	85.3 ± 2.2
70% H	12	9.7 ± 1.0[c]	77.4 ± 1.0[c]

[a] Groups: I, intact; S, sham operated; H, hepatectomized.
[b] Mean ± SEM.
[c] $P < 0.05$ from S group.

and 40% H groups suggest that the liver possesses a reserve capacity for those functions (e.g., hepatic Δ^4-steroid hydrogenase, synthesis of steroid binding proteins, etc.) involved in the balance of the HPA system and may be the reason others (17, 18) have reported normal total plasma corticosteroid levels in patients with chronic liver disease; that is, the liver damage in these patients may not have exceeded the liver's capacity to affect the HPA system.

Alterations in androgen metabolism, which occur following partial H (19), may in turn affect adrenocortical activity by inhibiting either adrenal Δ^4-5α-reductase (20) and/or hepatic Δ^4-steroid hydrogenase activity (13). Although the data are not presented herein, no significant differences were found in adrenocortical activity (e.g., adrenal weight, plasma and adrenal corticosterone levels) of 70% H (2 days post)-orchidectomized (8 days post)-rats, with or without testosterone (1 mg/day) replacement, when compared to the 70% H group. Thus, changes in androgen levels do not appear to play a major role in affecting the HPA system of 70% H rats.

Undoubtedly, the increase in plasma corticosterone levels following 70% H may in part be attributed to the reduced hepatic capacity, resulting from a decrease in liver mass, to inactivate corticosterone following 70% H. The present study has also demonstrated a significant depression in hepatic Δ^4-steroid hydrogenase activity in the remaining portion of the liver. These results are in agreement with those of Teebor *et al.*

TABLE II. HEPATIC Δ^4-STEROID HYDROGENASE ACTIVITY 2 DAYS POSTOPERATIVE

Fraction	Group[a] S	70% H
Whole	42.8 ± 2.3[b]	34.2 ± 3.3[c]
Microsomal	95.6 ± 9.6	42.9 ± 4.8[c]
Supernatant	14.7 ± 1.4	11.6 ± 2.1

[a] S, sham operated (N = 11/fraction); 70% H, 70% hepatectomized (N = 10/fraction).
[b] Mean ± SEM in μmole corticosterone metabolized/100 mg protein/hr. The wet liver weights of the S and H groups were 11.2 ± 4 g (103.4 ± 5.3 mg protein) and 5.8 ± 0.3 g (50.8 ± 2.5 mg protein), respectively.
[c] $P < 0.05$ from S group.

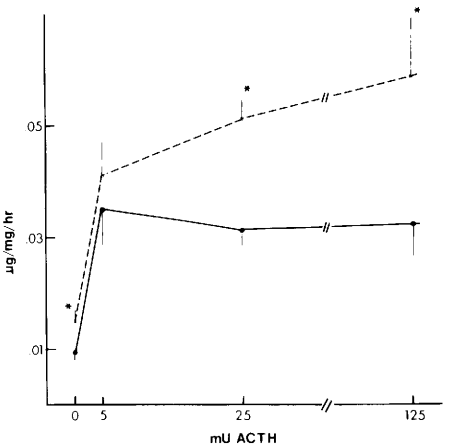

FIG. 3. *In vitro* adrenal corticosterone secretion of sham (solid line) and 70% hepatectomized (broken line) rats in response to 0, 5, 25, and 125 mU ACTH added to the incubation medium. Each point represents the mean of five to six rats, while the vertical line represents the SEM. Asterisk denotes $P < 0.05$ from the sham group at similar doses.

(21), who showed that the plasma clearance of cortisol, after its ip administration, was curtailed in rats following 70% H. Since the 70% H rat has not only a 50% decrease in liver mass on Day 2 post-H, but also a depression in the activity of hepatic Δ^4-steroid hydrogenase in the regenerating liver, it is apparent that both contribute to a decreased clearance of corticosterone. Other investigators have also reported that the regenerating liver has depressed activities in those enzymes involved in the degradation of other steroids, such as ring A and 20α-steroid reductases for progesterone (22) and 17β-hydroxysteroid dehydrogenase for androgens (19). These reductions in the hepatic capacity to inactivate steroids in the regenerating rat liver may be related to a decreased uptake of the steroid into the hepatocytes as found in the cirrhotic rat liver (23).

The increase in adrenal gland weight, corticosterone content, and production in the 70% H animals, however, all indicate that a heightened secretory activity of the adrenal gland is also contributing to the elevation in plasma corticosterone levels. Yet, this increase in adrenocortical activity on Day 2 post-70% H may not be attributed to an elevation in plasma ACTH levels since no significant differences were found between the S and 70% H groups. Therefore, the enhanced adrenocortical activity is probably due to the increased responsiveness of the adrenal glands of 70% H animals to ACTH. It is not surprising to find a dissociation between plasma corticosterone and ACTH levels, since it is known that various factors at the adrenal level can affect the activity of this gland. For example, it has been shown that previous *in vivo* exposure to ACTH will increase the *in vitro* adrenal responsiveness to ACTH 24 hr later (24). If the ACTH levels of the 70% H animals on the day of and/or the day following surgery were higher than in the S group, then this would contribute to the change in responsiveness noted on Day 2 post-H; however, the stress of ether, laparotomy, and/or the removal of a lesser amount of liver, most likely activated the HPA maximally and not unlike the 70% H group. Furthermore, it is possible that a factor produced as a result of liver regeneration or a substance whose level is changed due to the loss of liver function may be responsible for the altered adrenal responsiveness. Recently, a hepatic regenerative simulator substance (MW $\sim$ 10,000) has been described whose appearance is demonstrable for 3 days post-H (25), a temporal pattern corresponding with that of the adrenocortical changes noted post-H (1). An enhanced responsiveness of the adrenal gland to ACTH has also been described under other conditions, such as in some patients with Cushing's disease (26). In the latter, a possible corticotrophin potentiating factor has been considered (27). Thus, it appears that one cannot always attribute an alteration in adrenocortical secretory activity to a corresponding change in circulating ACTH levels since there are extra-ACTH mechanisms which can alter adrenocortical secretion. Furthermore, the failure of the elevated free plasma corticosterone levels in the 70% H animals to depress plasma ACTH levels below those observed in S animals may indicate an alteration in feedback regulation. Although it is difficult to compare the results of the present study with those performed on patients with chronic liver disease, it is interesting to note that McCann and Fulton (18) reported elevations in free plasma corticosteroids with normal cortisol secretory rates in such patients and proposed a resetting of the HPA feedback mechanism. The latter may be due to altered neuroendocrine control of the HPA system (28, 29) resulting from an imbalance in brain neurotransmitters observed in both hepatectomized (30) and portal-caval shunted (31) animals. Further investigation is necessary to test this hypothesis both in 70% H rats and liver diseased patients.

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