

# Alteration in Binding of Dexamethasone to Glucocorticoid Receptors in Regenerating Rat Liver after Partial Hepatectomy<sup>1</sup>

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## ABSTRACT

Changes in binding of dexamethasone (9 $\alpha$ -fluoro-11 $\beta$ , 17 $\alpha$ ,21-trihydroxy-16 $\alpha$ -methylpregna-1,4-diene-3,20-dione) to its receptors in regenerating rat liver after 70% hepatectomy were examined. Specific receptors for dexamethasone in the liver remnants of adrenalectomized rats decreased significantly during the period of DNA synthesis after 70% hepatectomy; then, they increased to above the control values between Days 4 and 7 after partial hepatectomy and subsequently returned to the control values. During the period of DNA synthesis, decreased binding was observed in partially hepatectomized rats with or without adrenals, but later enhanced binding was not prominent in rats with adrenals.

## INTRODUCTION

Glucocorticoid hormones inhibit mitosis in regenerating liver of partially hepatectomized rats (5, 16). They also greatly decrease the rate of DNA synthesis in the regenerating liver when given before or during the period of rapid increase in DNA synthesis (21), but they promote accumulation of lipids and glycogen with increase in cell size, so that restoration of tissue mass is near normal (*cf.* Ref. 5). On the other hand, administration of adrenocorticotropin or adrenocortical extract causes increase in the restoration of liver mass and protein in partially hepatectomized rats (22). Furthermore, glucocorticoid hormones result in increased synthesis of tryptophan oxygenase [L-tryptophan:oxygen oxidoreductase (EC 1.13.1.12)] by the hepatic cells (19). When given to adrenalectomized rats, the induction rate of tryptophan oxygenase by hydrocortisone was depressed in 70% hepatectomized rats during the period of DNA synthesis but not in sham-hepatectomized animals (27). The induction of tryptophan oxygenase by L-tryptophan is not reduced in regenerating liver (27).

Beato *et al.* (3) reported that the degree of saturation of soluble hepatic glucocorticoid receptors by the hormone is closely correlated with the extent of hormonal induction of the hepatic enzymes tryptophan oxygenase and tyrosine aminotransferase [L-tyrosine:2-oxoglutarate aminotransferase (EC 2.6.1.5)]. It is generally agreed that the binding of glucocorticoid to specific cytoplasmic receptors is an essential initial step in specific enzyme induction mediated by glucocorticoid and involves tissue responsiveness to the hormone (3, 11, 24). Thus, a change in the level of receptors may be an important mechanism for regulating the effects of hormones on target

cells and tissues (11, 17, 25).

Thus, we examined the levels of glucocorticoid receptors in the cytoplasm of regenerating rat liver after 70% hepatectomy. This study reports that the binding capacity decreased during the period of the initial rapid increase of DNA synthesis after 70% hepatectomy, then increased to above the control values, and finally returned to the normal level.

## MATERIALS AND METHODS

**Animals and Treatments.** Male albino Donryu rats, weighing 100 to 120 g (supplied by Kitayama Rabes Corp., Kyoto, Japan), were given laboratory chow and water *ad libitum* and were adapted to constant laboratory conditions (17, 18). Rats weighing 190 to 220 g were used for experiments. When specified, the rats were adrenalectomized 7 days before 70% hepatectomy. At the beginning of the experiment, the rats were subjected to 70% hepatectomy by the procedure of Higgins and Anderson (15) or to a sham operation (27). The animals were sacrificed at the times after partial hepatectomy or sham operation indicated in the charts.

**Preparation of Cytoplasmic Extracts.** Animals were killed by decapitation, and their livers were perfused with 30 ml of ice-cold 50 mM Tris-HCl buffer (pH 7.55) containing 250 mM sucrose, 25 mM KCl, 3 mM MgCl<sub>2</sub>, and 1 mM mercaptoethanol (1). The cytoplasmic supernatant fraction (cytosol) was prepared from a homogenate of the tissue by centrifugation at 105,000  $\times$  g for 1 hr at 2° as described previously (17, 18).

**Dexamethasone Binding Assay.** The binding of [<sup>3</sup>H]dexamethasone (28 Ci/mmol; The Radiochemical Centre, Amersham, England) to cytosol receptors of adrenalectomized rat liver was assayed as described by Beato and Feigelson (1), with the modifications reported previously (17, 18). Specific binding was estimated by subtracting the binding of 5  $\times$  10<sup>-8</sup> M [<sup>3</sup>H]dexamethasone plus 1000-fold excess of unlabeled steroid from that of 5  $\times$  10<sup>-8</sup> M [<sup>3</sup>H]dexamethasone (18). Radioactivity was measured as described elsewhere (17). The method of Giannopoulos (12) was used for measurement of the binding of the hormone to cytosol receptors of the liver of nonadrenalectomized rats with the modification that samples of 0.2 ml of cytosol were incubated for 20 hr at 0° with 5  $\times$  10<sup>-8</sup> M [<sup>3</sup>H]dexamethasone with or without 1000-fold excess of unlabeled glucocorticoid. Apparent equilibrium constants (K<sub>d</sub>'s) for dexamethasone were estimated by plotting the binding data according to the method of Scatchard (26).

Protein was estimated with bovine serum albumin as a standard (14).

## RESULTS

Chart 1 shows the effect of adrenalectomy on specific dex-

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amethasone binding. After adrenalectomy, the binding capacity increased steadily, reaching a maximum on Day 2 or 3 and remaining steady for a few days before it gradually decreased. Additional sham hepatectomy had little effect on specific dexamethasone binding. The apparent binding capacity of the liver of nonadrenalectomized rats did not change appreciably during the experimental period. When carried out, adrenalectomy was performed 7 days before partial hepatectomy, since this resulted in better survival than both operations simultaneously and since the binding capacity was not changed appreciably by adrenalectomy, although it decreased gradually.

The changes in dexamethasone binding in adrenalectomized 70% hepatectomized rats are shown in Chart 2. The binding capacities decreased significantly ( $p < 0.05$ ) as early as 12 hr after 70% hepatectomy and remained low during the period when DNA synthesis is known to occur. Then, it increased markedly and surpassed the prehepatectomy level between Days 4 and 7 after the operation.

Chart 3 shows the changes in the levels of binding in liver remnants after 70% hepatectomy in rats with intact adrenals. Decrease in binding capacity was also observed in the early period after partial hepatectomy. At 48 hr, the dexamethasone-binding capacity began to increase in regenerating liver of adrenalectomized rats (Chart 2) but remained low in rats with adrenals (Chart 3). Between Day 5 and Day 7, apparent dexamethasone binding increased to slightly higher than the control level ( $p < 0.05$ ) (Chart 3), but the increase was less than that in regenerating liver of adrenalectomized rats (Chart 2).

Chart 4 shows Scatchard plots of dexamethasone binding to receptors in the cytosol of regenerating liver of partially hepatectomized rats which had been adrenalectomized 7 days before 70% hepatectomy (Chart 4A) or which had intact adrenals (Chart 4B). The apparent equilibrium (dissociation) constants ( $K_d$ ) for dexamethasone were  $4.0$ ,  $4.4$ , and  $2.7 \times 10^{-9}$

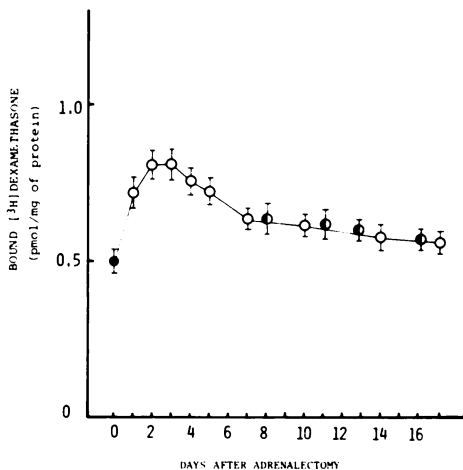


Chart 1. Influences of adrenalectomy alone and adrenalectomy plus sham hepatectomy on specific binding of  $[^3\text{H}]$ dexamethasone to liver cytosol protein. Some adrenalectomized rats were sham hepatectomized 7 days after the operation. Rats were sacrificed at the times indicated after adrenalectomy, and the liver cytosol was prepared. Samples (0.2 ml) of cytosol were incubated at  $0^\circ$  with  $5 \times 10^{-8}$  M  $[^3\text{H}]$ dexamethasone with or without 1000-fold excess of unlabeled dexamethasone. The incubation times were 2 and 20 hr for the liver cytosol of adrenalectomized and nonadrenalectomized rats, respectively. Specific binding was determined by the charcoal assay method and corrected for nonspecific binding. Points represent means of duplicate determinations of 4 to 6 animals; bars, S.D. ●, rats with intact adrenals; ○, adrenalectomized rats; ◐, sham-hepatectomized rats without adrenals.

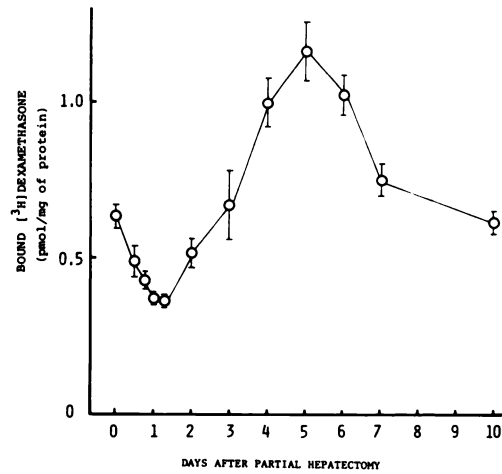


Chart 2. Changes in the level of specific dexamethasone binding of regenerating liver cytosol from adrenalectomized rats. All rats were adrenalectomized 7 days before hepatectomy. Specific dexamethasone binding was determined as described in Chart 1 and "Materials and Methods." Points, means of duplicate determinations on 4 and 6 animals; bars, S.D.

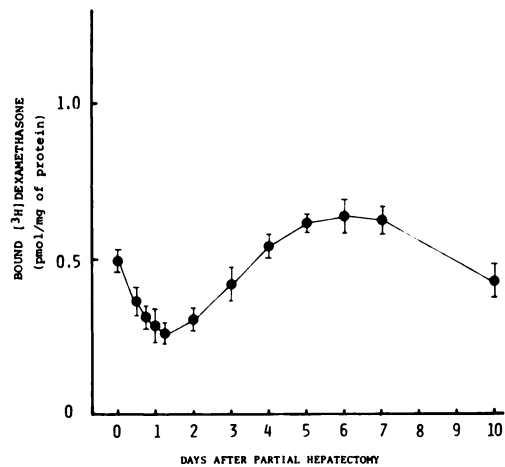


Chart 3. Changes in the levels of specific dexamethasone binding of regenerating liver cytosol from nonadrenalectomized rats. Specific dexamethasone binding was determined as described in Chart 1 and "Materials and Methods." Points, means of duplicate determinations of 4 to 6 animals; bars, S.D.

M at  $0^\circ$  in the cytosol of regenerating liver 0 hr, 24 hr, and 5 days, respectively, after partial hepatectomy in the adrenalectomized rats (Chart 4A). The  $K_d$  values for dexamethasone in the cytosol of regenerating liver of partially hepatectomized rats with intact adrenals were  $4.3$ ,  $5.3$ , and  $3.2 \times 10^{-9}$  M at  $0^\circ$  at 0 hr, 24 hr, and 5 days after the operation, respectively (Chart 4B). Although slight differences in the affinities of the dexamethasone receptors at different stages of liver regeneration were consistently observed, it is uncertain whether these differences were significant physiologically. Possible mechanisms of change in the  $K_d$  values were suggested in our previous paper (17).

## DISCUSSION

There is evidence, as reviewed in the papers of Bucher (5) and Bucher and Malt (6), that adrenalectomy does not fundamentally interfere with liver regeneration in animals but that it slows down the restoration of tissue mass and protein and

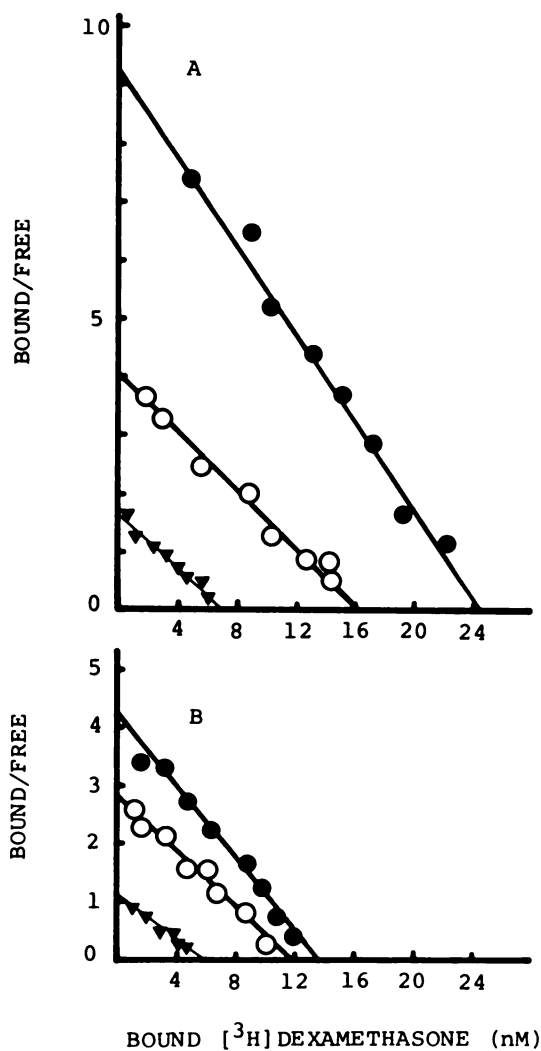


Chart 4. Scatchard plots of [ $^3\text{H}$ ]dexamethasone binding to receptors in cytosol from regenerating liver of partially hepatectomized rats. A, regenerating liver from rats that had been adrenalectomized 7 days previously; B, regenerating liver from rats with intact adrenals. O, control rats;  $\nabla$ , 24 hr after hepatectomy;  $\bullet$ , 5 days after hepatectomy.

reduces the usual pronounced fatty infiltration (10, 13, 22, 29), with little change, or possible increase in the mitotic index (16, 27). Within 3 days after bilateral adrenalectomy of male rats, we observed an increase in the level of specific dexamethasone binding of liver cytosol, which was maintained for several days and then gradually decreased to the level in the liver cytosol of control rats (Chart 1), in agreement with previous reports (2, 23). The rapid increase after adrenalectomy may be explained by decay of endogenous adrenal steroids leading to increased binding of radioactive steroid by the constant population of receptors (23), which are redistributed between the cytosol and nuclear compartment under the influence of the endogenous corticosterone level (2).

Charts 2 and 3 show that in rats with or without adrenals the time of the decrease in binding of dexamethasone to its receptors in regenerating liver corresponded to the initial period of DNA synthesis (7) and to the period when induction of tryptophan oxygenase by hydrocortisone was depressed (27). However, the increase in binding of dexamethasone to its receptors

4 to 7 days after partial hepatectomy was much greater in regenerating liver of adrenalectomized rats (Chart 2) than in that of rats with adrenals (Chart 3). This difference may be related in part to the high level of endogenous corticosterone in partially hepatectomized rats with adrenals,<sup>3</sup> since translocation of the receptor-steroid complex to the nucleus occurs in the presence but not in the absence of the hormone (4, 9). The relationship between the high level of glucocorticoid receptor between Days 4 and 7 after partial hepatectomy (Chart 2) and the induction rate of tryptophan oxygenase by glucocorticoid (27) is still unknown, because no information is available on the induction of tryptophan oxygenase by hydrocortisone between 48 hr and 8 days after partial hepatectomy in rats without adrenals (27).

Several published studies demonstrated that mitogen stimulation of human lymphocytes induced an increase in the number of glucocorticoid-binding sites per cell (20, 28). The increase in receptor number after mitogen stimulation may correlate with phases of the cell cycle. Cidowski and Michaels (8) reported that in synchronized HeLa cells the number of cytoplasmic glucocorticoid receptors per cell doubled between late G<sub>1</sub> phase and S phase and that during this period there was an increase in alkaline phosphatase activity that can be stimulated by glucocorticoid. In contrast to this, we found that cytoplasmic glucocorticoid receptors in regenerating liver of rats decreased significantly (Charts 2 and 3). This decrease of dexamethasone binding to the receptors is correlated with the fact that the induction of tryptophan oxygenase by a pharmacological dose of hydrocortisone was depressed during the period in which DNA synthesis occurred after 70% hepatectomy (27). The physiological meaning of the decrease and increase of glucocorticoid receptors after partial hepatectomy is unknown; but these changes during the period of rapid rise of DNA synthesis should be favorable for DNA synthesis *in vivo*, since glucocorticoid inhibits DNA synthesis and, if this inhibition is mediated by the steroid receptors, the observed high level of endogenous corticosterone would inhibit the synthesis. The subsequent increase of glucocorticoid receptors is probably associated with induction of the differentiated function of glucocorticoid (27) and slowdown of the rapid DNA synthesis and mitosis in the later part of liver regeneration.

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