

## Effect of an antiandrogenic H<sub>2</sub> receptor antagonist on hepatic regeneration in rats

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Because biochemical "feminization" of the liver in males is observed with hepatic regeneration and because the hepatic regenerative response in females is greater than that in males, the possibility that antiandrogens might potentiate liver regeneration was investigated. Before 70% hepatectomy, adult male Wistar rats were treated with cimetidine, an antiandrogenic H<sub>2</sub> antagonist, at doses up to 10 times greater than those used clinically. Control animals received either the saline vehicle or ranitidine, an H<sub>2</sub> antagonist without antiandrogenic properties. Treatment with cimetidine reduced the hepatic cytosolic androgen receptor content compared with ranitidine treatment. Hepatectomy caused a further reduction in androgen receptor activity in all groups. Hepatic cytosolic estrogen receptor activity was comparable in all groups throughout the study. Moreover, the rate of liver growth and the levels of ornithine decarboxylase and thymidine kinase activity induced as part of the regenerative response were similar in all groups. Thus, cimetidine, despite its ability to bind to androgen receptors, and ranitidine, an H<sub>2</sub> receptor antagonist without antiandrogen action, do not modulate the hepatic regenerative response to a 70% partial hepatectomy. (J LAB CLIN MED 1988;112:232-9)

**D**espite the fact that the liver is not generally considered a classical target for sex hormones, it has been shown to have a number of sexually dimorphic characteristics.<sup>1-3</sup> Recent data have demonstrated that during the regenerative response that follows partial hepatectomy, the liver in the male rat is "demasculinized" in that it loses many of its male-specific attributes.<sup>4</sup> Neither the regulation of this demasculinization process nor the biologic significance of hepatic demasculinization associated with hepatic regeneration is understood. Moreover, it is well known that the regenerative response of female animals is

greater than that of male animals to a wide variety of hepatotoxic stimuli. An extension of these data is that agents with antiandrogenic properties might have the potential to enhance the rate of hepatic regeneration after liver injury by augmenting the demasculinization of the liver. This hypothesis has been evaluated by using a standard rat model of hepatic regeneration after two-thirds hepatectomy.<sup>5</sup> Cimetidine was chosen as the drug for these experiments because of its widespread clinical use. In addition to its antagonism of H<sub>2</sub> receptor-mediated functions, it also has been shown to have antiandrogenic properties, including the ability to bind to androgen receptors.<sup>6-8</sup>

### METHODS

**Animals and supplies.** Adult male inbred Wistar rats (200 to 350 gm) were used in these studies. They were purchased from Harlan Sprague-Dawley, Indianapolis, Ind. Cimetidine (SK&F Co., Philadelphia, Pa.) and ranitidine (Glaxo Inc., Research Triangle Park, N.C.) were obtained from the pharmacy of the Presbyterian University Hospital (Pittsburgh, Pa.). Dupont NEN Medical Products, Boston, Mass., was the source for the carbon 14-labeled ornithine (57.6

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mCi/mmol), tritiated estradiol (99 Ci/mmol), tritiated R1181 (87 Ci/mmol), and unlabeled R1881 used in these experiments. Tritiated thymidine and ACS scintillation fluid were purchased from Amersham Corp., Arlington Heights, Ill. Absolute ethanol and DEAE-cellulose paper were purchased from U.S. Industrial Chemicals Co., Tuscola, Ill., and Bio-Rad Laboratories, Richmond, Calif., respectively. Unlabeled ornithine, pyridoxal phosphate, Tris base, diethylstilbestrol, adenosine triphosphate, sodium molybdate, nicotinamide adenine dinucleotide, and bovine serum albumin were purchased from Sigma Chemical Co., St. Louis. All other chemicals were purchased from Fisher Chemical Co., Pittsburgh, Pa.

**Drug dosages.** The cimetidine and ranitidine dosages used were based on the dosages used clinically in humans for the active treatment of duodenal ulcer disease. The dosage identified as "1×" in the text and figures corresponds to 17 mg/kg for cimetidine and 4.3 mg/kg for ranitidine, respectively. The dosages identified as "5×" and "10×" represent five times and 10 times the dosages used at "1×" for each of the two drugs. The drugs were diluted as necessary with saline solution, such that both experimental and control animals received 0.5 ml saline solution per injection.

**Animal treatment and tissue preparation.** Male Wistar rats were assigned randomly to the various treatment groups. Animals in the experimental groups were given the appropriate dose of either cimetidine or ranitidine dissolved in 0.5 ml saline solution administered intraperitoneally beginning 72 hours before partial hepatectomy and every 24 hours thereafter until the time the animals were killed. Animals used as placebo controls received 0.5 ml saline solution, which was administered intraperitoneally by using the same dosing schedule. A two-thirds partial hepatectomy was performed, as described by Higgins and Anderson,<sup>5</sup> between 9 and 12 AM with the animals under ether anesthesia. The liver tissue removed at the time of hepatectomy was used to determine the baseline values for all of the parameters assessed.

At 6, 12, 24, and 48 hours after hepatectomy, animals from the control group and both drug treatment groups were anesthetized with ether and weighed. The liver remnant was removed, weighed, and homogenized in 4 vol ice-cold buffer consisting of 0.25 mol/L sucrose, 1.5 mmol/L EDTA, 10 mmol/L mercaptoethanol, and 10 mmol/L Tris HCl (pH 7.4) by using a Polytron homogenizer. Cytosol was prepared by centrifugation at 103,000 g for 1 hour at 4° C. All cytosolic enzyme assays were performed immediately after preparation of the cytosol.

**Ornithine decarboxylase assay.** Ornithine decarboxylase activity was determined in vitro by measuring the release of <sup>14</sup>CO<sub>2</sub> from labeled ornithine.<sup>9</sup> In this assay, 0.4 ml cytosol was preincubated for 5 minutes at 37° C with a mixture containing 0.2 mmol/L pyridoxal phosphate, 5 mmol/L dithiothreitol, 1.5 mmol/L L-ornithine in 10 mmol/L Tris HCl (pH 8.0). At the end of this period, 1 μCi L-<sup>14</sup>C-DL-ornithine was added to the mixture and 250 μl ethanolamine-ethylene glycol (2:1) was added to a center well to act as a CO<sub>2</sub> trap. The assay flask was sealed and incubated at 37° C for 1 hour. The reaction was terminated by the injection of 0.1 ml 100% (wt/vol) trichloroacetic acid solution into the reaction mixture through the top. After the reaction flask had been maintained

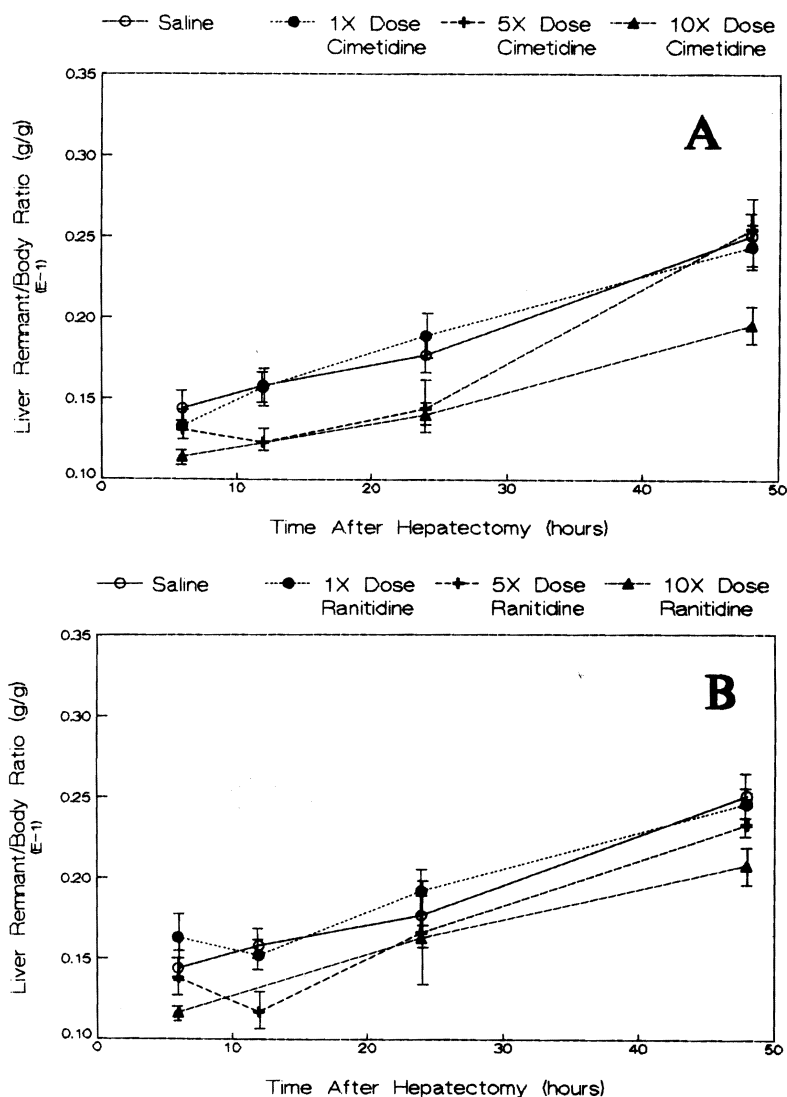
at 37° C for an additional hour, the CO<sub>2</sub> trapping solution was removed and placed directly into a glass scintillation vial containing 10 ml ACS scintillation fluid. Radioactivity was measured in a Packard Tri-Carb 460 CD liquid scintillation system (Packard Instrument Co., Downers Grove, Ill.).

**Thymidine kinase assay.** Thymidine kinase activity was determined by measuring the in vitro conversion of thymidine to thymidine phosphate.<sup>10</sup> Cytosol (0.1 ml) was mixed with 850 μl incubation buffer consisting of 5 mmol/L adenosine triphosphate, 3.6 mmol/L MgCl<sub>2</sub> in 50 mmol/L Tris HCl (pH 8.0), and 50 μl of 1 μmol/L tritiated thymidine. The reaction was maintained at 37° C for 10 minutes and terminated by immersion in boiling water for 2 minutes. After cooling in an ice bath, the mixture was centrifuged for 5 minutes at 4° C at 1500 g to remove denatured protein. An aliquot (0.1 ml) of the supernatant was spotted on a 3.8 × 3.8 cm piece of DEAE-cellulose paper. The paper was washed twice with 1 mmol/L ammonium formate for 5 minutes followed by distilled water for 3 minutes. Next, the paper was placed in a glass scintillation vial and the radioactivity bound to the paper was eluted into solution by the addition of 1 ml of 0.1 mol/L HCl–0.2 mol/L KCl. After 15 minutes, 10 ml ACS scintillation fluid was added, and the tritium present in the vial was determined.

**Cytosolic sex steroid receptor assays.** The activity of the cytosolic estrogen receptors was determined by measuring the specific binding at a saturating concentration of tritiated estradiol.<sup>11</sup> The cytosol, prepared as noted above, was diluted 1:1 with buffer consisting of 40 mmol/L sodium molybdate, 1.5 mmol/L EDTA, and 10 mmol/L Tris HCl (pH 7.4) to stabilize the receptors. To measure total binding of the ligand, 200 μl of this diluted cytosol was mixed with 25 μl of 30 nmol/L radioactive ligand and 25 μl ethanol. Nonspecific binding was measured in parallel assays in which the ethanol was replaced with 25 μl of 3 μmol/L unlabeled diethylstilbestrol dissolved in ethanol. After 2 hours at 4° C, the incubation was terminated by the addition of 0.4 ml 1% dextran-coated charcoal to each tube to remove unbound ligand. This suspension was centrifuged for 5 minutes at 1500 g at 4° C, and the supernatant was carefully transferred to a scintillation vial containing 8 ml ACS scintillation fluid. The radioactivity in the vial was measured.

The cytosolic androgen receptor assay used was similar in design to that described for the estrogen receptor assay.<sup>4</sup> Tritiated R1881, a synthetic androgen, was used as the labeled ligand, and unlabeled R1881 was used in the nonspecific binding assays. Triamcinolone acetonide (5 μmol/L) was included in all androgen assays to block binding of R1881 to glucocorticoid receptors. The only other difference between the two methods is that in the androgen receptor assay the cytosol was incubated at 0° C overnight rather than for 2 hours as in the estrogen receptor assay.

**Miscellaneous methods and procedures.** Protein concentrations were determined by the method of Lowry et al.<sup>12</sup> with bovine serum albumin being used as the standard. Statistical analysis of the data was performed with one-way analysis of variance followed by Scheffé's test through the Abstat program on an IBM PC-XT computer (IBM Corp., Valhalla, N.Y.). A *p* value of 0.05 or less was considered to represent



**Fig. 1.** Liver regeneration after partial hepatectomy. Animals from placebo-treated control, cimetidine-treated (A), and ranitidine-treated (B) groups were killed by exsanguination at times noted. Data are presented as ratio of liver remnant weight to body weight at time killed and as mean  $\pm$  SEM values with three to six animals per group.

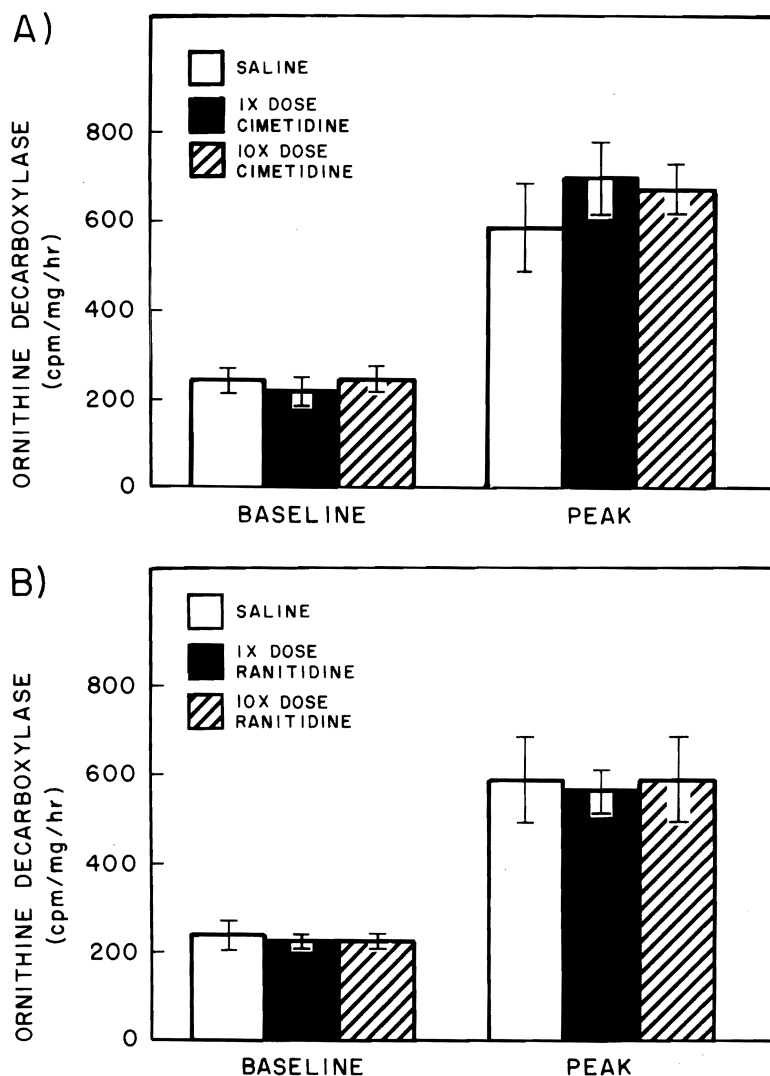
a significant difference. All results are presented as mean values  $\pm$  SEM.

## RESULTS

The increase in liver remnant weight measured during the course of regeneration after two-thirds hepatic resection in control, cimetidine-treated, and ranitidine-treated animals is presented in Fig. 1. No statistically significant difference between the three groups at 6 hours or at any other time examined in the study was noted. Moreover, in Fig. 1, both A and B, should be noted that the rates at which the liver increased in weight after partial hepatectomy in cimetidine- and ranitidine-

treated animals, denoted by the slopes of the lines in the figure, did not differ from those of the vehicle-treated controls, and no dose effect was seen for either agent.

Preliminary experiments performed by us and other investigators demonstrated that the maximum induction of ornithine decarboxylase after partial hepatectomy occurs 6 hours after surgery.<sup>9,13-15</sup> Pretreatment with either drug at both the 1 $\times$  or 10 $\times$  dose did not alter the hepatic ornithine decarboxylase activity measured initially or that observed at peak levels seen after partial hepatectomy (Fig. 2). A comparable threefold increase in ornithine decarboxylase activity was observed in all



**Fig. 2.** Induction of ornithine decarboxylase activity by partial hepatectomy. **A,** Data from cimetidine-treated animals. **B,** Data from ranitidine-treated animals. Data are presented as mean  $\pm$  SEM values with three to six animals per group and are presented as counts per minute per milligram of protein per hour.

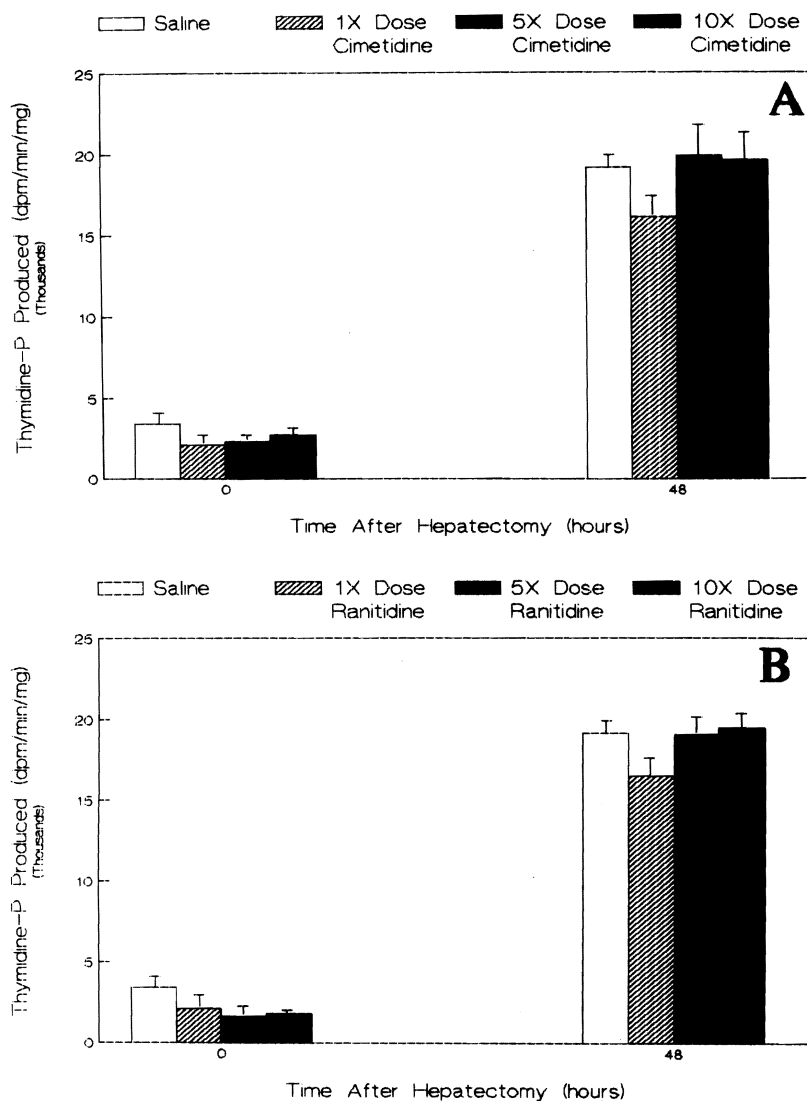
treatment groups at 6 hours after partial hepatectomy when ornithine decarboxylase levels were expected to be increased maximally (Fig. 2).

Thymidine kinase activity was induced also in all groups as a result of partial hepatectomy (Fig. 3). The activity of this enzyme reaches a maximum at 24 to 48 hours after hepatectomy.<sup>10</sup> No statistically significant difference in hepatic thymidine kinase activity either between the drug-treated groups and the vehicle-treated controls or between the various doses of either drug at any time point evaluated during the study was evident.

Before hepatectomy, administration of the antiandrogenic  $H_2$  receptor antagonist cimetidine caused a slight decrease in hepatic cytosolic androgen receptor

content (Fig. 4). Conversely, ranitidine treatment resulted in a significant increase in the hepatic cytosolic androgen receptor content. All differences between two groups of drug-treated animals and the vehicle-treated controls disappeared at 24 hours and remained no different through the next 24 hours.

In contrast with the observations on the androgen receptor activity, pretreatment with the lowest dose of cimetidine caused a twofold increase in the hepatic cytosolic content of estrogen receptor compared with that seen in the controls at time zero (Fig. 5, A), an effect not observed in the ranitidine-treated groups (Fig. 5, B). Partial hepatectomy was followed by a sharp reduction in the cytosolic estrogen content in all animals



**Fig. 3.** Induction of thymidine kinase activity by partial hepatectomy. Data are presented as mean  $\pm$  SEM values with three to six animals from the cimetidine (A) and ranitidine (B) groups. Ordinate is in disintegrations per minute per milligram protein  $\times$  1000.

studied within 6 hours, reaching a nadir at 12 hours after partial hepatectomy and abolishing any difference between the treatment groups.

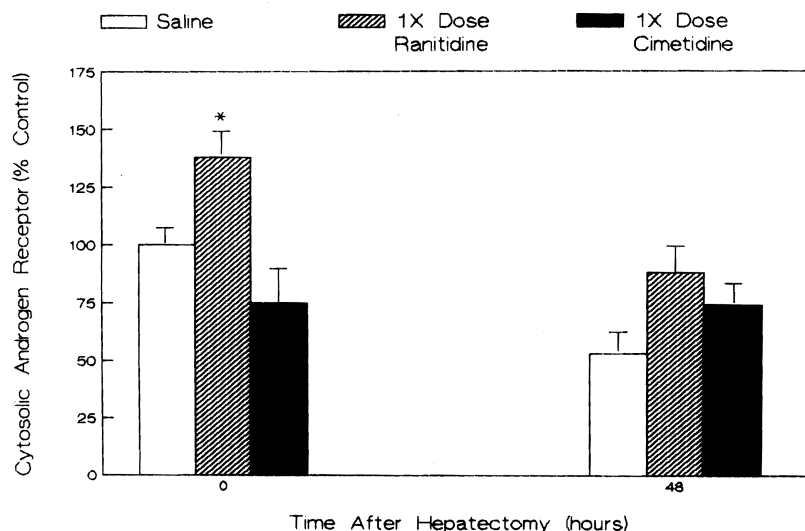
## DISCUSSION

This study was designed to examine the effect of hepatic sex hormone receptor modulation by two commonly used  $H_2$  antagonists, cimetidine and ranitidine, on the rate of hepatic regeneration that occurs after two-thirds partial hepatectomy in the rat. It was hypothesized that cimetidine, acting as an antiandrogen and augmenting hepatic demasculinization during regeneration after partial hepatectomy, might increase the rate of subsequent hepatic regeneration.<sup>4</sup> Ranitidine was included in the study as a control for the  $H_2$  antagonist

effects of cimetidine, because this drug has no known antiandrogenic effects.

Significantly, neither drug was found to alter the rate of regeneration after partial hepatectomy. These results were somewhat unexpected for two reasons. First, as noted above, the regenerating liver has been reported to be more feminized than the nonregenerating liver.<sup>4</sup> Second, studies have been published claiming an inhibitory effect of both cimetidine and ranitidine on hepatic regeneration in rats.<sup>16,17</sup> Conversely, data also have been published that suggest that cimetidine at suprapharmacologic doses enhances hepatic regeneration *in vitro*.<sup>18</sup>

Our data demonstrate that the administration of cimetidine and ranitidine alters the cytosolic androgen



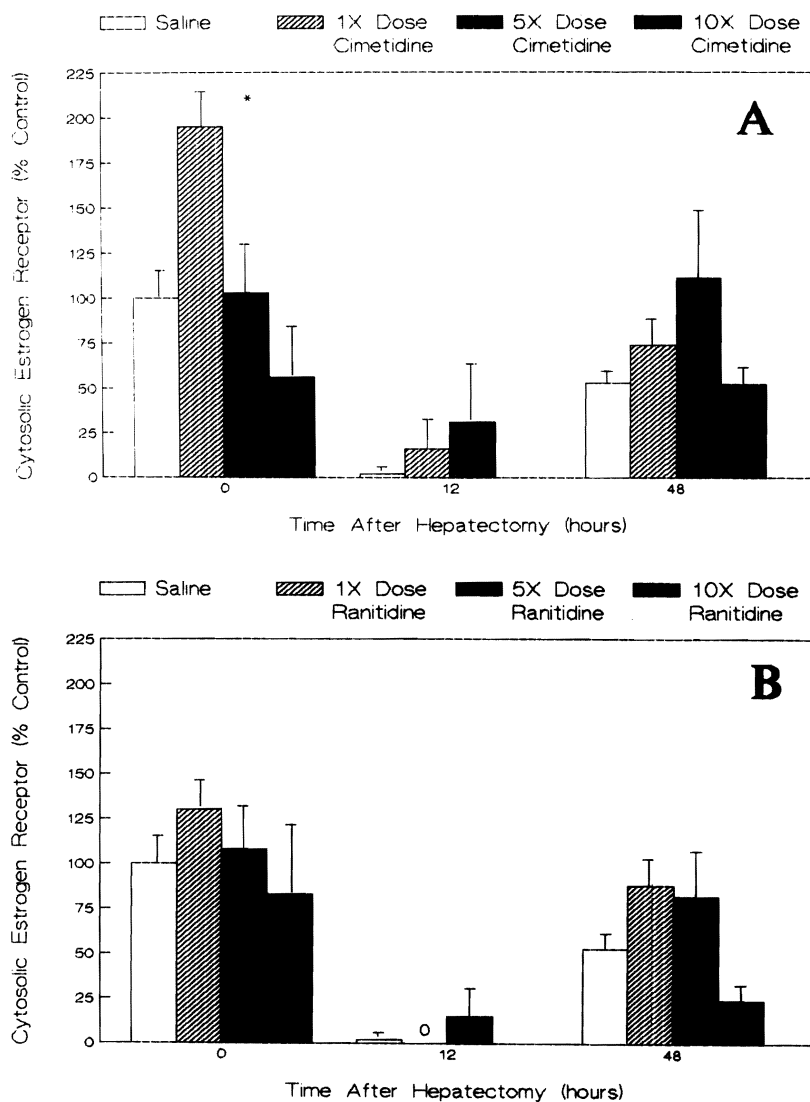
**Fig. 4.** Hepatic cytosolic androgen receptor content during hepatic regeneration. Bars represent mean  $\pm$  SEM values with three to six animals per group. \* $p < 0.05$  versus controls.

receptor status of the liver before hepatic resection, although in different directions. Cimetidine treatment produced a reduction in androgen receptor activity, and ranitidine produced a small, and probably unimportant, increase in the androgen receptor activity of the liver. Cimetidine treatment also produced a significant increase in the basal cytosolic estrogen receptor content of the rat liver when used at the dose used clinically but had either no effect or a paradoxical effect when used at the two larger doses. Ranitidine had no effect on the basal level of estrogen receptor activity within the liver before hepatectomy. After two-thirds hepatectomy, the androgen and estrogen receptor activities of the liver of the three groups of animals studied changed in a direction characteristic of the regenerative process. Prior and continuing  $H_2$  receptor antagonist therapy had no effect on the changes produced by the regenerative process per se, which have been described previously and which were replicated in the vehicle-treated control animals.<sup>4</sup> Because it is generally believed that sex hormones, like other hormones, exert their effects through an interaction with specific receptors for the hormones in question, the changes observed in the concentrations of the two hepatic cytosolic sex hormone receptors studied before hepatectomy and induced by the two  $H_2$  receptor antagonists used should represent alterations in the potential responsiveness of the liver to both androgens and estrogens in response to prior drug treatment. It should be noted, however, that the changes in sex hormone receptor activities induced as a result of  $H_2$  antagonist therapy were only modest.

Despite these initial changes in the basal (time zero) receptor concentrations in the liver, the subsequent rate

of hepatic regeneration and the changes in the cytosolic content of androgen and estrogen receptors after partial hepatectomy was not affected, as evidenced by parallel growth curves, regardless of the drug or dosage used in the experiments (Fig. 1) and data relative to the effects of the hepatic regenerative response on androgen and estrogen receptor activity within the liver (Figs. 4 and 5). These data conflict somewhat with the conclusions reached by earlier investigators, who have reported an inhibition of regeneration in vivo with these two drugs, and others have reported an enhancement of the regenerative response, at least with cimetidine, when studied in vitro.<sup>16-18</sup> The claim for an inhibition of the regenerative response was based primarily on the finding of a slight delay in the increase in the mitotic index and a lesser increase in the level of serum aspartate aminotransferase activity observed after partial hepatectomy in the animals studied. It should be noted that the rate of liver growth in the drug-treated animals in these earlier studies was not significantly different from that of the controls at any time point examined. This latter observation is consistent with the data obtained in the present study.

The present study extends the previous studies in terms of the number of rats used, the number of drug dosages investigated and the variety of hepatic parameters examined. An important difference between the present study and the three previous studies<sup>16-18</sup> is that thymidine kinase activity was used instead of the mitotic index as a measure of DNA replication. It is well known that there is a good correlation between these two procedures in a variety of systems<sup>10,19,20</sup> and that the variance for the mitotic index is greater than that



**Fig. 5.** Hepatic cytosolic estrogen receptor content during hepatic regeneration. Hepatic cytosolic estrogen receptor concentrations in control, cimetidine-treated (A), and ranitidine-treated (B) rats were measured in same cytosolic extracts as androgen receptor concentrations. Ordinate is in disintegrations per minute per milligram protein  $\times 1000$ . \* $p < 0.05$  versus controls.

of thymidine kinase activity. Nonetheless, it should be pointed out that the measurement of levels of thymidine kinase assumes similar precursor pools for the various groups of animals being studied, although the mitotic index relies less heavily on this assumption.

In the present study, no difference between peak levels of hepatic thymidine kinase activity was observed between animal groups, regardless of the drug or dosage used. Moreover, the similar levels of hepatic ornithine decarboxylase activity, an indicator of the increase in polyamine synthesis thought to signal hepatic regeneration, between the various groups studied further supports a lack of an effect of either  $H_2$  antagonist on the regenerative process of the liver.<sup>13</sup>

An unexpected observation noted during the course of this study was that although pretreatment with both drugs induced alterations in the prehepatectomy concentration of hepatic cytosolic androgen and estrogen receptors, these drug effects on the cytosolic content of steroid hormone receptors disappeared within as little as 6 hours after partial hepatectomy. Presumably, some unknown overriding mechanisms are initiated or take over after partial hepatectomy, such that hepatic regeneration occurs regardless of the basal sex hormone receptor status of the liver, and the sex hormone receptor activities change as a consequence of regeneration rather than in response to earlier and continued  $H_2$  receptor antagonist treatment.

On the basis of the present data, it is not possible to determine whether this overriding mechanism originates within the liver itself or whether it is extrahepatic in origin. Some of the effects originally attributed to the interaction of sex hormones with their receptors within the liver have been shown to be mediated by the pituitary.<sup>3,21-23</sup> An alternative liver-based mechanism that could be active in these studies is the production of a putative growth-stimulating factor thought to be released into the serum by injured or regenerating liver tissue.<sup>24-26</sup> No investigations have yet been published on the effect of such a material or materials on the activity of hepatic sex hormone receptors present within liver cells.

In summary, the data presented do not support the earlier published conclusion that either cimetidine or ranitidine inhibits the rate of hepatic regeneration observed after a two-thirds hepatectomy in the rat. Neither agent had a lasting detectable effect on a variety of parameters associated with hepatic regeneration, including thymidine kinase activity, ornithine decarboxylase activity, and the level of two major sex hormone receptor levels within the liver after partial hepatectomy.

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