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Sex Hormone-Related Functions in Regenerating Male Rat Liver

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Sex hormone receptors were quantitated in normal male rat liver and in regenerating liver at several different times after partial (70%) hepatectomy. Both estrogen and androgen receptor content were altered dramatically by partial hepatectomy. Total hepatic content and nuclear retention of estrogen receptors increased, with the zenith evident 2 days after partial hepatectomy, corresponding to the zenith of mitotic index. Serum estradiol increased after 1 day, and reached a maximum at 3 days after surgery. In contrast, total and nuclear androgen receptor content demonstrated a massive decline at 1, 2, and 3 days after resection. Serum testosterone displayed a parallel decline. In addition, hepatic content of two androgen-responsive proteins was reduced to 15% and 13% of normal values during this period. The activity of these various proteins during regeneration of male rat liver is comparable to that observed in the liver of normal female rats. Taken together, these results indicate that partial hepatectomy induces a feminization of certain sexually dimorphic aspects of liver function in male rats. Furthermore, these data provide evidence that estrogens, but not androgens, may have an important role in the process of liver regeneration.

Mammalian liver of both sexes is responsive to sex hormones. Liver is estrogen responsive in that it contains receptors for this hormone and responds by the synthesis of specific proteins (reviewed in Reference 1). Liver contains androgen receptors as well (2–5). In fact, the sexually dimorphic hepatic content of certain receptors and microsomal enzymes observed in male rats is maintained by testosterone (reviewed in References 1 and 6). Ultimate control over these sex differences in hepatic function resides in the pituitary gland. In particular, growth hormone secretion patterns, also sexually dimorphic in nature, appear to regulate hepatic levels of many microsomal steroid and drug metabolizing enzymes as well as steroid receptor levels (1,7,8).

Since Higgins and Anderson originally reported (9) the technique of partial hepatectomy (PH) in rats, investigators have been studying the effect of hepatic resection and the remnant liver with the goal of identifying the mechanisms that regulate hepatic regeneration. During the last 10 yr, hormones have assumed a prominent role as factors related in some way to hepatic regeneration after PH. It has been suggested that hormonal agents such as insulin, glucagon, and epidermal growth factor may initiate the process of regeneration (10–17); however, other hormones such as parathyroid hormone (18), calcitonin (14), iodothyronines (19), and glucocorticoids (20) also have been shown to influence the hepatic regenerative response.

There are several lines of evidence suggesting that estrogens have a potential role in liver cell proliferation. First, estrogens have been implicated in certain liver diseases characterized by increased mitogenic activity (reviewed in References 1 and 21).

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Abbreviations used in this paper: AR, androgen receptor; E_2, estradiol; E2-OHase, estrogen 2-hydroxylase; ER, estrogen receptor; [^{3}H]R1881, 17 α-methyl-[^{3}H]-trienolone; MEB, male-specific [illegible]; PH, partial [illegible]

Furthermore, livers containing hepatic adenoma and focal nodular hyperplasia have demonstrated increased estrogen receptor (ER) content (22). Thus it was of interest to investigate potential influences of estrogens in regeneration; in a recent study our group reported changes in cytosolic and nuclear ERs in regenerating rat liver during the first 72 h after hepatectomy (23). These data demonstrated that the proliferative activity of the regenerating liver is concurrent with a significant increase in nuclear localization of hepatic ERs. However, this study did not examine possible increases in serum estradiol content that might account for the increased nuclear ER localization, nor was total hepatic content of ER examined. We have now extended the study to answer these questions, and in addition, to determine what effects PH may have on serum testosterone and hepatic content of androgen receptor (AR) and certain androgen-responsive proteins.

Materials and Methods

Animals

The male Sprague–Dawley rats (350 g) used in these studies were maintained on standard rat laboratory diet and water ad libitum in a temperature- and light-controlled room (lights on, 6:30 AM–6:30 PM). Partial hepatectomy was performed according to the method of Higgins and Anderson (9). All operations were performed between 7:30 AM and 9:00 AM using ketamine anesthesia (10 mg/100 g body wt). In the sham-operated animals used as controls, the liver was manipulated in the same manner as were the livers of animals undergoing hepatic resection, but was returned intact to the abdomen. The animals were killed by decapitation at 12, 24, 48, 72, 96, and 144 h after PH.

Materials

Estrone and estradiol (E_2) were purchased from Steraloids, Wilton, N.H. Diethylstilbestrol, leupeptin, benzamidine, bovine serum albumin, sodium molybdate, protamine sulfate, and other buffer components were purchased from Sigma Chemical Company, St. Louis, Mo. Norit A and dextran C were obtained from Fisher Scientific Company, Pittsburgh, Pa. Radioactive (2, 4, 6, 7, 16, 17)-[^{3}H]estradiol, ([^{3}H]E_2), 151 Ci/mmol; [^{3}H]methyl S-adenosylmethionine, 10 Ci/mmol; 17 α-methyl-[^{3}H]methyltrienolone ([^{3}H]R1881), nonradioactive R1881, and Econofluor scintillation fluid were obtained from New England Nuclear, Boston, Mass. Aqueous Counting Scintillant was purchased from Amersham, Arlington Heights, Ill. The radiolabeled steroids used in these studies were assayed periodically for purity by thin-layer chromatography on silica gel G in ethyl acetate/hexane/ethanol (85:10:5), and were used only if purity was 95%. The sources of other material have been described (23,24).

Buffers

Unless otherwise stated, all experiments were performed at 0°–4°C using the following buffers: 0.01 M Tris HCl, 1.5 mM ethylenediaminetetraacetic acid, pH 7.4 (TE buffer); TE buffer with 5 mM dithiothreitol (TED buffer); TE buffer with 20 mM sodium molybdate (TEM buffer); TE buffer with 0.25 M sucrose (TES buffer); and both of the latter additions (TEMS buffer); and 0.25 M sucrose, 3 mM $MgCl_2$, 10 mM HEPES, pH 7.4 (SMgH buffer); and SMgH with 20 mM sodium molybdate, pH 7.4 (SMgHM buffer). Leupeptin (0.15 mM) and benzamidine (1.0 mM) were added to all buffers used in preparation of nuclei and cytosol, and to those used for gel filtration chromatography.

Estrogen Binding Studies

Protamine sulfate assay of cytosolic estrogen receptor. The protamine sulfate precipitate method was used to assay cytosolic ER; this method avoids interference of [^{3}H]E_2 binding to the high-capacity male specific estrogen binder (MEB) of male rat liver cytosol (23,24). Cytosol and protamine sulfate precipitates thereof were prepared as described previously (23). Protamine sulfate precipitates of 200-μl aliquots of hepatic cytosol were incubated with varying concentrations of [^{3}H]E_2 over a range of 0.15–5.0 nM in the absence (total binding) and presence (nonspecific binding) of 100-fold excess of unlabeled E_2 for 18 h at 0°C. These conditions yield maximum binding and represent equilibrium conditions (23). Specific binding was obtained by subtracting nonspecific binding from total binding.

Exchange assay for nuclear estrogen receptor. Liver (5 g) was homogenized in 3 vol of TEM buffer using a Brinkman PT 10-35 polytron (Brinkman Instruments, Inc., Westbury, N.Y.), and nuclei were prepared and washed as previously described (23). Cytosolic contamination of nuclei was assayed by determining the activity of alcohol dehydrogenase (25) in the homogenate, cytosol, and the washed nuclei preparation and was found to be <0.5% in the nuclear preparation. The average recovery of DNA in the nuclear preparation was 72.8% of that in the homogenate. Nuclear ER was quantitated by incubating nuclear suspensions (0.2 ml) in the presence of 10 nM [^{3}H]E_2 in SMgH buffer with and without a 100-fold excess concentration of unlabeled diethylstilbestrol at 30°C for 1 h. Each assay was performed in triplicate. The reaction was stopped by chilling the tubes on ice for 5 min; free steroid was removed by washing the nuclear pellet with 2 ml SMgH containing 0.1% Triton X-100 followed by centrifugation at 800 g for 10 min. The nuclear pellet was washed three more times with 2 ml of SMgH. The bound steroid was extracted from the nuclear pellet with 2 ml of absolute ethanol at 30°C for 30 min. The ethanol extract was counted in 10 ml of aqueous counting scintillant in a Packard Tri-Carb spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.). The remaining nuclear pellet was used for determination of DNA content.

Androgen Binding Studies

Preparation of subcellular fractions. To prepare all subcellular fractions from the same liver, the liver was homogenized in 3 vol of TES buffer. Nuclei were sedi-

mented by centrifugation of the homogenate at 800 g for 15 min. The crude nuclear pellet was washed five times by resuspension of the pellet in SMgHM buffer and recentrifugation. The pellet was resuspended in SMgHM buffer to a final volume equal to that of the original homogenate. Nuclei prepared this way appeared rounded under light microscopy and stained blue with hematoxylin and eosin. The final nuclear preparation contained no detectable cytosolic contamination as judged by lack of specific cytosolic staining (above) and by assay for alcohol dehydrogenase activity (25).

For the preparation of cytosolic and microsomal fractions, the supernate from the crude nuclear pellet was centrifuged at 27,000 g for 15 min; the pellet was discarded. The decanted supernate was centrifuged again at 150,000 g for 30 min. The supernate from this step was considered to be the cytosolic fraction. Sodium molybdate (20 mM) was added immediately after the final centrifugation to the portion of cytosol to be used for receptor determinations, as omission of this salt results in a 50%–70% reduction in androgen binding activity. The microsomal pellet was washed by resuspension in SMgH, centrifuged again as before, and resuspended in SMgH containing 0.1 mM dithiothreitol. Final protein concentration of the microsomal suspension was typically 6–8 mg/ml, whereas that of cytosolic fraction was 25–30 mg/ml.

Androgen binding assays in cytosol. Cytosol prepared as described above was diluted with 1 vol of TEM buffer before use. All incubations for determination of AR included 500 nM triamcinolone acetonide to block any potential contribution of the glucocorticoid receptor to [^{3}H]R1881 binding. For quantitation of the cytosolic receptors, aliquots (200 μl) of cytosol were incubated overnight at 4°C with 0.2–5.0 nM [^{3}H]R1881 in the absence (total binding) and presence (nonspecific binding) of a 100-fold excess of unlabeled R1881. The difference between these two values was considered to be specific binding. Characterization of the [^{3}H]R1881 binding activity as a cytosolic AR has been reported elsewhere (2,3). The androgenic ligand commonly used in other tissues, [^{3}H]dihydrotestosterone, could not be used in liver cytosol because of extensive metabolism of this substrate; however, no metabolism of [^{3}H]R1881 could be detected under these conditions. Bound steroid was separated from free steroid at the end of the incubation period using dextran-coated charcoal as described previously (24).

Nuclear binding assays. To determine the best concentration of steroid for a one-point assay, aliquots (200 μl) of nuclear suspensions from livers of normal male and castrated rats were incubated with 0.2–10 nM [^{3}H]R1881 and 1 μM triamcinolone acetonide at 4°C overnight in the absence (total binding) and presence (nonspecific binding) of a 100-fold excess of unlabeled R1881. Specific binding was calculated as the difference between total and nonspecific binding. The single concentration at which the specific binding best agreed with the binding value calculated from the saturation curve analysis data using [illegible] was 5 nM [^{3}H]R1881 (r = 0.92); these [illegible] minimized nonspecific binding while specific [illegible] were [illegible]

described for the nuclear ER, with the exception that the wash buffer did not contain detergent. The washed pellet was then extracted with 2 ml of ethanol for 1 h at 30°C; the entire pellet and extract were transferred to a 20-ml scintillation vial and 8 ml of Aqueous Counting Scintillant was added. Evidence that [^{3}H]R1881 binding represents nuclear AR has been described elsewhere (2,3).

Assay of androgen-responsive hepatic proteins. Assays for the determination of cytosolic content of the MEB and of microsomal estrogen 2-hydroxylase (E2-OHase) activity have been described previously (4). Briefly, MEB is separated from other cytosolic estrogen binding proteins by gel filtration chromatography in TED buffer on Sephadex G-100, followed by incubation of the fractions with a saturating dose of [^{3}H]E_2 (5 nM) at 4°C overnight. This assay is quantitative for MEB and is linear with protein concentration over a broad range including those of the column fractions used in this assay.

Microsomal E2-OHase was assayed by the method of Paul et al. (26) with modifications described previously (4). Briefly, estrone is used as substrate for the E2-OHase reaction. The product, 1,3,5(10)-estrien-17-one-2,3,-diol, is labeled in a second reaction at the 2-position with a tritiated methyl group obtained from [^{3}H]methyl *S*-adenosylmethionine and transferred by partially purified catechol-O-methyltransferase to form the product [^{3}H]2-methoxy-1,3,5(10)-estrien-17-one-3-ol. The activity of E2-OHase observed is proportional to the rate of formation of the tritiated product. The quantity of product was determined by two-phase scintillation counting (4).

Other methods. Protein concentrations were determined by the method of Bradford (27); DNA concentrations of homogenates and nuclear preparations were determined by the method of Burton (28). Cytosolic receptor content for each animal was calculated on the basis of the amount of ^{3}H-labeled steroid bound per milligram cytosol protein, and then corrected for yield of cytosolic protein per gram of liver. Nuclear receptor content was calculated on the basis of the amount of ^{3}H-labeled steroid bound per milligram DNA recovered in the nuclear preparation, and then corrected for the DNA content of the original homogenate. Each calculation included appropriate volume corrections. For each animal, the total liver content of receptor represents the sum of the cytosolic and nuclear content based on 1 g of liver. Serum testosterone and estradiol were determined by specific radioimmunoassays as described previously (29). Equilibrium dissociation constants and the concentration of binding sites were calculated by the method of Scatchard (30). Unweighted linear regression analysis of Scatchard plots was performed on a Texas Instruments TI55 calculator (Texas Instruments, Inc., Houston, Tex.). Statistical analyses were performed using the Student's *t*-test program available on the Hewlett-Packard 9815S (Hewlett-Packard Co., Palo Alto, Calif.). Radioactivity content of samples was determined using a Packard TriCarb 4530 with automatic dpm conversion. Aqueous Counting Scintillant was used for single-phase scintillation counting. Econofluor scintillation fluid with an acidified aqueous phase (4) was used for two-phase scintillation counting.

Results

Figure 1 displays the total content of ER (A), as well as its distribution as cytosolic ER (B) and nuclear ER (C) in rat liver at different times after 70% hepatectomy. A previous study [23] indicated that PH induces a significant alteration in subcellular localization of hepatic ER, resulting in a decrease in cytosolic ER and a concomitant increase in nuclear ER. A recalculation of this data to express receptor number as a function of liver weight is depicted in Figures 1*B* and 1*C*. Cytosolic ER decreases rapidly during the first 12 h (0.3 pmol/g liver) and remains near this level until 48 h after hepatectomy, when the lowest recorded cytosolic ER is reached (0.24 ± 0.02 pmol/g liver). The values for cytosolic ER at 12, 24, and 48 h are significantly lower ($p < 0.005$) than that at 0 h. After this time the cytosolic ER [illegible] returns to a level somewhat greater than that at 0 and then normalizes for the remainder of the observation period [illegible]. The affinity of cytosolic ER for E_2 did not vary as a function of the change of receptor level. The equilibrium dissociation constant values for cytosolic ER at all time points studied were similar (0.5–2.5 nM). In contrast, the level of nuclear ER (Figure 1C), after a small decrease during the first day after surgery, increased rapidly, reaching a zenith at 48 h (35 pmol/g liver), a value significantly different ($p < 0.005$) from that observed at 0 h. Beginning at 72 h, the hepatic content of nuclear ER slowly returned to normal levels (10 ± 1 pmol/g liver). Figure 1A indicates that total hepatic ER also increased signif.

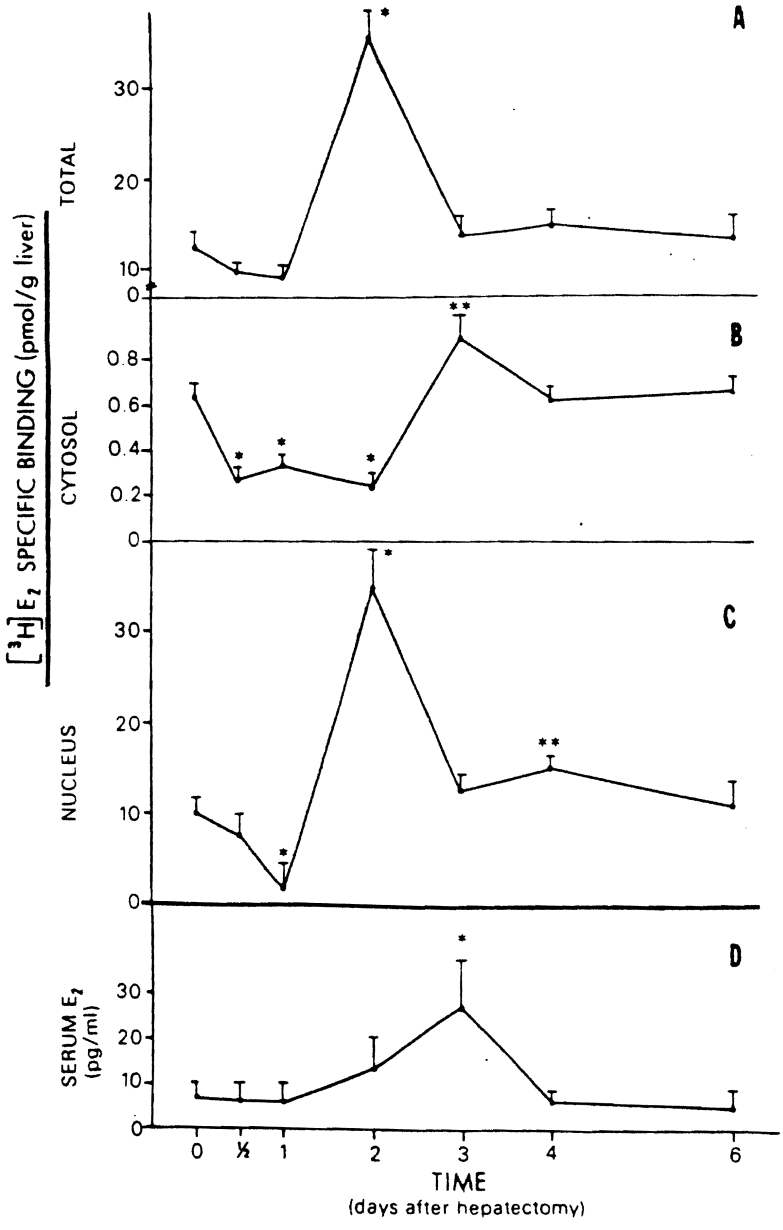

Figure 1. Variations in hepatic estrogen receptor activity after partial hepatectomy of male rats. Specific $[^3H]E_2$ binding was measured in cytosolic and nuclear fractions prepared from livers of partially hepatectomized rats (days after 70% hepatectomy). The values are expressed as picomoles per gram liver for total liver content (*A*), cytosolic binding (*B*), and nuclear binding (*C*). *B* and *C* represent recalculations of data previously described in Reference 23. Serum E_2 levels (picograms per milliliter) as measured by specific radioimmunoassay are shown in *D*. The values are expressed as mean ± SD. Values differing from those at time 0 are indicated: *$p < 0.005$, **$p < 0.01$. Sham-operated animals killed at 24 and 48 h displayed receptor and serum E_2 levels identical to those animals killed at time 0.

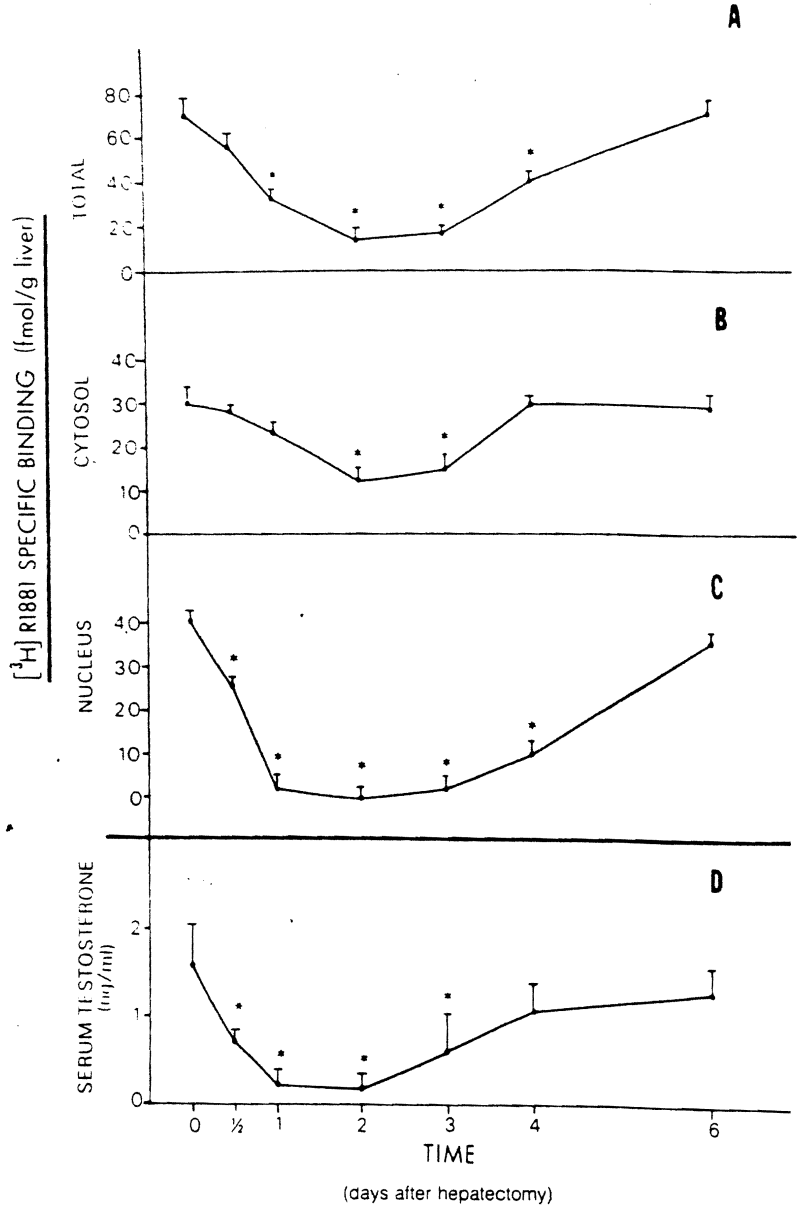

Figure 2. Variations in hepatic androgen receptor activity after partial hepatectomy of male rats. Specific [^{3}H]R1881 binding was measured in cytosolic and nuclear fractions prepared from livers of sham-operated (time 0) and partially hepatectomized rats (days after partial hepatecto[illegible] The values are expressed as femton[illegible] per gram liver for total liver con[illegible]nt (*A*), cytosolic binding (*B*), and nuclear binding (*C*). Serum testosterone levels are shown in *D*. The values expressed as mean ± SD and values differing from those at time 0 are indicated: *$p < 0.005$.

icantly at 48 h after PH; this increase in total ER parallels the increase in nuclear ER, as the nuclear form of ER predominates in liver. One potential explanation for the increase in nuclear ER observed in this previous study might be that PH resulted in an increase in serum E_2 content. Thus, we measured serum E_2 levels to determine if this was the case. These results are shown in Figure 1*D*. Values for serum E_2 rose at day 2 after surgery, and were significantly greater than basal values (0 time) at 3 days ($p < 0.005$). These changes in ER distribution after PH correlate temporally with the period of maximum mitotic indices and DNA synthesis in the regenerating liver [23]. In other experiments, receptor levels were quantitated in sham-operated animals at 24 and [illegible] after surgery; the receptor [illegible] was not different from that observed in the time 0 controls, nor did these livers display increased mitotic indices or DNA synthesis.

Other studies also demonstrated variations in hepatic AR after PH. Figure 2 shows the total hepatic content of AR (*A*), and the distribution of cytosolic AR (*B*) and nuclear AR (*C*) in rat liver at different times after 70% hepatectomy. Cytosolic AR levels (Figure 2*B*) decreased slowly, beginning 24 h after hepatectomy and reaching minimum levels at 48 h (10 fmol/g liver). From this nadir, the value for cytosolic AR returned to normal at 4 days after hepatectomy (30 fmol/g liver) and remained stable at this level during the remainder of the study period. At no time was a significant variation detected in the affinity of cytosolic AR for the androgenic ligand. Specifically, equilibrium dissociation [illegible]stant val-

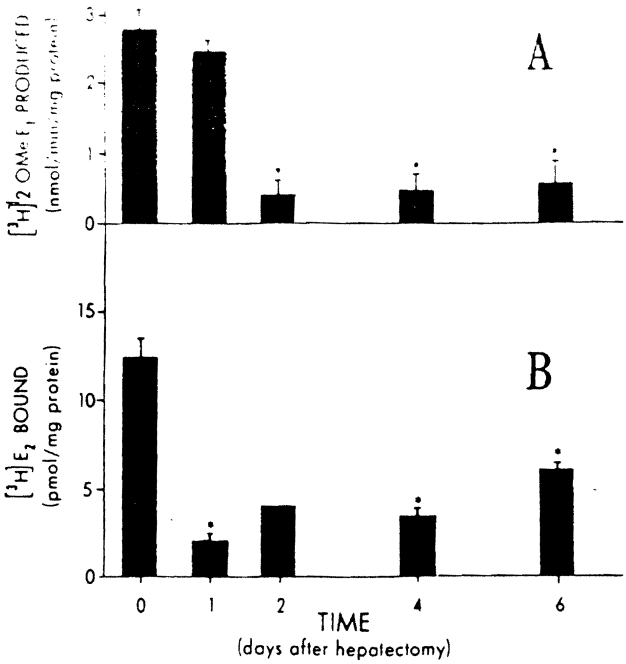

Figure 3. Activity of two hepatic androgen-responsive proteins after partial hepatectomy. Microsomal E2-OHase (*A*) activity was measured as the amount of [^{3}H]2-methoxy-1,3,5(10)-estrien-17-one-3-ol product formed, and cytosolic MEB (*B*) was quantitated by its [^{3}H]E_2 binding activity, as described in Materials and Methods. All groups consisted of at least 3 animals except for the MEB determination on day 2, which consisted of a single rat. The values are expressed as mean + SEM, and values differing from those observed at time 0 are indicated by $^*p < 0.005$. Sham-operated animals were also tested at 24 and 48 h after surgery; the values for MEB and E2-OHase from these animals were identical to those of the time 0 control group.

ues did not vary, having a range of 0.55–1.8 nM. In contrast to the increase noted in nuclear ER (Figure 1C), hepatic nuclear AR content decreased rapidly during the first day after hepatectomy to reach a value near 0 (3.0 ± 0.2 fmol/g liver), and remained at this level for the next 48 h (Figure 2C). Beginning at 72 h the nuclear AR level increased and returned to normal 6 days after hepatectomy. As a result of these negative changes in both cytosolic AR and nuclear AR, the total androgen binding capacity of the regenerating liver (Figure 2*A*) is extremely reduced, especially during the period of maximum rate of DNA synthesis and mitotic index (days 1 and 2). Serum testosterone was also measured in this study, as loss of both forms of AR suggested that PH may result in a decrease in available testosterone. The serum content of testosterone is shown in Figure 2*D*; it is apparent that serum testosterone content also showed a significant decrease during the period when the total hepatic binding capacity for androgen is greatly reduced.

Because of the dramatic decrease in serum testosterone and hepatic AR content, we assayed the liver of these animals for their androgen responsiveness. Figure 3 illustrates the effect of regeneration on the level of two hepatic androgen-responsive proteins. The activity of the microsomal enzyme E2-OHase displayed a time-dependent decline (Figure 3A) during regeneration. On day 1 after hepatectomy, the activity of E2-OHase was essentially normal, but decreased drastically on day 2 to an activity 13% of normal values and did not rise appreciably over the next 4 days. The activity of the enzyme on days 2–6 was comparable to that displayed by castrated male or normal female rats (4). The level of a second androgen-responsive protein, MEB, also rapidly declined to a level about 15% of normal values within 24 h of PH (Figure 3*B*). The level of MEB increased gradually during the next 5 days, but did not reach normal levels even by day 6 after PH. In other experiments, the livers of sham-operated animals were examined for MEB and E2-OHase content 24 and 48 h after operation. The activities of both proteins were identical to those observed in the livers of the time 0 animals.

Discussion

The data reported herein show significant variations in serum androgen and estrogen levels and changes in the hepatic content of their specific cytosolic and nuclear receptors during the course of liver regeneration after 70% hepatectomy. These changes were profound and occurred in opposite directions. Serum testosterone decreased rapidly and significantly, beginning 24 h after hepatectomy, and remained at very low levels until 72 h. Coincidentally, the total AR content of the liver decreased due to a loss of both cytosolic, but in particular, nuclear AR content. We also measured hepatic content of two androgen-responsive proteins to correlate these findings. Estrogen 2-hydroxylase is a microsomal enzyme that rapidly metabolizes estrogen in male liver; this androgen-responsive enzyme displays an activity in male rat liver that is eight times higher than in female rat liver (4). The MEB is a cytosolic protein that has a moderate affinity for E_2, high binding capacity and specificity for steroidal estrogens (24), and is also androgen responsive in that its activity is virtually undetectable in the liver cytosol of female rats (4,24). We have hypothesized that the MEB serves to bind free estrogen and estrogenic metabolites in the hepatocyte of the male (1,24); however, the physiologic role of this unique protein remains a question. Together, these proteins may complement each other to promote rapid binding and metabolism of excess estrogen, which could compromise the sexual integrity of the male rat.

Therefore, the decrease in activity of these two proteins not only represents a loss of androgen responsiveness, but also, because they metabolize or bind estrogens, may provide a mechanism by which intracellular estrogen levels may be increased.

The apparent failure of the androgen-responsive mechanisms, specifically the reduction in serum testosterone, hepatic AR activity, and the hepatic content of two androgen-responsive proteins, is remarkable in that these changes occurred within 24–48 h after PH. In contrast, the loss of these hepatic activities as a result of castration is not apparent for 8–10 days (3). The decrease of serum androgen and the corresponding failure of AR and androgen-dependent proteins in the liver could be due to a mechanism not yet elucidated, such as a decreased synthesis or an increased metabolism of testosterone. On the other hand, the hepatic androgen system failure might be a response to the increase in serum estrogen, and thus an increased action of estrogen receptor, particularly at the level of the pituitary. Administration of estrogens to male rats can result in feminization of sexually dimorphic liver function (reviewed in References 7 and 8). This effect presumably results from feminization of sexually dimorphic patterns of pituitary growth hormone secretion, as growth hormone appears to be the predominant determinant of sexually dimorphic patterns of hepatic enzymes and receptors (7,8,31).

Whereas a significant decline in serum androgen levels and AR activity occurred after PH, an increase in estrogen-related functions was noted. Serum estrogen levels increased as soon as 24 h after hepatectomy and reached the maximum level 72 h after surgery. Equally important, ER activity also increased; the increase noted in total ER content was essentially due to an increase in those receptors retained in the nuclear fraction. The increase in serum E_2 is likely to result in nuclear translocation of existing receptors. However, the increase in total ER content of the liver may be a result of induction of new receptor synthesis by direct action of E_2 on the liver or an indirect action on liver via stimulation of the pituitary. It is also possible that E_2 might activate any latent ER that may be present. The observed receptor redistribution started at the same time as the stimulation of DNA synthesis and reached its maximum at 48 h, a time that coincides with the high values of mitotic index (23). The relationship between these phenomena and hepatocyte division is also demonstrated by the fact that a normal distribution of ERs within the hepatocytes is reestablished 72 h after PH, a time at which few mitoses are found in the liver (23). Moreover, these hepatic receptor variations correlate well with the other markers of [illegible] such as DNA polymerase, protein synthesis, and deoxythymidine kinase as reported by others (reviewed in Reference 1). From the present data we cannot draw a definite cause-and-effect relationship between hepatic regeneration and the observed increase in serum E_2 and ER activity, but the data suggest that an important role for estrogens in the regenerative process might exist.

In light of the dramatic decrease in androgen-mediated hepatic responses, one has to wonder whether androgens contribute at all to the process of regeneration after PH. In contrast, the increases in E_2 levels and nuclear ER activity might be important during cell division or in the induction of specific proteins necessary during the process of hepatic regeneration. In this regard, estrogens are known to be inducers of the hepatic synthesis of many serum transport proteins (32), a function that is likely to be critical during regeneration. Moreover, the presence of a massive estrogenic effect during the proliferative phase of regeneration is reminiscent of the observations that estrogens promote development of certain hepatic neoplasms associated with an increased mitogenic activity (1,21,33).

In summary, the results reported herein document a rise in serum E_2 and a distinct feminization of certain sexually dimorphic liver functions as a result of PH of male rats. These findings suggest an important role for estrogen in the regenerative process. The observed reduction in serum testosterone, along with the reduced activity of hepatic ARs and androgen-responsive proteins during the proliferative period, leads to the conclusion that androgens have no positive role in the process of hepatic regeneration.

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