Relation of Estrogen and Its Receptor to Rat Liver Growth and Regeneration

Bernard Fisher, Nurten Gunduz, Elizabeth A. Saffer and Shu Zheng

Department of Surgery, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261

Abstract

A variety of hormones have been implicated in the process of liver regeneration. Despite the demonstration of specific estrogen receptors (ER) in mammalian liver and the identification of responses to estrogen which occur in liver, there has been little or no investigation of the relation of that hormone or its receptor to liver regeneration or liver growth. This report provides information which indicates: (a) the effect of 17β-estradiol on the mass of both intact and regenerating rat liver; (b) the percentage of hepatocytes in neonatal and adult normal intact livers which contain ER, i.e., the estrogen receptor index (ERI); (c) changes in ERI and in nuclear ER occurring in that cell population following partial hepatectomy (PH) and/or 17β-estradiol administration; and (d) the temporal relation of the changes in ER with those related to DNA synthesis and liver regeneration and to liver growth.

Approximately 55 to 60% of parenchymal cells from intact livers in adult rats were found to contain ER which was entirely located in the cytoplasm. No nuclear ER was evident in such cells. By 1 hr, and subsequently following 17β-estradiol administration to such animals, there resulted a depletion in the number of ER-containing cells and the identification of nuclear ER. These changes were followed by the onset of increased DNA synthesis and an increase in liver weight (p < 0.001). Subsequent to 70% PH, a similar series of events occurred in liver remnants. Not only was there a decrease in the ERI from 60% at the time of PH to 40% 3 hr later and 30% after 72 hr, there was a decrease in cytosol ER as well. Accompanying the decrease was an increase in the number of cells with nuclear ER. By 24 hr post-PH, 29% of the cells with ER displayed that receptor in their nuclei. At that time, DNA synthesis was at its peak, and liver regeneration was taking place. When 17β-estradiol was administered at the time of PH, there was a more rapid onset of with ER to the nuclei of parenchymal cells. At PH, when 17β-estradiol was given, no cells displayed nuclear ER. One hr later, 18% of cells with ER had nuclear ER, in contrast to the finding that only 3% of cells had nuclear ER 1 hr post-PH when 17β-estradiol was not administered. Regeneration was greater (59 versus 73%; p = 0.003) when animals received 17β-estradiol prior to PH.

Findings in parenchymal cells from livers of growing rats were consonant with those observed in remnants after PH. The ERI of 5-day-old rats was only 9%. That value increased with maturity, reaching adult levels at 1 month (65%). Concomitantly, the labeling index, indicative of DNA synthesis, decreased from 30% in 5-day-old rats to 5% in 1-month-old animals.

The temporal sequence of events, i.e., the appearance of nuclear ER, onset of DNA synthesis, and restoration of liver mass, found in these investigations, when they are considered in light of the observations of others, permits formulation of a hypothesis which relates estrogen and its receptor to both the onset of hepatic regeneration after PH and liver growth in the neonate.

Introduction

Ever since Higgins and Anderson (30) reported, in 1931, a technique for PH in rats, investigators have been interested in identifying changes occurring in residual liver following PH and in determining factors which might be responsible for the initiation of liver regeneration following that operation (3, 5). Research by us (21, 24–27) and by others (4) has attempted to discern whether regeneration might be related to a humoral agent(s), alteration of blood flow, or to stimulation of remaining hepatic cells by an increased work load. Despite considerable effort, the role of those factors in the initiation of regeneration has not been defined clearly. Interest currently centers upon the significance of the hormonal agents insulin, glucagon, and EGF (5, 6, 9, 13, 34, 40). It has been suggested that they initiate the process of regeneration by acting directly upon liver cells. Parathyroid hormone (41), calcitonin (34), iodothyronines (7), and glucocorticoids (8) are other hormones which have also been found to affect hepatic proliferation under special conditions. Investigations have indicated that specific changes in blood hormone levels occur rapidly after PH and that altered hepatic uptake of these peptide hormones takes place by their interaction with cellular receptors. How insulin, glucagon, and EGF produce their signals by combining with receptors and how these signal-interactions lead subsequently to enhanced frequencies of DNA synthesis and cell division remains obscure. Despite these deficiencies in knowledge, it has been proposed that it may be possible to unify the diffuse experimental results obtained regarding those agents into a theory which relates liver regeneration to endocrine control (34).

Despite the demonstration of specific receptors for estrogen in mammalian liver (10, 17, 18, 19, 39, 44, 46) and the identification of responses to estrogen which are known to occur in the liver (15, 20, 33, 38, 42, 44, 45), there has been, to our knowledge, little or no investigation of the relation of that hormone (estrogen) or its receptor to liver regeneration. Moreover, all information relative to ER in liver and in other tissues, i.e., uterus, tumor, etc., has been obtained by determining the binding of 17β-[3H]estradiol with receptor protein in the cytosol and/or nuclear fractions. Such methodology measures the total receptor

1 Supported by USPHS Grant CA-14972 from the National Cancer Institute and by the Stuttm Pharmaceutical Co.
2 To whom requests for reprints should be addressed at University of Pittsburgh, 914 Scaife Hall, Pittsburgh, PA 15261.
3 Visiting scholar at University of Pittsburgh School of Medicine. President of Zhejiang Medical University, Hangzhou, China. Received February 5, 1982; accepted March 7, 1984.
content of tissue, but it fails to identify the proportion of cells containing ER.

In our recent studies (22, 23, 29) using 17-FE, we have determined the percentage of cells in both human and murine breast cancer that demonstrate 17-FE binding to ER, i.e., the ERI. Consequently, we considered it worthwhile to use the same technique to identify the proportion of hepatic parenchymal cells which contain receptor and to determine whether changes in the number of cells displaying 17-FE ER binding might occur in rapidly growing parenchymal cells during regeneration after PH. Information is presented in this report regarding: (a) the effect of 17β-estradiol on the mass of both intact and regenerating rat liver; (b) the percentage of hepatocytes from neonatal and adult normal intact livers which demonstrates 17-FE binding (ERI); (c) changes occurring in that cell population after PH and/or 17β-estradiol administration; and (d) correlation of these changes with those related to DNA synthesis.

MATERIALS AND METHODS

Studies were carried out using female Fischer Mai 344 rats varying in age from 5 days to 4 months. A 70% hepatectomy was performed according to the method of Higgins and Anderson (30). Liver regeneration was calculated by subtracting the weight of the liver remnant after PH from the weight of the liver at sacrifice and dividing that value by the weight of the liver removed at PH; the result, multiplied by 100, indicated the percentage of regeneration. Suspensions of hepatocytes for determination of fluorescence were prepared immediately after liver removal. Cells were exfoliated from the cut surfaces with Dulbecco’s PBS (pH 7.4), placed in tubes, and centrifuged at 500 × g for 3 min.

The ligand used was 1-(4′-fluoresceinylestrone thiosemicarbazone, which consists of a fluorescein moiety coupled to position 17 of estrone. All ligand was prepared and supplied by W. B. Dandliker, University Research Foundation, San Diego, CA and C. Y. Meyers, Department of Chemistry, Southern Illinois University, Carbondale, IL. The stock solution of 17-FE (2 × 10^−5 μM) was diluted with PBS just prior to its use. Cells were incubated with 2 × 10^−7 μM ligand for 1 hr at 37°C. Following incubation, cells were washed twice with cold PBS (15 min each), and resuspended in 0.5 ml of cold PBS. The single-cell suspensions were maintained in a light-free ice bath. Two drops of suspension were placed on a slide and coverslipped. The cells were examined immediately with a Zeiss epifluorescence microscope with a halogen light source, utilizing an objective. A minimum of 500 cells/sample was counted by each of 2 viewers to determine the proportion of 17-FE bound cells (ERI). More than 95% of the cells in the liver were hepatocytes.

For determination of LI, liver cells were suspended in McCoy’s medium with 20% fetal calf serum (Grand Island Biological Co., Grand Island, NY) at room temperature and filtered through a nylon screen. The single-cell suspension (3 to 5 × 10^5 cells/ml) was incubated for 1 hr at 37°C in fresh medium with 2.5 μCi of [3H]dThd/ml (14 to 17 Ci/mmol; New England Nuclear). Labeling was terminated by inserting tubes containing cells into ice. The cells were washed with cold Ca2+- and Mg2+-free Eagle’s minimal essential medium (Grand Island Biological Co.). After the cells were washed, they were digested in 0.25% Bacto-trypsin (Difco Laboratories, Inc., Detroit, MI) plus DNase (0.1 mg/ml; Sigma Chemical Co., St. Louis, MO) and incubated for 10 min at 37°C. They were centrifuged at 500 × g for 3 to 5 min and resuspended in fresh medium, and viability tests were performed for each sample. The single-cell suspension (3 to 5 × 10^5 cells/ml) was incubated for 1 hr at 37°C in fresh medium with 2.5 μCi of [3H]dThd/ml (14 to 17 Ci/mmol; New England Nuclear). Labeling was terminated by inserting tubes containing cells into ice. The cells were washed with cold Ca2+- and Mg2+-free Eagle’s minimal essential medium (Grand Island Biological Co.). After the cells were washed, they were digested in 0.25% Bacto-trypsin (Difco Laboratories, Inc., Detroit, MI) plus DNase (0.1 mg/ml; Sigma Chemical Co., St. Louis, MO) and incubated for 10 min at 37°C. They were centrifuged at 500 × g for 3 to 5 min and resuspended in fresh medium, and viability tests were performed for each sample. The cell suspension (3 to 5 × 10^5 cells/ml) was incubated for 1 hr at 37°C in fresh medium with 2.5 μCi of [3H]dThd/ml (14 to 17 Ci/mmol; New England Nuclear). Labeling was terminated by inserting tubes containing cells into ice. The cells were washed with cold Ca2+- and Mg2+-free Eagle’s minimal essential medium (Grand Island Biological Co.). After the cells were washed, they were digested in 0.25% Bacto-trypsin (Difco Laboratories, Inc., Detroit, MI) plus DNase (0.1 mg/ml; Sigma Chemical Co., St. Louis, MO) and incubated for 10 min at 37°C. They were centrifuged at 500 × g for 3 to 5 min and resuspended in fresh medium, and viability tests were performed for each sample. The cell suspension (3 to 5 × 10^5 cells/ml) was incubated for 1 hr at 37°C in fresh medium with 2.5 μCi of [3H]dThd/ml (14 to 17 Ci/mmol; New England Nuclear). Labeling was terminated by inserting tubes containing cells into ice. The cells were washed with cold Ca2+- and Mg2+-free Eagle’s minimal essential medium (Grand Island Biological Co.). After the cells were washed, they were digested in 0.25% Bacto-trypsin (Difco Laboratories, Inc., Detroit, MI) plus DNase (0.1 mg/ml; Sigma Chemical Co., St. Louis, MO) and incubated for 10 min at 37°C. They were centrifuged at 500 × g for 3 to 5 min and resuspended in fresh medium, and viability tests were performed for each sample.

To intensify the latent image, slides were incubated in KAuC4 for 20 min at 15°C and rinsed in distilled water just prior to development. The slides were then developed in Kodak Amidol developer for 15 min at 15°C, fixed, washed, air dried, and stained with hematoxylin. Gold activation reduces the exposure time to 1 day. With this method, background labeling was approximately 4 grains/cell, while the grains over labeled cells were usually too dense to count. To determine LI, 700 to 1500 cells were counted for each sample.

DNA synthesis, [3H]dThd uptake, was determined by injecting [methyl-3H]dThd (10 μCi; specific activity, 3 Ci/mmol New England Nuclear) i.p. 1 hr before sacrifice. The liver was removed, blotted free of blood, and weighed. The portion was frozen immediately on dry ice. DNA was extracted from the liver with hot 5% trichloroacetic acid, and the extract was assayed for DNA content with diphenylamine. Radioactivity was determined with a scintillation spectrometer. Activity is expressed as 10^6 dpm/mg of DNA.

Estrogen receptor-binding protein was determined by a modification of the methods described by Eagon et al. (16) and by Chamness et al. (10). Samples were kept at 4°C throughout the procedure. The tissue was weighed and homogenized in 6 to 8 volumes of Tris-EDTA buffer (0.01 M Tris-HCl − 0.0015 M EDTA, pH 7.4). The homogenate was centrifuged for 30 min at 8000 × g. The supernate was removed carefully to avoid lipid contamination and recenterrifuged for 60 min at 150,000 × g. The protein concentration in the cytosol was determined by the method of Lowry et al. (35). A 200-μl aliquot of liver cytosol was mixed with 25 μl of 17β-[3H]estradiol (New England Nuclear, 90 to 100 Ci/mmol) with or without 100-fold excess cold 17β-estradiol (Steraloids). The cytosol-containing tubes were inserted in ice and kept at 4°C overnight. The unbound hormone was removed by adding 400 μl of dextran-coated charcoal suspension (5 g of Norit A and 500 mg of dextran C in 100 ml of Tris-EDTA buffer) with vortexing. After 10 min of exposure, the sample was centrifuged at 800 × g for 10 min. The supernate was removed, and the radioactivity was determined in a Packard Tri-Carb liquid scintillation spectrometer after mixing of samples with 10 ml of Aquasol (New England Nuclear). Scatchard analysis was performed to determine the specific binding values by utilization of 10 concentrations of labeled and unlabeled 17β-estradiol (43). Values are expressed as fmol/mg of cytosol protein.

When estradiol (1, 3, 5, 10) (estratrien-3,17β-diol; Steraloids, Inc., Wilton, NH) was used, it was administered s.c. in sesame oil, 5.0 mg as a single dose or on 2 successive days with a third dose 1 week after the first. Student’s t test was used in the statistical analyses.

RESULTS

Effect of Estradiol on the Weight of Intact or PH Liver. When animals receiving 3 doses of estradiol were sacrificed 7 days after the last dose, their livers were found to be 27% greater in weight than the livers of animals receiving only sesame oil (Table 1). The increased weight was accompanied by an increase in total liver DNA. The DNA content per g was found to be similar in both treated and control animals, indicating that the weight gain was not the result of increased water, glycogen, or fat.

When animals were subjected to 70% PH 7 days after the last dose of 17β-estradiol and were sacrificed 7 days after operation, livers displayed greater regeneration [72.8 ± 3.41% (S.E.)] than did livers of animals receiving no 17β-estradiol (58.9 ± 1.56%) (p = 0.003) (Table 2). While, at the time of PH, livers in 17β-estradiol recipients were 30% heavier than in controls, at sacrifice, the weight of the increment, i.e., newly formed liver, was 61% greater in hepatectomized animals. As found in intact livers, there was no decrease in DNA/g of liver (3.6 ± 0.08 in controls and 3.4 ± 0.04 in 17β-estradiol recipients), indicating that the increase in weight was due to regenerated liver.

JUNE 1984

2411
Relation of Age to ERI and LI in Intact Livers. The proportion of ER-positive cells in livers increased rapidly during the growth of animals (Table 3). At 5 days of life, ~10% of cells demonstrated 17-FE binding. By 35 days, the ERI was equivalent to that demonstrated by mature (4-month-old) adult animals (~60%). The proportion of DNA-synthesizing cells (LI) was inversely related to the age of animals and the percentage of parenchymal cells with ER, decreasing from 30% at 5 days after birth to 2% at maturity. In virtually all cells, the 17-FE ER binding was confined to the cytoplasm. 

Effect of Estradiol on ERI, Nuclear Translocation, and DNA Synthesis in Intact Liver. In mature rats, within 1/2 hr after administration of a single dose of 17β-estradiol, there was evidence of translocation of ER from the cytoplasm to the nucleus. There was a decrease in the ERI, which remained depressed for at least 24 hr (Table 4). By 2 hr, nuclear ER had increased from 0% at time of 17β-estradiol administration to 7.9%, and the percentage of cells with fluorescence which displayed nuclear ER had increased from 0 to 18.9%, after which there was a gradual decrease. All livers examined from 1 to 24 hr after 17β-estradiol displayed some degree of nuclear fluorescence, as did 3 of 4 examined at 1/2 hr. During the period when translocation was evident, there was no increase in DNA synthesis. At 24 hr, however, a greater than 4-fold increase was observed.

Effect of PH on ERI and Cytosol ER of the Liver Remnant. The percentage of hepatocytes in liver remnants displaying 17-FE ER binding was initially determined 1, 2, 3, 5, and 7 days after 70% PH (Table 5). The ERI decreased in all livers, reaching a nadir by 72 hr after PH. At that time (72 hr), there was a 47% reduction in hepatocytes demonstrating 17-FE ER binding. Whereas, at hepatocyte, livers displayed fluorescence in 60.6% of parenchymal cells, at sacrifice, only 32.2% of the cells demonstrated 17-FE binding. At 7 days (168 hr) after PH, the proportion of marker-positive cells had returned to prehepatectomy levels. Livers were examined for ER content at the time of and at intervals after PH by means of the incorporation of 17,β-[3H]-estradiol using the dextran-coated charcoal method (Table 5). Findings were concordant with those of studies using 17-FE.
Effect of partial hepatectomy on nuclear ER, DNA synthesis, and regeneration of the liver remnant

Table 6

<table>
<thead>
<tr>
<th>Time after PH (hr)</th>
<th>No. of livers</th>
<th>No. with nuclear translocation</th>
<th>% of all cells</th>
<th>% of cells with ER</th>
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<td>0</td>
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<td>1</td>
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<td>3</td>
<td>1.4 ± 0.49a</td>
<td>2.7 ± 0.8</td>
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<td>3</td>
<td>8.5 ± 0.72</td>
<td>21.9 ± 1.30</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>8</td>
<td>10.2 ± 1.85</td>
<td>27.3 ± 7.50</td>
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<td>18</td>
<td>10</td>
<td>10</td>
<td>12.5 ± 0.92</td>
<td>26.1 ± 3.42</td>
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<tr>
<td>24</td>
<td>8</td>
<td>3</td>
<td>11.1 ± 1.64</td>
<td>29.0 ± 3.35</td>
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<td>4</td>
<td>1.3 ± 0.39</td>
<td>4.0 ± 1.13</td>
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<td>0</td>
<td>0</td>
</tr>
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<tr>
<td>188</td>
<td>4</td>
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*Mean ± S.E.

Table 7

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<tr>
<th>Time post-PH (hr)</th>
<th>ER (%)</th>
<th>Nuclear ER (%)</th>
<th>DNA synthesis ([3H]dThd uptake) (dpm/mg of DNA × 10³)</th>
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</thead>
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<tr>
<td></td>
<td></td>
<td></td>
<td>% of all cells</td>
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<td>60.2 ± 1.07a</td>
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<td>0</td>
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<tr>
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<td>46.6 ± 2.93</td>
<td>8.2 ± 1.08</td>
<td>18.0 ± 4.50</td>
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<tr>
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<td>27.1 ± 2.39</td>
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<td>11.6 ± 2.16</td>
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<td>44.7 ± 2.92</td>
<td>8.9 ± 1.12</td>
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</tr>
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<td>41.3 ± 2.82</td>
<td>9.2 ± 0.69</td>
<td>23.4 ± 8.25</td>
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<td>24</td>
<td>35.9 ± 4.62</td>
<td>6.2 ± 0.60</td>
<td>18.6 ± 4.09</td>
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</table>

*Mean ± S.E. of 4 livers.

18 hr after PH, the ER was reduced from 20 to 3 fmol. This value remained low at 1 and 2 days but had returned to normal by 7 days.

Effect of PH on Nuclear Translocation of ER, DNA Synthesis, and Regeneration of the Liver Remnant. At 24 hr, after PH, nuclear fluorescence was noted occasionally in a few hepatocytes from some livers. These findings prompted investigations to determine whether nuclear 17-FE ER might be more evident at times earlier than 24 hr after PH. Consequently, animals were sacrificed at intervals from 1 hr to 7 days after hepatectomy with the express purpose of determining the presence of 17-FE ER in the nucleus. Of 37 animals sacrificed between 1 and 48 hr, livers of 31 (84%) had hepatocytes demonstrating nuclear ER (Table 6). None of the livers from animals sacrificed after 48 hr had cells displaying nuclear fluorescence. When the number of cells with nuclear fluorescence was related to the total number of hepatocytes examined, about 12% of cells from livers obtained between 6 and 24 hr contained nuclear ER. At those times, between 25 and 30% of cytoplasmic ER-positive cells demonstrated the presence of ER in the nucleus.

Increased DNA synthesis was first observed at 18 hr after PH and, by 24 hr, there was a 30-fold increase (163.5 × 10³ dpm/mg of DNA) over that observed in livers at the time of PH (5.6 × 10³ dpm/mg of DNA). While [3H]dThd uptake remained elevated for several days after PH, it approached prehepatectomy levels by the seventh day.

Increase in liver mass was first observed at 18 hr after PH and steadily progressed so that, by 7 days, 60% of the removed liver had been restored. That event was preceded by the changes in ER translocation and DNA synthesis.

Effect of Estradiol Administered at Time of PH on Nuclear Translocation of ER. A single dose of 17β-estradiol was administered at the time of PH, and rats were sacrificed 1, 3, 6, 18, and 24 hr later. At 1 hr after operation, the ERI had decreased from 60.2 to 46.6%. That reduction persisted for at least 24 hr. Whereas there was no evidence of nuclear ER in liver removed at PH 1 hr after operation and at subsequent times, all liver remnants displayed nuclear ER (Table 7). During the first 24 hr after PH, ~10% of all parenchymal cells and ~25% of all cells with ER had nuclear receptor.

DISCUSSION

The findings presented amplify the concept that liver regeneration may be regulated hormonally. For the first time, to our knowledge, they implicate estradiol and its receptor in hepatic parenchymal cells with the phenomenon. Moreover, they indicate that our findings obtained using 17-FE ligand binding regarding ER in individual parenchymal cells are compatible with those noted by others (1, 32, 36) using 17β-estradiol binding by hepatic cell cytosol and/or nuclei in experiments evaluating the modulating effects of estrogen on liver function. Several of those studies have particular relevance to our observations.

Marr et al. (36) noted that rat hepatic receptor content and its distribution between cytosol and nucleus did not fluctuate in concert with known (physiological) cyclical changes in the concentration of plasma estrogen which occur during estrus. It was observed, however, that there was a substantial translocation of receptor to the nucleus following administration of hyperphysiological doses of estrogen and that there was also a substantial decrease in cytosol ER. Others have similarly observed increased translocation of receptor to the nucleus and a decrease in total hepatic ER following estradiol administration to rats with intact livers (1, 32). Marr et al. concluded from their findings that, since liver is the main site for the conversion and deactivation of steroids, it is probable that the rapidity of estrogen metabolism regulates the interaction of the steroid with its receptor, a concept also proposed by Eisenfeld and Aten (17). The constancy of the nuclear receptor content during the cycle implies a limited but sustained stimulation of the hepatic genome by estrogen, whereas high concentrations of steroid could overcome the normal regulatory inhibitory mechanism(s), allowing estrogens or their metabolites to interact with the cytoplasmic receptors. Subsequent to their binding with specific sites, the resultant complex translocates to the nucleus, and an increase in the concentration of certain plasma proteins follows. Indeed, Kneefel and Katzenellenbogen (32) observed a definite correlation be-

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tween the onset and maintenance of elevated levels of PRS and the time course of movement of cytoplasmic ER sites to the liver nucleus.

It is of interest to consider our findings relative to changes in ER occurring after 70% PH with those findings after estradiol administration to animals with intact livers, as described above. Our observations, both that the proportion of cells demonstrating 17-FE binding in the cytoplasm becomes reduced and there is a decrease in cytosol ER following PH, are concordant with the ER changes noted after estradiol administration to animals with normal livers. Evidence of translocation of receptor for 17-FE to the nucleus as early as 3 hr after PH, and regression of this event at about 18 hr is also in keeping with the findings described after estradiol administration to animals with intact livers. Moreover, our observation that administration of estradiol to rats at the time of PH results in evidence of ER translocation in parenchymal cells at earlier times than is observed after PH alone suggests that excess estradiol is related to the initiation of translocation.

Since there are similarities in the receptor changes occurring after PH with those noted after 17β-estradiol administration to animals with intact livers, we postulate that, after PH, there is an alteration in the capability of the liver remnant to metabolize plasma 17β-estradiol to the same extent as in the intact liver prior to PH. Consequently, there may be a relative increase in plasma estradiol. In effect, there is created a circumstance similar to that which occurs after exogenous estradiol administration, i.e., the elevated estradiol provides a stimulus for ER receptor translocation and translocation. The decrease in the total cytosol ER and in the proportion of cells displaying 17-FE binding could be the result of saturation of all available binding sites by the excess 17β-estradiol, or it could relate to a failure of renewal of cytoplasmic ER.

It has been postulated that the increase in concentrations of certain plasma proteins known to be synthesized and secreted by the liver (e.g., clotting factors, PRS, lipoproteins) after estradiol administration is related to translocation of the steroid-receptor complex into the nucleus and its binding with chromatin, with a resulting increase in mRNA. The present studies provide no information relative to changes in plasma protein after PH. It is unlikely, however, that similar alterations occur as a consequence of the receptor in parenchymal cell nuclei, i.e., translocation, which we observed, since, as Bucher and Malt (4) have stated, "activities associated with specialized hepatic functions may be suppressed while those leading to cell proliferation are stimulated." Evidence for the latter is supplied by our finding that receptor translocation preceded the onset of increased DNA synthesis, which was maximal at 24 hr, and that the administration of estradiol prior to PH resulted in enhanced liver regeneration. While it is highly likely that the initiation of DNA synthesis was the consequence of receptor translocation, the findings from these studies cannot entirely eliminate the possibility that the events were coincidental.

Information from a variety of sources suggests to us that the mechanism responsible for the growth of liver may be similar to that postulated for liver regeneration. Our observation that the ERI of 5-day-old rats was only 9% and that it increased with maturity, reaching adult levels at 1 month, is completely consistent with those who noted low concentrations of ER in supernates of liver from immature rats (12, 19, 31). That observation is similar to our findings relative to the ERI in liver remnants following PH. Moreover, in both situations, there is an inverse relationship between ER content and DNA synthesis. Just as it was postulated that the changes in ERI following PH may be related to a relative increase in plasma estradiol, so may the findings in the neonatal animals be postulated to be the result of elevated maternal estrogen that is associated with pregnancy and that persists in the neonate (2) because of inadequate liver mass for its metabolism. Our observation that administration of 17β-estradiol to adult animals with intact livers results in increased liver mass which is due to DNA synthesis and the observation by Gershbein (28) that the extent of liver regeneration in PH rats is markedly elevated by pregnancy, which is associated with increased estradiol, seems to be relevant to the findings. The finding by Eisenfeld et al. (19) that, unlike the adult, the prepubescent rat has no increase in PRS after estrogen administration, is also in keeping with that which occurs after PH. In both circumstances, biosynthetic mechanisms necessary for cell replication seem to take precedence over those related to other hepatic functions.

While a precise explanation for each of the observations may be unavailable at present and may require refinement, elaboration, and confirmation, when considered overall, in concert with findings by others, the observations lead us to hypothesize that estrogen and its receptors may play a significant role in liver regeneration after PH and in liver growth during maturation. The temporal nature of the findings suggests that an imbalance in favor of estrogen over its metabolism results in ER translocation to the nucleus and an increase in DNA synthesis and liver growth.

It has been claimed by some investigators that nonspecific binding limits the worth of cytochemical methods for ER determination (11, 37). The findings from the current studies provide additional support to those presented by us previously (22) relative to the credibility of the use of 17-FE for the determination of ER in individual cells. The inverse relationship noted in the liver of maturing animals and in those after PH, including nuclear translocation, 17-FE ER binding, and DNA synthesis, are in accord with changes in those parameters noted previously to occur after primary tumor removal, radiation, or cyclophosphamide administration. It is extremely unlikely that the specific relationship between the proportions of LI and fluorescing cells in all of these diverse circumstances could have been defined so clearly unless the ligand was binding specifically to ER.

Further evidence indicating the specificity of binding of 17-FE by ER and the lack of nonspecific binding at the concentrations needed to bind ER has been supplied by Dandliker et al. (14). To compare 17β-[3H]estradiol to 17-FE as probes for ER, a fluorometric dextran-coated charcoal assay with 17-FE showed 210 fmol of specific sites/mg of protein as compared to 170 fmol of specific sites/mg of protein obtained with the use of 17β-[3H]-estradiol. Findings by Dandliker and ourselves have indicated that the binding observed is not due to free fluorescein, because this material does not bind, even at concentrations at orders of magnitude larger than that used for 17-FE. It is important to consider the recent report of McCarty et al. (37) suggesting that findings from histochemical analyses with "7-FE" were poor predictors of clinical outcome of patients with advanced breast cancer and, consequently, not an accurate determinant of ER. The material termed "17-FE" by McCarty is in fact 17β-estradiol.
17-TSC-BSA-FITC, a molecule drastically different from the 17-FE used by us and roughly 100 times as large. The finding in all of our studies indicating that 17-FE binding is cell selective, i.e., that there are nonstained cells, tends to discredit the contention that nonspecificity is involved in the process. Aside from these studies lending additional support to the credibility of the use of 17-FE for the determination of ER in individual cells, they indicate the usefulness of the method for obtaining information regarding the role of ER in a variety of biological processes.

ACKNOWLEDGMENTS

We are grateful for the support given by W. B. Dandlikar, Ph.D., of the University Research Foundation, San Diego, CA, in these studies. We thank Betty Richey, Morton Levine, Judy Benson, and Barbara Breidenbach for their technical assistance and P. K. Eagon, Ph.D., of the University of Pittsburgh, School of Medicine, Pittsburgh, PA, for advice on the cytosol ER determination.

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