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Promoting effect of estrogen on regeneration of the liver transplanted to an ectopic site in mice*

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Summary. A single oral administration of a pharmacological dose of estriol (E3) immediately after transplantation of small liver fragments of mice under the kidney capsule induced a remarkable growth of regenerating liver tissue. The hepatocytes were successfully arranged in cords with well developed sinusoids between them. The cytoplasm of the hepatocytes showed prominent basophilia. In mice injected with carbon intravenously, large numbers of carbon-laden endothelial lining cells and Kupffer cells apeared in the newly building sinusoids. E3 raised the mitotic activity of the regenerating hepatocytes markedly and for a long period. The act of E3 on mitosis was much more effective on the regenerating hepatocytes than on the recipient's own hepatocytes.

Key words: Liver transplantation – Regeneration promoting effect – Estrogen – Mouse.

Introduction

It is well known that partial hepatectomy induces proliferation of hepatocytes (Phillips and Steiner 1965; Stenger and Confer 1966). It has also been reported recently that oral administration of estrogen, either natural (Fujii et al. 1985) or synthetic (Cole and Sweeney 1980) induces proliferation of hepatocytes without any pathological lesions, in the liver of adult animals. If estrogen accelerates the proliferation of hepatocytes in the transplanted liver, it may be clinically useful in liver transplantation. To determine whether estrogen acts on the transplanted hepatocytes, causing proliferation, small fragments of mouse liver were transplanted to an ectopic site under the kidney capsule of syngeneic recipients. Estrogen was then administered orally.

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Materials and methods

Female inbred C57BL/6J strain mice (10–16 weeks old) of our own colony were used throughout this study. The animals were fed a laboratory chow (MR, Nihon Nosan, Yokohama) and given water ad libitum. In total, 18 recipients for liver transplantation were used in each group of animals treated with and without hormone.

The liver from the donor mouse was minced into small fragments of approximately 8 cu·mm in cold saline. Fragments were transplanted into the subcapsular space of the recipient mouse kidney, exposed through a flank incision under ether anesthesia. Each kidney received one fragment.

Estriol (E3, Merck, Darmstadt, FRG) was dissolved in dimethylsulfoxide at a concentration of 50 mg/ml and then diluted 1:5 with saline to obtain the final concentration (10 mg/ml) of an aqueous suspension of E3. The mice of the E3 group were given a single dose of 10 mg/25 gm of body weight through a stomach tube immediately after liver transplantation. This dose of E3 has the most effective potence for the proliferation of hepatocytes of adult mice (Fujii et al. 1985). The control mice were given an equal volume of solvent only.

The recipient mice were killed 4, 5, 6, 8, 10 and 20 days after liver transplantation. The liver grafts from three mice at each time point in each of the control and E3 groups were excised with the underlying kidney tissue, fixed in Carnoy's solution, sectioned serially at 7 μm-thickness, and stained with haematoxylin and eosin (HE). The recipient liver was also sectioned at 7 μm and stained with HE.

Six hours before sacrifice, the recipients were injected subcutaneously with colchicine at a dose of 0.1 mg/100 gm of body weight. The ratio of the number of cells in the metaphase of mitosis to the total (182–206) regenerating hepatocytes observed in 24–30 serial sections of the control group and to 500 cells of the E3 group was calculated. The mitotic index was also calculated for 5,000 hepatocytes of each recipient liver.

In order to examine the phagocytic function of the reticuloendothelial system of the transplanted liver, Pelican ink (16 mg/ml carbon, Günther Wagner, Hannover) of 1.0 ml per 100 gm body weight was injected intravenously into two recipient mice in each of the control and E3 groups on day 20 after transplantation, one hour prior to sacrifice.

Results

After transplantation of small fragments of the liver under the kidney capsule, regeneration of the liver tissue was observed immediately outside the kidney parenchyma in both groups of animals, with and without E3. However, the growth of regenerating tissue was distinctly different between the two groups.

Histologically, differences between the two groups were found as soon as 4 days after grafting, as shown in Figs. 1 and 2. The majority of hepatocytes in the control group contained huge vacuoles in their cytoplasm in the HE sections, resulting in peripheral displacement of the nuclei. Development of the hepatic cords and sinusoids was not observed. However, the cytoplasm of the hepatocytes was strongly basophilic and had no vacuoles in the E3 group. Sinusoids developed between the hepatocytes which were arranged in rows to form cords. The difference in growth of regenerating liver tissues between the control and E3 groups became more conspicuous in both width and thickness of the cords as time went on. Twenty days after transplantation, the regenerating liver was composed of about 10 layers of hepatocytes at the thickest part in the E3 group, in contrast to only 2 layers in the control group, as shown in Figs. 3 and 4. The basophilia of the cytoplasm of the hepatocytes was much more prominent in the E3

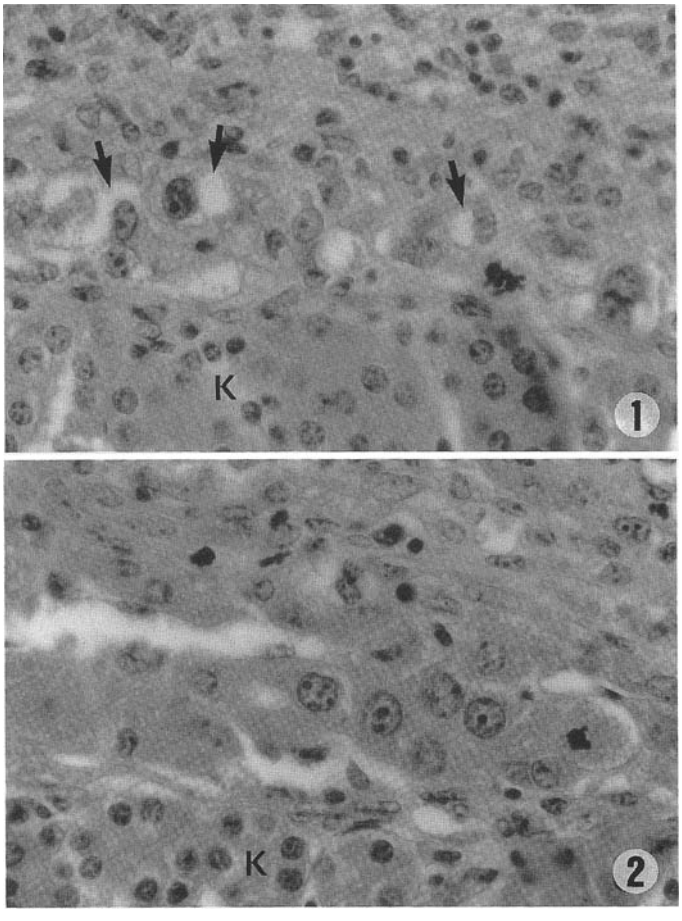

Fig. 1. The thickest part of the regenerating liver tissue in the control group 4 days after transplantation. Nuclei of the majority of the hepatocytes are displaced to one side due to huge vacuoles in their cytoplasm (*arrows*). A mitotic figure of the hepatocyte can be seen. *K* kidney. HE × 580

Fig. 2. The thickest part of the regenerating liver tissue in the E3 group 4 days after transplantation. Hepatocytes are already arranged in cords with well developed sinusoids between them. Their cytoplasm shows prominent basophilia. There is a mitotic hepatocyte. *K* kidney. HE × 580

group than in the control group. No vacuole was observed in the cytoplasm of hepatocytes in the E3 group, whereas the cytoplasm of many hepatocytes in the control group contained various sized vacuoles. In the E3 group, the hepatocytes were arranged in cords with well developed sinusoids between them. In the control group, however, the hepatocytes were partly arranged in cords, but the sinusoids were hardly defined. The difference in the development of sinusoids between the two groups was more evident when the animals received an intravenous injection of carbon (Figs. 5 and 6). In the E3 group, well developed sinusoids were clearly lined by endothelial

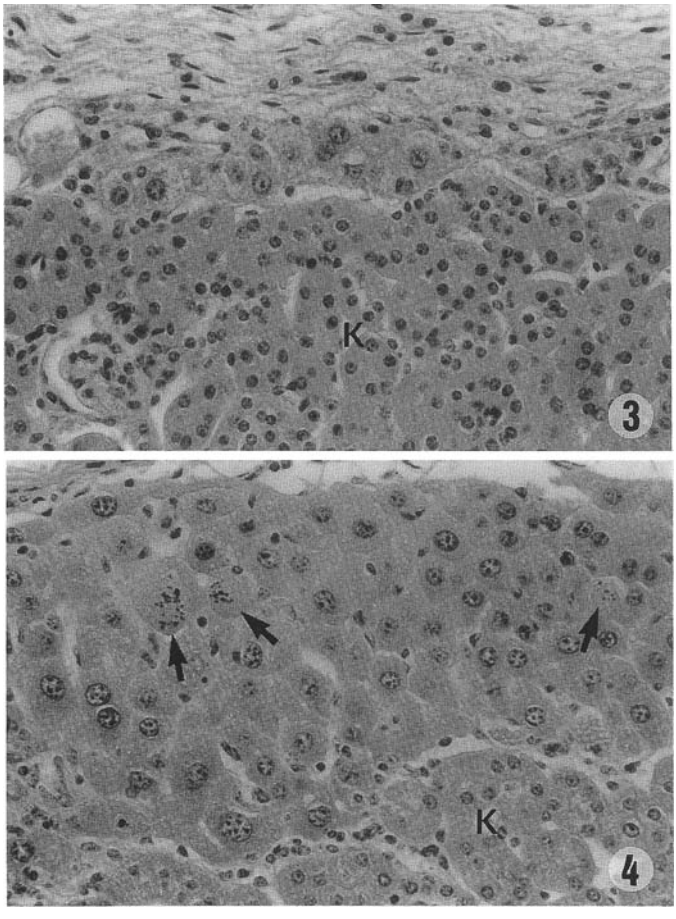

Fig. 3. The thickest part of the regenerating liver tissue in the control group 20 days after transplantation. Although hepatocytes are partly arranged in cords, sinusoids can not be definitely found. Hepatocytes contain various sized vacuoles in their cytoplasm. *K* kidney. HE ×290

Fig. 4. The thickest part of the regenerating liver tissue in the E3 group 20 days after transplantation. Regenerating liver tissue comprises 8–10 cell layers in contrast to 1–2 cell layers in the control group in Fig. 3. Sinusoids are between hepatic cords. The cytoplasm of all hepatocytes shows prominent basophilia. Three hepatocytes in mitosis are seen (*arrows*). *K* kidney. HE ×290

cells with carbon particles. Moreover, many Kupffer cells stained black by abundantly phagocytized carbon appeared in the sinusoids. In contrast, carbon-laden cells were scarce in the control group.

The mitotic index of regenerating hepatocytes after liver transplantation in the control and E3 groups is shown in Fig. 7. In two groups, the mitotic index rose sharply in a short time between day 4 and day 5 after transplantation and reached its peak on day 5. The peak value on day 5 did not differ statistically ($P > 0.50$) between the two groups, although the mean of the

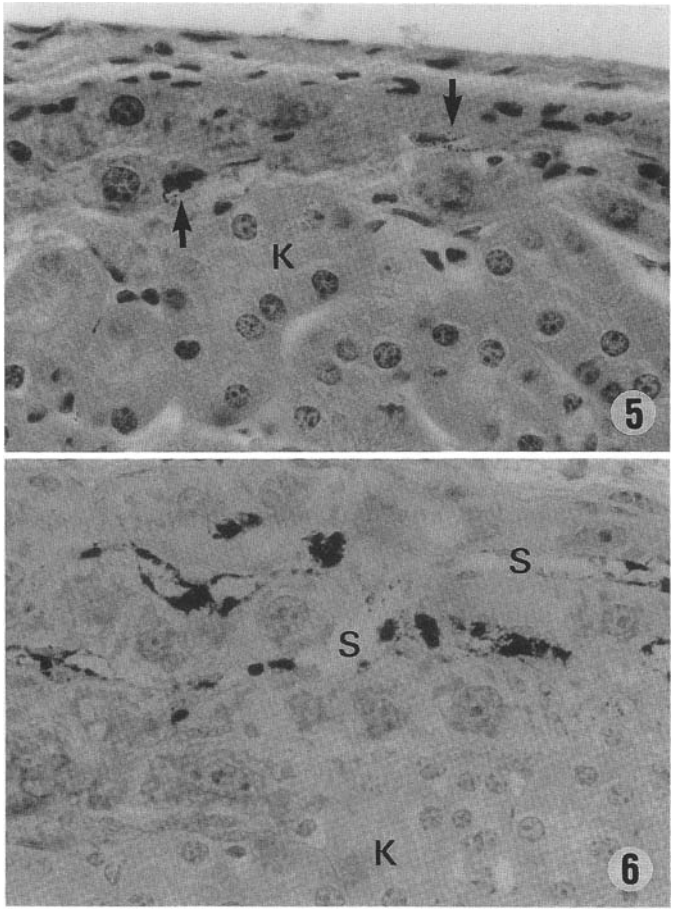

Fig. 5. The thickest part of the regenerating liver tissue in a control mouse injected intravenously with carbon 20 days after transplantation. Carbon-laden cells are scarce (*arrows*). *K* kidney. HE × 580

Fig. 6. The thickest part of the regenerating liver tissue in a mouse of the E3 group, receiving an intravenous injection of carbon 20 days after transplantation. Well developed sinusoids (*S*) are lined by carbon-laden endothelial cells. Many Kupffer cells stained black due to abundant phagocytized carbon can be seen in sinusoids. *K* kidney. HE × 580

E3 group ($7.61 \pm 1.43\%$) was slightly higher than that of the control group ($5.81 \pm 0.45\%$). However, the difference in the mitotic index between the two groups appeared after day 5. In contrast to a rapid decrease in the control group after the peak, the high mitotic index persisted for a further 3 days in the E3 group. After day 8, the mitotic index of the E3 group declined sharply during the next 2 days to $2.77 \pm 0.60\%$. In contrast, the mitotic index of the control group on day 10 was under 0.10%. This difference in the mitotic index between the two groups of animals treated with and without E3 was found for a long period, at least during the 20 days

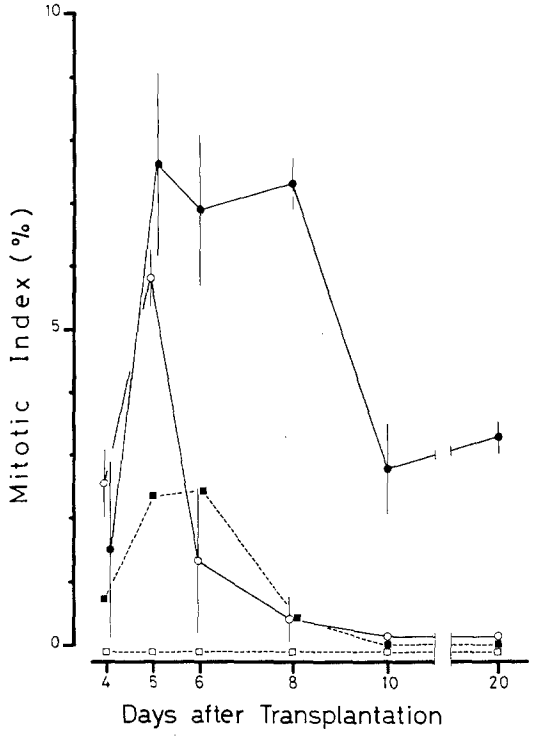

Fig. 7. Time course of the mitotic index of hepatocytes. Each point represents a mean value of 6 transplants and of 3 recipient's liver specimens and vertical bar shows standard error. —●—, the regenerating liver tissue in the E3 group; —○—, the regenerating liver tissue in the control group; —■—, the recipient's liver of the E3 group; —□—, the recipient's liver of the control group

of examination. The mitotic figures of the regenerating hepatocytes are illustrated in Figs. 1, 2 and 4.

The mitotic index of the hepatocytes of the recipient liver in the two control and E3 groups is also shown in Fig. 7. The mitotic index of the recipient's own hepatocytes also rose transiently after the E3 administration. However, its peak was markedly lower than that of the regenerating hepatocytes. Moreover, the duration of the mitotic activity of the recipient's own hepatocytes was considerably shorter than that of the regenerating hepatocytes. In the control group, the mitotic index of the recipient's own hepatocytes was under 0.10% throughout this experiment.

Discussion

Recently, we have reported that natural estrogens induce mitoses of hepatocytes without any pathological lesions in the liver of adult mice (Fujii et al. 1985) as others have reported for a synthetic estrogen (Cole and Sweeney 1980) in the adult rat liver. The present study demonstrated an induction of a high mitotic index of regenerating hepatocytes by E3 after ectopic transplantation of a small fragment of the adult liver. It was also demonstrated that the mitotic activity following a single oral administration of E3 persisted for a long period, at least for the 20 days examined. The prolonged duration of high mitotic activity of the regenerating hepatocytes

by E3 resulted in a striking difference in growth between the control and E3 groups. The high level of serum estrogen concentration after oral administration lost for 1 or 2 days as previously reported (Fujii et al. 1985). The reason why E3 causes such a long duration of high mitotic activity in the regenerating hepatocytes is uncertain at present.

Furthermore, the important problem of whether estrogen acts on the hepatocytes directly or not is also unresolved. Since hepatocytes have estrogen receptors (Eisenfeld et al. 1976; Beers and Rosner 1977; Francavilla et al. 1984) it is thought that the proliferative effect of E3 on the hepatocytes may be a direct action. However, the sinusoidal vascular supply is essential to the survival and proliferation of regenerating hepatocytes (Tavassoli and Crosby 1970). The effect of estrogens on the proliferation of sinusoidal endothelial cells and Kupffer cells in the adult liver has been previously reported (Widmann and Fahimi 1976; Fujii et al. 1985). In this study, the development of sinusoids in the regenerating liver tissue was much more progressive in the E3 group than in the control group and many Kupffer cells also appeared in the sinusoids in the E3 group. Therefore, it is also supposed that the active proliferation of the regenerating hepatocytes may be due to E3 action on the acceleration of vascularization and the stimulation of the reticuloendothelial system (Vernon-Roberts 1969; Sljivic and Warr 1973).

In this study, E3 had more effect on the mitotic activity of the regenerating hepatocytes when comparison with the recipient's own hepatocytes. Ectopic hepatocytes may be more sensitive to estrogen than the recipient's own hepatocytes or out of regulation due to some factors, e.g. liver chalone (Sekas and Cook 1976; Kuo and Yoo 1977; Lype and McMahon 1981). According to Mito et al. (1979) no regulatory interaction is present between the host liver and the hepatic tissue transplanted in the spleen.

There is an older paper reporting that various attempts to promote an increase in the amount of ectopically transplanted liver have not been successful (Seller 1972). Here, we demonstrate a remarkable effect of E3 on the promotion of growth of the transplanted liver, suggesting a potent clinical application of estrogen in liver transplantation.

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