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Tumor Growth in Liver Atrophy and Growth. An Experimental Study in Rats*

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Abstract—*Despite vast knowledge on liver regeneration, little is known about the effect of active liver atrophy and regeneration on tumor growth. Ligation of a branch of the portal vein to the one or two anterior lobes was performed in inbred Wistar rats. This induces acute atrophy of the anterior and regeneration of the other lobes. During the same operation a tumor cell suspension (NGW_1 — adenocarcinoma) was inoculated in liver lobes undergoing atrophy and regeneration. Tumor volume and weight were measured and the histologic appearance was assessed. During the early and active phases the tumor growth was significantly accelerated in regenerating lobes and partially inhibited in rapidly atrophied segments. After the regeneration and atrophy was completed the normal pattern of growth was re-established in both parts of the liver. The results suggest that tumor growth is affected in proportion to regenerative response. They further suggest that portal branch ligation is of limited value in surgical palliation of liver tumors. The risk for further induction of growth of clinically undetected tumor foci in the remaining liver tissue appears to be small, although a significant, but short-lasting, stimulatory response was found.*

INTRODUCTION

INFORMATION on the regulation of hepatocyte proliferation is important when dealing with problems related to management of hepatic malignant disease. Despite a vast knowledge on liver regeneration little is known about the effect of active liver atrophy and regeneration on tumor growth. Acceleration of tumor growth has been demonstrated following partial hepatectomy and tumor cell proliferation has been noted to be partially restrained after implantation into atrophic liver tissue [1–3].

In this study two doses of tumor cell suspension were simultaneously inoculated into ligated and unligated parts of the rat liver. The experiment was undertaken to re-examine the rationale of portal branch ligation (PBL) in surgical palliation of an advanced unresectable carcinoma of the liver. We also wanted to study if any stimulatory effect on growth of clinically undetected tumor foci could be expected in patients undergoing hepatic resection.

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MATERIALS AND METHODS

The experiment was carried out on 60 inbred male Wistar rats weighing 250–350 g. They were housed four per cage with artificial light 12 hr per day (6 a.m.–6 p.m.). The rats were maintained on standard laboratory food and water *ad libitum*. All operations were performed using clean but not sterile apparatus.

Portal venous branch to the left anterior (LAL) or both anterior (AL) lobes was dissected under an operating microscope (magnification × 10–15), and was ligated following identification of an intact arterial branch. By such a procedure the whole portal flow perfused the residual unligated lobes.

Sham-PBL involved laparotomy and dissection of the relevant portal branch without ligature.

The tumor was an *N*-methyl-*N*-nitrosoguanidine-induced adenocarcinoma of the colon transplanted into the kidney of a Wistar rat every tenth day. The tumor was kindly supplied by Professor H. O. Sjögren, The Wallenberg Laboratory, University of Lund, Sweden. The number of passages before this generation was ten. A cell suspension containing 1.0×10^6/0.1 ml viable tumor cells was used.

In all rats at the time of portal branch ligation (PBL) 2 × 0.05 ml of the tumor cell suspension was injected under the liver capsula into the periphery of the unligated right anterior lobe and into ligated or sham-ligated LAL.

There were five experimental groups: group I (13 rats), PBL of the LAL (about 30% of the liver); group II (8 rats), PBL of the two AL (about 70% of the liver mass); group III (9 rats), sham-PBL; group IV (20 rats), PBL of the LAL; and group V (10 rats), sham-PBL.

Animals in groups I–III were killed 6 days following operation and in groups IV and V 14 days after operation.

At termination the abdomen and chest were opened and macroscopic observation of tumor growth was carried out. Rats with ruptured tumors and with extrahepatic metastates were excluded from the study.

The liver was removed, blotted and both tumors extirpated, weighed and measured by Vernier calipers. The tumor volume was calculated according to the formula $\frac{\pi}{6} \times A \times B \times C$ (A, B, C: the smallest, largest and third diameters). Both ligated and unligated parts of the liver were separated for measuring their relative weights (weight to body weight proportion).

The liver and tumor biopsy specimens were fixed in neutral buffered formalin, embedded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin.

For statistical analysis student's test for non-paired data was used.

RESULTS

Tumor growth was obtained in all inoculated lobes.

There was no mortality; but 6/20 rats of group IV and 4/10 of group V were excluded from the study because of ruptured tumors and/or extrahepatic tumor growth.

All the ligated lobes showed acute atrophy. This was confirmed by low values of the relevant liver weight ratios and by histological examination.

In group I rats the tumors removed from the ligated lobes were significantly smaller and those from regenerating lobes larger than in sham-PBL controls (Fig. 1).

In group II rats, in which the two AL were ligated, the mean weight and volume of the tumors growing in the atrophied lobes were found to be slightly smaller than in the group I rats. On the other hand, tumors grown in regenerating lobes were significantly larger than in groups I and III. Finally, it was found that the total tumor weight and volume in group II rats were significantly greater than in group I and III rats (Fig. 1).

Two weeks following PBL the mean tumor weight and volume in the unligated lobes were greater than in atrophic segments; however, the differences were not significant. Also, in relation to sham-PBL controls (group V) no differences were found (Fig. 2).

Histological examination did not reveal any essential differences of the tumors from the different experimental groups (Fig. 3a, b).

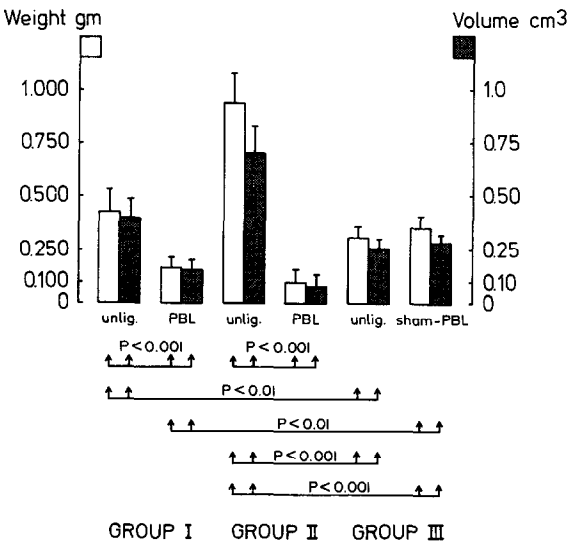

Fig. 1. Tumor weight and volume from ligated and unligated liver lobes. The tumors were extirpated 6 days following PBL and tumor cell inoculation. Values are given as mean ± S.D.

DISCUSSION

In 1975 Honjo *et al.* reported their preliminary experience with PBL alone in 20 patients with unresectable carcinoma of the liver [4]. The clinical effects were encouraging and closely related to the degree of tumor vascularity, its malignancy and concomitant disease such as cirrhosis and portal hypertension.

PBL was also suggested to be useful as the first step of so-called 'extensive hepatectomy in two stages'. In the experiments of Kozaka extirpation of an atrophied liver tissue seemed to be easier and safer to perform than using the conventional approach [5].

In experimental animals PBL induces acute atrophy of the lobe deprived of portal flow and hepatocyte proliferation in the residual unligated segments. The intensity of regeneration as measured by incorporation of labeled thymidine into hepatic DNA and by mitotic activity was found to be in proportion to the loss of liver parenchyma and to a similar extent as following extirpation of the corresponding liver mass [6].

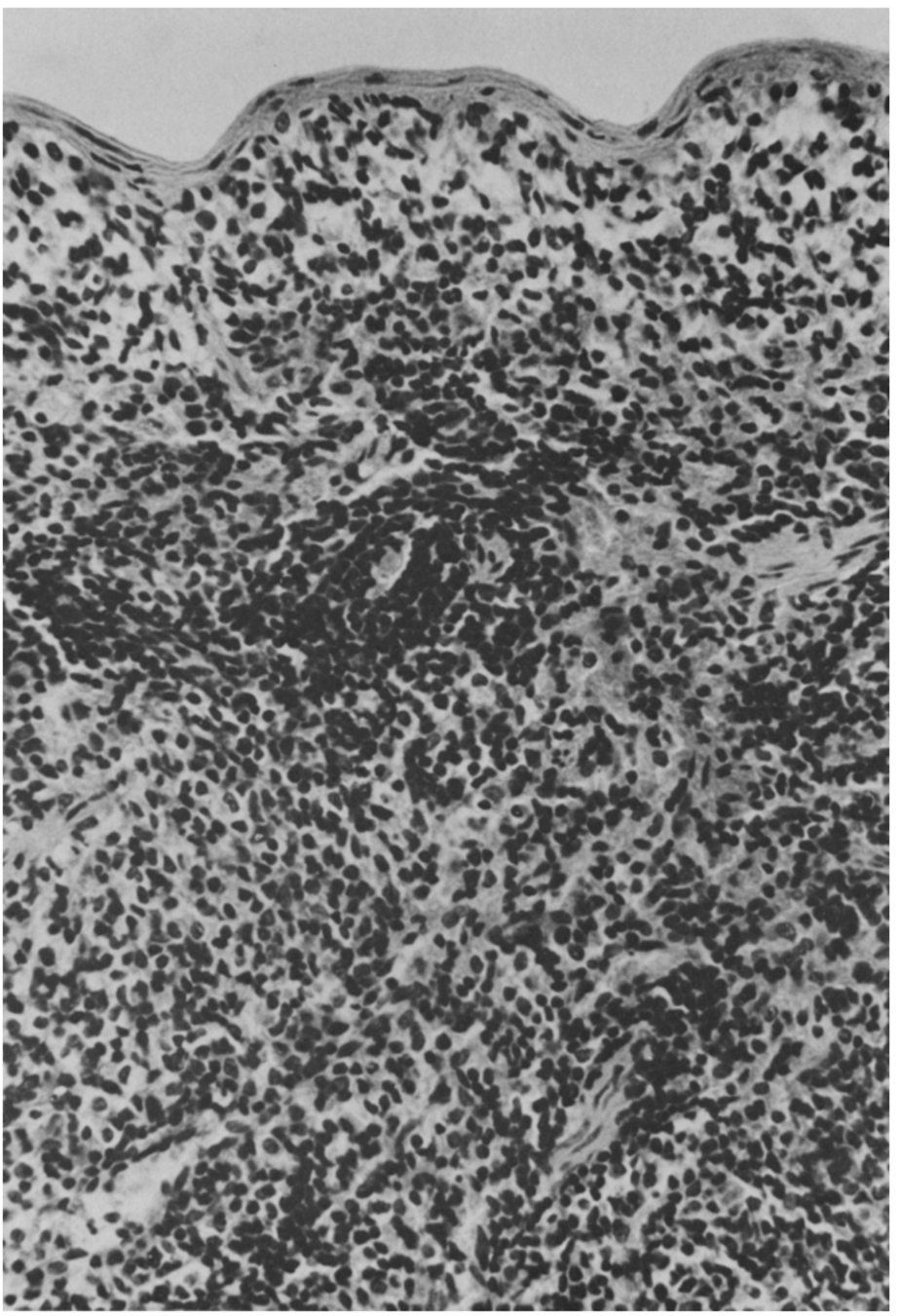

Fig. 3(*a*).

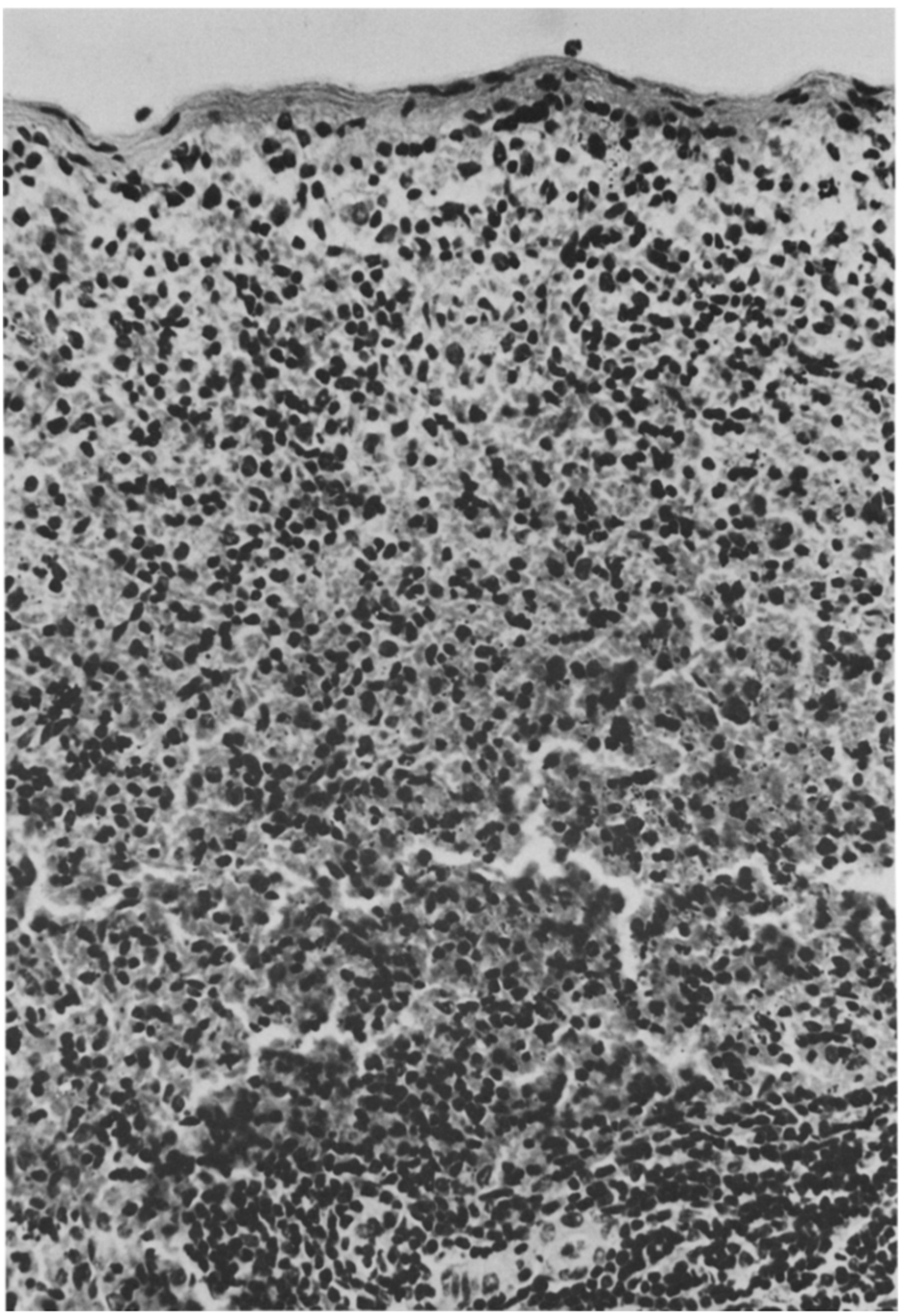

Fig. 3(*b*).

Fig. 3. *Histologic picture of tumor growth from ligated and unligated liver lobes of the same rat.* (*a*) *Tumor from ligated lobe* 6 days *after inoculation — hematoxylin and eosin,* × 125; (*b*) *tumor from unligated lobe of the same liver — hemotoxylin and eosin,* × 125.

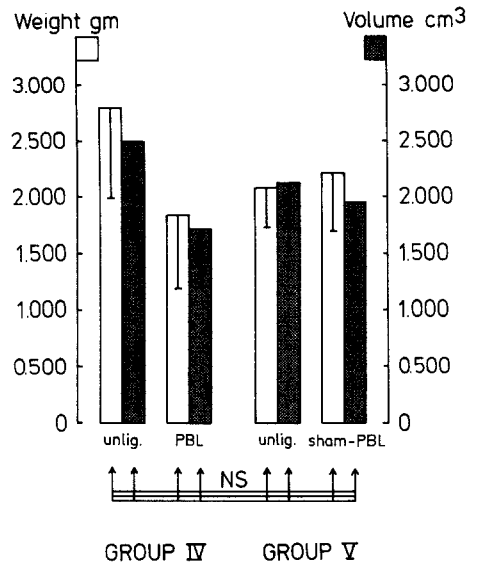

Fig. 2. Tumor weights and volumes from ligated and residual liver lobes 2 weeks *following PBL. Values are given as mean ± S.D.*

Similar findings were seen in the present series of PBL rats.

When the experiment was terminated after 6 days the rate of tumor growth in the regenerating lobes was closely related to the proliferative stimulus being higher in rats subjected to PBL of the two AL (70% of liver mass) than found in rats with ligation of one lobe (30%). In contrast, in all of the ligated rapidly atrophied lobes the growth was partially inhibited. Therefore, during the regeneration process the tumor growth is enhanced in proportion to the regenerative stimuli and in the course of active atrophy diminished.

However, results obtained in groups IV and V suggest that these phenomena occurred transiently. During a longer exposure tumor growth in regenerating liver lobes decreased. Moreover, in the atrophic lobes the mean weight of the tumor and its volume did not differ from those found in sham-PBL lobes, and also in relation to those growing in previously regenerating segments. Such equilibrium between the atrophic and hypertrophic lobes stressed the likelihood of accelerating growth in the atrophic parts from day 6 to day 14.

There are several possible explanations to the findings of the experiment. The unligated lobes were overperfused and supplied from both portal venous and arterial sources, as has already been established in the case of small liver tumors [7]. As long as the regeneration response continued the tumor was affected by hepatotrophic factors, which probably modified the growth to some extent.

On the other hand, tumors of the ligated lobes were initially affected by ischemia, especially their peripheral parts. After a while hepatic arterial supply increased and the normal pattern of growth was reconstituted. The cause of acceleration of growth from day 6 to day 14 remains obscure.

The present study provides support that regeneration response and acute liver atrophy (portal blood ischemia) actually affect tumor growth, but only during the early and active phases of the two processes. After a certain period of time, when the regeneration is completed and the full-grown arterial vascularity developed, the normal pattern of growth is re-established. Consequently, it might be assumed that segmental interruption of the portal flow in itself is of limited value in the management of hepatic malignant disease.

The results of the present work suggest that even if some regression of the tumor could be obtained by deviation of the portal blood supply, there is a risk of inducing the growth of a small, clinically undetectable tumor focus in the remaining liver tissue. This may also be applicable to undetected lesions in the remaining liver lobe after hepatic resection. The stimulatory response is, however, of short duration and may be counteracted by the concomitant administration of cytotoxic drugs.

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