THE POSSIBLE ROLE OF RETICULO ENDOTHELIAL SYSTEM IN HEPATIC REGENERATION

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Summary: Calciphylactic blockade of Reticulo endothelial system (REB) was used to study the role of reticulo-endothelial system (RES) in hepatic regeneration. REB was induced by i.v. egg yolk administration in DHT sensitized animals, which were subsequently partially hepatectomized. Mitotic index and percentage regeneration were studied in these animals 48 hr after partial hepatectomy. REB produced a significant depression of the mitotic index and percentage regeneration in regenerating livers. This suggests that functional integrity of the RES is essential for hepatic parenchymal proliferation after partial hepatectomy.

Key words: liver regeneration reticulo endothelial blockade

INTRODUCTION

The restoration of the functional loss of the hepatic tissue involves complex processes of growth and differentiation, their initiation and control. The changes in the immediate cellular environment which are necessary for the induction of hepatic regeneration are largely unknown; although available evidence indicate that alterations in the hepatic milieu are the prime factors in its initiation.

Glinos (5) observed that fall in serum proteins initiated and rise in serum proteins inhibited the parenchymal proliferation in the resting and regenerating livers respectively. It has been suggested that plasma proteins may participate as information carrying agents in a negative feed back system controlling the growth of liver cells.

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Since reticulo-endothelial system has been partly implicated in the production of plasma proteins and its role in hepatic regeneration has not been elucidated, this study has been undertaken.

**MATERIALS AND METHODS**

Male albino rats of Wistar strain with a mean body weight of 100±10 gms were used in this study. The animals were fed with Hind Lever small animal feed and fed *ad libitum* with water.

The animals were divided into 4 groups consisting of 12 animals in each group.

Group A are control animals in which partial hepatectomy was performed.

Group B animals received intragastric Dihydrotachysterol (DHT) 1 mg/100 gm of body weight on the first day and partial hepatectomy was performed on the third day.

Group C animals received intravenous egg yolk (1 ml of 50% egg yolk) and partially hepatectomized 24 hours later.

Group D animals received DHT on the first day, egg yolk on the next day and partial hepaectomy was performed on the third day.

All these animals were sacrificed 48 hours after partial hepatectomy, their livers removed, blotted dry, weighed and the weight gain during regeneration was calculated and expressed as percentage regeneration. Small bits of liver tissues from identical region were processed for histological study and mitotic index (Metaphases/1000 parenchymal cells) worked out (11).

*Partial hepatectomy*: Partial hepatectomy was carried out according to the procedure of Higgins and Anderson (6). The animals were anaesthetized with ether and partial hepatectomy was performed through a ventral midline incision. The abdomen was closed in layers. About 65-70% of the liver was removed during this procedure.

*Reticuloendothelial blockade*: Reticuloendothelial blockade (REB) was brought about by calciphylactic procedure (10). Vegetable oil suspension of Dihydrotachysterol (DHT) in a dose of 1 mg/100 gm of body weight was administered by gastric gavage as a sensitizing agent on the first day. The challenger 1 ml of 50% egg yolk was given intravenously 24 hr later. Calcification of Kupffers cells and other R.E. cells set in as judged by Von Kossa’s staining and carbon clearance studies on the third day.
Colloidal Carbon Clearance:

Colloidal carbon clearance was studied in all the experimental and control animals just before sacrifice using the method of Benacerraf (1). This test consists of intravenous injection of 15 mg of colloidal carbon/100 gm of body weight and determination of its half life in the blood by means of samples taken after 5, 10, 15 and 20 min. The carbon content in the serum was measured spectrophotometrically at a wave length of 600 nm. The clearance constant K was calculated from these values and used as an index of R.E. phagocytic activity.

RESULTS

The mitotic index of the regenerating control livers (Group A) in this study is 18.25±3.06, 48 hours after partial hepatectomy (Fig. 1).

MITOTIC INDEX IN RETICULO ENDOTHELIAL BLOCKADE

Fig. 1: Histogram of the Mitotic index in the experimental animals. Hep-Xp-Partial Hepactectomy. DHT=Didydrotachysterol (1 mg/100 gm). Yolk-egg Yolk (50% 1 ml IV). The Vertical lines denote S.D.
Administration of DHT (Group B) 48 hrs before the partial hepatectomy decreases the mitotic index to 13.08±1.727 which is significant at 5% level.

In the egg yolk treated animals (Group C) the mitotic index does not differ from the control value (18.9±2.08).

DHT followed by egg yolk produces calciphylactic blockade of reticuloendothelial cells (Group D). Partial hepatectomy in these animals results in depressed hyper plastic and hypertrophic responses as evidenced by a mitotic index of 7.33±1.72 which is significantly lower than the control value (P<.01). The percentage regeneration an index of weight gain and hypertrophic response is also depressed in these animals which is significant at 5% level (Fig. 2).

**Fig. 2:** Histogram of the percentage regeneration of the liver after partial Hepatectomy in experimental animals. The vertical lines denote S.D.
The percentage regeneration has not been influenced by DHT or egg yolk alone.

The carbon clearance studies have shown that partial hepatectomy decreases the clearance constant $K$ from $0.625\pm0.0214$ to $0.0324\pm0.0005$. This decrease in $K$ is due to the loss of phagocytic R.E. Cell population entrapped in the excised liver lobes (Fig. 3).

**CARBON CLEARANCE IN REB**

![Graph showing carbon clearance constant](image)

Fig. 3 : Histogram of the Carbon clearance constant in Reticuloendothelial Blockade.
Normal - Clearance constant in unoperated animals.

A - partial Hepatectomy.
B - DHT+Partial Hepatectomy
C - YOLK+Partial Hepatectomy
D - DHT+YOLK+Partial Hepatectomy (REB)

Hepatectomy following the administration of DHT alone (Group B) or Yolk alone (Group C) has no further significant depressant action on the clearance constant. Hepatectomy following the sequential administration of DHT and Yolk (Group D) depresses the clearance value to $0.264\pm0.0005$ indicating a very low level of phagocytic activity of the RE Cells.
Histologically calcium deposits can be identified in the sinusoidal cells using Von Kossa’s technique in Group D animals only (Fig. 4).

DISCUSSION

The administration of DHT, a vitamin D derivative increases the serum calcium level. This induced hypercalcemia is probably responsible for the depression of mitotic index seen in the group B animals. Even though the exact steps leading to this are not clearly known, the probable sequence involve the decreased permeability of the hepatic...
parenchymal cell membrane leading to decreased rate of entry of essential nutrients into the cell which in its turn suppresses the DNA duplication. Interference with mitochondrial metabolism and alterations in the nuclear membrane permeability may also be contributory for this depression of mitosis.

The calciphylactic procedure used in this study brings about selective calcification of the RES. This is due to the fact that lipid particles of yolk are preferentially taken by the phagocytes of this system which subsequently undergo calcification. Though yolk given alone is also taken up by the RE Cells, is neither capable of causing calcification of the RE cells nor depressing the Mitotic index of the regenerating parenchymal cells.

Similarly DHT or Yolk given singly has no depressant action on the carbon clearance values, indicating a normal or near normal R.E. Phagocytic function. Mitotic index, carbon clearance and percentage regeneration are all depressed only when there is calciphylactic blockade of the R.E. cells suggesting that there is more than a causal relationship between the RES and Hepatic regeneration.

Moreover, administration of egg yolk following DHT did not produce further exacerbation of hyper calcemia, (more than that produced by DHT alone) the significant depression in mitotic index observed in the REB animals could not be attributed to hyper calcemia. This implicates the co- incidental R.E. blockade seen in these animals as the responsible factor for the mitotic depression.

In this connection, it is pertinent to review briefly the concept of humoral regulation of hepatic regeneration. Christensen and Jacobson (3), Wenneker and Sussmen (11), Bucher, Scott and Aub (2), Fisher, Fisher and Elizabeth Saffer (4), Levi and Zeppa (7) and others have shown that regeneration after partial hepatectomy is dependent upon the appearance of a humoral agent which stimulates the mitotic division of the partially hepatectomised liver.

Morley and Kingdon (8) reported a factor isolated from the serum of rats 24 hr after partial hepatectomy which is capable of stimulating DNA synthesis in the livers of normal mice. A similar factor has also been isolated by Morley (9), from the foetal calf serum which enhances the activity of ornithine decarboxylase, an enzyme necessary for the initial stages of DNA synthesis.

Since the existence of the humoral factor has been firmly established, it is postulated that either the release of the humoral factor or its ultimate site of action is dependent
upon the reticulo endothelial system. The reticulo endothelial cells especially the Kupffers cells are strategically situated in the sinusoids of the liver where they can monitor the chemical and physical changes in blood more closely than the parenchymal cells. Blockade of these cells may interfere with the efflux of humoral signals or with their reception.

This hypothesis of introducing reticuloendothelial cells as an intermediary in the hepatic regenerative process need not be looked upon as a new scheme, since such mechanisms exist in other areas like haemopoietic system where the anoxic stimulus releases erythropoietin from the kidney. Similar mechanisms have been postulated for leucocytes and platelets also.

REFERENCES