

^{I131} ROSE BENGAL CLEARANCE DURING HEPATIC REGENERATION IN RATS

A. NAMASIVAYAM* AND N. PADMANABHAN**

*Institute of Physiology,
Madras Medical College, Madras-600 003*

Summary: Intravenous ^{I131} Rose bengal clearance was conducted in partially hepatectomized rats with a dose of 1 μ Ci/100 gm body weight. Blood samples were taken at regular intervals upto 25 minutes which were suitably diluted to count the remaining radioactivity. From these values $t_{1/2}$ and the clearance constants were worked out upto a period of 120 hr after partial hepatectomy. An analysis of the results show that there is a linear correlation between the rose bengal clearance and the regenerating hepatic mass which may be used for quantitative assessment of functional hepatic mass.

Key words: ^{I131} Rose bengal clearance regenerating liver mass

INTRODUCTION

Estimation of regenerating hepatic mass after partial hepatectomy or after toxic hepatic injury is difficult, since none of the routine liver function tests like serum albumin, globulin levels or their ratio show linear correlation with the functional mass of the hepatic tissue. The hepatic clearance method is more specific and quantitative for estimating functional capacity of the liver. For routine work the clearance can be calculated by measurement of the decrease of plasma concentration of an injected substance. Clearance of substance such as bilirubin, rose bengal and Bromsulphthalein (BSP) which are mostly bound to serum albumin and excreted through biliary ducts are dependent on the total mass of the parenchymal cells available to do the clearance, and their functional efficiency (1). ^{I131} rose bengal clearance technique is ideal because, dose of this compound is too small to have any toxic effect and its estimation is not affected by lipemia, jaundice or haemolysis.

The present investigation was, therefore, undertaken to verify the correlation between the clearance of this dye and the regenerating mass of hepatic tissue in rats.

Present address: *Department of Physiology, Chengalpattu Medical College, Chengalpattu (TamilNadu).

**Department of Physiology, Madurai Medical College, Madurai (Tamil Nadu).

MATERIALS AND METHODS

Male albino rats of Wistar strain with a mean body weight of $100 \text{ gm} \pm 10$ were used. The animals were nourished with Hind Lever small animal feed and fed *ad libitum* with water. Seventytwo rats were used for this study, which were divided into two equal groups. The test animals were subjected to partial hepatectomy and in the control group sham hepatectomy was done.

1. *Partial hepatectomy*

Partial hepatectomy was carried out according to the procedure of Higgins and Anderson(3). 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.

2. *I¹³¹ Rose bengal clearance*

The partially hepatectomized and the controls were anaesthetized with ether. I¹³¹ rose bengal supplied by Isotope Division, Bhabha Atomic Energy Corporation was suitably diluted in sterile saline and injected intravenously into the animals in a concentration of $1 \mu\text{Ci}/100 \text{ gm}$ of body weight. Blood samples were taken from the opposite jugular vein at 2.5, 5, 10, 15, 20 and 25 min intervals after the injection. 0.5 ml of blood diluted in 5 ml of distilled water was used to count the remaining radioactivity in the blood, in a gamma ray spectrograph adjusted for I¹³¹ measurement. From these values $t_{\frac{1}{2}}$ was calculated and the disappearance constant 'K' worked out.

The partially hepatectomized animals were sacrificed at regular intervals after rose bengal clearance test upto 120 hr and the regenerating livers were removed blotted dry, weighed and the percentage regeneration was calculated (5).

RESULTS

I¹³¹ rose bengal clearance is a sensitive index of the functional changes after partial hepatectomy (Fig.1). The normal clearance constant $K 0.128 \pm 0.016$ decreases after partial hepatectomy to a value of 0.0237 ± 0.003 at 6 hr and gradually rises to reach 0.0856 ± 0.026 at 120 hr indicating that the functional restoration is not complete even at that period. The slow elimination of the dye from the circulation due to the decreased hepatic mass is also evidenced by the prolongation of the half life of the dye in the partially hepatectomized rats.

The maximum prolongation is seen at 6 hr at which time the half life is 29.38 ± 5.42 mts. (4.66 ± 0.433 mts. for normal animals) and the half life has not come back to normal even at 120 hr.

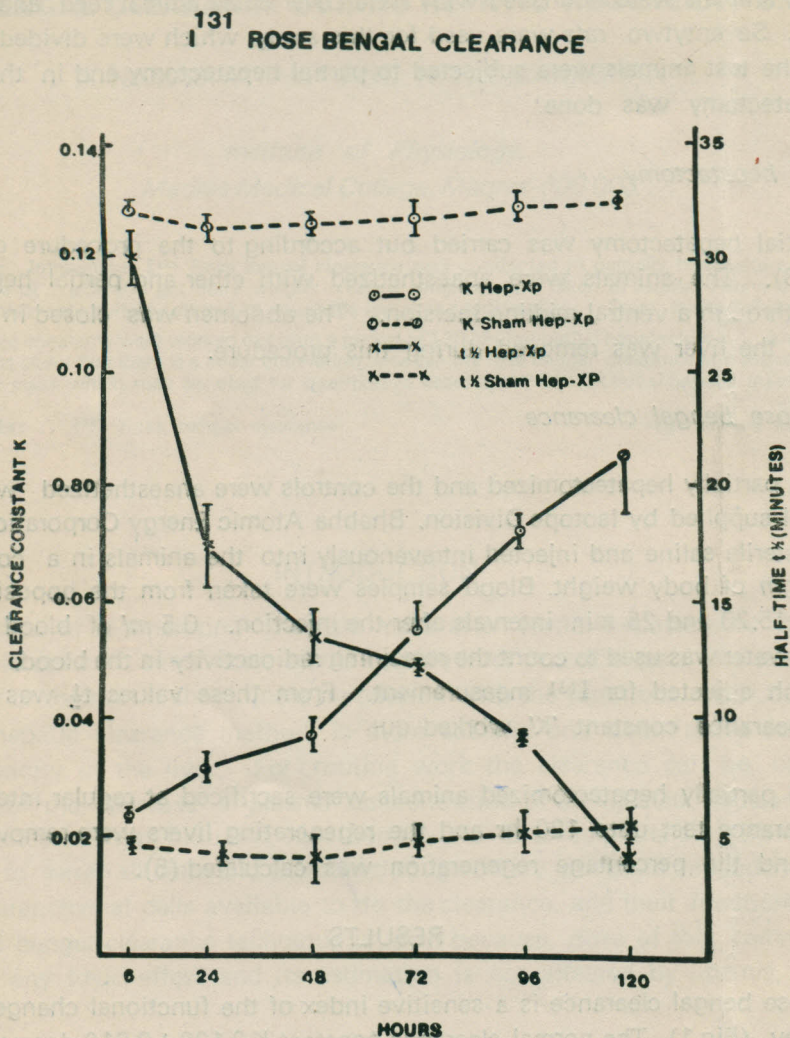


Fig. 1: Rose bengal clearance constant and $t_{1/2}$ of Rose bengal in partially hepatectomized and sham operated animals. Each point in the graph represents the mean value of six animals.

The percentage of regeneration which is an index of growth of the liver closely correlates with the recovery of clearance constant after partial hepatectomy (Fig.2).

FUNCTIONAL AND MORPHOLOGICAL RECOVERY AFTER HEP-XP

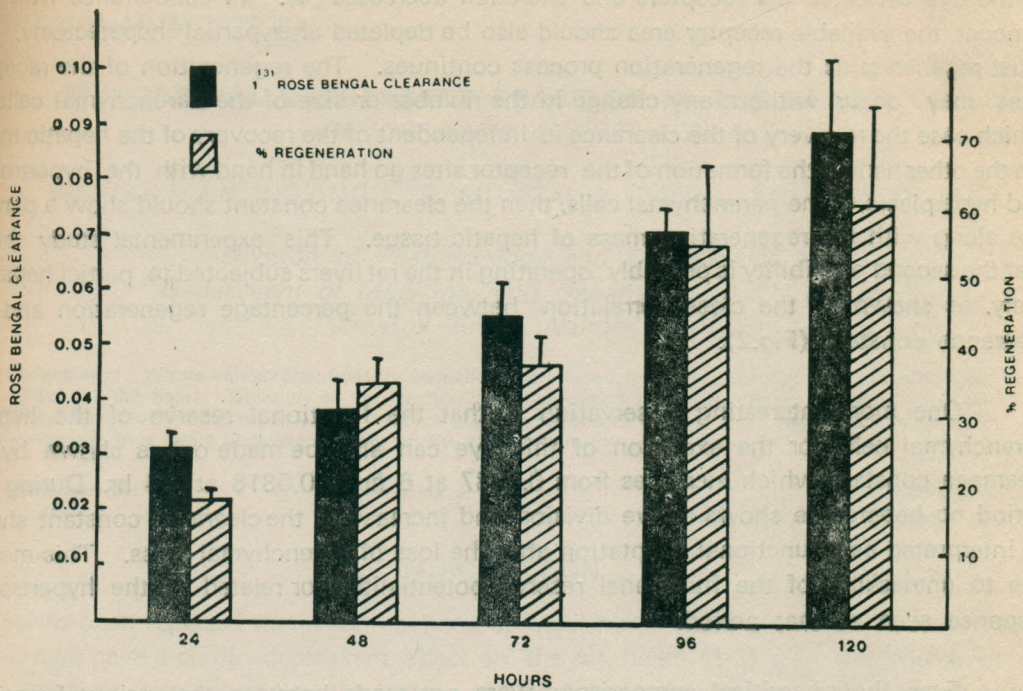


Fig. 2 : Correlation between morphological and functional recovery in livers after partial hepatectomy.

DISCUSSION

The primary idea of this study was to evaluate the usefulness of the Rose bengal clearance as a measure of the morphological recovery (weight gain) during the regenerative process of the liver after partial hepatectomy.

Previous workers (2,5), in this field resorted to the autopsy weights of the regenerating liver at varying intervals to calculate the growth, after partial hepatectomy, and used the percentage regeneration as an index to measure growth. Hepatic I^{131} rose bengal clearance when compared with the percentage regeneration shows a good correlation and the recovery of the Rose bengal clearance parallels the morphologic recovery of the hepatic mass (Fig.2). Taplin, Meredith and Kade (4) also showed a similar recovery pattern in the clearance after partial hepatectomy but these authors have not correlated the recovery of the clearance values with the percentage regeneration. Moreover a perusal of literature also failed to show any such previous study correlating functional recovery with the morphological recovery of the hepatic mass.

Further the hepatic clearance of the I^{131} rose bengal from the blood is mediated through receptor mechanism present in the parenchymal cell membrane. Large doses of the dye saturated the receptors and excretion decreases (4). In concurrence with this concept, the available receptor area should also be depleted after partial hepatectomy, and must regenerate as the regeneration process continues. The regeneration of the receptor sites may occur without any change in the number or size of the parenchymal cells, in which case the recovery of the clearance is independent of the recovery of the hepatic mass. On the other hand if the formation of the receptor sites go hand in hand with the hypertrophy and hyperplasia of the parenchymal cells, then the clearance constant should show a parallel rise along with the regenerating mass of hepatic tissue. This experimental study shows that the second possibility is probably operating in the rat livers subjected to partial hepatectomy, as shown by the close correlation between the percentage regeneration and the clearance constant (Fig.2).

One more interesting observation is that the functional reserve of the hepatic parenchymal cells for the excretion of this dye can also be made out as shown by the clearance constant which increases from 0.0237 at 6 hr to 0.0316 at 24 hr. During this period no hepatocyte shows active division and increase in the clearance constant should be interpreted as a functional adaptation after the loss of parenchymal mass. This may be due to unmasking of the functional reserve potentialities or related to the hypertrophic response seen at that period.

Even though critical comparisons were not made between the various functional tests, our observations show that I^{131} rose bengal clearance test may give more accurate idea about the functional and morphological recovery of the liver, during regeneration.

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