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DECREASE OF THE ACTIVITY OF THE MIXED FUNCTION OXIDASE SYSTEM IN REGENERATING RAT LIVER: AN ALTERNATIVE EXPLANATION.

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SUMMARY

Partial hepatectomy and sham-operation reduce to a similar extent the activity of two enzymes of rat liver mixed function oxidase system, aniline hydroxylase and aminopyrine demethylase. On this basis it is proposed that the decreased functional specialization of regenerating liver depends on events associated to the stress of intra-abdominal surgery and not, as previously hypotized, to cellular multiplication. This view is confirmed by the fact that regenerating liver retains its ability to respond to phenobarbitone administration with an increased activity of the two enzymes.

INTRODUCTION

On the basis that the activity of the enzymes of liver mixed function oxidase system is reduced in conditions characterized by rapid cellular proliferation, as in liver regenerating after partial hepatectomy (1,2), in hepatomas (3) and in liver of newborn animals (4), it is accepted that the process of cell proliferation competes with the capacity for functional specialization (1,2,5). Preliminary results obtained in our laboratory on the capacity of regenerating liver to respond functionally to phenobarbitone (PB) administration have however cast some doubt on this assumption. The capacity for functional specialization of the dividing hepatocyte was hence reinvestigated by measuring the activity of two enzymes of liver microsomal mixed function oxidase system, aniline hydroxylase (AH) and aminopyrine demethylase (APD) in sham-operated and in partially hepatectomized rats.

ABBREVIATIONS

PB, phenobarbitone, disodium salt; AH, aniline hydroxylase; APD, aminopyrine demethylase.

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Since PB administration induces an increase of the activity of the enzymes of liver mixed function oxidase system in normal liver (6), to further define the influence of regeneration on the functional specialization of the hepatocyte, the activity of AH and APD was studied also in sham-operated and in partially hepatectomized rats treated with this inducer. The results demonstrate that cell division does not compete with functional specialization since i) the reduction of AH and of APD activity in regenerating liver depends mainly on the surgical stress and not on cellular proliferation and ii) the capacity to respond functionally to PB is maintained in proliferating hepatocyte.

MATERIALS AND METHODS

Animals: Male albino rats (Wistar strain) weighing 150-200 g fed on a diet of laboratory chow (Piccioni, Brescia, Italy) were used. The animals were distributed at random in five groups: control, sham-operated, partially hepatectomized, sham-operated treated with PB, partially hepatectomized treated with PB. Partial hepatectomy (70%) was performed according to Higgins and Anderson (7) and sham-operations according to Fouts et al. (1) by the same investigator, between 9.00 and 10.00 a.m. Each operation, taking 3-4 min, was performed under ether anesthesia; drinking water after surgery was not supplemented with glucose. PB was injected i.p. at a dose of 8 mg/100 g b.wt. (8)30 min after surgery and thereafter once daily allthroughout the experimental period. The animals were killed by cervical dislocation 24,48,72,96 h after surgery and their livers quickly removed and transferred to ice-cold medium A. All subsequent operations were performed at a temperature between 0° and 4°C.

Cellular fractionation: The livers were weighed and passed through a tissue press made to the design of Porterfield (9). The liver mince was homogenized in 2.0. vols. of medium A consisting of 0.25 M Sucrose in TKM buffer [50mM-TrisHCl (pH 7.8 at 20°C); 25 mM-KCl; 5 mM-$MgSO_4 \cdot 7\ H_2O$]. Microsomal fraction was prepared as previously described (B procedure, Ragnotti, 10). The sedimented microsomal fraction was resuspended in 10 mM-potassium phosphate buffer, pH 7.5. This washing procedure was repeated twice. Aliquots were then taken for the determination of protein concentration and of the activity of AH and of APD.

Assay of AH: This was performed as described previously (11) with the only modification that the reaction substrate (aniline) was prepared in acetone (13); p-aminophenol in acetone was used as a standard.

Assay of APD: This was performed as described previously (11); incubation was stopped by adding 0.25 ml of 25% $ZnSO_4$ and 0.25 ml of saturated $Ba(OH)_2$ solution (12).

Protein assay: This was performed using the Bio Rad Protein Assay Kit.

Statistical treatment: The differences of the means were tested for statistical significance by the analysis of variance.

RESULTS AND DISCUSSION

Body weight: The activity of the enzymes of rat liver mixed function oxidase system has been shown to be influenced by the nutritional status of the animal (14). The variation of body weight was hence determined in our experimental groups. The results (Fig.1) demonstrate that intra-abdominal surgery per se greatly alters the dietary habitus of the rat for at least 48 h. PB administration does not influence the variation of body weight in sham-operated rats, while it has a dramatic effect in hepatectomized animals. Since starvation has been shown to alter many aspects of liver metabolism (14,15,26) our results suggest that evaluation of parameters in liver of rats subjected to intra-abdominal surgery must take into account also the alimentary status of the animal.

AH and APD activity. Fig. 2 shows that in the absence of PB administration AH and APD activity in liver of sham-operated and of hepatectomized rats is equally reduced in respect to the controls at all times studied, except at 48 h after surgery, when the activity of both enzymes is significantly lower in hepatectomized in respect to sham-operated animals. The decrease of the activity of liver mixed function

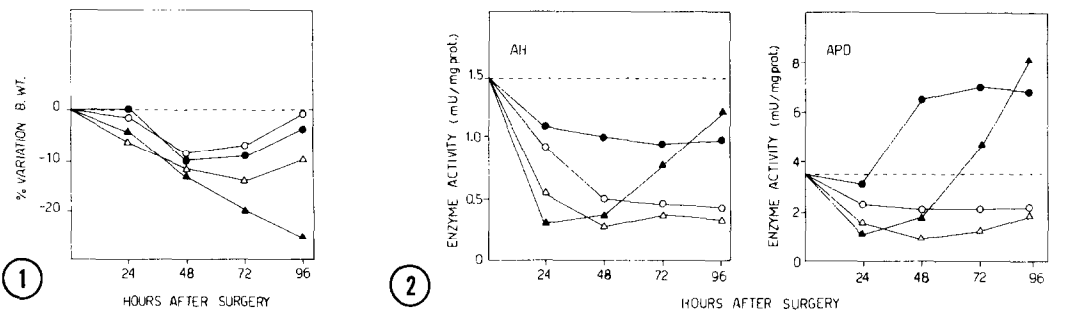

FIG. 1 Variation of rat body weight. For the details of surgery and PB administration see the Materials and Methods section.
Each point represents the mean of 5-9 experiments.
- - - -: control animals; O—O: sham-operated animals; ●—●: sham-operated PB-treated animals; Δ—Δ: hepatectomized animals; ▲—▲: hepatectomized PB-treated animals.

FIG. 2 AH and APD activity in microsomes isolated from rat liver.
For the details of surgery, PB administration, microsomes preparation and assay of AH and APD activity see the Materials and Methods section.
Each point represents the mean of 5-9 experiments.
- - - -: control animals; O—O: sham-operated animals; ●—●: sham-operated PB-treated animals; Δ—Δ: hepatectomized animals; ▲—▲: hepatectomized PB-treated animals.

oxidase system in partially hepatectomized rats has been hypothized to depend on a competitive effect of cellular proliferation on functional specialization (1,2,5). Our results suggest instead that the decrease of the activity of AH and APD in regenerating rat liver is not related to the process of cellular multiplication, depending instead on the "stress" caused by the surgical manipulation. The possible sources of stress in our experimental conditions are the anesthesia and/or the surgical trauma, with the consequent alteration of the dietary habitus and/or of the hormonal homeostasis (16,17,18,27,28). Since ether anesthesia does not influence the activity of AH and of APD (results not shown) and since our results show that the patterns of these enzymatic activities is not related to the variation of body weight, we suggest that the decrease of the activity of AH and of APD which occours in liver of sham-operated and of hepatectomized rats depends on the hormonal modifications, such as an increase in adrenocortical activity (17,27) and in glucagone release (28) and a decrease of insuline release (18), occurring as a consequence of surgery. These hormonal modifications cause infact an increased concentration of cAMP into the hepatocyte (29,30,31,32), condition which has been demonstrated (19) to decrease the activity of the mixed function oxidase system both for a type I substrate, such as aminopyrine, and for a type II substrate, such as aniline.

PB has been reported to increase the activity of the mixed function oxidase system in the liver of normal rats (6). To further define the influence of cellular proliferation on functional specialization, the capacity of liver of sham-operated and of hepatectomized rats to respond to the increased functional demand caused by PB administration was studied (Fig.2). The administration of the inducer to sham-operated animals greatly reduces the fall of activity of AH and completely prevents that of APD which, 48 h after surgery, is also significantly higher than that of the controls. The activity of AH and of APD in hepatectomized PB-treated rats after an initial fall occurring within the first 24 h, similar to that seen in the hepatectomized PB-untreated counterpart, increases exceeding at 96 h the value of the sham-operated PB-treated animals.

These results therefore demonstrate that the capacity of the hepatocyte to respond to PB is not impaired by the process of cellular proliferation, supporting the view that the decrease of AH and of APD activity observed in regenerating liver is the consequence of the mere surgical stress.

The lag of the phenobarbitone induction of AH and of APD activity in hepatectomized in respect to sham-operated animals (Fig.2) may then be interpreted as the consequence of the more severe surgical stress caused by partial hepatectomy in respect to sham-operation. This view is supported by the fact that, in respect to normal animals (6), also sham-operation per se causes a lag of 24 h in the onset of PB-dependent stimulation of AH and APD activity.

Our results, together with those obtained by Fouts et al.(1), Gram et al.(25) and Lloyd et al.(33), provide a clearcut instance of the dramatic effect of sham-operation on some aspects of liver metabolism.

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