Metabolic Restoration in Rat Liver After Partial Hepatectomy

After partial hepatectomy the tissue mass in rat liver is promptly restored through regeneration. The early periods of regeneration, especially the first 2 days, are marked by several metabolic and enzymic changes[1-4]. Generally the metabolic pattern returns to normal as the restoration of the liver nears completion[5]. The liver mass is regained sometimes between 10 and 20 days after partial hepatectomy. Therefore, while the second day is important for recording the metabolic changes that occur as a consequence of partial hepatectomy, the twentieth day is important for the examination of any functional or enzymatic alterations retained by the liver after restoration of the weight.

In this study an increase in the metabolism of galactose during the period of regeneration was observed. This increase in galactose metabolism apparently diminished upon complete restoration of the liver. Since ethanol strongly inhibits oxidation of galactose, its effect was also studied during these periods.

Materials and methods. Wistar albino rats (160–180 g) were partially hepatectomized[6] removing 70% of the liver; the animals were fed ad libitum for 2 to 20 days, sacrificed and the livers removed. Sham operated animals were used as the controls and will be referred to as 'normal' animals.

Liver slices were prepared and incubated in 3 ml Krebs-Ringer phosphate medium (pH 7.4) containing 1 m*M* 1-^{14}C-galactose (specific activity 0.5 μc/3 μmoles) and ethanol (5 m*M*) when indicated in Warburg vessels for 1 h at 37 °C using O_2 as gas phase[7]. The labeled CO_2 generated from the incubated tissue was trapped by hyamine in the center wells of the Warburg vessels. The resulting hyamine carbonate was transferred to Scintillation vials containing 15.0 ml scintillation liquid consisting of 10 g, 2, 5-diphenyloxazole (PPO), 0.6 g l, 4-*bis* (4-methyl -5 phenyloxazolyl) benzene in 2 l toluene. The radioactivity was assayed using a Tricarb Liquid Scintillation Spectrometer. The values of $^{14}CO_2$ yields are expressed as corresponding to ng atoms of ^{14}C derived from 1-^{14}C galactose per 100 mg wet weight of the tissue[8].

In another set of experiments approximately 1 h following surgery, both controls and partially hepatectomized rats were injected via the tail vein with 100 μg actinomycin D in physiological saline (Merck, Sharp and Dohme, Montreal). Liver slices were prepared and galactose metabolism was studied by incubation in Warburg apparatus as described above.

Results and discussion. From the results in Table I, there is a two-fold increase in the oxidation of 1-^{14}C-galactose to $^{14}CO_2$ by regenerating rat liver slices on the second day following partial hepatectomy. This increase may be due to accelerated rate of operation of the hexose-monophosphate shunt in this tissue[9]. The increased metabolism of galactose is reversed by the 20th day when restoration of the liver is completed and the rate of galactose oxidation is similar to the controls. It is interesting to note that ethanol inhibits the metabolism of 1-^{14}C-galactose to $^{14}CO_2$ by 25% on the second day of regeneration compared to 80% inhibition in the controls. On the 20th day the inhibition of galactose oxidation is only 67%, showing a tendency to return to normal metabolic pattern.

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Table I. Metabolism of 1-^{14}C-galactose to $^{14}CO_2$ in regenerating rat liver after complete recovery from partial hepatectomy

Days after partial hepatectomy	$^{14}CO_2$ yields [c]	$^{14}CO_2$ yields [c] when 5 m*M* ethanol added	Inhibition by ethanol (%)
Sham operated controls	20.10 ± 0.46 [a]	5.10 ± 0.40 [b]	75.0
2	40.00 ± 1.43	30.00 ± 0.70	25.0
20	20.70 ± 0.60 [a]	6.90 ± 0.43 [b]	67.0

The values of $^{14}CO_2$ yields are expressed as corresponding to ng atoms of ^{14}C carbon derived from 1-^{14}C-galactose per 100 mg wet weight. [a] $p > 0.05$. [b] $p < 0.01$. [c] Mean ± S.E.

Table II. Effect of in vitro administration of actinomycin D on $^{14}CO_2$ yields from 1-^{14}C-galactose and ethanol inhibition in regenerating rat liver slices

Actinomycin D	Normal rat liver slices $^{14}CO_2$ yields [c]	Regenerating rat liver slices $^{14}CO_2$ yields [c]	Ethanol added (5 m*M*) Normal $^{14}CO_2$ yields [c]	Regenerating $^{14}CO_2$ yields [c]
Nil	21.0 ± 0.46 [a]	38.0 ± 0.72	4.0 ± 0.42 [a]	30.0 ± 0.60
100 μg	24.0 ± 1.23 [a]	21.0 ± 0.28	5.0 ± 0.45 [b]	8.0 ± 0.35 [b]

The values of $^{14}CO_2$ yields are expressed as corresponding to ng atoms of ^{14}C carbon derived from 1-^{14}C-galactose per 100 mg wet weight. [a] $p > 0.05$; [b] $p < 0.001$; [c] Mean ± S.E. of 6-10 experiments.

The amount of $^{14}CO_2$ produced from 1-^{14}C-galactose is unaffected in normal liver slices by the administration of actinomycin D; whereas, in regenerating rat liver there is a marked decrease approaching the control values (Table II). Furthermore, in regenerating rat liver slices ethanol inhibition is attenuated approaching the normal values if the antibiotic is injected at the beginning of partial hepatectomy (Table II).

The observations correlate with the reports that the activities of mitochondrial and microsomal enzymes are altered during the early phase of regeneration and return to normal levels upon the restoration of liver mass[5]. However, this is not true with all biological processes; for instance, polyploidy remains high after complete restoration of tissue mass by the liver[10].

The return of normal metabolic activity in the regenerated liver may result from either regulation of enzyme activity or newly synthesized proteins. The abolition of the increase in galactose metabolism and decrease in ethanol inhibition by actinomycin D would indicate that the synthesis of nucleic acids and thus, the elaboration of a new protein might be needed for metabolic adaptation of the regenerating rat liver.

This experimental approach of inhibiting nucleic acid synthesis has been successfully used to demonstrate de novo formation of several enzymes. For instance, the injection of actinomycin D to animals suppressed the adaptive synthesis of glucokinase[11], glucosyl transferase[12] and tryptophan pyrrolase[13]. It should be emphasized that the evidence obtained by these experiments, however valuable, can only be deemed tentative until the actual turnover of enzyme-proteins is demonstrated. With this limitation in mind, it is concluded that the activities of enzymes of galactose metabolism are adapted to the process of regeneration, being regulated by the synthesis of newly formed proteins.

Zusammenfassung. Es zeigt sich während des frühen Regenerationsstadiums bei der teilweise hepatektomierten Rattenleber, dass ein Anstieg im Galaktose-Metabolismus mit gleichzeitiger Abnahme seiner Hemmung durch Ethanol einhergeht. Nach vollständiger Geweberegeneration kehrt der normale Metabolismus zurück.

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[14] I wish to thank Professor J. H. Quastel for providing me with the facilities and for his encouragement during my work at McGill University, Montreal (Canada).

Fibrinogen-Fibrin Conversion as Determined by Polymer Bead Sedimentation Technique

A newly-developed, sensitive method for determining the clotting time of fibrinogen involves addition of suitably-sized bead particles to a fibrinogen solution followed by exposure of the mixture to coagulating enzyme. The time required for gel formation to occur is related to the extent of particle sedimentation observed and can be readily assessed at any time after clot formation by measuring the absorbance of the coagulated system. The influence of several parameters on the results obtained with this technique suggests that it may be useful in the determination of low fibrinogen concentrations and low levels of thrombin clotting activity.

Methods. Bovine fibrinogen (65% clottable, Pentex, Kankakee, Illinois, USA) was dissolved in 0.05 *M* Tris/HCl buffer, pH 7.4, containing 0.15 *M* NaCl (hereafter referred to as 'buffered saline') to give a final concentration of 0.125% (w/v) (in terms of 100% clottable fibrinogen) and briefly centrifuged. Bio-Gel P-2 (200–400 mesh, Bio-Rad

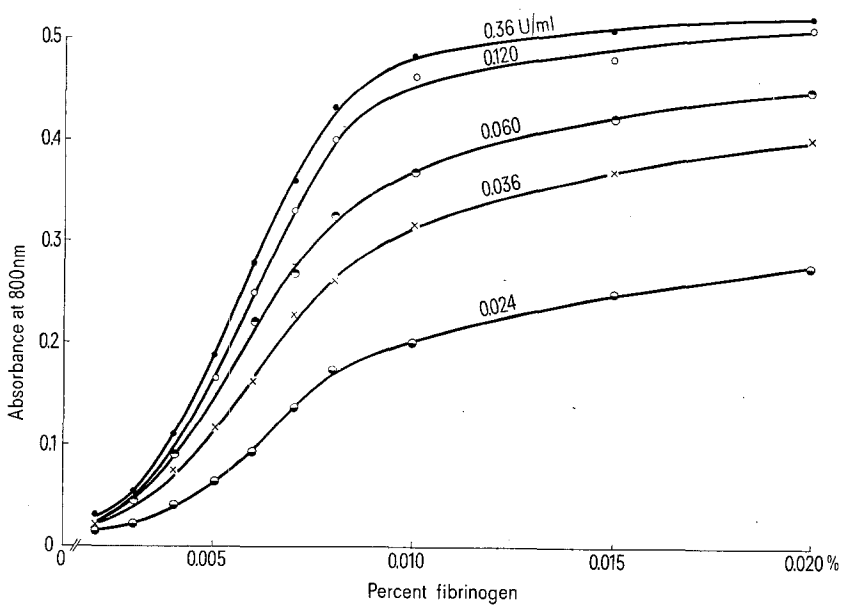

Fig. 3. Effect of fibrinogen concentration on clot absorbance at several thrombin levels, with fixed concentration of Bio-Gel P-2 (1%) in 'buffered saline' at 22 °C. *Legend:* Final thrombin concentrations. ●, 0.36 U/ml; ○, 0.12 U/ml; ◓, 0.060 U/ml; ×, 0.036 U/ml; ◒, 0.024 U/ml.