PURINE METABOLISM IN REGENERATING LIVER-BEARING RATS

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INTRODUCTION

Many biochemical events have been described in the regenerating liver, and several studies have focused on the control of RNA, DNA and protein metabolism (1,2). The gluconeogenic function of the regenerating liver has also been studied revealing a fall in glycaemia, a rapid recovery (3)and gluconeogenesis from lactate or aminoacids (4,5). Little is known about nitrogen metabolism under such conditions, especially purine and pyrimidine metabolism. A number of enzyme studies have been performed. Adenine phosphoribosyltransferase has been reported to increase in regenerating rat liver extracts for 4 days after partial hepatectomy, and to still be above control values after 14 days (6). Hypoxanthine-phosphoribosyltransferase activity has been found not to increase in extracts of regenerating rat liver until the second day after the operation, and to begin to decrease on the fourth day (6). The activity of xanthine oxidase does not undergo any particular variation up to 96 hour after operation (7). However, the behavior of other enzymes involved in the de novo synthesis or catabolism of purine nucleotides, purine nucleotide content, and the pattern of plasma and urinary purine bases -all important parameters of purine nucleotide metabolism- have not been extensively investigated.

In order to improve our knowledge of the behavior of purine nucleotide metabolism in regenerating liver-bearing rats, we studied the behavior of three catabolic products of purine metabolism, free oxypurines, uric acid and allantoin, in the urine of regenerating liver-bearing rats.

MATERIALS AND METHODS

Animals and treatment

Male Albino Wistar rats (Nossan Company, Corezzano, Milano) were kept under controlled temperature (21-23°C) and light periods (07.00 - 19.00 h). They received water ad libitum and a standard pellet diet (Nossan Company) with 22% protein.

The rats were partially hepatectomized according to the method of Higgins and Anderson (8). The operations were carried out between 9.00 and 11.00 a.m. with the animals under diethyl-ether anaesthesia, in aseptic conditions. 65-75% of the liver was removed, leaving the right lateral lobe and the small caudate lobe. The animals were immediately allowed a normal diet.

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The postoperative effects of the anaesthesia wore off very rapidly (1 h), and this was taken as zero time for subsequent observations. Sham operated rats having normal food intake were used as controls.

Food intake was standardized in both groups of animals. They were given the same diet in aliquots which according to our experience, were completely eaten:

10 gr of the standard pellets during the first 24 h after partial hepatectomy; 20 gr of pellets during the subsequent 24 h; after 48 h, pellets were allowed ad libitum. Temperature and light period remained above. Urine was collected every 24 hours and analyzed for oxypurines, uric acid and allantoin.

Uric acid was determined according to Praetorius & Poulsen (9). Allantoin was estimated by Rimini's reaction (10). Oxypurines were evaluated by high pressure liquid chromatography. We used a Beckman Instrument mod 332, equipped with an UV detector at 254 nm and a Supelcosil LC-18 column (250x4.6mm). Isocratic elution, was performed with 10mM KH2PO4 (pH 5.5) and the oxypurines were identified on the basis of retention time, when coeluted with internal standards and treated with xanthine oxidase.

RESULTS AND DISCUSSION

The results are shown in the following figures and tables. Figure 1 shows a typical chromatogram of urinary oxypurines, and the same chromatogram with internal standards.

A strong decrease in the urinary excretion of hypoxanthine and xanthine was observed. This could be explained by the fact that the regenerating liver shows a very high activity of APRT (6), the enzyme of the purine salvage pathway. The reutilization of adenine would decrease the formation of hypoxanthine and xanthine through adenase and xanthine oxidase.

![Figure 1](image-url)

Fig. 1. A: Typical chromatogram of urinary oxypurines. B and C: the same chromatogram with internal standards.