

## Antipyrine metabolism during hepatic regeneration in the rat

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**ABSTRACT** - We investigated antipyrine clearance and urinary excretion of 4-hydroxyantipyrine, 3-hydroxymethylantipyrine and nor-antipyrine in rats after 70% partial hepatectomy. Antipyrine clearance recovered more slowly than liver weight but after 240 h both liver weight and antipyrine clearance had reached control values. During hepatic regeneration conversion of antipyrine to the three major metabolites was reduced, conversion to 3-hydroxymethylantipyrine being reduced more than conversion to the other metabolites. Antipyrine clearance was closely correlated to liver weight ( $r=0.81$ ), indicating a close relation between the functional hepatic mass and the liver weight during hepatic regeneration.

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Phenazone (Antipyrine®) is eliminated almost exclusively by the liver (1, 2). The drug is metabolized by the hepatic mono-oxygenase enzyme system, which consists of several isozymes each presumed to be responsible for the formation of different antipyrine metabolites (3). The antipyrine clearance is used as a quantitative measure of the functional hepatic mass (2). In the present study we investigated 1) antipyrine clearance, 2) clearance of antipyrine to 4-hydroxyantipyrine, 3) clearance of antipyrine to 3-hydroxymethylantipyrine and 4) clearance of antipyrine to nor-antipyrine. These investigations were performed at various times after surgical removal of 70% of the rat liver in order to determine which of these measures was related most closely to the liver mass during hepatic regeneration.

### Material and methods

Female Wistar rats weighing about 200 g were fed Altromin pellets and tap water *ad libitum*. Partial hepatectomy

of 70% of the liver (48 animals) was performed between 7 and 11 a.m. during diethyl-ether anesthesia (4). One group of six animals was untreated and considered as a control group, and another group of six animals was subjected to diethyl-ether anesthesia and sham-operation, consisting of laparotomy, exteriorization and manipulation of the anterior lobes of the liver. The animals were divided into groups of six animals. After 3, 6, 12, 24, 48, 72, 96, and 240 h antipyrine (AP) (Hoechst) 4 mg in 1 ml isotonic saline was injected into a tail vein and the jugular vein catheterized (5) under light diethyl-ether anesthesia. Each animal was then placed in a metabolic cage and urine was collected for 24 h in a plastic bottle kept in dry ice and then transferred to  $-20^{\circ}\text{C}$  until analysis for AP metabolites. Five hours after the AP injection 200  $\mu\text{l}$  blood was drawn from the jugular catheter, heparinized plasma was separated and stored at  $-20^{\circ}\text{C}$  until analysis for AP as earlier described (6). After the 24-h urine collection, the animals were killed, the liver was blotted on filter paper and weighed. All animals were treated and examined in random order over a period of about 2 months.

The urine samples were assayed after hydrolysis with glucuronidase/arylsulfatase (Boehringer) for 3 h at  $37^{\circ}\text{C}$ . Alkaline extraction with dichloromethane was performed for analysis of AP and 3-hydroxy-methyl AP

(3HMA), and acid extraction for analysis of 4-hydroxy AP (4OHA) and nor AP (NA) with dichloromethane/pentane (30/70 v/v). After evaporation to dryness and redissolving the residue in 100- $\mu$ l mobile phase, 25  $\mu$ l was injected into a high pressure liquid chromatographic (HPLC) system, consisting of a Waters pump and injection loop, a Waters  $\mu$  Bondapak C18 column and a Waters UV detector (Model 440) with a fixed wavelength of 254 nm. The mobile phase was 0.01 M phosphate buffer/methanol (65/35, v/v) and the flow rate was 2 ml/min. Retention times for metabolites and internal standard (phenacetin) ranged from 2.6 to 7.1 min. Reference metabolites were used for standard curves: NA, (EGA-Chemie), 4OHA (EGA-Chemie), and 3HMA (kindly donated by Drs. Danhof, Eichelbaum, and Yoshimura). The coefficient of variation of duplicate analyses of the AP metabolites was about 3%, except for 3HMA, for which it was about 10%.

Because of technical difficulties, one animal was excluded from the 6-, 12-, and 48-h groups.

AP clearance was estimated as the single point AP clearance (APC) (6). Clearance of AP to each of the metabolites was calculated as APC times fraction of AP dose excreted as the metabolite.

## Results

The time course of the single point AP clearance (APC) is depicted in Fig. 1 as the mean  $\pm$  SEM of the various groups (solid line). The interval from hepatectomy to antipyrine administration was assigned to the clearance value. There was no difference between APC of untreated control animals and sham-operated animals 3 h after surgery. APC was reduced from 0.83 to 0.34 ml/min (0.41%) estimated immediately after hepatectomy, and the liver weight was reduced from 8.31 to 3.68 g

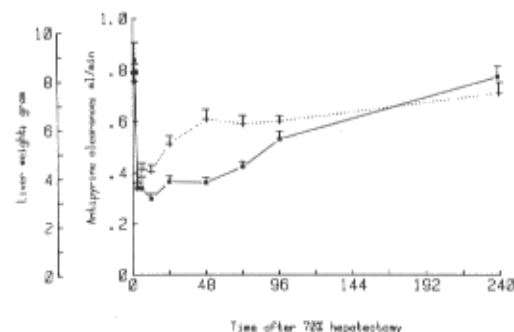


Fig. 1. Time course of antipyrine clearance (solid line) and liver weight (dotted line) after 70% partial hepatectomy. Symbols indicate mean, and bars SEM of 5-6 rats.

(0.44%) immediately after the hepatectomy. In Fig. 1 the time course of the liver weight (dotted line) is also given, showing that liver weight increased more rapidly than APC during the first 72-96 h of the hepatic regeneration ( $p < 0.01$ , two-way analysis of variance).

There was no change in the per cent of dose excreted as NA and 4OHA ( $p = 0.19$  and  $p = 0.71$ , respectively, one-way analysis of variance). The excretion of 3HMA varied at various times after the partial hepatectomy ( $p = 0.002$ , one-way analysis of variance) and by multiple tests with correction for mass significance, it was demonstrated that sham-operation increased 3HMA excretion ( $p < 0.05$ ) and that the excretion was reduced 3, 12, and 24 h after hepatectomy ( $p < 0.05$ ).

The clearance of AP to NA, to 4OHA and to 3HMA, is given in Table 1. The clearance of AP to the three metabolites was reduced according to the reduction in liver weight by hepatectomy, and at 240 h the three clearances recovered according to the recovery of liver weight. However, during the period of the most intensive liver cell proliferation, i.e. 12-72 h after partial hepatectomy (7), liver weight recovered more rapidly than the clearances. After longer periods, i.e. longer than 96 h, this dissociation disappeared. Considering all data as one group, the correlation ( $r$ ) between liver weight (LW) and APC was 0.81; between LW and clearance of AP to 3HMA it was 0.68; between LW and clearance of AP to 3OHA it was 0.36;

Table 1

Clearance of antipyrine to the major antipyrine metabolites at time periods after 70% partial hepatectomy. 3HMA denotes clearance of antipyrine to 3-hydroxymethyl-antipyrine, NOR clearance to nor-antipyrine and 4OH clearance to 4-hydroxyantipyrine

Group	3HMA	NOR	4OH
Control	117 $\pm$ 15	19 $\pm$ 06	66 $\pm$ 11
Sham opr.	189 $\pm$ 20	17 $\pm$ 03	60 $\pm$ 11
3 h	39 $\pm$ 03	10 $\pm$ 03	31 $\pm$ 05
6 h	55 $\pm$ 08	08 $\pm$ 01	40 $\pm$ 09
12 h	34 $\pm$ 04	06 $\pm$ 02	28 $\pm$ 04
24 h	44 $\pm$ 06	09 $\pm$ 02	40 $\pm$ 09
48 h	56 $\pm$ 05	09 $\pm$ 02	32 $\pm$ 02
72 h	70 $\pm$ 06	12 $\pm$ 02	52 $\pm$ 06
96 h	88 $\pm$ 03	16 $\pm$ 02	56 $\pm$ 07
240 h	102 $\pm$ 12	20 $\pm$ 06	57 $\pm$ 10

Values are given as  $\mu$ l/min, mean  $\pm$  SEM of 5-6 rats.

and between LW and clearance of antipyrine to NA it was 0.28.

The renal excretion of unmetabolized AP was higher after hepatectomy compared to the untreated control group ( $p < 0.05$ ), but not compared to the sham-operated group ( $p > 0.05$ ). In the control group,  $3.1 \pm 0.7\%$  of the dose was found as AP in urine collected 24 h after AP administration. Six hours after partial hepatectomy,  $13.5 \pm 2.7\%$  of the dose was found as AP; thereafter, this value decreased, and it was  $5.3 \pm 0.6\%$  after 240 h.

## Discussion

Antipyrine clearance (APC) was reduced as much as the liver weight (LW) immediately after the partial hepatectomy. The clearance of antipyrine (AP) to 3-hydroxy-methyl AP (3HMA), to nor AP (NA) and to 4-hydroxy AP (3OHA) was also reduced according to the LW. These findings support the concept that antipyrine is eliminated almost exclusively by the liver. The renal excretion of unmetabolized AP was about 2% in the untreated animals, and about 14% in the animals examined 6 h after partial hepatectomy, i.e. the animals with the lowest AP clearances, demonstrating that AP elimination by the renal route is of minor importance.

AP clearance represents a period of time, in the present study about 5 h after antipyrine administration, and the formation of metabolites represents 24 h. Changes most probably take place during these periods, and it has to be decided which time point should be assigned to the estimated clearance value. In the present study we chose the time of antipyrine administration because this is a well-defined time point, and to allow comparison with other studies (8).

From 12 to 72 h after partial hepatectomy, LW, which corresponds closely to total hepatic DNA (9), increased earlier than APC and earlier than the clearance to the three metabolites. This demonstrates a reduced capability to metabolize AP in the early stage of hepatic regeneration where hepatocyte division is at a maximum (7). The proliferation of hepatocytes takes place predominantly in the periportal region, which has a lower concentration of cytochrome P-450 (10). New he-

patocytes may therefore have a relatively reduced P-450 content, which in part could explain the reduced metabolic activity. Sham-operation appeared to have a stimulating effect on the clearance of AP to 3HMA, which was not present for the other two metabolic pathways. The reason for this remains obscure.

The metabolism of antipyrine to its metabolites has been the subject of many studies. However, mapping of the metabolic pathways is still incomplete. The recovery of antipyrine and antipyrine metabolites in urine varies greatly in different studies. In a study on rats, with identical strain and sex to the rats in the present study, a recovery of 38% was found, a value close to the 30% found in the present study. Other investigators, using different strains and sex, have recovered up to 75% of the dose given (11-13). The reason for the low recovery is not clear, but obvious explanations are technical difficulties with the assay and metabolism of antipyrine to unknown metabolites. Antipyrine metabolites are known to adhere to various materials, like glass, which is why silanized glass test tubes are most often used. Using the same technique as in the present study, we recovered about 70% of the antipyrine dose given to human volunteers (14). The low recovery in the present animal study is therefore presumably due to differences in the metabolic pathways giving rise to unknown metabolites rather than to systematic assay problems. More important, the conclusions in the present study rest on relative changes which are independent of such systematic errors.

In conclusion, we found that APC closely reflects the hepatic mass with the exception that during intense hepatic regeneration the hepatocytes have a reduced ability to metabolize antipyrine. The major metabolic pathways of antipyrine are unchanged, except that 3HMA formation is reduced. The closest correlation was found between liver weight and APC.

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