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Inhibition of heme synthesis in the kidney by organic mercurials

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MERCURIAL diuretics, although much reduced in clinical importance, remain of investigative interest. Their action on the renal tubule is to block sodium reabsorption by inhibiting active transport.[1] The mechanism of action is not defined, but has been presumed to be associated with the inactivation of protein-bound sulfhydryl groups.[2] Mercury (Hg) is also an inhibitor of heme synthesis, producing blockade of a series of sulfhydryl-dependent enzymes in the synthetic sequence.[3] This brief report documents the fact that mercaptomerin is able to inhibit heme synthesis in the kidney *in vivo* in the rat at a dosage level that suggests such inhibition may be associated with its use in other animals and in man.

Sprague–Dawley rats weighing between 250 and 300 g were given mercaptomerin (7 mg Hg/kg), *p*-chloromercuribenzoate (PCMB; 7 mg Hg/kg), furosemide (2 mg/kg, and chlorthiazide (10 mg/kg) or saline (control) subcutaneously. After a period of 1·5 hr, they were given 1 μc 4-^{14}C-delta aminolevulinic acid (ΔALA; Calatomic sp. act., 45 mc/m-mole) intravenously by tail vein injection and killed 90 min later. The livers and kidneys were removed and perfused with 0·1 M phosphate buffer; the kidney was dissected macroscopically into cortex and medulla, homogenized, and heme extracted as described previously.[4] The number of counts recovered as heme was expressed as a percentage of the total injected counts.

At a dosage of 7 mg Hg/kg, mercaptomerin significantly reduced heme synthesis from precursor ΔALA in both kidney cortex and medulla ($P < 0·05$), but did not inhibit heme synthesis in liver (Table 1). This inhibition of renal heme synthesis may not be related to its diuretic effects, as PCMB, which is a poor diuretic, has a similar effect on heme synthesis. Other nonmercurial diuretics such as furosemide (2 mg/kg) and chlorthiazide (10 mg/kg) had no effect on heme synthesis.

TABLE 1. PER CENT INCORPORATION OF ^{14}C-ΔALA INTO HEME PER GRAM OF TISSUE*

Tissue	Saline	Mercaptomerin (7 mg Hg/kg)	PCMB (7 mg Hg/kg)	Furosemide (2 mg/kg)	Chlorthiazide (10 mg/kg)
Kidney cortex	7·8 ± 1·9	3·9 ± 2·3	3·1 ± 0·78	8·5 ± 2·0	9·6 ± 2·2
Kidney medulla	5·0 ± 1·8	2·0 ± 1·6	1·4 ± 0·87	4·9 ± 1·2	5·4 ± 1·9
Liver	0·74 ± 0·13	0·95 ± 0·04	0·83 ± 0·12	0·82 ± 0·20	0·9 ± 0·16

* There were five rats per group.

To determine the direct effects of mercaptomerin and PCMB *in vitro*, homogenates of liver, kidney medulla and cortex were prepared in 0·25 M sucrose. The fresh homogenates were preincubated with the mercurial for 30 min before adding 0·2 μc ^{14}C-ΔALA. After 30 min of incubation with the tracer, the homogenates were extracted for heme.

It can be seen in Fig. 1 that mercaptomerin is a better inhibitor of heme synthesis *in vitro* than is PCMB. It is also seen that there is greater inhibition of heme synthesis in the liver homogenate than in renal cortex or medulla. The latter finding suggests that the observation *in vivo* of inhibition of heme synthesis in kidney, but not in liver, may be due to the higher concentration of organic mercurials achieved in the kidney than in the liver. Kessler *et al.*[5] using 12 organic mercurials, including PCMB but not mercaptomerin, observed that all were accumulated in the kidney at a higher concentration than in the liver. For PCMB, this concentration difference was 10-fold.

The inhibitory effect of mercury on heme synthesis relates to its inhibition of a series of enzymes in the synthetic pathway including ΔALA dehydrase, uroporphyrinogen I synthetase, uroporphyrinogen decarboxylase and ferrochelatase. These are sulfhydryl-dependent enzyme systems and inhibition can be overcome by the addition of reduced glutathione or cysteine.[6–9]

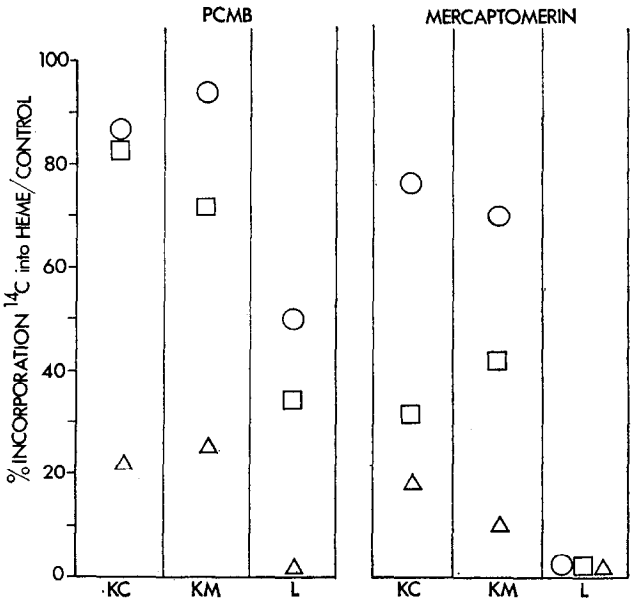

FIG. 1. Incorporation of ^{14}C-ΔALA into heme in liver (L), kidney medulla (KM) and kidney cortex (KC), expressed as a percentage of the control. To the homogenates were added: 0·01 mg ○; 0·10 mg, □; and 1·0 mg, △ (as Hg), of PCMB and mercaptomerin per gram of wet tissue, 30 min before ^{14}C-ΔALA.

The usual single diuretic dose of mercaptomerin in man is 1–2 mg Hg/kg which may be given repetitively. That this mercurial can totally inhibit heme synthesis at a concentration of 0·01 mg/g of liver *in vitro* and that *in vivo* as a single dose of 7 mg/kg it markedly reduces heme synthesis in the kidney suggest that this inhibition may be an important aspect of the pharmacology and toxicology of mercury and its compounds.

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