



## TISSUE LEVELS OF GLUTATHIONE, CYSTEINE AND COENZYME A IN EXPERIMENTAL MERCURIALISM

D. C. SHARMA, P. K. SHARMA and P. P. SINGH

Department of Biochemistry, R.N.T. Medical College, Udaipur 313 001, India

THE toxicity of mercurials has been known for centuries but the precise biochemical mechanism of their toxic action is not known with certainty. Since mercurials have an exceptionally high affinity for sulphhydryl groups<sup>1</sup> and predominant low molecular weight intracellular sulphhydryl compounds are cysteine, coenzyme A, glutathione, lipoate and thioglycolate<sup>2</sup>, it is argued that if binding of mercury with these compounds is responsible for toxicity, it should be possible to demonstrate their depleted tissue levels on mercurial administration and their protective effect against mercurial toxicity. The protective effect of cystine<sup>3</sup> and lipoic acid amide<sup>4</sup> against mercury compounds has been reported. Recently, we had found significant protection of toxicities of mercuric chloride and methylmercuric chloride by cysteine and coenzyme A<sup>5</sup>, but the tissue levels of these thiols could not be measured on account of small organ size of goldfish. The aim of the present study was to measure the tissue content of coenzyme A, glutathione and cysteine after administration of mercuric chloride in rats.

Twenty male albino rats (*Rattus norvegicus*) (weighing 200–250 g) were divided into two groups, equalising them for litter-mates and weight. The experimental group rats were intraperitoneally injected a dose (5 mg/kg) of mercuric chloride. The control rats received 0.2 ml of normal saline. As the peak values of mercury in blood were obtained at 3 h post-injection<sup>6</sup> and the renal distribution of mercury was also quite extensive at this time (unpublished observations) these rats were sacrificed under ether anesthesia after 3 h. The blood was collected directly from heart in heparinized vials. Liver and kidneys were then removed, washed in ice-cold normal saline, blotted dry, and a weighed piece was homogenized in ice-cold 5% perchloric acid in glass homogenizer. The homogenate was centrifuged at 3000 r.p.m. for 20 min at 5°C in a refrigerated centrifuge.

The homogenate was immediately assayed for its coenzyme A (CoASH), reduced glutathione (GSH) and cysteine content. CoASH was assayed by phosphotransacetylase method<sup>7</sup>. Reduced GSH was

**Figures 2 and 3.** 2. An interpretive drawing of the chromosome of figure 1. Centromere of each chromosome is marked by its number which corresponds to that of idiogram. Bar represents 10  $\mu$ ; 3. An idiogram of *C. forskohlii* pachytene chromosomes; the nucleolus (N) is not drawn to the scale. Bar represents 10  $\mu$ .

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**Table 1** Tissue levels of some mercaptans upon mercury administration

Mercaptan	Group	Liver	Kidney	Blood
GSH ( $\mu\text{mol/g}$ )	Control	$5.24 \pm 0.60$	$2.86 \pm 0.31$	$25.81 \pm 1.55^a$
	Hg-treated	$3.84 \pm 0.39$ ( $P < 0.001$ )	$1.96 \pm 0.34$ ( $P < 0.001$ )	$18.26 \pm 2.38^a$ ( $P < 0.001$ )
Cysteine ( $\text{nmol/g}$ )	Control	$118.4 \pm 20.2$	$93.8 \pm 19.7$	$35.2 \pm 9.5^b$
	Hg-treated	$85.9 \pm 22.5$ ( $P < 0.005$ )	$72.5 \pm 16.6$ ( $P < 0.025$ )	$37.4 \pm 10.2^b$ ( $P > 0.60$ )
CoASH ( $\text{nmol/g}$ )	Control	$179.3 \pm 14.4$	$130.0 \pm 15.5$	$38.6 \pm 9.9^b$
	Hg-treated	$110.6 \pm 17.5$ ( $P < 0.001$ )	$99.9 \pm 22.9$ ( $P < 0.005$ )	$36.8 \pm 9.5^b$ ( $P > 0.70$ )

<sup>a</sup> $\mu\text{mol/g}$  Hb; <sup>b</sup> $\text{nmol/ml}$  blood; all values are mean  $\pm$  S.D. of 10 observations on different rats.

estimated by the method of Beutler<sup>8</sup> using 5,5'-dithiobis-(2-nitrobenzoic acid). Cysteine was determined by the method of Gaitonde<sup>9</sup>. CoASH and GSH in blood were similarly determined by the above methods. Cysteine was estimated in blood after deproteinization with 5% perchloric acid. Haemoglobin was measured as cyanmethaemoglobin.

The tissue levels of CoASH, GSH and cysteine after administration of a toxic dose of mercuric chloride are presented in table 1. The level of GSH was significantly decreased in liver, kidney and blood while cysteine and CoASH were significantly lowered only in kidney and liver. GSH in blood was expressed as  $\mu\text{mol/g}$  haemoglobin as it is found only in red blood cells

The decrease in GSH, cysteine and CoASH may be due to their direct binding with mercury which explains the highly significant protection afforded by cysteine and CoASH against mercury toxicity<sup>5</sup>. The present data also support our hypothesis that mercury toxicity is partly due to its combination with CoASH which interferes in its functions<sup>5</sup>.

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#### **ORTHIS AFF. RUSTICA FROM THE DEVONIAN QUARTZ-ARENITE OF THE MUTH FORMATION, KHIMOKUL LA (KINNAUR), HIMACHAL HIMALAYA**

U. K. BASSI

*Geological Survey of India, SCO 98-100, Sector-17C, Chandigarh 160 017, India.*

This note records the discovery of a well-preserved *in situ* fossil referable to *Orthis* aff. *rustica* from the quartz-arenite of the Muth Formation of the Kinnaur Basin<sup>1</sup> (figure 1a). The fossil was recorded from the C horizon (figure 1b) exposed 1 km west of Khimokul La along the foot track.

The Muth Formation, extending from Kashmir to Kumaun, comprises a sequence of snow-white to white mottled quartz-arenite with local dolomite interbeds in its basal and upper parts. The quartz-arenite in the Spiti, except for an orthoceratid and an ill-preserved coral in a rolled boulder<sup>2</sup>, has not yielded any fossil so far. The other fossils reported from the Muth Formation are from the calcareous bands<sup>3, 5</sup>.