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Binding of bismuth in the kidneys of the rat: The role of metallothionein-like proteins*

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In recent years much attention has been paid to the role of metallothionein in binding of heavy metals in the liver and kidneys, especially of cadmium, mercury and zinc [1–3]. All these metals enhance the organ-levels of metallothionein, presumably through induced *de novo* synthesis of this protein [4–6].

More recently it has been found that the level of metallothionein-like proteins in the kidneys may also be elevated by repeated administration of bismuth and that the metal shows ability to bind with these proteins *in vivo* [7].

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The aim of the present report is to confirm the above finding in experiments with ^{206}Bi and to evaluate its role in the metabolism of bismuth.

The experiments were carried out on white female rats of the Wistar strain, body weight 200–220 g, fed standard LSM diet. Three groups of animals were administered subcutaneously with $BiCl_3$-labelled ^{206}Bi, in doses of 2.5 mg Bi/kg body wt (single dose) and five successive doses of 0.5 and 2.5 mg Bi/kg, respectively, every other day. Rats were sacrificed 24 hr following the last dose. Skin was discarded and bismuth was determined in the kidneys, liver and in the remaining carcass. The determination was based on gamma-counting of ^{206}Bi in a USB-scintillation counter. Homogenates of kidneys (20% w/v) were prepared in 0.25 M sucrose/0.01 M Tris–HCl buffer, pH 7.4, and

Table 1. The distribution of ^{206}Bi in the body of rats following subcutaneous administration of $BiCl_3$

Organ	Dosage of $BiCl_3$ (a) 2.5 mg Bi/kg 1 × (μg Bi/ organ)	(% of total)	(b) 2.5 mg Bi/kg, 5 × (μg Bi per organ)	(% of total)	(c) 0.5 mg Bi/kg, 5 × (μg Bi per organ)	(% of total)
Kidneys	10.4 ± 3.7	8.3	122.6 ± 33.3	17.9	41.8 ± 4.7	29.6
Liver	1.6 ± 1.0	1.3	7.5 ± 1.64	1.1	1.6 ± 0.2	1.1
Remaining carcass	113.6 ± 37.0	90.4	556.5 ± 198.2	81.0	98.9 ± 13.6	69.3

Calculation based on whole body retention (except of skin). Means of 5 rats (groups a and c) and 7 rats (group b).

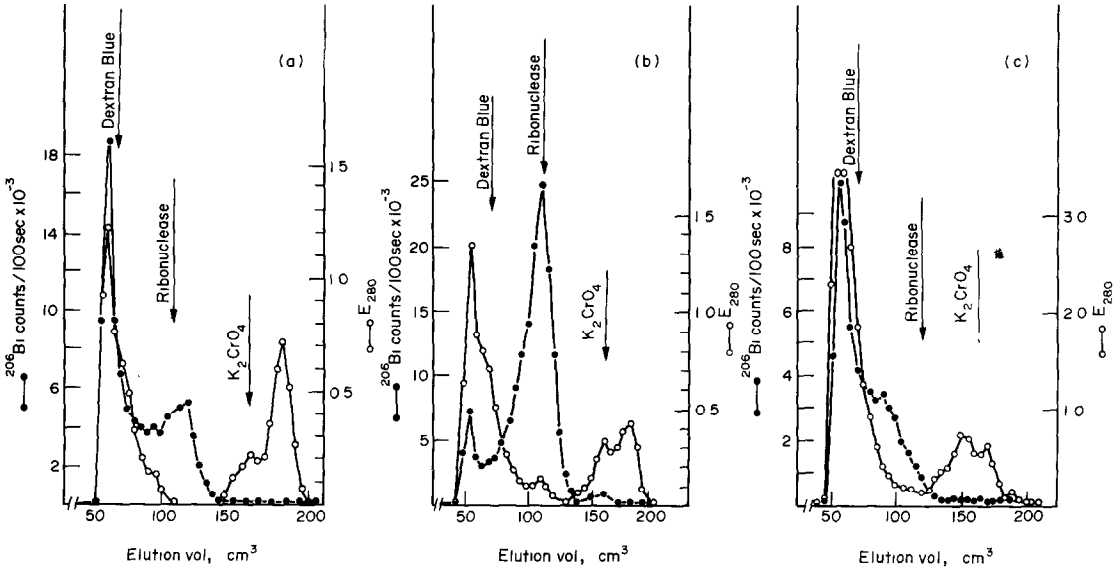

Fig. 1. The chromatography of bismuth contained in the kidneys: (a) 105,000 *g* supernatant from kidneys of rats, administered a single dose of 2.5 mg Bi/kg. (b) 105,000 *g* supernatant from kidneys of rats administered bismuth repeatedly 5 × 2.5 mg Bi/kg. (c) 105,000 *g* sediment, solubilized from kidneys of rats administered bismuth repeatedly, 5 × 2.5 mg Bi/kg. Sephadex G-75, formate-buffer pH 8.0, fractions 5 cm^3 each. Columns calibrated with: Dextran blue (void volume); ribonuclease, mol. wt. 13,700 (elution volume close to metallothionein, mol. wt. 10,000); K_2CrO_4 (marker of ionic and peptide-bound metal).

were filtered through a gauze. The filtrate was centrifuged at 105,000 *g* [8]. The sediment was solubilized by re-homogenization in water. Both the soluble fraction and the solubilized sediment were subjected to molecular filtration on columns filled with Sephadex gel G-75.

Table 1 shows that the fraction of ^{206}Bi retained by the kidneys increased from 8.3 per cent after a single dose to 17.9 per cent in repeated injections of ^{206}Bi. Following repeated injections of the low dose of Bi (0.5 mg Bi/kg) the kidneys retained 30 per cent of the total body burden. This experiment shows that the relative role of kidneys in storing bismuth increases after repeated administration.

Fig. 1. (a and b) shows that the chromatographic pattern of bismuth contained in the soluble fraction differs depending on the type of exposure. Following a single dose (a), binding by high-mol. wt proteins prevails and only a limited amount of Bi is bound by metallothionein-like proteins. However, practically the whole increment of bismuth accumulated in the kidneys in the course of repeated administration (b) is contained in fraction of metallothionein-like proteins.

The latter proteins are contained in the soluble fraction of the kidneys (b). Bismuth contained in the organelle is bound mainly with high mol. wt proteins (c). In this respect bismuth resembles mercury [9].

From the above observations, it follows that the elevated capacity of kidneys for bismuth, observed if the metal is administered repeatedly, is connected with metallothionein-like proteins the level of which is enhanced by this element [7]. Whether these proteins are identical with those playing role in the storage of mercury in the kidneys requires further studies.

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REFERENCES

1. G. F. Nordberg, M. Piscator and E. Lind, *Acta pharmac. tox.* **29,** 456 (1971).
2. M. Webb, *Biochem. Pharmac.* **21,** 2751 (1972).
3. J. K. Piotrowski, B. Trojanowska, J. M. Wiśniewska-Knypl and W. Bolanowska, *Toxic. appl. Pharmac.* **27,** 11 (1974).
4. M. Piscator, *Nord. Hyg. Tidskr.* **45,** 76 (1964).
5. J. K. Piotrowski, B. Trojanowska and A. Sapota, *Arch Toxic.* **32,** 351 (1974).
6. B. Trojanowska and J. K. Piotrowski, *Bromat. Chem. Toksykol.* **7,** 137 (1974).
7. J. K. Piotrowski and J. A. Szymańska, *J. Toxic. environ. Hlth.*, in press.
8. W. C. Schneider, *J. biol. Chem.* **176,** 259 (1948).
9. E. M. Komsta-Szumska, J. Chmielnicka and J. K. Piotrowski, *Biochem. Pharmac.* **25,** 2539 (1976).