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Binding of gold in the kidney of the rat*

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The protein binding of several metals in animal tissues has recently been the subject of interest in numerous reports. Special emphasis was put on the role of metallothionein-like proteins in binding of Cd, Zn, Cu, Hg and Bi (for review see [1]). Gold has not been investigated in this respect. The present report gives preliminary data on the binding of gold in the kidney of the rat with reference to protein classes of various molecular weights.

METHODS

Female rats of the Wistar strain, body weight 220–260 g were used in the experiments. Animals were fed standard LSM† diet. Solutions of soluble gold were prepared by dissolving chloroauric acid (FOCh, Gliwice), neutralized to pH about 4, to reach the concentrations 2 and 4 mg Au/ml. The solutions were administered i.v. in a single dose of 880 μg per rat and in repeated doses: 3 × 880 μg Au per rat every second day, 5 × and 7 × 440 μg Au per rat every second day. Rats were sacrificed 24 hr following last injection. Liver and kidneys were removed, the remaining carcass (except the skin) was homogenized with water to reach a homogenous suspension from which samples were taken for gold determination.

Kidney homogenates (20%), were prepared in a solution containing 0.25 M sucrose and 0.01 M Tris–HCl buffer, pH 7.4. Homogenates were centrifuged at 12.000 *g* for 1 hr. The supernatants were subjected to column chromatography on Sephadex G-75 gels. Elution was performed using formate buffer, pH 8.0.

For the determination of gold, samples were dry ashed at temperature of 650–700°. The residue was dissolved in aqua regia, evaporated almost to dryness and immediately dissolved in 0.8 N HCl. In the final solution gold was determined spectrophotometrically with rhodamine B (The British Drug Houses Ltd) [2, 3]. The coloured complex of gold with rhodamine B in diisopropylether was measured at 553 nm on the VSU-2G spectrophotometer. The limit of detection was 2 μg Au in a sample, precision 7.5%. The recovery of gold following dry ashing was about 85 per cent.

Proteins were determined nephelometrically with tannic acid (Anal. Lab., Katowice) using bovine albumine (Serva) as a standard [4]. In the eluates from column chromatography absorbance at 250 nm was measured on the VSU-2P spectrophotometer.

RESULTS

Following repeated exposure rats accumulated gold to an extent not exceeding 200 per cent of the individual dose (Table 1). Taking the wholebody retention of gold as 100 per cent it could be shown that the share of the kidney in binding gold had a rising tendency, from about 13 per cent following single administration of 880 μg Au to about 35 per cent following the longest repeated exposure (Fig. 1). Similarly, the gold content in the liver became stabilized at the level of about 30 per cent of the wholebody retention. The content of gold in the remaining carcass dropped from about 70 per cent following single dose of 880 μg Au per rat to about 30 per cent following the longest repeated exposure to doses of 440 μg Au per rat.

Table 1. The whole body retention of gold and its concentration in the kidney and liver, depending on magnitude and number of doses

Doses	Whole body retention μg Au	Concentration, μg Au/g of tissue Kidney	Liver
1 × 880 μg Au/rat	604 ± 94	52.3 ± 15.5	15.2 ± 4.9
3 × 880 μg Au/rat	1153 ± 182	126 ± 9	26.3 ± 6.8
5 × 440 μg Au/rat	741 ± 106	103 ± 28	26.4 ± 4.6
7 × 440 μg Au/rat	782 ± 138	158 ± 15	34.5 ± 11.0

5–7 animals in a group; means ± standard deviation.

In the kidney, the 12,000 *g* supernatant contained 35 per cent of gold in the case of single dose of 880 μg Au and about 50 per cent after 5–7 doses of 440 μg Au. The respective concentrations expressed per mg of protein were 0.5 and 1.1 μg Au/mg respectively. Figure 2 shows a typical chromatography of the kidney supernatant following repeated exposure to gold. In this case ½ of gold was bound by the fraction of low molecular weight proteins. The latter proteins bound only 10 per cent of gold in experiment with single exposure. The above changes

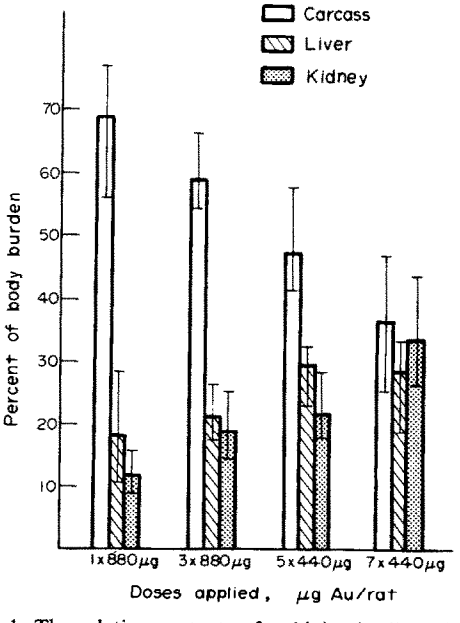

Fig. 1. The relative contents of gold in the liver, kidney and in the remaining carcass depending on the magnitude and number of gold doses.

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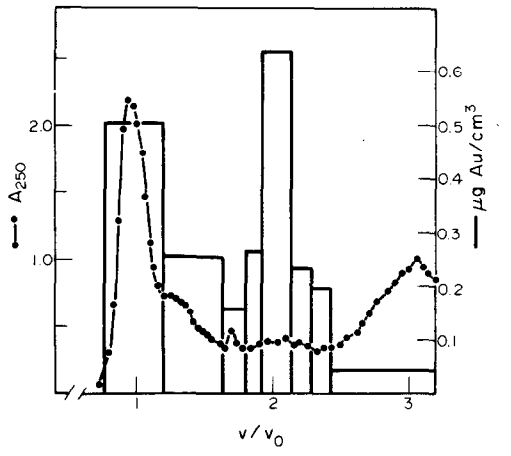

Fig. 2. The chromatography of the 12,000 g supernatant of the kidneys. Sephadex G-75, column 65 × 2.5 cm, formate buffer pH 8.0; fractions 3 ml each. Rat given 7 i.v. doses, 440 μg Au each. Markers of the molec.wt: dextran blue (molec.wt = 2 mln, $V_t:V_0 = 1$); ribonuclease (molec.wt = 12,700, $V_t:V_0 = 2.0$), K_2CrO_4(molec.wt = 194, $V_t:V_0 = 2.8$).

occurred at the cost of the proteins of the highest molec.wt which bound about 60 per cent of gold following single exposure and only about 35 per cent after repeated exposure to gold. About $\frac{1}{4}$ of gold was contained in the fraction of proteins of intermediate molec.wt ($V_t:V_0 \simeq 1.5$), independently on the kind of exposure. Table 2 shows the concentration of gold expressed per mg of protein in joint eluates from column chromatography corresponding to various elution volumes. With prolonged exposure an increase of concentration was seen in the fraction of low molecular weight proteins, the concentrations in the remaining fractions being basically constant. The data of Table 2 refer to mean values in joint fractions. Within the fraction of the lowest molec.wt the peak concentrations were found up to 26 μg Au/mg of protein.

DISCUSSION

From the existing data it follows that the critical organ for gold may be either liver or kidney, depending on the preparation applied [5, 6]. Liver seems to concentrate gold especially efficiently if colloidal gold is administered, whereas soluble gold compounds are localized mainly in the kidney. High levels of gold in the kidney were still found after more than 10 days following injection [6]. In the present report we applied soluble gold and the share of both organs, liver and kidneys, in the storage of gold was approximately equal, however the concentrations in the kidney were several times higher. The relative share of the kidneys rose with the duration of exposure.

The mechanism owing to which the accumulation of gold in the kidney occurs so efficiently has been obscure. Some light is shed on this problem by the study of binding by various protein classes. Table 2 shows that in prolonged exposure the high molec.wt proteins soon reach a plateau. Consequently the increase of gold content in this organ

Table 2. The concentration of gold (μg Au per mg proteins) in the protein fractions of different molecular weights (V_t/V_0) depending on magnitude and number of doses (supernatant of kidneys)

V_t/V_0 \ Doses	1 × 880 μg Au/rat	5 × 440 μg Au/rat	7 × 440 μg Au/rat
1.0 (0.8 – 1.3)	0.41	0.64	0.61
1.5 (1.3 – 1.8)	0.37	0.64	0.57
2.0 (1.8 – 2.2)	1.90	9.20	15.70

is due to the rise of gold chelated by a fraction of proteins of the molec.wt about 10,000. This fraction of proteins binds in prolonged exposure about 20 per cent of the total gold contained in this organ. As compared with mercury or bismuth the role of this protein fraction is relatively less pronounced. Nevertheless, some analogy is obvious: as it is known, mercury and bismuth are efficiently bound in the kidneys due to a fraction of low molec.wt, inducible proteins, classified as metallothionein-like proteins [7–9]. Whether the gold binding proteins contained in the soluble fraction of rat kidneys are identical with those binding Hg or Bi remains to be elucidated. Also, it is far from being clear whether the increasing capacity for Au of this protein fraction is due to induction of the protein synthesis, as it is the case with mercury [7] or bismuth [9].

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