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Uptake of Mercury and Mercury–Amino Acid Complexes by Rat Renal Cortex Slices[1] (39024)

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Experiments on the renal deposition of mercury in the rat led to the hypothesis that mercury–amino acid complexes might be involved in the transport of mercury into kidney cells via the same mechanism as amino acids themselves are transported (1).

It has been shown that copper is actively accumulated by kidney slices in the form of amino acid complexes, largely of histidine and threonine (2). Since kidney accumulates mercury to a greater extent than any other tissue *in vivo* (3) and amino acid transport in kidney is enormously active (4), it would not be unreasonable to expect at least a component of the mercury uptake in kidney to be amino acid-mediated, as in the case of copper intake.

In this study, rat renal cortex slices were used to assess effects of metabolism or complexation by amino acids on mercury uptake. Attention was focused on the complex with cysteine in view of the large stability constants of mercury–cysteine complexes (5) and the observation that parenteral administration of mercuric cysteine to rats resulted in higher levels of mercury in kidney than when equimolar mercuric chloride was given (3).

Methods. Animals. Male Holtzman rats were used. Throughout the range of experiments body weights were 175–380 g; in single experiments the range of body weights was no more than 20 g. Animals were housed in an air-conditioned room with an automatic 12-hr light cycle and fed Purina Rat Chow (Ralston Purina Co., St. Louis, Mo.) and water *ad libitum*.

Chemicals. Analytical reagent grade chemicals were used in the preparation of buffers and other solutions unless specified otherwise. Solutions were made in deionized distilled water.

Mercuric nitrate [^{203}Hg] in 10 m*M* HNO_3 (New England Nuclear, Boston, Mass.) was diluted with 10 m*M* HNO_3 to an activity of 1000 μCi/ml. This stock solution was further diluted with unlabeled mercuric chloride in phosphate/HCl buffer (pH 7.4) to give a final specific activity of 1.0 μCi/50 μM/ml. In all experiments, 100 μl of this solution was added to make a final volume of 5.0 ml. Each flask then contained a total of 1.0 μg of mercury and 0.10 μCi of activity. The final mercury concentration was 1.0 μM.

Mercury complexes with amino acids or glutathione were prepared by diluting 100 μM mercuric solutions (predominantly mercuric chloride) with equal volumes of 100 or 200 μM amino acid or glutathione, thus yielding 1:1 or 1:2 molar complexes. Cysteine and glutathione solutions were prepared within 15 min of use. L-cysteine hydrochloride monohydrate and glutathione were obtained from Calbiochem, Los Angeles, Calif. L-histidine, L-serine, and L-lysine were obtained from Eastman Kodak Co., Rochester, N.Y. 2,4-Dinitrophenol (DNP) was synthesized in the Department of Nutrition, Harvard School of Public Health. Solutions added to incubation flasks were adjusted to pH 7.4 with HCl or NaOH shortly before use.

Tissue slices. Rat renal cortex slices were prepared as described by Rosenberg *et al.* (6). The average thickness obtained with the Stadie–Riggs tissue slicer was 0.50 mm. Each kidney yielded four cortical slices. Prior to incubation, slices were taken from ice-cold

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Krebs–Ringer buffer, gently blotted on filter paper moistened with cold buffer, and weighed by suspending three slices at a time from a wire placed inside a Mettler analytical balance. The average wet-weight of tissue per flask (three slices) was 100–120 mg. After weighing, slices were placed in 25-ml conical flasks containing Krebs–Ringer buffer and kept on ice until the start of the incubation interval.

Incubations. Slices were incubated at pH 7.4 either in Krebs–Ringer bicarbonate (KRB) or Krebs–Ringer phosphate (KRP) buffer containing 10 m*M* glucose. Incubations were carried out in a Dubnoff incubator agitating at about 85 oscillations/min. Flasks were covered by an air-tight hood and gassed with 95% O_2/5% CO_2 when KRB was used, or 100% O_2 when KRP was used. In anaerobic experiments 95% N_2/5% CO_2 (for KRB) was used. Flow rate was adjusted to 4 ft^3/hr, sufficient to produce bubbling from under the hood. Temperature was maintained at 37 ± 2° or at 25 ± 1° in reduced temperature experiments. Before addition of test substances, slices were preincubated for 15 min. Times of incubation varied from 15 to 120 min in the time-course experiment, to a standard 60 min incubation for single time-point experiments. At the end of the incubation interval, flasks were removed from the incubator to an ice bath and the slices quickly removed with forceps, dipped briefly into fresh cold buffer, blotted with buffer-moistened filter paper and placed into 10 × 70 mm glass test tubes.

Mercury assay. The glass tubes containing the tissue slices were placed into plastic gamma-well counting tubes with screw-on caps. Counting was done in a dual-channel Nuclear Chicago gamma scintillation spectrometer against a standard ^{203}Hg solution in order to compensate automatically for radioactive decay. Standards and blanks were counted also to correct for background radiation and to provide a basis for calculating mercury concentrations and recoveries. Mercury concentration in tissue was expressed as μg Hg/g (wet weight). Aliquots of incubation medium were counted and mercury content expressed as μg Hg/ml. Results are presented as the ratio of slice concentration to medium concentration, S/M, or as a percentage of the S/M ratio of mercuric chloride under standard conditions.

Partition coefficients. Radio-labeled mercuric chloride or mercuric cysteine complexes (100 μl of 50 μ*M*) were added to a mixture of 2.4 ml sodium phosphate buffer (pH 8.0) and 2.5 ml *n*-heptane in stoppered test tubes. The solutions were shaken vigorously several times and allowed to equilibrate for 20 min. Aliquots (1.0 ml) were taken from each phase for gamma scintillation counting and ratios of counts in heptane to counts in buffer were computed.

Statistics. Data are expressed as means ± SE based on the number of incubation flasks. Significance of differences between means was determined using Student's *t* test, with $P < 0.05$ being regarded as significant.

Results. Time-course of mercury uptake. Mercuric chloride added to the medium at zero time was accumulated by the slices as shown in Fig. 1. The uptake approached a plateau between 60 and 120 min of incubation. The leveling-off of uptake was not due to depletion of extracellular mercury since the concentration of mercury in the medium remained constant throughout the incubation interval. Recovery of radioactivity was 96.7 ± 1.1%.

A standard incubation interval of 60 min was selected for studies of the effects of amino acids and metabolism on mercury uptake.

Influence of amino acids or glutathione. Mercury complexes of amino acids or glutathione were formed by premixing mercuric chloride with two equivalents of complexing agent during the preincubation interval and adding the complex to the incubation flask at zero time. The final concentration of mercuric chloride or mercury complex in the medium was always 1.0 μ*M*. Some of the media contained additional amino acids which were added at the start of the preincubation interval. The uptake of mercury under these different conditions is shown in Table I. The uptake of mercuric chloride without added amino acids or glutathione was assigned a value of 100% to serve as a basis of comparison.

The histidine complex was not taken up to

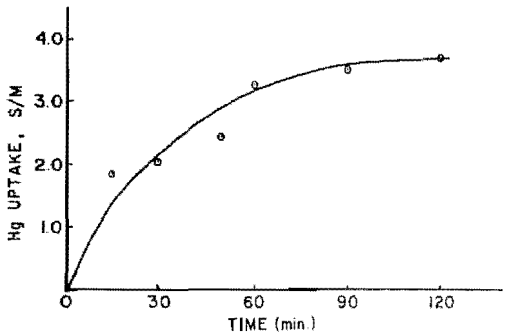

FIG. 1. Time-course of mercury uptake into renal cortex slices. Slices were preincubated in KRB buffer for 15 min before addition of uncomplexed mercuric chloride at zero time to give a final mercury concentration in the medium of 1.0 μM. Incubations at 37° under 95% O_2/5% CO_2 were carried out for the times shown. Data are expressed as slice:medium ratios of mercury concentration (S/M = [μg Hg/g] slice/[μg Hg/ml] medium). Each point is the mean of two flasks containing three slices each.

a significantly higher degree than mercury alone. Uptake of the cysteine and glutathione complexes was enhanced over 50% above that of mercury alone. Addition of a tenfold molar excess of histidine over the mercury–cysteine complex abolished enhancement of the uptake of the complex. Increasing the ratio of histidine to mercuric cysteine to 100 or 1000 resulted in increasing the mercury uptake, but the level of uptake of the pure cysteine complex was not attained. Addition of serine in tenfold molar excess over mercuric cysteine had no effect on uptake. Lysine, however, significantly reduced uptake of mercuric cysteine. The most pronounced effects occurred when the cysteine concentration was increased. Molar ratios of cysteine to mercury–cysteine complex of 10, 100, and 1000 gave mercury uptake levels of 275, 350, and 370% of mercury alone, respectively.

Partition coefficients. Partition coefficients between equal volumes of *n*-heptane and phosphate buffer (pH 8.0) were measured for mercuric chloride and mercury–cysteine complexes produced by premixing mercuric chloride with either one or two equivalents of cysteine. The mean ± SE (n = 3) heptane:buffer partition coefficients were: 0.1330 ± 0.0240 for $HgCl_2$, 0.0190 ± 0.0010 for the 1:1 Hg:Cysteine complex and 0.0065 ± 0.0005 for the 1:2 complex.

TABLE I. INFLUENCE OF AMINO ACIDS OR GLUTATHIONE ON MERCURY UPTAKE BY KIDNEY CORTEX SLICES *IN VITRO*.

Additions to slice incubation flasks[a]	Hg^{2+} uptake[b] (Percentage of S/M of Hg^{2+} alone)
Hg^{++} only	100 ± 8
Hg $(His)_2$	110 ± 15
Hg $(GSH)_2$	155 ± 8
Hg $(Cys)_2$	158 ± 5
Hg $(Cys)_2$ + 10 His	100 ± 10
Hg $(Cys)_2$ + 100 His	120 ± 3
Hg $(Cys)_2$ + 1000 His	128 ± 10
Hg $(Cys)_2$ + 10 Ser	163 ± 2
Hg $(Cys)_2$ + 10 Lys	135 ± 10
Hg $(Cys)_2$ + 10 Cys	275 ± 10
Hg $(Cys)_2$ + 100 Cys	350 ± 15
Hg $(Cys)_2$ + 1000 Cys	370 ± 15

[a] Mercuric chloride (Hg^{2+}) or mercury compound with histidine (His), glutathione (GSH), or cysteine (Cys) was added to preincubated slices in KRB buffer at zero time. The final total concentration of mercury was 1.0 μM in each case. Incubations were continued for 60 min at 37° under 95% O_2/5% CO_2. Some flasks contained histidine, serine (Ser), lysine (Lys), or cysteine which was added at the beginning of the 15 min preincubation interval in the molar excess over mercuric cysteine indicated.

[b] Data are presented in terms of the percentage of S/M ratios for mercuric chloride alone. Figures are means ± SE's from three flasks.

This measurement verified that cysteine complexes of mercury are much less lipid soluble than mercuric chloride itself. Increased uptake of complexed mercury in the kidney slice is thus not due to increased lipid solubility of the complexes.

Metabolic influences. Figure 2 summarizes results of experiments carried out under standard conditions of 37° and 100% O_2 compared with incubations under 100% N_2, reduced temperature, or in media containing 2,4-dinitrophenol (DNP). In each case, a comparison was made between the uptake of mercury alone and the uptake of the mercury–cysteine complex. In the control situation, mercuric cysteine accumulates to a level over 150% that of mercury alone. Under

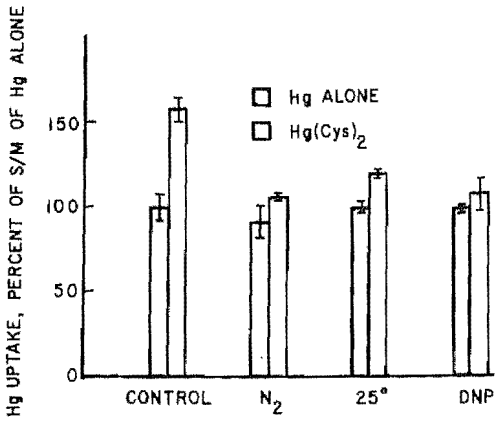

FIG. 2. Metabolic influences on mercury uptake. Control flasks were incubated for 60 min at 37° in KRB buffer under 95% O_2/5% CO_2 or in KRP under 100% O_2. There was no significant difference in relative mercury uptake under these two control conditions. Uptake of mercury due either to added mercuric chloride or mercuric cysteine is expressed as percent of S/M ratio of control mercuric chloride uptake. Anaerobic conditions (N_2) were done in KRB at 37° under 95% N_2/5% CO_2. Reduced-temperature incubation (25°) was done in KRP under 100% O_2. DNP (100 μM) was added at the start of the 15 min preincubation period with mercury added at zero time and incubation continued at 37° in KRB under 95% O_2/5% CO_2. Each bar represents the mean ± SE of three flasks.

anaerobic conditions, uptake of mercury alone was not significantly altered, while the uptake of mercuric cysteine was depressed to the level of mercury alone. Reduction of the temperature to 25° had no effect on the uptake of mercury alone, but mercuric cysteine accumulation was significantly reduced. Addition of DNP failed to affect uptake of mercury alone, but it reduced mercuric cysteine uptake to the level of mercury alone.

Discussion. Mercuric cysteine is clearly accumulated by kidney slices to a greater extent than mercuric chloride. Since measured recovery of total added mercury from media and slices was high (96.7%) for mercuric chloride alone and the amount of mercury available in the medium was not limiting in these experiments, the effect of cysteine was not simply to sequester mercury from binding sites on the glass walls of the flask.

Complexation of mercury with polar amino acids would be expected to produce molecular species with low oil/water partition coefficients. This was verified for mercuric cysteine, thus excluding the possibility that the complexes may have become more permeable to membranes by virtue of increased lipid solubility.

The fact that retardation of metabolism reduces the uptake of mercuric cysteine, but not mercuric chloride suggests that mercury compounds enter kidney cells by at least two processes. One component of the uptake is presumably diffusion; another component is dependent upon metabolic energy. The metabolic component seems to act only on the amino acid complex of mercury and not mercury itself.

Clarkson and Magos (3) observed reduced renal uptake of mercury *in vivo* by administering DNP to rats. A greater effect was seen with mercuric cysteine than with mercuric chloride, but a significant reduction of accumulation was seen with both compounds. Cysteine would be available to form complexes with administered mercury compounds *in vivo*, thus partially or totally masking differences that might be seen between mercuric chloride and mercuric cysteine uptake *in vitro*.

Histidine and lysine are known to compete with cysteine reabsorption in kidney (7, 8). These amino acids significantly reduced uptake of mercury when added to the medium in tenfold excess over mercuric cysteine. Excess cysteine markedly increased mercury uptake. From equilibrium calculations of the major species present in Krebs-Ringer buffer containing added mercuric chloride (5), virtually all of the mercury would be expected to be in the form of Hg $(cysteine)_2$, even at the lowest cysteine concentration. If the complex were being accumulated by amino acid transport mechanisms for cysteine, the rate and level of uptake might be expected to increase with increasing cysteine concentration, just as occurs in renal amino acid transport when the external concentration of the same amino acid is increased (6).

Richardson and Murphy (1) have observed suppression of renal deposition of

mercury following renal glutathione depletion. It was suggested that the effect could be due to reduced uptake of mercury–amino acid complexes which share the amino acid transport system mediated by the γ-glutamyl cycle (4). The present work supports the hypothesis that amino acids and mercury–amino acid complexes may be accumulated by kidney cells by a common mechanism. Further work will be required to substantiate or refute this hypothesis and to clarify the role that may be played by intracellular glutathione.

Summary. This study was undertaken in order to assess the effects of metabolism and complexations with amino acids on the renal uptake of mercury using rat renal cortex slices as the experimental system.

Mercury levels attained in the slices after 60 min of incubation were 50% higher with mercuric cysteine than with mercuric chloride. This enhancement of uptake with mercuric cysteine was reduced in the presence of a tenfold molar excess of histidine or lysine, but not by serine. Excess cysteine markedly increased mercury uptake. Incubation at 25° significantly reduced uptake of mercuric cysteine, but not mercuric chloride. Anaerobic conditions and incubation in the presence of DNP each reduced mercuric cysteine uptake to the control level of mercuric chloride without affecting uptake of mercuric chloride.

The differential aspects of metabolism on the uptake of mercuric cysteine and mercuric chloride and the competitive effects obtained with amino acids known to compete with cysteine in renal reabsorption support the hypothesis that a portion of the renal uptake of mercury operates through amino acid transport mechanisms acting on mercury–amino acid complexes.

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