Brit. J. industr. Med., 1972, **29**, 74-80

Biliary excretion of ^{203}Hg, ^{64}Cu, ^{52}Mn, and ^{210}Pb in the rat

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Cikrt, M. (1972). *Brit. J. industr. Med.*, **29**, 74-80. **Biliary excretion of ^{203}Hg, ^{64}Cu, ^{52}Mn, and ^{210}Pb in the rat.** The biliary excretion of ^{52}Mn, ^{64}Cu, ^{203}Hg, and ^{210}Pb after intravenous administration of $^{52}MnCl_2$, $^{64}CuCl_2$, $^{203}HgCl_2$, and $^{210}Pb(NO_3)_2$ in non-toxic doses was studied in rats. Cumulative biliary excretion reached by 24 hours after administration in the case of ^{64}Cu 31·06%, of ^{52}Mn 26·7%, of ^{203}Hg 3·8%, and of ^{210}Pb 6·7% of the administered dose. The excretion curve for ^{203}Hg differed significantly from those of the other three metals. The maximum rate of excretion was reached at different periods after administration for each metal. The excretion of the metals via the wall of the gastrointestinal tract during 24 hours after administration was also studied. The excretion into faeces occurred mainly via the bile; to a lesser extent there was excretion through the wall, probably chiefly of the upper segments of the digestive tract.

The gastrointestinal tract has repeatedly been shown to be one of the main pathways of excretion of heavy metals. Little is known of the mechanisms responsible for the transport of metal into the intestinal lumen. In principle, there are two origins of the metal detected in faeces after parenteral administration—the bile or pancreatic fluid, and the intestinal wall. In most investigations, either one route or the other has been studied, and often only on one metal. This is a reason for the discrepancies in estimates of the relative importance of the two routes (for example, for lead, Witschi (1964) and Castellino, Lamanna and Grieco (1965)). For mercury, most attention has so far been paid to excretion in urine because of the marked tendency of mercury to be excreted by this route (Friberg, 1956). For manganese and copper, it is known that both these metals accumulate in the liver and that they are mainly excreted via bile (for manganese—Cotzias (1962); Papavasiliou, Miller, and Cotzias (1966); Bertinchamps, Miller, and Cotzias (1966); for copper—Owen (1964); Gaballah, Abood, Caleel, and Kapsalis (1965); Farrer and Mistilis (1968); Marceau, Aspin, and Sass-Kortsak (1970)). The transport of manganese and copper via the intestinal wall into the intestinal lumen has not been studied. The excretion of some heavy metals into faeces is complicated due to the enterohepatic circulation which takes place (for manganese—Bertinchamps *et al.* (1966); for copper—Mistilis and Farrer (1968)).

We have studied the excretion of ^{203}Hg, ^{64}Cu, ^{52}Mn, and ^{210}Pb into the bile during the 24 hours after intravenous administration. The total amount of metal excreted into the lumen of the gastrointestinal tract during the same period was also determined.

Material and methods

Female Wistar rats, mean weight 200 g (180-220 g), fed on a pellet diet were used in the experiments. Rats were starved for 24 hours before study but had free access to water.

Under light ether anaesthesia the bile duct was cannulated with PE-10 tubing (i.d. 0·28 mm, o.d. 0·61 mm) at a point about 5 or 6 mm below the hilus of the liver and just above where pancreatic tissue usually no longer surrounds it (Grossman, 1958). The cannula was brought through the skin at the back of the animal. Secretion pressure in the bile duct is sufficient to overcome this

small difference in height (Bohdal and Novák, 1958). A glass test tube, connected with the cannula by a rubber stopper, was fixed on the animal's back by means of a 'jacket'. This allowed the animal completely free movement in the cage. The animal drank and accepted food quite soon after the introduction of the cannula. The test tube on the back of the animal was easily changed every hour. The bile in the test tube was weighed, and in the same test tubes radioactivity was measured on a scintillation well-type gamma counter. No further handling of samples was therefore required and possible losses were avoided.

One hour after cannulation of the bile duct, when the bile flow had become stabilized, a radioisotope of the metal under study was injected into the tail vein of the rat. The volume of the injected solution was 1 ml.

Substances administered

The following substances were administered:

Manganese, ^{52}Mn (Institute of Nuclear Research, Prague, Czechoslovakia) in the form of $^{52}MnCl_2$ (30 μg Mn^{2+} per rat)

Mercury, ^{203}Hg (Amersham, England) in the form of $^{203}HgCl_2$ (120 μg Hg^{2+} per rat)

Lead, ^{210}Pb (Institute of Nuclear Research, Prague, Czechoslovakia) in the form of ^{210}Pb nitrate (125 μg Pb^{2+} per rat)

Copper, ^{64}Cu (Institut für Kernforschung, Dresden) in the form of $^{64}CuCl_2$ (40 μg Cu^{2+} per rat).

The radioactivity of the administered isotopes amounted to 8 to 10 μCi per rat.

For separation of urine and faeces we used metabolism cages with perforated floors, as described by Östlund (1969). Faeces fell through the mesh bottom against an obliquely placed filter paper, which transmitted the faeces to a glass container for collection. The filter paper was previously dusted with crystalline methylene blue and sprayed with a 0·4% (w/v) solution of dithizone in carbon tetrachloride. When the urine, falling through the mesh bottom, hit the filter paper, it was readily absorbed. The particles of methylene blue dissolved in the urine, thus giving bright blue spots indicating the localization of the absorbed urine. The dithizone in the paper combined with metal in the urine to give non-volatile dithizonates and thus prevented losses of activity. The paper was renewed after 12 hours. The spots containing urine were cut out and their radioactivity was measured.

At the end of the 24 hours experiment the animals were decapitated and the blood was collected and heparinized. Plasma was separated by centrifugation and its radioactivity was measured. The liver (entire organ) and one kidney were homogenized and their radioactivity was estimated. The complete gastrointestinal tract was carefully removed from the abdominal cavity and divided into six parts according to anatomical segments—stomach, duodenum, jejunum, ileum, caecum, and colon. The radioactivity of the individual segments including intestinal contents was determined (A). Afterwards segments were washed in saline solution and their radioactivity was again measured (B). The amount of metal which was excreted into the lumen was given by B minus A. The radioactivity of the faeces which we collected during the experiment was added to the radioactivity in the caecum.

The measurement of radioactivity was carried out for ^{52}Mn, ^{64}Cu, and ^{203}Hg on a well-type scintillation counter (Frieseke-Hoepfner type). Measurement time was selected after radioactivity sampling had demonstrated significant differences from the background. Counts were within a $\pm 5\%$ error.

^{210}Pb was measured by gamma-spectrometry at 47 keV (scintillation crystal NaI/Tl, 5 in $\times$ 4 in).

The results were expressed as percentages of the administered dose. For plasma, the results were expressed in terms of the entire plasma volume (8·3 ml per rat). For ^{210}Pb the bile samples of the individual animals were pooled and measured as a single sample.

The number of experimental animals varied from five to nine. Results are expressed as means $\pm$ standard errors of the means.

Results

Biliary excretion

Excretion of ^{64}Cu Figure 1 shows the excretion of

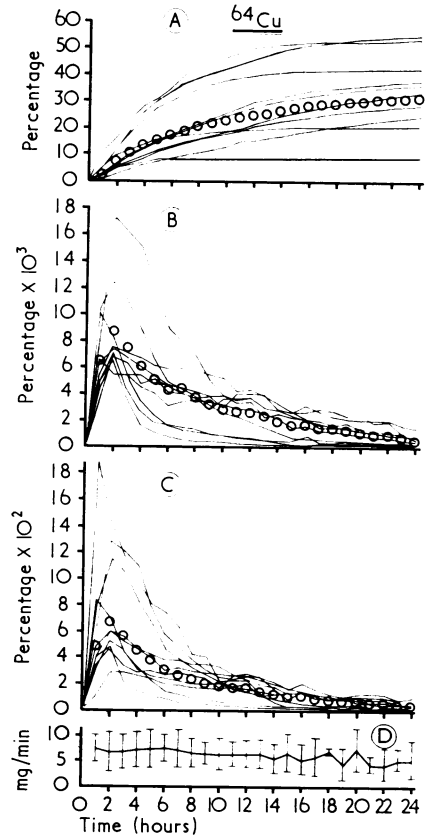

FIG. 1. A. Cumulative biliary excretion of ^{64}Cu as percent of the administered dose. B. Percentage of excreted ^{64}Cu per milligramme of bile. C. Excretion of ^{64}Cu as percent of the administered dose per minute. D. Bile flow (mg/min). Solid lines—results from individual animals. Open circles—mean values.

^{64}Cu into bile in the individual animals during the 24 hours experiment. The cumulative biliary excretion of ^{64}Cu reached a mean of 31·06 ± 9·96% (± S.E.M.). Figure 1B and C show that the maximum rate of ^{64}Cu excretion was reached two hours after administration.

Excretion of ^{52}Mn The cumulative excretion of ^{52}Mn during 24 hours after administration represented a mean of 26·7 ± 9·3% (Fig. 2A). The maximum rate of ^{52}Mn excretion was already reached by the time of the first bile collection, i.e., one hour after administration (Fig. 2B and C). In order to find out more exactly when ^{52}Mn excretion was maximal we repeated the experiment but collected bile every two minutes for the first hour (Fig. 3). The rate was maximal 8 to 16 minutes after injection.

Excretion of ^{210}Pb The mean values of ^{210}Pb excretion into bile during the 24 hours after administration are shown in Figure 4. The samples from five rats were pooled and measured as a single sample. The cumulative excretion of ^{210}Pb 24 hours after administration reached 6·7%. The maximum rate of ^{210}Pb excretion, after recalculation to milligrammes of excreted bile, was reached two hours after administration (Fig. 4B). The higher value in the first hour (Fig. 4C) was caused by the higher bile flow during this period of collection.

Excretion of ^{203}Hg Figure 5 shows ^{203}Hg excretion into the bile. During the 24 hours 3·8 ± 2·1% of the administered dose was excreted into the bile. Both the cumulative excretion curve (Fig. 5A) and the curves of the rates of excretion were quite unlike any others. Figures 5B and 5C show two waves of ^{203}Hg excretion. The first wave appeared two to three

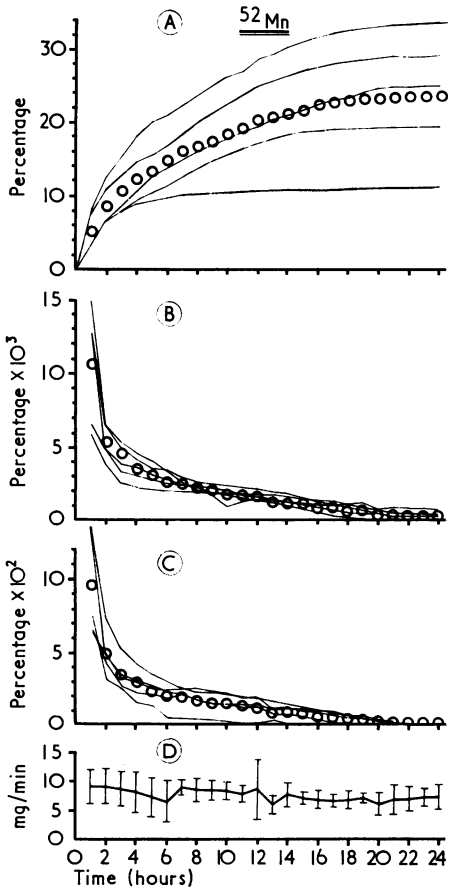

FIG. 2. A. Cumulative biliary excretion of ^{52}Mn as percent of the administered dose. B. Percentage of excreted ^{52}Mn per milligramme of bile. C. Excretion of ^{52}Mn as percent of the administered dose per minute. D. Bile flow (mg/min). Solid lines—results from individual animals. Open circles—mean values.

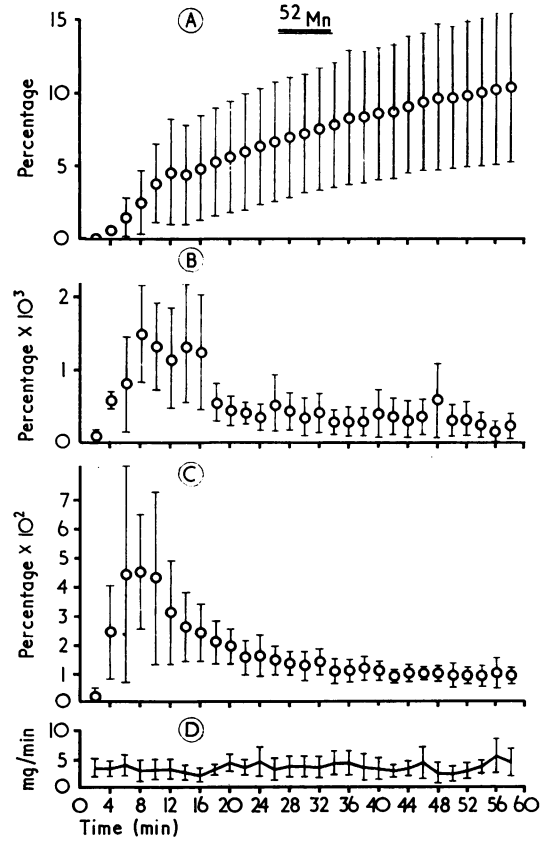

FIG. 3. A. As for Fig. 2, but with a different time scale.

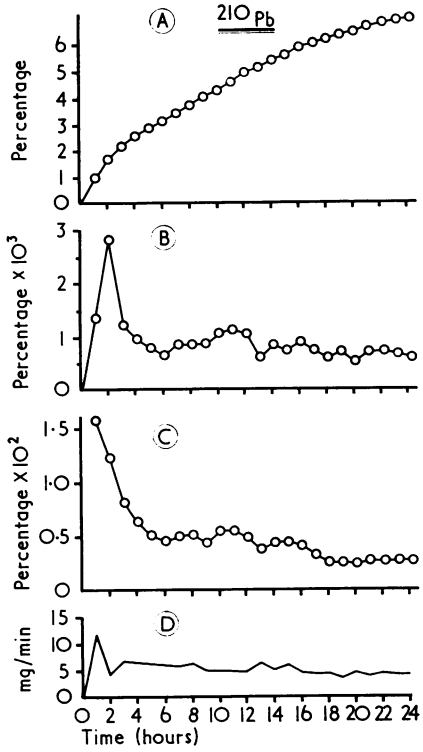

FIG. 4. A. Cumulative biliary excretion of ^{210}Pb as percent of the administered dose. B. Percentage of excreted ^{210}Pb per milligramme of bile. C. Excretion of ^{210}Pb as percent of the administered dose per minute. D. Bile flow (mg/min). Open circles—the bile samples of five rats were pooled and measured as single samples.

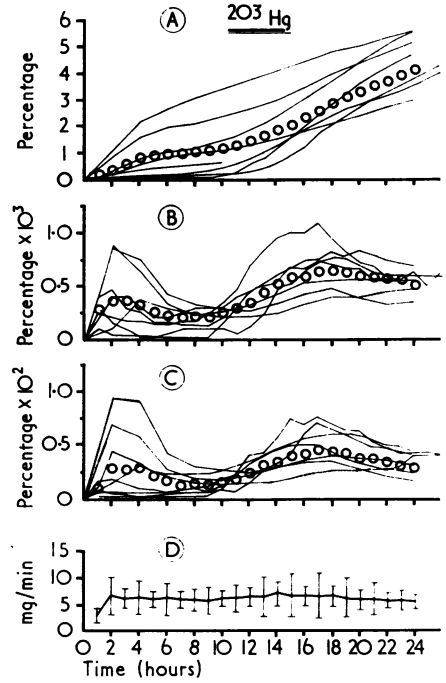

FIG. 5. A. Cumulative biliary excretion of ^{203}Hg as percent of the administered dose. B. Percentage of excreted ^{203}Hg per milligramme of bile. C. Excretion of ^{203}Hg as percent of the administered dose per minute. D. Bile flow (mg/min). Solid lines—results from individual animals. Open circles—mean values.

hours after injection and the second wave was 16 to 20 hours after injection. From 4 to 10 hours was a 'latency' period during which a comparatively small amount of ^{203}Hg was excreted into the bile.

Liver and kidneys

Figure 6 shows the percentages of the radioisotopes in the liver and both kidneys 24 hours after injection. The liver contained more of each metal except for mercury than the kidneys. For mercury the reverse was found.

Urine

Figure 7 summarizes the results of measurements of the metals excreted in the urine during 24 hours. The highest values (6·8 ± 3·1%) were measured for ^{203}Hg. Of copper 1·3 ± 0·29% ^{64}Cu was excreted in urine. The quantities of ^{52}Mn in the urine were very low (0·015 ± 0·008%). The ^{210}Pb excretion (not shown in Fig. 7) was measured in four animals only and was 1·81 ± 0·96% of the administered dose.

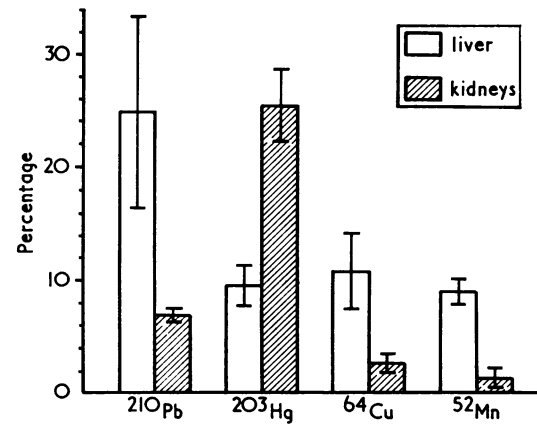

FIG. 6. Amounts of metal in both kidneys and in the liver as percent of the administered dose 24 hours after administration.

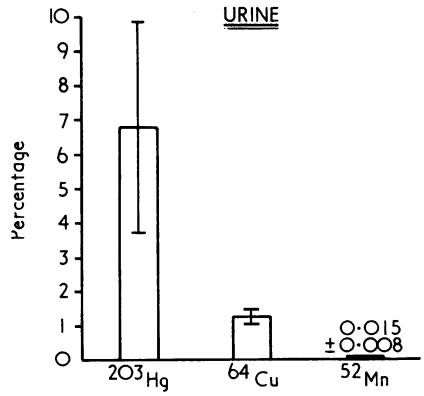

FIG. 7. Amount of metal excreted in urine during 24 hours after administration as percent of the administered dose.

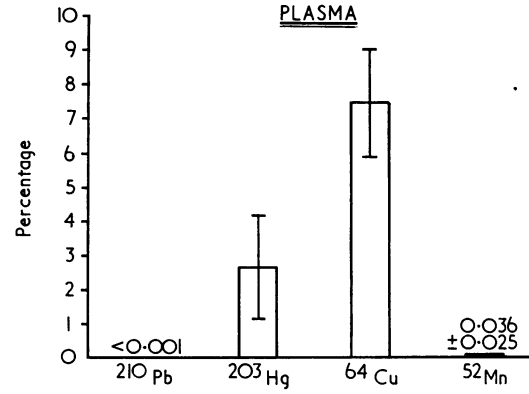

FIG. 8. Plasma levels 24 hours after administration. The values are calculated to 8·3 ml of plasma and expressed as percent of the administered dose.

Plasma

At the end of the experiment the level of ^{64}Cu in the plasma was the highest (7·41 ± 1·53), and the ^{52}Mn and ^{210}Pb levels were again very low (Fig. 8).

Gastrointestinal tract

Figure 9 shows the distribution of each isotope between the anatomical segments of the intestine and their contents after 24 hours. Of all metals the highest 'wall' concentrations were found in the jejunal segment, and the highest quantities in the intestinal contents were in the caecum except for ^{210}Pb, which was maximal in the jejunum.

The radioactivity measured in faeces was added to that of the intestinal contents of the colon. In order to compare the amount of metal excreted during the same period via the intestinal wall, we added up the contents in all the anatomical segments of the gastrointestinal tract. There were no great differences between the metals except for ^{52}Mn, for which the results showed considerable variability (Table). The quantities in the wall of the entire gastrointestinal tract also did not differ significantly.

Discussion

Our experiments show the differences in the biliary excretion of four metals studied. Only ^{52}Mn and ^{64}Cu resembled each other closely in the shape of the excretion curve as well as in the total amount of metal excreted into bile within 24 hours of injection. Of these, ^{52}Mn was excreted most rapidly 8 to 10 minutes after injection (Fig. 3) whereas copper did not attain its maximum rate until two hours after injection (Fig. 1). Bertinchamps and his colleagues

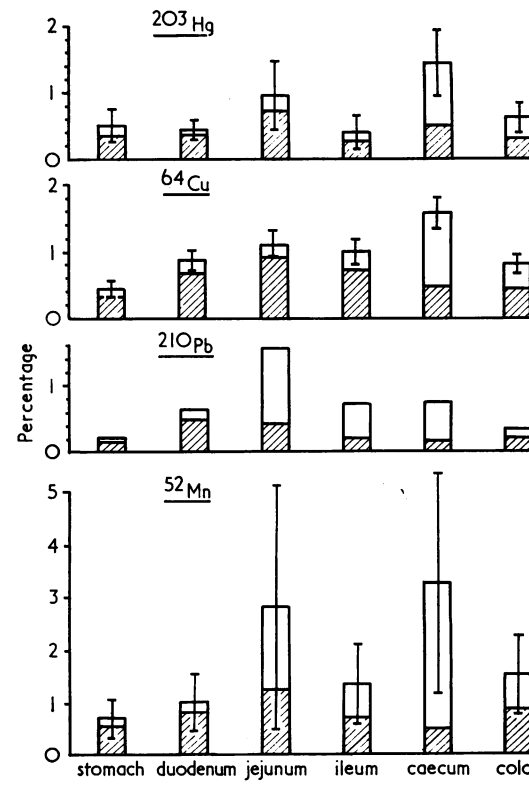

FIG. 9. Amount of metal in the wall (hatched column) and in the lumen (open column) of the individual segments of the gastrointestinal tract 24 hours after administration as percent of the administered dose.

TABLE

SUMMARY OF RESULTS 24 HOURS AFTER INTRAVENOUS ADMINISTRATION OF EACH METAL

		Bile (cumulative excretion)	*Plasma (mean vol. 8·3 ml)*	*Liver*	*Kidneys*	*Urine*	*Gastrointestinal tract*	
							Wall	*Content + faeces*
^{203}Hg	$\bar{x}$	3·8 ±2·1	2·7 ±1·5	9·6 ±1·9	25·5 ±3·3	6·8 ±3·1	2·4 ±0·7	1·6 ±0·9
^{64}Cu	$\bar{x}$	31·06 ±9·96	7·41 ±1·53	10·8 ±3·42	2·48 ±0·7	1·3 ±0·29	3·55 ±0·73	2·35 ±0·61
^{52}Mn	$\bar{x}$	26·7 ±9·3	0·03 ±0·02	8·9 ±1·9	1·4 ±0·8	0·015 ±0·008	3·8 ±2·8	6·0 ±5·5
^{210}Pb	$\bar{x}$	6·7	0	24 8 ±8·5	7·1 ±0·6	1·81 ±0·96	1·6 ±0·8	2·6 ±1·3

The results are expressed as percentages of the dose administered.

(1966) detected two waves of excretion of manganese in the bile after intravenous ^{54}Mn injection. The first wave appeared 7 to 10 minutes after intravenous administration of the radioisotope, and they suggested that it corresponded to direct passage of the metal from the plasma into the bile. After 60 to 120 minutes the second wave appeared and it corresponded to an acceleration of the enterohepatic circulation. This wave was present in experiments on animals kept on a diet with a high manganese content but it was absent in experiments on animals kept on a diet without manganese for several days before the experiment. In our experiment we did not find the second wave in any rat but the manganese content in the diet of our animals was lower than in that used by Bertinchamps *et al.* (1966), and our animals had been starved for 24 hours before the experiment. In our experiments we collected bile from unnarcotized animals, which may also have led to different results.

The cumulative ^{64}Cu excretion into bile which we determined agrees with the results of Farrer and Mistilis (1968).

The cumulative ^{210}Pb and ^{203}Hg excretion into bile during the 24 hours after intravenous administration did not differ significantly, but the character of the ^{203}Hg excretion curve differed strikingly from that of ^{210}Pb as well as from those of ^{64}Cu and ^{52}Mn. The excretion curve of ^{203}Hg into the bile was characterized by two waves (Fig. 5). The first wave with its peak between the second and third hours after injection was followed by a comparatively long 'phase of latency' (six hours) when the excretion of ^{203}Hg was constantly slow. This was followed by a slow rise to the second wave, with its peak between the 16th and 20th hours. There is some evidence (our unpublished results) that the relative sizes of the two waves changed with the dose. As the dose increased the first wave became larger and the second wave smaller.

It is very difficult at present to explain the reason for two waves. The first wave probably corresponds to the excretion of mercury complexed with some diffusible plasma component, constituting a fairly high proportion of the diffusible ^{203}Hg in the circulation. As time goes on, the level of this diffusible fraction in the blood decreases, leading to a 'latency phase'. About 10 hours after administration, mercury probably starts to be released from the non-diffusible components into the circulation and from there into the bile, giving rise to the second wave. This hypothesis needs to be verified by further experiments.

The quantities of ^{210}Pb excreted in the bile agree with the results of Castellino and his co-workers (1965).

The question of metal excretion through the intestinal wall is very interesting. There are several possible pathways for the passage of a substance from the circulation through the intestinal wall to the intestinal lumen. Most substances come directly or indirectly from plasma, either passing in some way through the epithelium or being incorporated into cells and later lost with them into the lumen. Some substances are secreted by goblet cells and Paneth cells (Loehry *et al.*, 1970). The turnover of the intestinal mucosal cells in rats occurs on 1·4 to 1·6th day (Leblond and Stevens, 1948), so this pathway should not manifest itself during our 24 hours' experiments. One must be aware that the radioisotope contents of the lumen of the individual intestine segments do not entirely correspond to the metal excreted in these segments. The transit time of the intestinal contents in the different segments

must also be considered. A relatively rapid rate of transit is seen in the duodenum and upper jejunum, while a marked reduction in rate of passage occurs in the lower jejunum and in the ileum (Marcus and Lengemann, 1962). This means that the higher quantities of radioisotope in the caecum do not correspond only to the metal excreted in this segment but also to the accumulation of metal excreted in the upper segments of the digestive tract. Also the relatively large quantities of metal found in the lumen of the ileal segment indicate the participation of the higher segments of the gastrointestinal tract in this excretion. During the 24 hours experiment there may well occur reabsorption of already excreted metal. We assume this mechanism to occur mainly for manganese, as we have already demonstrated its easy passage through the intestinal wall in vitro (Cikrt, 1970).

On comparing the amounts of metal excreted via bile and via the wall of the digestive tract during 24 hours it is found that excretion via bile predominated for all the metals studied.

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Received for publication April 5, 1971