

EXCRETION AND DISTRIBUTION OF MERCURY
IN RATS, ANTIDOTES FOR MERCURY
AND EFFECTS OF EGGPRODUCTION
AND FERTILITY OF HENS AFTER MERCURY
ADMINISTRATION

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The results of investigations of the distribution and excretion of organic and inorganic mercury compounds in albino rats and white leghorn hens conducted over a period of ten years are surveyed. The storage of mercury in eggs as well as its effects on the egg-lay-frequency and hatchability of the laid eggs have also been studied. All investigated mercury compounds were labelled with the radioactive mercury isotope ^{203}Hg and the mercury level was measured with a scintillation technique. Since antidotes used in the treatment of mercury poisoning influence not only the excretion of mercury, but also its distribution in the body, the effects of nine antidotes on the metabolism of different mercury compounds were also investigated. The results of the survey are presented graphically.

The results which are presented in the following report have been obtained during about ten years of cooperation with Dr. Ake Swensson. Most of them have been published in various journals (figures within brackets refer to the list of publications). The distribution and excretion of organic and inorganic mercury compounds have been studied on two species of animals: albino rats weighting about 200 g and white leghorn hens. The mercury compounds were labelled with the radioactive mercury isotope ^{203}Hg . All studied mercury compounds were soluble in water and were intravenously injected as water solutions or fed to the animals through the drinking water or in some cases (hens) in the form of seed treated with mercury. Organs or whole animals were analysed for mercury by a scintillation technique. Further details about the synthesis of labelled mercury compounds, administration of mercury, dissection and analyses are given in the publications. Organic mercury compounds of the general type RHgX and inorganic mercury compounds, HgX_2 have been investigated, where R stands for an organic radical methyl, ethyl, propyl methoxy ethyl or phenyl and X for a monovalent inorganic anion,

usually hydroxide or nitrate. The carbon mercury bond in different organic mercury compounds is of different stability. Alkyl mercury compounds are very stable in the body especially the methyl mercury ion, while the phenyl mercury ion is very unstable and decomposes almost immediately after the injection. The stability of the methoxy ethyl mercury ion is between those two extremes. The bond to the anion X is usually an ionic bond. Let us see first what happens when a mercury compound is injected to an animal.

If we look at the whole body concentration, it decreases at a rate which varies with the mercury compound in question (Fig. 1). Methyl mercury compounds are slowly excreted, mainly in the faeces (Fig. 2) (1).

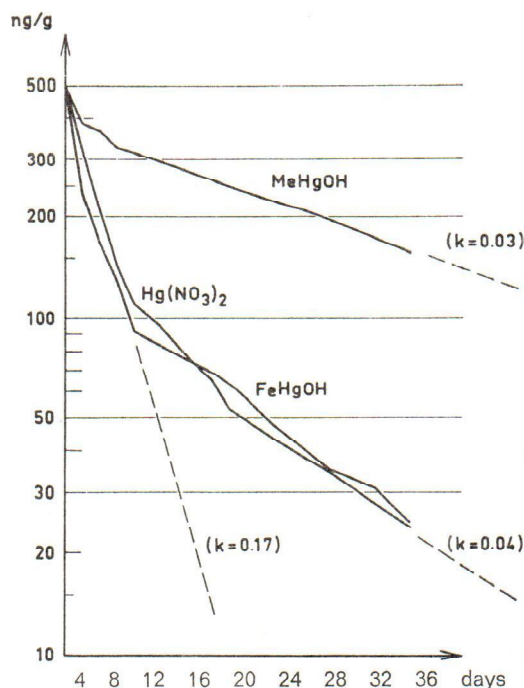


Fig. 1. Whole-body concentrations of mercury in rats after single injections of various mercury compounds. The k -values refer to the time constant in the simplest form of equation 1. Logarithmic scale for mercury concentration (3)

Other organic compounds and the inorganic ones are much more rapidly excreted both in the faeces and urine. If we look at the distribution between the organs we find for methyl mercury (Fig. 3) (3) that a short period after the injection it is distributed between the organs and — especially interestingly — slowly accumulates in the brain as first reported by Berlin and Ullberg in 1963. After this period the distribution

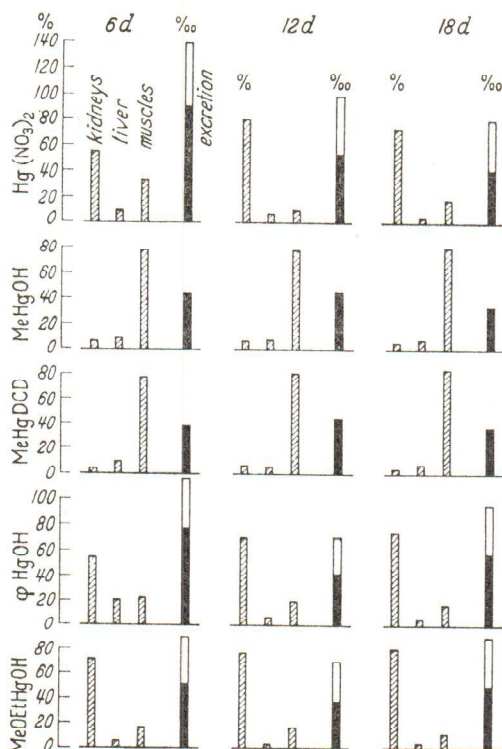


Fig. 2. Distribution and excretion of mercury in rats. The animals were injected subcutaneously with 100 ng Hg/g of body weight with the mercury compound in question every other day during the experimental period. The distribution is given in per cent of the total-body content of mercury found in each organ, on the 6th, 12th and 18th day respectively. The excretion is given, as the mercury eliminated on the 6th, 12th and 18th day after the injections started, in pro mille of the total body content on the same days (1)

stabilizes. It was also shown, that it was rather independent of the dose (Fig. 4) (5). Inorganic mercury (Fig. 5) (3) shows a different picture. Initially mercury is distributed in a certain ratio in the kidney, liver and blood and then much more rapidly it is eliminated in the liver and blood than in the kidney. A similar picture is found for phenyl mercury compounds (Fig. 6) (3). The distribution of inorganic mercury was later found to be very dose depending, indicating saturation effects (Fig. 7) (5). The distribution of methoxy ethyl mercury compounds on the other hand is probably not dose depending, but time depending (Fig. 8) (5). Before discussing these findings another feature of the mercury compounds should be mentioned. When alkyl mercury compounds with the same organic radical and different anions were administered and distribution studied it was found that distribution was influenced by the organic ra-

dical but probably not at all by anion, although the anions more than the organic radical are likely to account for those properties of the mercury compounds which affect the distribution (Fig. 9) (1). Therefore it is concluded that the anions are dissociated from the cations after the compound has entered the organism. Hence the anion is not supposed to have any importance for the distribution or excretion after the administration to the organism. We therefore analyse and discuss the results with various mercury compounds with reference only to the mercury containing the cation in question.

The distribution of mercury compounds within the rat organism is obviously a complicated process which depends both on how mercury is bound in various organs and on the stability of the mercury compounds. I make the following assumptions: We have four types of mercury compounds to deal with, namely 1) stable, lipophilic, monovalent compounds, that is the alkyl compounds; 2) stable hydrophilic, divalent compounds, that is Hg^{2+} ; 3) moderately stable, lipophilic, monovalent compounds, that is methoxy ethyl mercury compounds, and finally 4) unstable lipophilic monovalent compounds, that is the phenyl mercury compounds

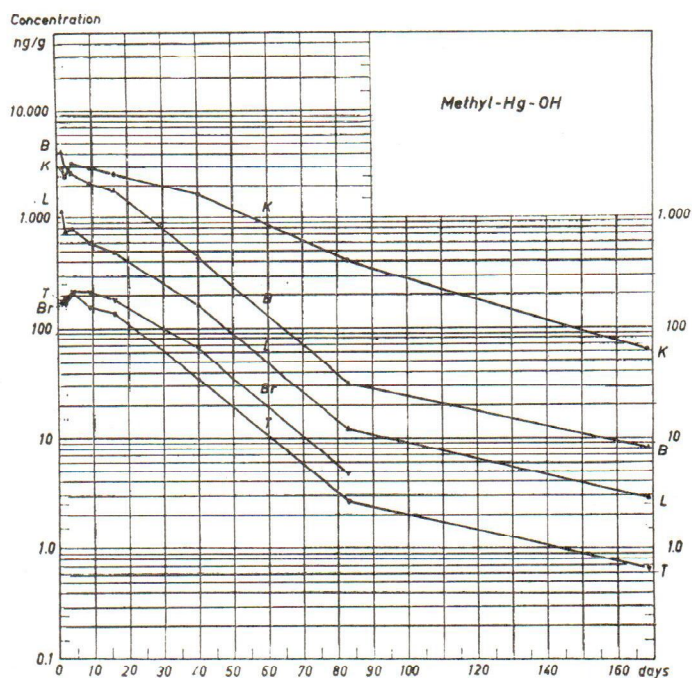


Fig. 3. The concentration of mercury in different organs (Br = brain, B = blood, L = liver, K = kidney, T = testis) at various times after intravenous injection of methyl mercury hydroxide. Logarithmic scale for mercury concentration (3)

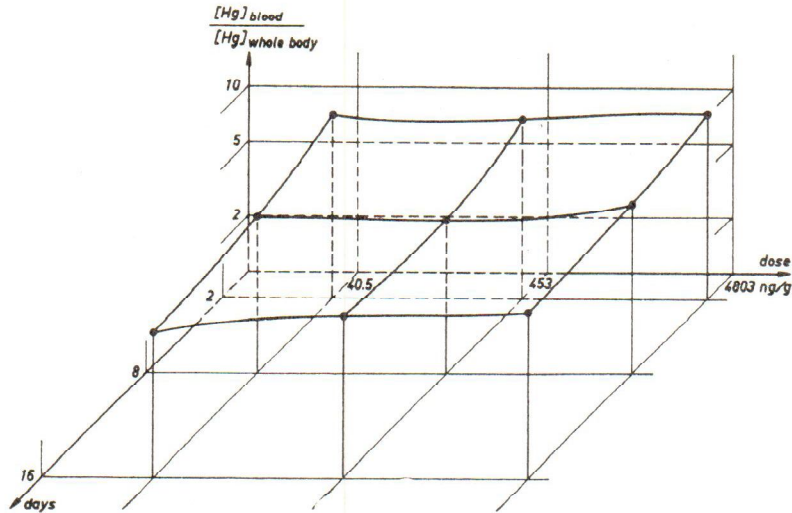


Fig. 4. The quotient, between the mercury concentration in blood and in whole body of rats intravenously injected with methyl mercury hydroxide, as a function of the given dose and the time after injection. Logarithmic scale for quotient and dose

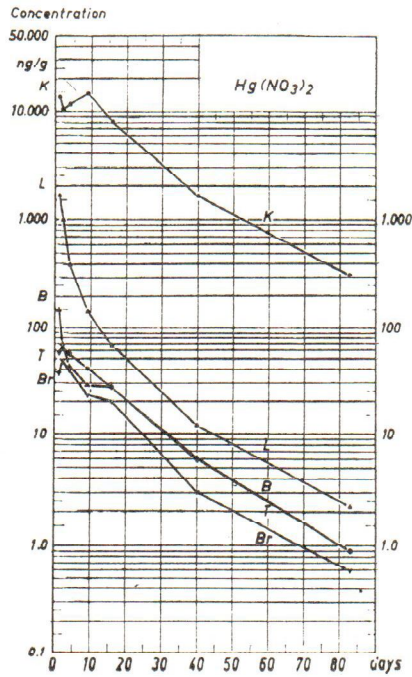


Fig. 5. The concentration of mercury in different organs (Br = brain, B = blood, L = liver, K = kidney, T = testis) at various times after intravenous injection of mercuric nitrate. Logarithmic scale for mercury concentration(3)

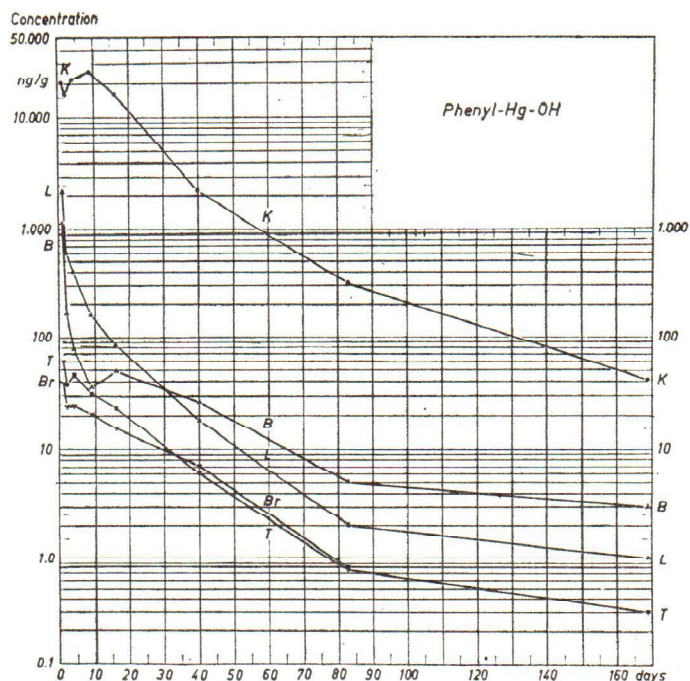


Fig. 6. The concentration of mercury in different organs (Br = brain, B = blood, L = liver, K = kidney, T = testis) at various times after intravenous injection of phenyl mercury hydroxide. Logarithmic scale for mercury concentration (3)

The first type is rather evenly distributed in the body between the organs and the relative distribution is influenced only to a small extent by the size of the dose and the time after the administration. Since it is both lipophilic and watersoluble it can penetrate all types of organs, since it is monovalent it occupies only one binding site per molecule, since it is stable its properties do not change and therefore this type of compound shows a stable relative distribution.

Type No 2 is hydrophilic and therefore has a limited penetration into some of the organs. It is divalent and will therefore occupy two binding sites per molecule. It is therefore inclined to show saturation effects above a certain concentration. The saturation effect means that the primary binding sites with a high stability constant are all occupied and therefore some mercury cations will be more loosely bound to binding sites with lower stability constants. This means a higher rate of elimination from those organs which have been saturated and a distribution to organs which are richer in primary, binding sites.

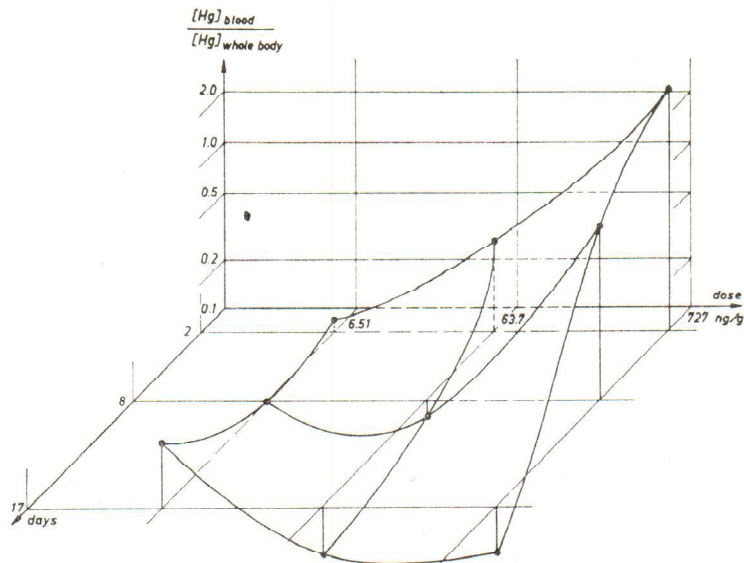


Fig. 7. The quotient between the mercury concentrations in blood and in whole body of rats intravenously injected with mercuric nitrate, as a function of the given dose and the time after injection. Logarithmic scale for quotient and dose

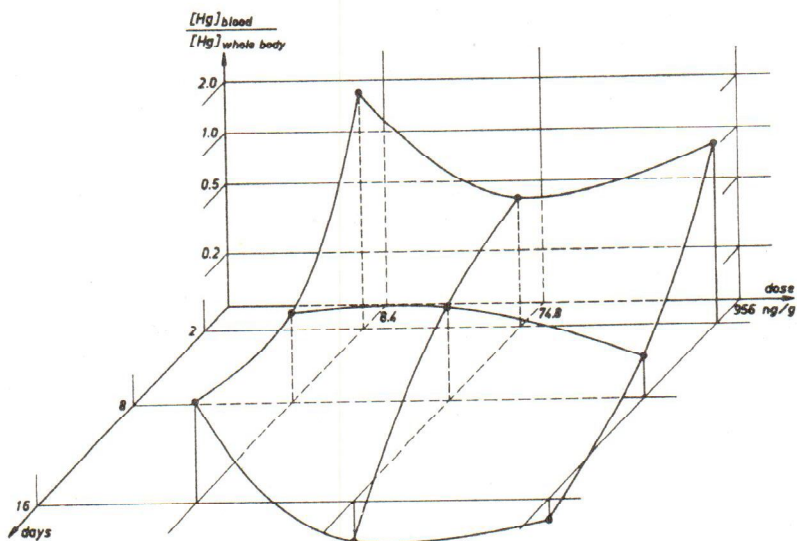


Fig. 8. The quotient, between the mercury concentrations in blood and in whole body of rats intravenously injected with methoxyethyl mercury hydroxide, as a function of the given dose and the time after injection. Logarithmic scale for quotient and dose

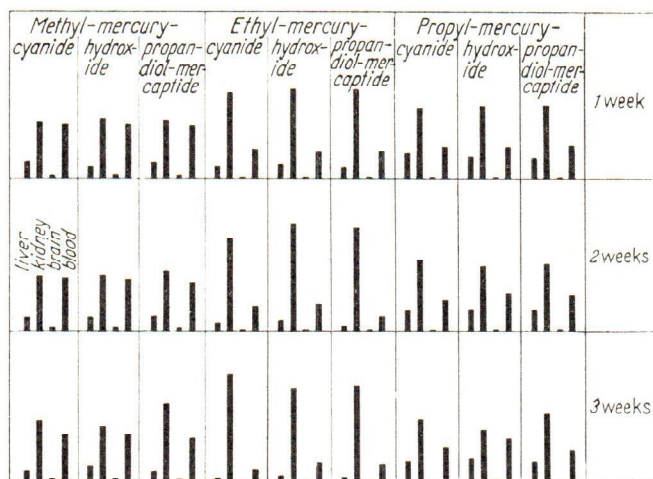


Fig. 9. Distribution equilibrium, for alkyl mercury compounds, between some organs of the experiment animals. The ratio between organ concentrations and sum of all organ concentrations at a given moment (1)

The 3rd type of mercury compound which is lipophilic, monovalent and moderately stable is less likely to show saturation effects. It is assumed that when it is decomposed the end result is the divalent inorganic mercury cation and that before it is formed some of the administered amount of mercury will already have been excreted. The relative distribution will however be affected by the decomposition since the physical properties of the mercury »particle« will change and it will lose its lipophilic properties in that process.

The 4th type of mercury compound will decompose to inorganic divalent mercury cation almost immediately after the administration to the organism and therefore shows a pattern which is almost similar to the 1st type.

Antidotes for mercury poisoning are usually compounds which bind to mercury and therefore compete for mercury with the binding sites of the organism. Hence they will protect the biochemical processes from the disturbances of mercury and also increase the excretion of mercury. Due to this the time during which a dangerous mercury concentration is found in the body will decrease. Since antidotes change the concentration of possible binding sites within the organism the administration of antidotes will affect not only the excretion but also the distribution between the organs. For orientation we tested 9 different antidotes on different mercury compounds as regards the effects on distribution and excretion and the antidote effect. It was found that although distribution and excretion were always influenced with the exception of ascorbic acid, the antidote effect was only found in some cases. In one case the antidote had an adverse effect on the animals (Table 1) (2, 5).

BAL increased the excretion of methoxy ethyl mercury hydroxide at low mercury doses, but the animals died immediately after the administration of toxic amounts of mercury together with the antidote.

In this informatory investigation, D-penicillamine was not found to be an antidote against methyl mercury poisoning. Since other authors had reported such an effect of D-penicillamine further investigations were started and it was later found, that to get the antidote effect of D-penicillamine the treatment had to be extended for a longer time (6). A marked increase in excretion was found with penicillamine at different doses of mercury (Figs. 10, 11) (6). The mercury is eliminated more rapidly from blood, liver, kidney and brain after administration of D-penicillamine (Fig. 12) (6), and it was also found, that the deposition in skin and hair was decreased when D-penicillamine was administered. At high concentrations of mercury the animals got sick and lost weight. This of course had an effect on the concentration of mercury, which seems to indicate a delayed elimination during the first week whether the antidote was administered or not. In the brain on the other hand there is a delayed elimination at all concentrations or even an accumulation. The penicillamine cannot change this process, but the maximum concentration in the brain is probably lower and the elimination thereafter faster when the antidote is administered. Since the mercury is distributed to some extent to skin and hair, cf Fig. 12, the concentration in each organ related to the body concentration is an uncertain measure of the redistributions within the body caused by the antidote. Therefore the quotient between organs and blood should also be investigated. This was also done and it was found that except for the quotient between the kidney and the blood (Fig. 13) (6), no systematic redistribution seems to be caused by the administration of antidote. Fig. 13 shows that at high doses the antidote causes a relative transfer from the blood towards the kidney, while at low doses the opposite is found. However, these effects are small.

Differences between the investigated mercury compounds as regards excretion and distribution were found in hens too. Methyl mercury cation is excreted slowly, the other compounds more rapidly. Together with Dr. Kivimäe at Uppsala, we investigated the effect of feeding hens with mercury-treated seed on mercury concentrations in the eggs, egg-lay-frequencies and hatchability of the laid eggs (4). Mercury was fed to hens in the form of methyl mercury hydroxide, phenyl mercury hydroxide, methoxy ethyl mercury hydroxide and mercury nitrate at two dose levels, 0,4 mg per hen/day and 1,6 mg per hen/day for 140 days, after which time the surviving hens were given normal feed. The hens were inseminated with sperma from cocks which were fed on normal feed. Eggs were sampled and analysed for mercury or hatched. A high dose of the methyl mercury compound caused the death of seven of the eight hens in the group after between 32 and 37 days feeding with the treated wheat. Except for the group that was given the higher dose of the methyl mercury compound the individual case of death could not be attributed

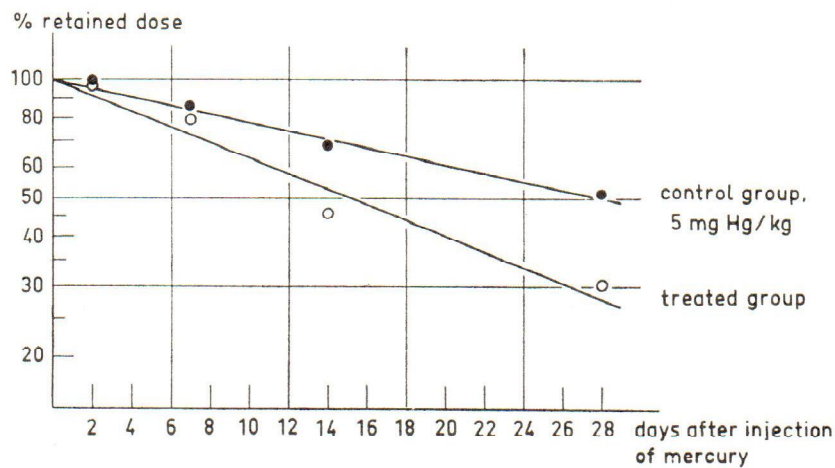


Fig. 10. Whole body retention of mercury at different times after single administration of methyl mercury hydroxide. Treatment: D-penicillamine

to the mercury diet. Nor could any effect on the weight of the hens be established. The consumption of wheat showed only random variations. In general the consumption of laying mash and egg production decreased. The fertility was not adversely affected and this applied also to the hatching-frequency. With the exception of the group that received the high methyl mercury dose, where on the other hand the material was

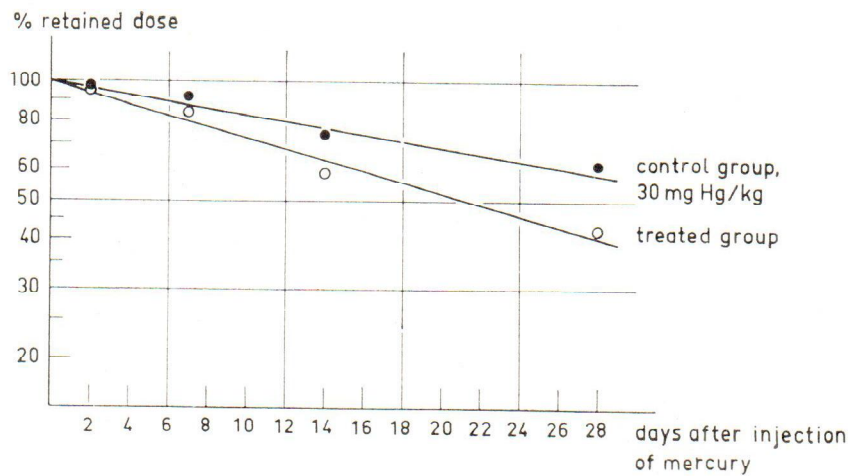


Fig. 11. Same text as in Figure 10, lower dose

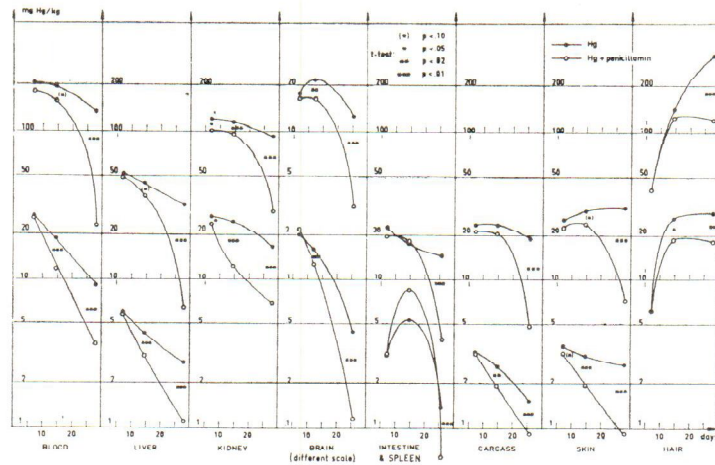


Fig. 12. Concentrations of mercury at different times after single administration of methyl mercury hydroxide. Treatment: D-penicillamine

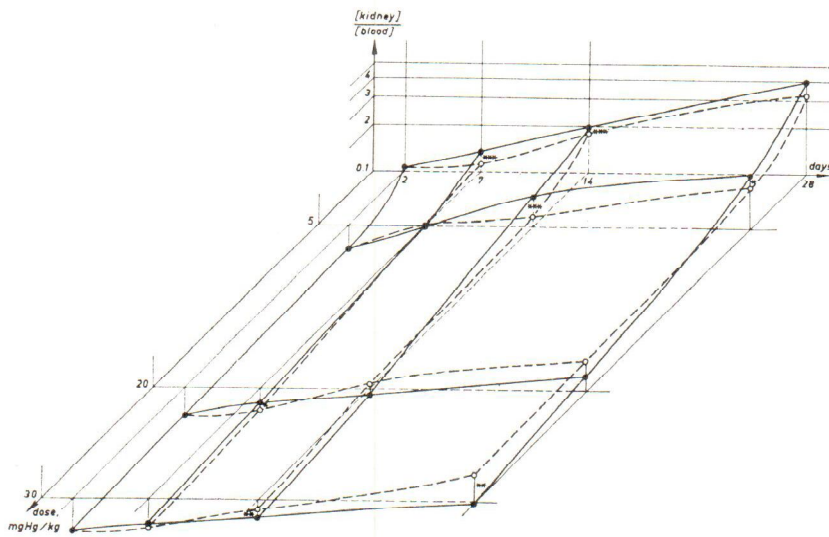


Fig. 13. Change with dose and time of the quotient between mercury concentration in kidney and whole blood in rat after injection of methyl mercury hydroxide, with and without administration of D-penicillamine. Control group had filled rings. Logarithmic scale for the quotient. The significance of the difference is tested with t-test

too small to enable definite conclusions, the weight development of the chicken was not affected. After feeding was started the mercury concentration in the eggs showed a rapid increase and a subsequent stabilisation after between one week and one month. The slow rate of elimination of methyl mercury caused a very high concentration of mercury in the hens and in the eggs (Fig. 14). The concentration in the white was

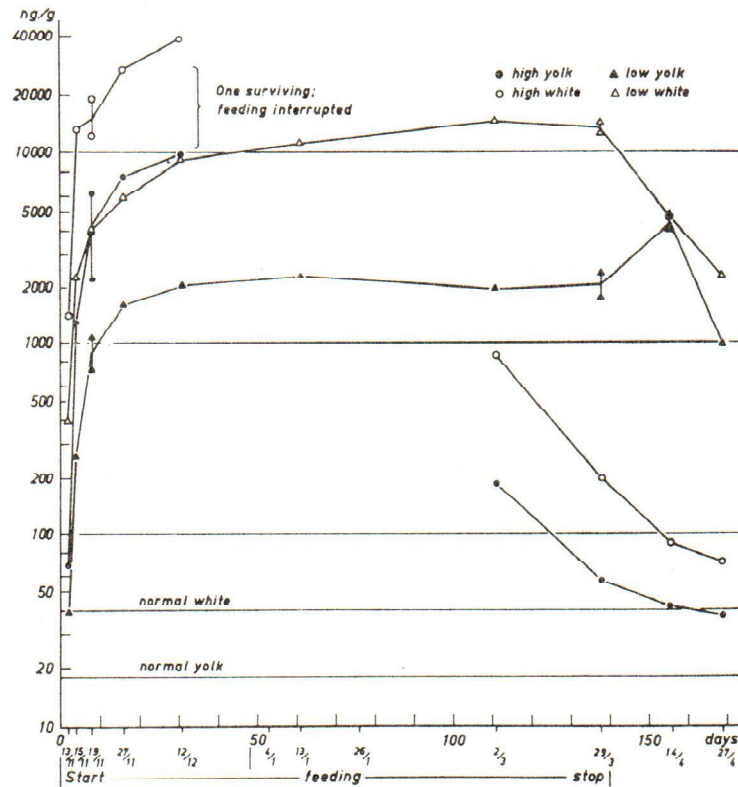


Fig. 14. The concentration of mercury in yolk and white of eggs laid by hens secd treated with methyl mercury hydroxide. The two doses correspond to a mercury intake of 400 and 1600 ng Hg per hen per day. Logarithmic scale for mercury concentration (4)

always higher than in the yolk with methyl mercury and this was found also in the controls. In contrast to this the concentration of mercury after administration of other mercury compounds was always higher in the yolk than in the white.

In the group which received the low dose of mercury the concentration in the eggs was still greatly elevated four weeks after the administration of the mercury was discontinued. With other compounds the mercury concentration in the eggs was close to that of the control eggs four weeks after mercury administration, e. g. Fig. 15.

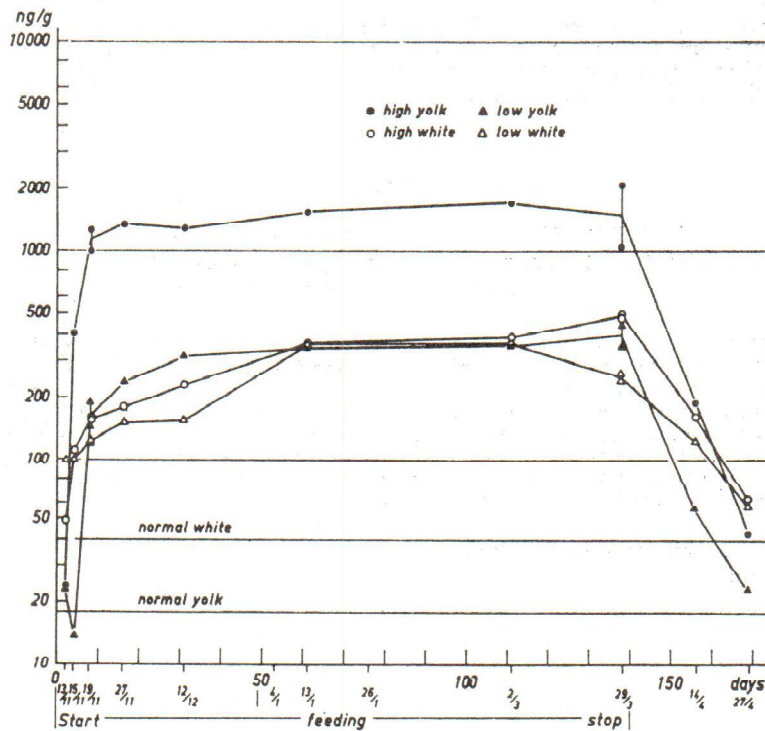


Fig. 15. The concentration of mercury in yolk and white of eggs laid by hens fed seed treated with mercuric nitrate. The two doses correspond to a mercury intake of 400 and 1600 ng Hg per hen per day. Logarithmic scale for mercury concentration (4)

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Sažetak

IZLUČIVANJE I RASPODJELA ŽIVE U ŠTAKORA,
ANTIDOTI ZA ŽIVU I UČINCI PRIMJENE ŽIVE
NA NESIVOST I PLODNOST U KOKOŠI

Prikazani su rezultati desetogodišnjeg istraživanja raspodjele i izlučivanja organskih i anorganskih spojeva žive na albino-štakorima i leghorn-kokošima. Istraživano je i odlaganje žive u jajima nesilica, učinci na nesivost i oplodnost jaja. Živini spojevi obilježavani su radioaktivnim izotopom ^{203}Hg , a živa mjerena scintilacijskom tehnikom. Budući da antidoti što se primjenjuju pri otrovanju živom utječu ne samo na izlučivanje već i na raspodjelu žive u organizmu, izneseni su i rezultati učinaka devet antidota na prijetvor pojedinih živinih spojeva u organizmu pokusnih životinja.

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