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Kaposvári Egyetem, Állattudományi Kar, Kaposvár
University of Kaposvár, Faculty of Animal Science, Kaposvár

Study in pigs of the protective effect against mercury of anion exchange resin in chloride form

T. Rétfalvi, I. [1]Sarudi, A. [1]Szabó
University of West Hungary, Institute of Chemistry, Sopron, 9400 Bajcsy-Zs. u. 4
[1]Kaposvár University, Faculty of Animal Science, Institute of Chemistry
Kaposvár, 7400 Guba S. u. 40

ABSTRACT

This experiment was performed on castrated boars of 16 to 18 kg live weight, with $HgCl_2$ labelled with Hg-203 isotope and VARION AD anion exchange resin in the form of Cl^- (NIKE, Balatonfűzfő). The mercury was administered to the pigs in a single dose (2.5 mg Hg per animal), while the resin was consumed continuously, mixed in with the diet (20 g or 40 g resin per kg feed). This experimental feeding proceeded for 10 days. The results obtained indicate that in the digestive system the resin bound the greater part of the Hg (II) ions, which were then excreted in the faeces together with the resin. Although, owing to the inhibiting of mercury absorption, the excretion of mercury in the urine was also reduced, there was a substantial increase in total mercury excretion. This was of course accompanied by slight accumulation of mercury in the various organs. The protective effect for which evidence was provided by the above findings can be attributed to the fact that Hg (II) ions form fairly stable chloro-complexes with Cl^- ions, and therefore VARION AD (Cl^-) also probably acts as a complex-forming entity in the digestive tract. With respect to the selectivity and effectiveness of the protective agent used, it is highly favourable that the ions of the alkali metals and the alkaline earth metals do not form chloro-complexes, thus restricting somewhat the range of competing metal ions present in the alimentary canal.
(Keywords: mercury, anion exchange resin, protective effect, pig)

ÖSSZEFOGLALÁS

Klorid formában levő anioncserélő műgyanta higannyal szembeni védőhatásának tanulmányozása sertéseken

Rétfalvi T., [1]Sarudi I., [1]Szabó A.
Nyugat-Magyarországi Egyetem, Kémiai Intézet, Sopron, 9400 Bajcsy-Zs. u. 4.
[1]Kaposvári Egyetem, Állattenyésztési Kar, Kémiai Intézet
Kaposvár, 7400 Guba S. u. 40.

A kísérletet 16-18 kg élőtömegű ártányokon folytattuk le Hg-203 izotóppal jelzett $HgCl_2$ és Cl^--formában levő VARION AD (NIKE, Balatonfűzfő) anioncserélő műgyanta felhasználásával. A higanyt a sertések egy dózisban kapták (2,5 mg Hg per állat), a műgyantát pedig a takarmányhoz keverve folyamatosan fogyasztották (20, ill. 40 g műgyanta per kg takarmány). A kísérleti etetés 10 napon át tartott. A kapott eredmények szerint az emésztőcsőbe került Hg(II)-ionok nagy része a műgyanta által megkötődött, s

azzal együtt a bélsárral eltávozott. Bár a Hg-felszívódás visszaszorulása révén a vizeleten keresztüli Hg-exkréció is lecsökkent, az összes Hg-exkréció jelentősen fokozódott. Az utóbbi tény együttjárt természetesen azzal, hogy a szervekben kevesebb higany akkumulálódott. A fentiek alapján bizonyítást nyert védőhatás annak tulajdonítható, hogy a Hg(II)-ionok a Cl^--ionokkal meglehetősen stabil klorokomplexeket képeznek, tehát a VARION AD (Cl^-) is valószínűleg komplexképzőként viselkedik az emésztőcsőben. A felhasznált védőanyag szelektívitása és hatékonysága szempontjából nagyon kedvező, hogy az alkáli- és az alkáliföldfémek ionjai nem képeznek klorokomplexeket, tehát az emésztőcsőben levő konkurrens fémionok köre meglehetősen leszűkül.
(Kulcsszavak: higany, anioncserélő műgyanta, védőhatás, sertés)

INTRODUCTION

In a previous paper (*Sarudi et al., 1999*) the authors gave an overview of the harmful consequences of anthropogenic mercury emission, and also offered experimental evidence that anion exchange resin in the form of EDTA or Cl^- added to contaminated livestock feed greatly inhibits the absorption of inorganic Hg (II). The experiments outlined in this earlier paper were performed on broilers, while the work dealt with in the present paper involved an investigation in pigs of the protective effect exerted by anion exchange resin in Cl^- form.

In the present study the authors again anticipated that a protective effect might arise from Hg (II) ions forming chloro-complexes in the alimentary canal; these complexes would also contain chlorine atoms which would bind to the resin. This would evidently be accompanied by a proportion of the mercury entering the solid phase, subsequently to be excreted from the organism with the faeces.

Of the heavy metals occurring in livestock feeds (Fe, Mn, Zn, Cu, etc., and as contaminants Cd, Pb and Hg) mercury establishes the most stable chloro-complexes; the gross stability indices of $[HgCl]^+$, $HgCl_2$, $[HgCl_3]^-$ and $[HgCl_4]^{2-}$ are 6.74, 13.22, 14.04 and 15.05 respectively (*Barcza, 1983*). With respect to the objective of this study it seemed particularly favourable that neither the alkali nor the alkaline earth metals form chloro-complexes, and therefore there are no such complexes to repress the formation of mercury chloro-complexes. Regarding the alkaline earth metals this is also worthy of note due to the fact that EDTA establishes chelates with both calcium and magnesium, and therefore a more selective effect was to be expected from the anion exchange resin in Cl^- form than from the EDTA form.

The authors also attached significance to the fact that the stability of chloro-complexes, in contrast with that of EDTA complexes, does not decrease even in the acidic section of the digestive tract; in fact, it is beyond doubt that these complexes actually become more stable in the stomach, due to the high concentration of chloride ions.

MATERIALS AND METHODS

Experimental animals, keeping conditions and feeding

The experiment was conducted with 12 Hungarian Large White x Pietrain castrated boars, aged 60-65 days and of 16-18 kg live weight. The animals were housed individually in metabolic cages (60×35 cm ground area, each fitted with a self-drinker) for 13 days, including a three-day acclimatisation period; throughout the experiment they were fed a commercially available piglet feed, the nutrient content of which corresponded to the

specifications of the Hungarian Livestock Feed Code (1990). (The mercury content of this diet was lower than 0.05 mg/kg.) Both feed and water were available *ad libitum*.

The mercury used

The mercury was administered to the animals in the form of $HgCl_2$ labelled with Hg-203 isotope (T_1 =46.6 days). The solution made up for this purpose contained the radioactive mercury preparation available (Izotóp Kft., Budapest), together with $HgCl_2$ of analytical purity (Fluka Chemie AG) and distilled water; this solution contained 0.25 mg/cm^3 mercury, and had an activity concentration of 53.0 kBq per cm^3.

The protective agent used

The protective agent used was VARION AD strongly basic anion exchange resin in Cl^- form (produced by NIKE, Balatonfűzfő). This resin was prepared with sodium chloride, according to the procedure described by *Inczédy (1962).* If kept in well-sealed polyethylene bags the protective agent, once prepared, maintains its effectiveness almost indefinitely.

Experimental procedure

The animals were divided into three equal groups, which were to be a control and two treatment groups (groups K, C2 and C4; n=4 in each group). Feeding with the diet including the protective agent began three days prior to the administration of the mercury, to be continued for 13 days; i.e., the duration of the experimental period was essentially 10 days. The treatment involved supplementation, with anion exchange resin in Cl^- form, of the diet given to groups C2 and C4, in quantities of 20 g and 40 g, respectively, per kg feed; the pigs of group K, which was to serve as the control, were given no protective agent. As the acclimatisation period ended 2.50 mg of mercury labelled with Hg-203 isotope was given to each animal. For this purpose 10 cm^3 radioactive $HgCl_2$ solution was soaked up into approximately 100 g of feed containing no protective agent; subsequent to air-dry homogenisation the feed was distributed as appropriate. To ensure that the feed containing the radioactive mercury was consumed quickly and in its entirety, no other feedstuff was provided for the animals at that time.

During the experimental period the selectively collected samples of faeces and urine were weighed and their radioactivity concentration recorded daily. Prior to the measuring of faecal activity concentration each sample was mixed by thorough grinding with a pestle and mortar. After the 10th day of experimental feeding the animals were slaughtered and tissue samples were taken from the *M. longissimus dorsi*, the hepatic lobe and the renal cortex of each carcass. The samples were then pulped in a suitable crushing device, after which their activity concentration was measured.

Nuclear activity measuring technique

The measuring system used consisted essentially of three units manufactured by GAMMA (Budapest): a 256-channel NK 370 type amplitude analyser and a 321 type measuring head fitted with a scintillation detector, connected to an NZ 138 type hollow measuring unit. A computer, printer and colour TV were also linked to the system. The programme which was used to process the signals was developed by the Central Physics Research Institute of the Hungarian Academy of Sciences (Budapest). This system was used for the purpose of measuring the 0.279 MeV γ-radiation of the Hg-203 radioisotope. Measuring times varied between 100 and 1000 seconds, depending on activity.

Processing of the data

Instead of the activity values actually recorded, the values used for data processing were in each case those obtained by calculating back to the point in time at which the mercury was administered. The activity of the faeces and urine for each day was calculated from the corresponding weight and activity concentration, while the concentration of mercury accumulated in the various organs was determined from the activity of the mercury administered (530 kBq) and the activity concentration of the sample. Excretion values were expressed as percentages: activity of faeces or urine in relation to the activity of the mercury administered.

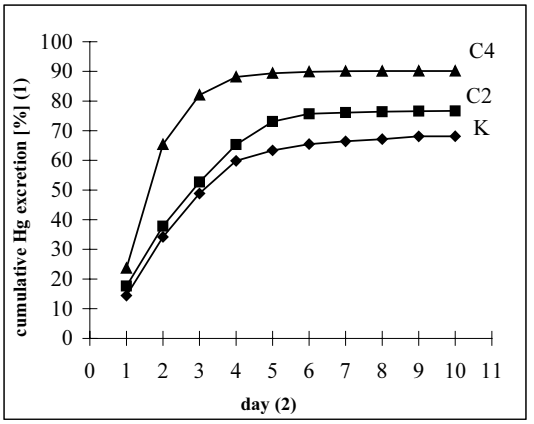
The mathematical and statistical processing of the experimental data consisted partly of two-way regression analysis and partly of the method of regression analysis most commonly used for the purposes of investigating the effect of individual factors (*Baráth et al., 1996*). The *SAS (1985)* software package was used for the processing of the experimental data.

RESULTS AND DISCUSSION

Figures 1 and *2* show time-dependence in cumulative mercury excretion via the faeces and the urine respectively, while *figure 3* illustrates changes with time in the cumulative values for total mercury excretion. (In this paper *total mercury excretion* refers to the entire quantity of mercury excreted with faeces and urine together.) These graphs show mean values corresponding to the respective treatments and durations of time elapsed. In each case the greatest deviation was observed in the second-day mercury excretion of the animals of group K; the values for this were 6.7% and 1.2% for faeces and urine respectively, and 4.9% for total mercury excretion.

Figure 1

Time dependence of cumulative Hg excretion via faeces



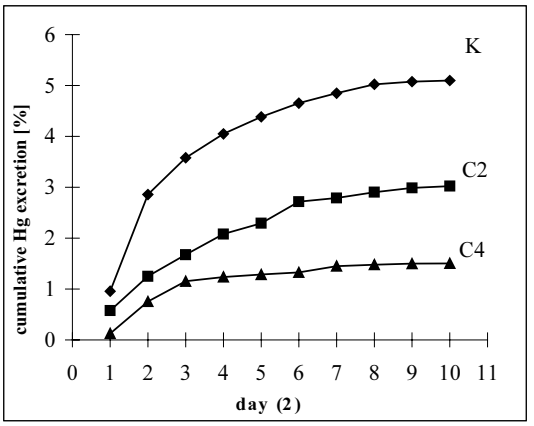
K: control (*kontroll*), C2 and C4: 20 or 40 g VARION AD (Cl^-) added to 1 kg feed. [*C2 és C4: 20, ill. 40 g VARION AD (Cl^-) hozzáadva 1 kg takarmányhoz.*]

1. ábra: A bélsáron keresztüli kumulatív Hg-ürítés időfüggése

Kumulatív Hg-ürítés(1), Nap(2)

Figure 2

Time dependence of cumulative Hg excretion via urine



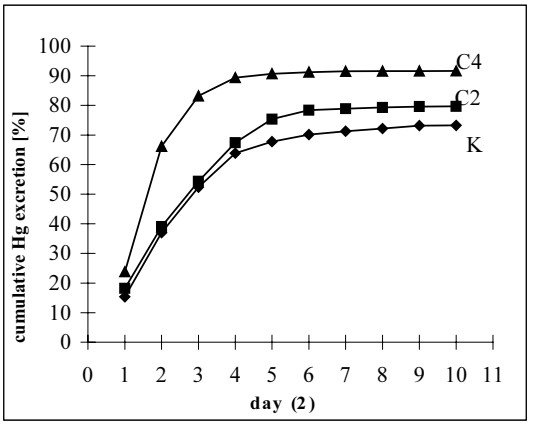
See *figure 1* (*Lásd 1. ábra.*)

2. ábra: A vizeleten keresztüli kumulatív Hg-ürítés időfüggése

Kumulatív Hg-ürítés(1), Nap(2)

Figure 3

Time dependence of the cumulative value of the total Hg excretion



See *figure 1* (*Lásd 1. ábra*.)

3. ábra: Az összes Hg-ürítés kumulatív értékének időfüggése

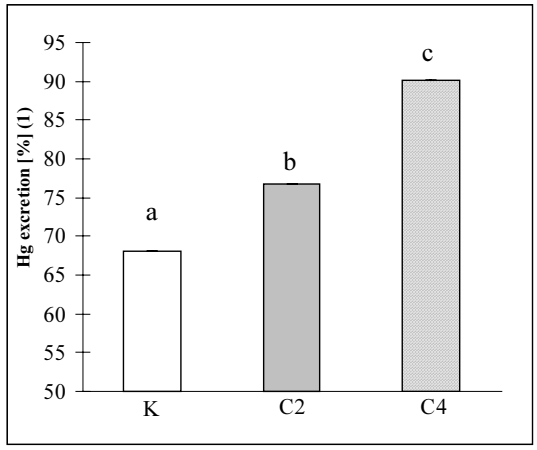
Kumulatív Hg-ürítés(1), Nap(2)

Whichever treatment was examined, mercury excretion via both the faeces and the urine was found to take place predominantly in the first three or four days, and after the fifth day had decreased to an almost negligible level. Although the nature of the time-dependence investigated was not altered by the VARION AD (Cl^-) added to the diet, the resin did exert a substantial influence on the level of mercury excretion and the distribution between faeces and urine of the mercury excreted. The protective agent used increased faecal mercury excretion significantly (*figures 1* and *4*), while decreasing the quantity of mercury excreted in the urine (*figures 2* and *5*). This is in accordance with the finding that a very close negative correlation existed between faecal and urinary mercury excretion (*figure 6*). This could be explained by the theory that it was precisely by reducing the absorption of mercury that the protective agent also enabled mercury excretion via the kidneys to decrease.

An important fact with respect to the accomplishment of the objective of this research is that, even in the case of the control group, it was with the faeces that the greater part of the mercury was discharged from the organism (*figures 1, 2, 4* and *5*). Owing to this, despite the reduction in urinary mercury excretion outlined above, a substantial protective effect was still demonstrable (*figures 3* and *7*).

Figure 4

Hg excretion via faeces in 10 days



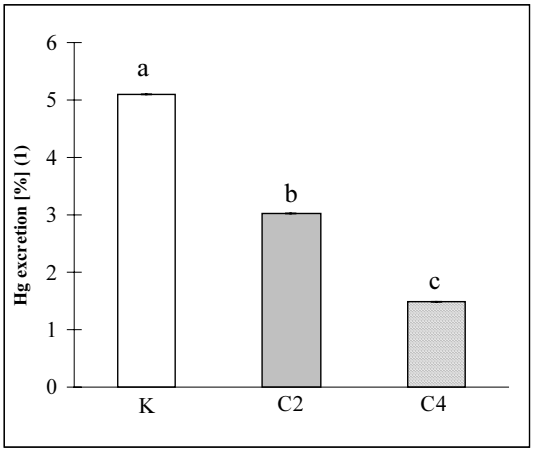
K, C2 and C4: see *figure 1* (*K, C2 és C4: lásd 1. ábra*.) Columns represent mean±S.D. (*Az oszlopok az átlagokat és a megfelelő szórásértékeket mutatják*.) Mean values with different letters differ significantly (P<0.05). (*Az eltérő betűkkel jelölt középértékek szignifikánsan különböznek*.)

4. ábra: 10 nap alatti Hg-ürítés a bélsáron keresztül

Hg-ürítés(1)

Figure 5

Hg excretion via urine in 10 days



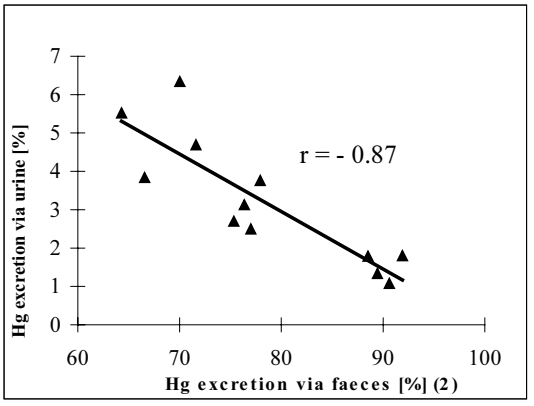
See *figure 4* (*Lásd 4. ábra*.)

5. ábra: 10 nap alatti Hg-ürítés a vizeleten keresztül

Hg-ürítés(1)

Figure 6

Relationship between Hg excretions via faeces and urine in 10 days

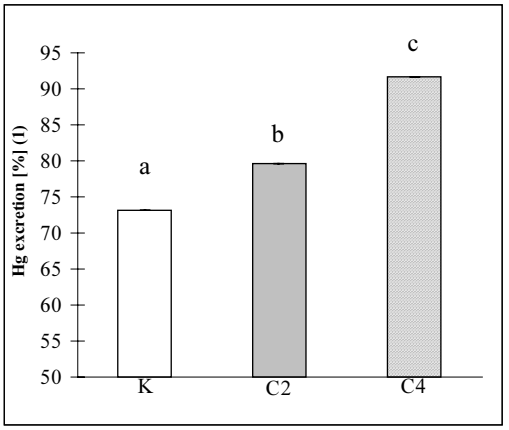


6. ábra: Összefüggés a bélsáron és a vizeleten keresztül 10 nap alatt végbement Hg-ürítés között

Hg-ürítés a vizeleten keresztül(1), Hg-ürítés a bélsáron keresztül(2)

Figure 7

Total Hg excretion in 10 days



See *figure 4* (*Lásd 4. ábra*.)

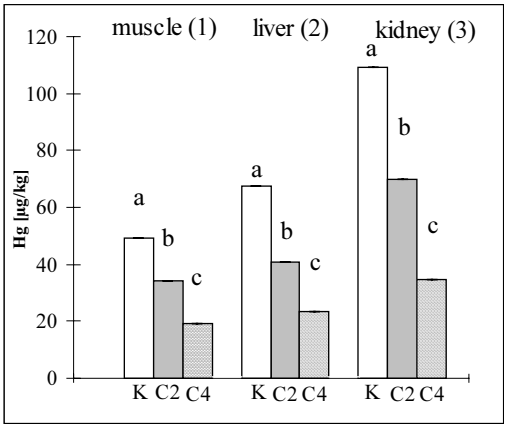
7. ábra: Összes Hg-ürítés 10 nap alatt

Hg-ürítés(1)

Figure 8 shows the average levels of mercury which accumulated in the muscle, the liver and the kidneys, and also the corresponding range of deviation values. It can be seen from this graph that supplementation with the anion exchange resin led to a significant reduction in mercury concentration in these organs.

Figure 8

Concentrations of Hg accumulated in the organs



See *figure 4* (*Lásd 4. ábra*.)

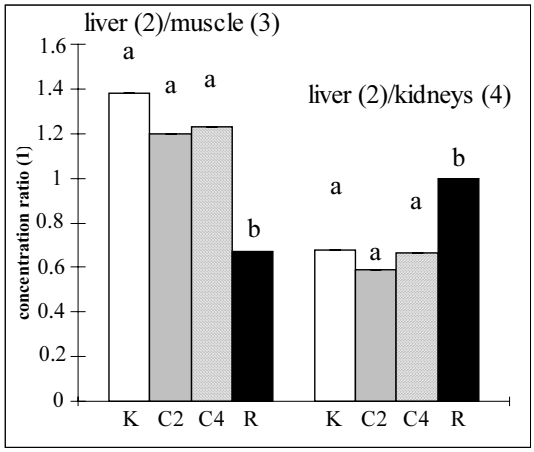
8. ábra: A szervekben akkumulálódott Hg koncentrációja

Izom(1), Máj(2), Vese(3)

Figure 9 illustrates the ratio between the concentrations of mercury which accumulated in the liver and in the muscle, and the corresponding ratio between the liver and the kidney. The results of the analysis of variance procedure for this indicate that the protective agent probably did not make a difference to the distribution of mercury within the organism. However, it is striking that these ratios diverged strongly from those calculated by the authors on the basis of concentration data, also relating to the organs of pigs, obtained by *Rader and Spaulding* (*1979*). Nevertheless, it should be noted that the mercury levels recorded by the above authors were measured under 'practical conditions'.

Figure 9

Ratio of the concentration of Hg accumulated in the liver and the muscle, and in the liver and the kidneys



K, C2 and C4: see *figure 1* (*K, C2 és C4: lásd 1. ábra*.) R: calculated from data published by *Rader and Spaulding, 1979*). (*Rader és Spaulding* (*1979*) *adataiból számolva*.) Mean values with different letters differ significantly (P<0.05). (*Az eltérő betűkkel jelölt középértékek szignifikánsan különböznek*.)

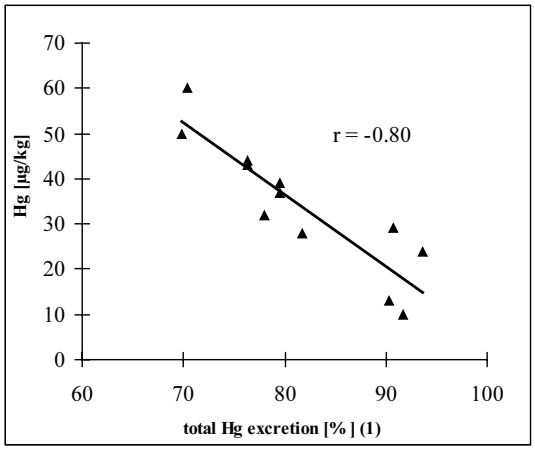
9. ábra: A májban és az izomban, valamint a májban és a vesében akkumulálódott Hg koncentrációk aránya

Koncentráció-arány(1), Máj(2), Izom(3), Vese(4)

A very close negative correlation was found to exist between the mercury levels in the organs examined (or, to be more precise, the concentrations of labelled mercury in these organs) and total mercury excretion (*figures 10* to *12*). Although the nature of these relations can also be deduced from the above data, the high absolute values of the correlation coefficients merit particular attention. Their significance lies partly in the fact that they give an indication of the reliability of the data for excretion and concentration, and partly in that they provide evidence that the muscle, the liver and the kidneys may be regarded as indicator organs with respect to mercury. Other authors have also drawn attention to this aspect of the above two secretory organs (*Szabó et al., 1994*).

Figure 10

Dependence of the concentration of Hg accumulated in the muscle on total Hg excretion

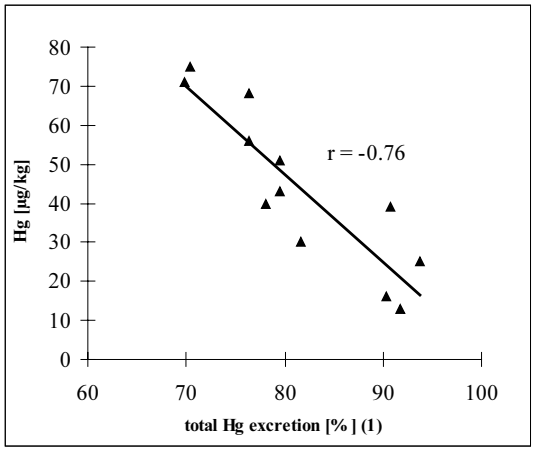


10. ábra: Az izomban akkumulálódott Hg koncentrációjának függése az összes Hg-ürítéstől

Összes Hg-ürítés(1)

Figure 11

Dependence of the concentration of Hg accumulated in the liver on total Hg excretion

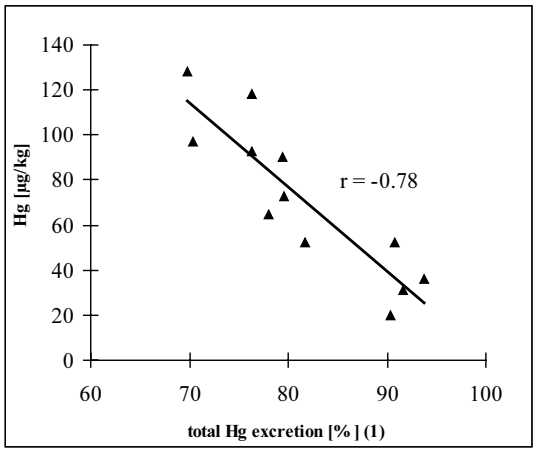


11. ábra: A májban akkumulálódott Hg koncentrációjának függése az összes Hg-ürítéstől

Összes Hg-ürítés(1)

Figure 12

Dependence of the concentration of Hg accumulated in the kidneys on total Hg excretion



12. ábra: A vesében akkumulálódott Hg koncentrációjának függése az összes Hg-ürítéstől

Összes Hg-ürítés(1)

CONCLUSIONS

Faecal mercury excretion was found to rise substantially by the effect of the anion exchange resin in Cl^- form which was used, while the quantity of mercury excreted with the urine decreased to a certain degree. Despite this, total mercury excretion increased significantly, and thus less substantial amounts of mercury accumulated in the various organs ($P<0.05$). The above findings justify the conclusion that the study gave evidence of the protective effect against mercury of the anion exchange resin used. One observation also conforming to the concept of this research is that the increased levels of faecal mercury excretion lasted only a short time after the mercury was administered. This suggests that the protective effect was exclusively attributable to a reduction in the absorption of mercury. Another observation in support of this conclusion is that the use of the protective agent resulted in no change to the ratios between the mercury concentrations measured in the organs examined.

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Corresponding author (*levelezési cím*):

Tamás Rétfalvi
University of Western Hungary, Institute of Chemistry
H-9400 Sopron, P.O. Box 132
Nyugat-Magyarországi Egyetem, Kémiai Intézet
9400 Sopron, Pf. 132.
Tel.: 36-99-518-176, Fax: 36-99-311-103