Possibilities of accelerated elimination of uranium upon its introduction into the body (An Experimental Study)

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To characterize the complex-forming ability of uranyl in combination with various pharmacological medications, the authors derive a function comprising all the main properties of complex-formation: stability constants of the complexes being formed, dissociation and concentration coefficients of the compounds being used, and the pH of the medium. The *in vivo* experiments on acute uranium poisoning show that there is a relationship between this function and the decorporating ability of medications; therefore the suitability of the compounds for decorporation can be estimated according to the value of the function F_c, which can be determined easily and rapidly.

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[English Abstract]

Possibilities of accelerated elimination of uranium in its penetration into the body (experimental studies)
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To characterize the complex-forming capacity of uranium in combination with various pharmacological preparations the authors derive a function comprising all the main effects of complex-formation: constants of the forming complexes stability, dissociation and concentration factors of the used preparations, pH of the medium.
In *in vivo* experiments in acute uranium poisoning it was shown that there was a relationship between the mentioned function and the decorporating capacity of preparations, and therefore the fitness of preparations for decorporation may be estimated by the value of the function which can be easily and quickly determined.

At the present time, the most promising technique for the elimination of radioactive elements from the body is [the use of] chelation therapy, *i.e.*, the use of the compounds which can form strong complexes with these elements, and can thus help eliminate them (V. S. Balabuha; Chalabreysse; Lafuma).

In this work, we studied the possibility of uranium elimination after acute uranium poisoning with the help of various medications widely used in the medical practice.

Thus, we pursued two directions in our work: on one hand, it was necessary to establish the stability of complexes of uranium with those medications; on the other, it was necessary to use preliminary experiments on animals to determine the uranium-decorporating ability of these medications and to connect these results with the data on the complex formation.

1. *In vitro* experimental determination of the suitability of a pharmaceutical medication for decorporation.

Dependence among the distribution (partition) coefficients and stability constants of the forming complexes MeL_n can be expressed in a following equation (F. F. Rossottii, Froneaus):

$$D = \frac{D_0}{1 + \beta_1[L^-] + \beta_2[L^-]^2 + \ldots + \beta_n[L^-]^n}, \tag{1}$$

where β_1, β_2, $\ldots\beta_n$ are step-wise stability constants of the forming complexes, D_o and D are distribution coefficients of uranium in the absence and in the presence of the medicine under investigation, [respectively]. Free concentration of ligands can be expressed through the concentration and dissociation constant of the compound under investigation. (The simplest kind of compound HL is taken, in order not to complicate the subsequent formulas):

$$HL \rightleftarrows H^+ + L^-, \; K_D = \frac{[L^-][H^+]}{[HL]} \tag{2}$$

$$[HL] = C, \tag{3}$$

where C is the concentration of the compound.

This last equation we write with the assumption that the organic compounds being investigated are predominantly hard-to-dissociate and their practical equilibrium concentration is equal to the overall concentration. Based on (2) and (3), we can write:

$$[L^-] = \frac{C \cdot K_D}{[H^+]}. \tag{4}$$

By substituting $[L^-]$ in equation (1) with its value from equation (4), we obtain:

$$D = \frac{D_0}{1 + \beta_1 \frac{K_D \cdot C}{[H^+]} + \beta_2 \left(\frac{K_D \cdot C}{[H^+]}\right)^2 + \ldots + \beta_n \left(\frac{K_D \cdot C}{[H^+]}\right)^n}. \tag{5}$$

After further transformations

$$D + D\beta_1 \frac{K_D \cdot C}{[H^+]} + D\beta_2 \left(\frac{K_D \cdot C}{[H^+]} \right)^2 + \ldots + D\beta_n \left(\frac{K_D \cdot C}{[H^+]} \right)^n = D_0 \qquad (6)$$

we obtain

$$\frac{D_0 - D}{D \cdot C} = \beta_1 \frac{K_D}{[H^+]} + \beta_2 \frac{K_D^2 \cdot C}{[H^+]^2} + \ldots + \beta_n \frac{K_D^n \cdot C^{n-1}}{[H^+]^n} \ . \qquad (7)$$

The right part of this equation contains the stability constants of the forming complexes ($\beta_1 \ldots \beta_n$), concentrations of the compounds being investigated (C), their dissociation constants in a particular (any given) medium (K_0), and the pH of the medium, so this part of the equation will quantitatively characterize all the complexation effects together, for a particular element in a solution. This function we simply call function F_c, which is:

$$F_c = \frac{D_0 - D}{D \cdot C} \ .$$

From the practical point of view, the introduction of this function makes sense, since its relative use is paid off by the much smaller investment of labor and time for its determination, compared to the determination of the true stability constants of the complexes. The meaning of stability constants can also be rather approximate under the conditions inside the body, even without mentioning the fact that the dissociation constant of the medicine being introduced (*i.e.*, the concentration of free ligands and the corresponding degree of complexation of an element) in body fluids would be analogous to the one extrapolated from the ideal solution conditions.

Determination of the function F_c values. Distribution coefficients D_0 and D were determined under the statistical conditions of the cation exchange. 20 mg of resin (cationite DOWEX 50×8) was weighed and, with shaking, was introduced into contact with 50 mL of the experimental solution (a physiological solution, containing uranium and various concentrations of the medicines under investigation). Preliminary determination of time necessary to establish the equilibrium for cation exchange showed that 1.5 hours of contact time was sufficient. Experiments were conducted using the following conditions:

1) DOWEX 50 cationite was taken in its Na form. Many researchers have shown that the use of the Na form of the cationite avoids the sorption of the positively charged complex ions, and also to avoid the sorption of the compounds being investigated. We chose the Na form primarily to avoid the change of pH of the experimental solution occurring because of the release of H^+ ions during the ion exchange;
2) pH of the solution was constant (we chose the pH value of 6.0 ± 0.2; it would be more appropriate to study these processes at the physiological pH, but in that case we would have to decrease the concentration of uranium so significantly, in order to avoid the colloid formation, that it would have negative effects on the precision of analytical determinations);
3) concentration of the element [uranyl] was much lower than the concentration of electrolytes (physiological solution, $\mu = 0.155$);

4) the amount of resin and the volume of solution was constant in all experiments.

During the evaluation of the results, we took the following facts into the account:

A. Under our experimental conditions, only ion exchange takes place. This issue should be discussed in more detail. First of all, it should be ascertained, whether the sorption of the positively charged complexes on the highly acidic cationite (chosen by us) is possible, and whether cationite, as a material with a large surface area can adsorb the compounds under investigation or their complexes.

Since the solution contains a significantly higher concentration of Na ions compared to the ions of the compounds under investigation, sorption of positively-charged complexes cannot happen. Furthermore, it should be taken into account that this resin is microporous and thus does not posses any specific properties with regard to the organic molecules (small pores prevent the internal diffusion of the large organic molecules and also prevent the "tamsit effect")

The question of the surface adsorption is decided by the choice of the resin type. Presence of only nonfunctional groups capable of ionic exchange practically excludes the formation of hydrogen bonds and van-der-Waals forces between the resin and the organic molecule. In addition, we studied the adsorbing ability of divinylbenzene polymer without active groups (resin matrix) with regard to the positively-charged complexes. The obtained value of the distribution coefficient is so low that it could be neglected.

B. Hydrolysis of the element and the related competitive complex formation should be excluded, since they complicate the exchange process. It is quite possible to assume the presence of the various hydrolysis products, but the absence of their significant influence on the experimental results can be proven by the fact, that the value of the distribution coefficient for an element (uranium) was determined by us for a cation exchange process in the pH range between 3.0 and 7.0, and this value at our concentration did not depend on the pH.

In conclusion we mention that the distribution coefficient of an element between phases was determined using the following formula:

$$D = \frac{C_0 - C_e}{C_e} \ ,$$

where C_0 is the initial concentration of the element, and C_e is the equilibrium concentration of the element.

Uranium was determined using the protocol previously described by us (Csövari and Molnar). The introduction of the F_c function significantly accelerated and simplified the investigation of the complex formation processes with the goal of choosing various medicines for chelation therapy of elements incorporated in the body, in general.

2. *In vivo* experimental determination of the suitability of a pharmaceutical medication for decorporation.

We studied 43 different pharmacological compounds. Here we present only some of our data (Table 1).

Table 1 shows that the highest values of function F_c was observed for oxytetracycline and arsotonin (an organic compound of arsenic). These compounds are more important than EDTA

and bicarbonates, which have been recommended for the elimination of uranium (V. N. Gus'kova).

For *in vivo* experiments, compounds from Table 1 were selected. The experiments were done using white rats (experimental hybrid CFY of the Agrarian University of the city of Gedelle), poisoned with uranium *via* the intramuscular introduction of uranyl acetate over 8 days, using 100 mcg of uranium per day. The average mass of rats before the poisoning was 450 g, the average mass after poisoning – 350 g. Decorporation lasted for 8 days, during which 5 intramuscular injections were administered, 40 mg per rat. Decorporation effect was evaluated by elimination of uranium with urine, by uranium concentration in the critical organics and in the whole body, and on the basis of certain physiological factors: change in weight, decrease in animal death. Uranium was determined using the protocol previously described by us (Csövari and Molnar).

Table 1. Complex formation characteristics of medicines

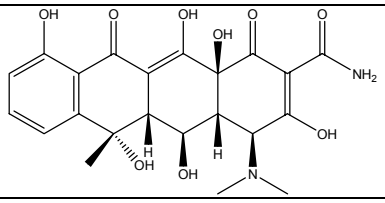
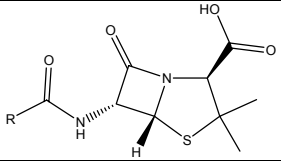
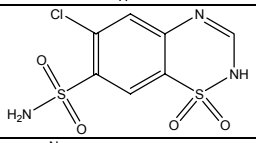
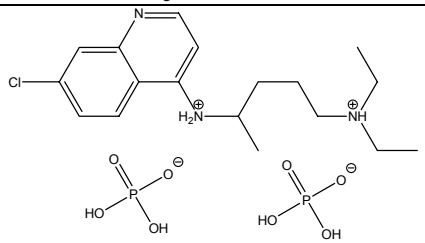
Name of the medicine	Value of F_c	Chemical structure
Oxytetracycline	$3.0 \cdot 10^4$	
Arsotonin	$3.6 \cdot 10^4$	$Ca[AsO_3CH_3]$
Sodium bicarbonate	$1.36 \cdot 10^4$	Na_2CO_3
Atriphos (ATP)	$1.22 \cdot 10^4$	(ATP)
"Edtakal"	$1.05 \cdot 10^4$	Potassium salt of EDTA
Penicillin	$0.81 \cdot 10^3$	
Chlorurit [Chlorothiazide, a diuretic]	$5.5 \cdot 10^3$	
Delagil	$0.24 \cdot 10^3$	

Figure 1 shows the changes in the animal weight during the treatment. When oxytetracycline and arsotonin were administered, the weight of the rats reached the weight before the uranium poisoning and even exceeded it, while the weight of the control rats fell sharply. Figure 2 shows the elimination of the uranium with urine during decorporation. When oxytetracycline and arsotonin were administered, uranium elimination is almost twice that of the control.

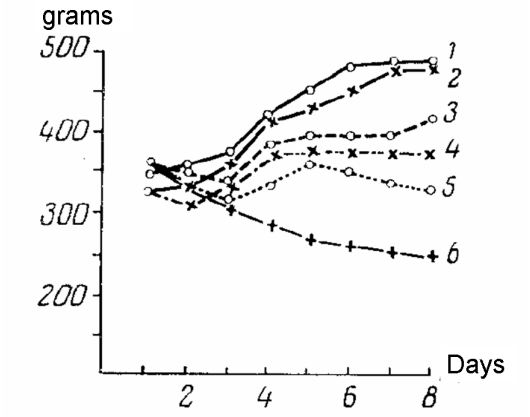


Figure 1. Changes in the animal weight during the course of treatment. 1-oxytetracycline; 2-arsotonin; 3-edtakal; 4-atriphos (ATP); 5-chlorurit; 6-control.

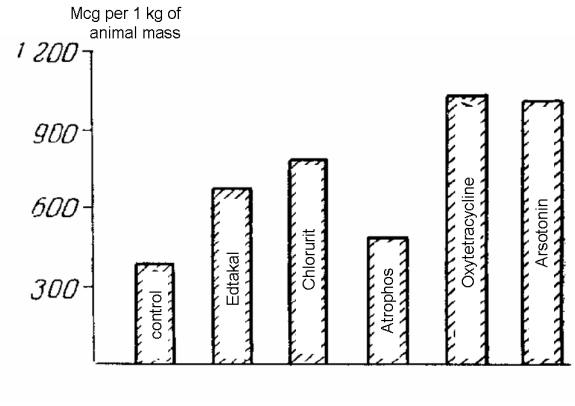


Figure 2. Elimination of uranium with urine during decorporation.

Table 2 consolidates the data on the uranium concentration in the body and on the animal mortality during the decorporation period. These data show that during the administration of chlorurit and tetracycline animal mortality was not observed, during the administration of EDTA it was 50 %, while in the control group it reached 65 %. Regarding the uranium concentration in the body, the maximum decorporation effect was observed during the administration of oxytetracycline and arsotonin: uranium concentration decreased almost 2 times, during the administration of EDTA – by 33 %, compared to the control.

Table 2. Decorporating characteristics of the medicines in animal experiments.

		Control	Edtakal	Chlorurit	Atriphos (ATP)	Arsotonin	Oxytetracycline
Mortality	abs.	13	10	–	5	5	–
	%	65	50	–	25	25	–
Uranium concentration							
Kidneys	mg	72±13	42±7	29±6	53±9	33±8	23±6
	%	100	58.1	40.4	73.6	45.8	32
Bones	mg	204±14	142±9	162±15	143±8	100±12	92±8
	%	100	69.5	79.5	70	49	45
Whole body	mg	396±26	267±39	290±21	358±15	210±12	214±15
	%	100	67.5	73	90	53	54

These data undoubtedly show that the compounds for which function F_c has a high value are also the most effective in *in vivo* experiments. Let us note the observed facts, connected first of all to the non-chelation action of the pharmacological compounds. During the chlorurit therapy not only no mortality was observed, but also the concentration of uranium in the kidneys significantly decreased, although the overall decorporation effect of this compound is not high. This positive action can be explained by diuretic properties of the compound. In the case of ATP, whose complex formation reactivity with uranium was not bad in *in vitro* experiments, the accelerated uranium elimination from the bones was accompanied by the accumulation of uranium in the muscle and brain tissues. It looks like the redistribution of uranium in the body is associated with the metabolic interconversion of ATP. These data indicate that during the administration of the pharmacological drugs, the specific effect of the drugs on the organism should be taken into the account as well.

Based on our experiments, we were able to show a good correlation between the complex formation function F_c, which was introduced by us and whose determination is very simple, and the decorporation effect of the particular compound.

Thus, for characterization of the complex formation ability of uranium with various pharmacological drugs, we introduced function F_c, describing and including in itself all the basic effects of the complex formation: stability constants for the complexes being formed, dissociation constants, and concentration of compounds being used, as well as the pH of the medium.

The *in vivo* experiments on the acute uranium poisoning showed that there is a mutual dependence [correlation] between the value of this function and the magnitude of the decorporating ability of the compounds, and thus the suitability of the compounds for decorporation can be evaluated using the value of the F_c function, which itself is easily and rapidly determined.