Complex Formation of Natural Uranium in Blood
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[English Abstract]
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Summary

Under study was the distribution of uranium in the blood. It is shown that this distribution is regular. It was found that uranium is bound with erythrocytic membranes. The authors calculated the constants of complex formation of uranil-ion with bicarbonate, plasma albumin and lipoprotein of erythrocytic membrane.

The studies of the complex formation of natural uranium in blood are very important in resolving various problems of uranium toxicology related both to the accumulation of the element in different organs and to its decorporation.

The existing data (L. Downs; V.S. Balabuha) lead to the conclusion that uranium introduced in the blood is basically distributed between the proteins and bicarbonate salts of the plasma. Concerning the bonding of uranium in the erythrocytes, there are no specific pathways [*i.e.*, non-specific binding occurs]. Fandl has studied in detail the interaction of multivalent metal cations with erythrocytes and has found that some of them (Pb, In, Be, La, Al, Th, Ti, Cr) form a bond with the membrane of the red blood cells. This bond can lead to an agglutination of the latter as a result of the decrease in the surface charge. However, the existing proteins in the blood protect the erythrocytes by adsorbing on their surface and forming complexes with the metal cations. Erythrocytes with a metal-protein complex on their surface are very sensitive and have a tendency towards deposition [and storage] in the spleen (Tompkins and Gustavson).

One can imagine that the uranyl ion behaves similarly with respect to the membrane of the erythrocytes. We decided to study the distribution of uranium in blood and the thermodynamic characteristics of this distribution. For this purpose, we used α-microphotography [autoradiography] of blood samples obtained from people exposed to uranium, which allowed the determination of the localization of uranium in the plasma and erythrocytes.

After this, the *in vitro* experiments were conducted using normal human blood (in a solution of heparin) and a 50% suspension of erythrocytes (rinsed four times with physiological solution). Uranyl nitrate was used in the experiments. The experiments were carried out at room temperature.

Uranyl nitrate was added to a known amount of blood or erythrocyte suspension. Upon achieving equilibrium, the mixture was centrifuged for twenty minutes at 4000 rpm. The blood plasma was subjected to ultrafiltration through a semipermeable membrane. In this way, the protein (albumin) part of the plasma was separated from the inorganic ions (proteins with molecular weights larger than 60,000 do not filter through). The erythrocytes were subjected to hemolysis using distilled water. Because the lipoprotein membrane is soluble in water, the concentration of NaCl was again brought to 0.9% using a 5% solution of table salt. Then the hemoglobin was separated from the membrane using centrifugation. We quantified the uranium [in] each fraction of interest. In order to oxidize the organic part, concentrated nitric acid and hydrogen peroxide were used. After oxidation, the sample was dried by evaporation and the sediment was washed with ammonium carbonate onto a metallic dish. The contents were evaporated under a strobe light [an infrared lamp, ???], then calcined, to transform the uranium into its oxide form and the α-activity was measured with a scintillation counter. In this way the distribution of different amounts of uranium was studied: 10, 15, and 20 μg of uranium in 5 mL of blood and the distribution of 10 μg of uranium in different volumes of blood. The data are shown in Tables 1 and 2.

Table 1

Distribution of different amounts of Uranium in 5 mL blood.

Starting Amount (in μg)	Analyzed Fraction	Amount of Uranium in each fraction	
		μg	%
10	Erythrocytes	2.0	20
	Proteins	3.2	32
	Inorganic part of plasma	4.7	47
15	Erythrocytes	3.4	23
	Proteins	4.8	32
	Inorganic part of plasma	6.4	42
20	Erythrocytes	5.0	25
	Proteins	5.8	29
	Inorganic part of plasma	8.4	42

Table 2

Distribution of 10 μg of Uranium in different volumes of blood.

Volume of blood (in mL)	Analyzed Fraction	Amount of Uranium in each fraction	
		μg	%
2	Erythrocytes	2.3	23
	Proteins	3.0	30
	Inorganic part of plasma	4.5	45
5	Erythrocytes	2.0	20
	Proteins	3.2	32
	Inorganic part of plasma	4.7	47
10	Erythrocytes	1.9	19
	Proteins	3.3	33
	Inorganic part of plasma	4.9	49

Note: Statistical error of α-activity measurements ± 10%.

From Tables 1 and 2, it is clear that uranium is distributed in blood not only between the protein and non-protein part of the plasma, but is also bound with the erythrocytes. The kinetics of the distribution of uranium is illustrated in Figure 1, where it can be seen that the equilibrium is achieved after a few minutes.

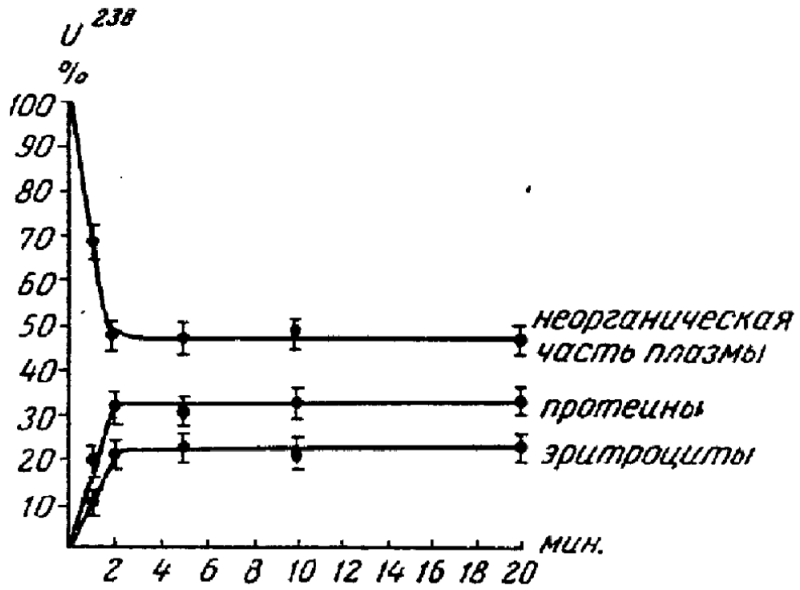


Fig. 1. Rate of distribution of U in blood.

In addition, experiments were carried out to investigate the interaction of uranium with the erythrocytes. Washed red blood cells were used in the experiments. The rate of the interaction of the uranyl ion with the erythrocytes is shown in Figure 2. From the figure, it is clear that that equilibrium is also reached in a few minutes. The hemolysis of the erythrocytes bound to uranium showed that this element is concentrated only in the membranes of the red blood cells. The dependence of the amount of uranium reacting with the erythrocyte membranes on the pH is shown in Figure 3.

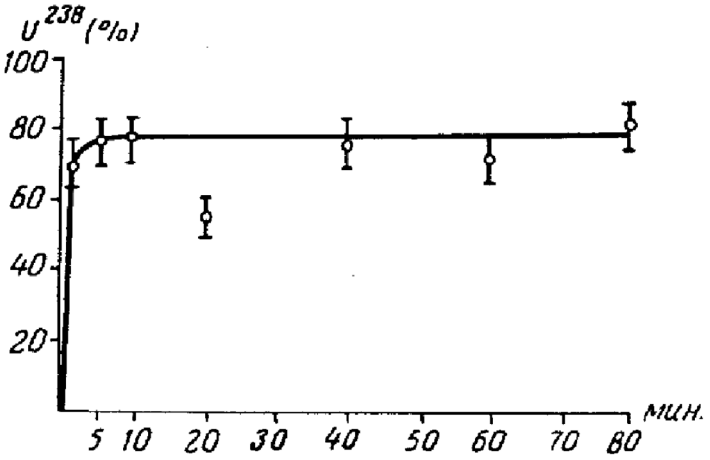


Fig. 2. Rate of bonding of Uranium with erythrocytes.

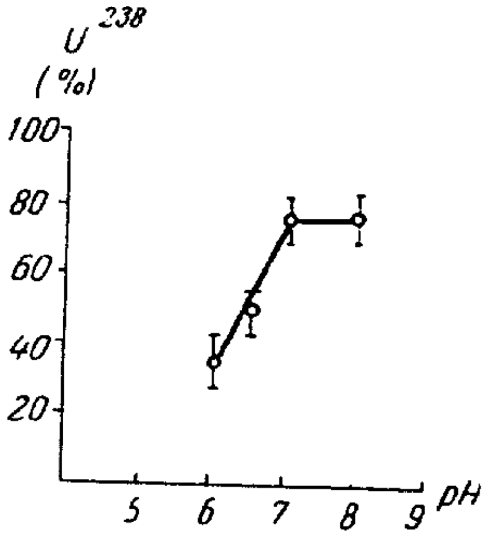
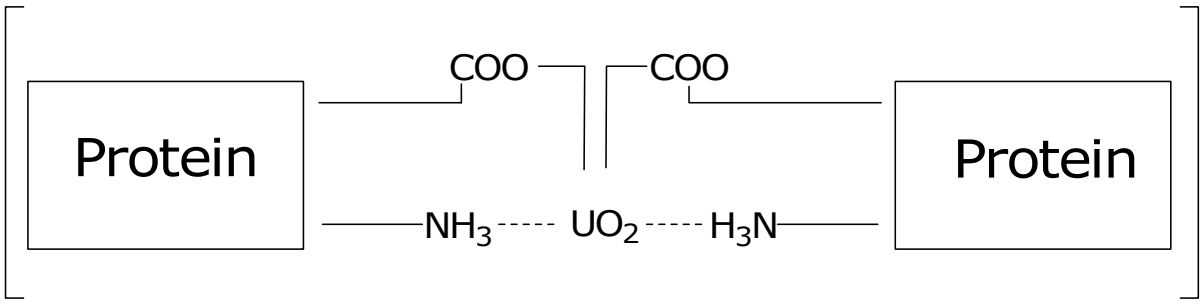


Fig. 3. Dependence of the bonding of Uranium with erythrocytes on pH.

From this Figure [3], it can be seen that the ability of the membranes to react with the uranyl ion decreases sharply upon decreasing the pH from 7.0 to 6.0. It seems that this can be explained [by noting] that the pH decrease leads to the decrease in the dissociation of the carboxylic groups of the lipoprotein, which composes the bulk of the erythrocytes. The maximum dissociation of the carboxyl group of proteins is reached at pH $\geq$ 7.0 (L. Downs).

On the basis of the experimental data, we calculated some thermodynamic constants of these reactions.

To simplify the calculations, we assumed, in full agreement with the literature (L. Downs; Bersen; V.S. Balabuha), that the distribution of uranium in the inorganic part of the plasma originates from the formation of the bicarbonate complex $[UO_2(HCO_3)_4]^{2-}$ [sic], while its distribution in the protein part of the plasma comes from the formation of uranyl-albuminate:



The bond of uranium with the erythrocyte membranes was considered as a uranyl-lipoprotein complex (by analogy with albumin).

It is necessary to mention that this interaction was greatly simplified. It needs to be considered that the complex formation of the uranyl ion in the blood is multifaceted and is not restricted to just these three complexes. Moreover, the mechanism of these reactions is more sophisticated. At the normal blood pH, the carboxylic groups of proteins are sufficiently ionized to be able to bind the existing ions, for example, calcium and

magnesium. It can be assumed that when uranium is introduced in the blood, the calcium and magnesium bonded to the protein carboxyl groups can be exchanged with divalent uranyl ions. The complexity of this type of reaction does not allow the determination of the exact mechanism of this phenomenon. Apparently, an isotopic exchange occurs that does not suppose a full chemical equivalency of the exchanging elements.

To calculate the thermodynamic constants and the Gibbs free energy we used the well known formulas (N.N. Zheligovskaia).

Equilibrium constant (dissociation [instability] constant): $\mathrm{K_{eq}} = \mathrm{K_{dissoc}} = \frac{[A]\,[M]}{[A_n M]}$

Stability constant: $\mathrm{K_{stab}} = \frac{1}{K_{dissoc}}$

Formation function: $\mathrm{n} = \frac{[C_A] - [A[}{[C_M]}$

Degree of complexation: $\Phi = \frac{[C_M]}{M}$

Degree of complex formation: $Q_m = \frac{[A_n M]}{[C_M]}$

The change of the Gibbs energy: $\Delta G = -2.303 RT \lg K_{eq}$, where $[C_A]$ is the total concentration of the ligand; $[C_M]$ is the total concentration of the uranyl ions; [A] is the equilibrium concentration of the ligand; [M] is the equilibrium concentration of the uranyl ions; $[A_nM]$ is the concentration of the complex.

Table 3

Complex Characteristics (in mol/L)

Complex	$[C_A]$	$[C_M]$	$[A]$	$[M]$	$[A_n M]$
Uranyl-bicarbonate	5×10^{-5}	13×10^{-6}	2.8×10^{-5}	7×10^{-6}	5×10^{-6}
Uranyl-albumin	2.2×10^{-6}	13×10^{-6}	1.4×10^{-5}	9×10^{-6}	4.3×10^{-6}
Uranyl-lipoprotein	2.5×10^{-5}	13×10^{-6}	1.9×10^{-5}	10×10^{-6}	3×10^{-6}

The data needed for the calculations are given in Table 3 for each of the complexes. These data were calculated on the basis of the investigations of the distribution of uranium in blood using ultrafiltration (V.S. Balabuha).

The thermodynamic constants of the complex formation are given in Table 4.

Table 4

Physical chemistry constants of complex formation

Complex	K_{dissoc}	K_{stab}	n	Φ	A_M	ΔG (kcal · mol^{-1})
Uranyl-bicarbonate	8.6×10^{-19}	0.11×10^{19}	1.7	1.9	0.46	-23.04
Uranyl-albumin	3.6×10^{-11}	0.3×10^{11}	0.6	1.4	0.31	-8.08
Uranyl-lipoprotein	1.2×10^{-9}	0.8×10^{9}	0.46	1.3	0.23	-7.32

Thus, calculated constants for the three complexes formed by uranium in blood show that the bicarbonate complex is the most stable, followed by the uranyl-albuminate and the uranyl-lipoprotein complexes. These three complexes are in equilibrium. The removal of the uranyl-bicarbonate complex from the blood (for example by filtration through the kidneys) perturbs the equilibrium in the system. But because in the blood there is always a significant amount of bicarbonate ions, which form more stable complexes with the uranyl ion than the proteins, it is possible to reestablish the equilibrium by breaking up the uranyl-protein complexes and forming uranyl-bicarbonate complexes (in amounts determined by the equilibrium). The uranyl-bicarbonate complexes are again removed from the plasma through the kidneys, which again perturbs the equilibrium and the system again tries to restore it. This process continues until the full removal of the uranium from the blood. The existence of such a mechanism can explain, for example, the quick liberation of uranium from the blood, which follows an exponential decay law (D.I. Zakutinski; Jackson and Luessenhop).

The determination of the stability constants of the complexes of the uranyl ion with bicarbonate and albumin in the plasma, and also with the lipoprotein membranes of the erythrocytes can, to a certain extent, help the search for effective ligands for the more rapid elimination of uranium from the blood and prevention of its accumulation in various organs.

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