Polarographic Determination of Uranium in Blood*

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A polarographic method has been developed for the determination of uranium in blood. Blood is destroyed with nitric acid and hydrogen peroxide and the uranium extracted with tetrahydropyrane in a micro liquid-liquid extractor. The polarographic determination is performed in a supporting electrolyte containing 0.16% salicylic acid, 0.4% v/v sulphuric acid, and 0.009% thymol. The method is applicable to the determination of uranium in blood in amounts ranging from 5 μg upwards.

Numerous methods have been employed for the determination of uranium but none of them seems to be satisfactory when small amounts of uranium in biological material are involved. According to J. F. Flagg[1] volumetric methods require a total amount of at least 500 μg of uranium, spectrophotometric methods using ferrocyanide 80 μg, polarographic methods 50 μg, and spectrochemical methods 10 μg (for pure aqueous solutions). Fluorometric methods, although very sensitive (0.5 μg) are not convenient, since a great number of fluorescence quenchers are always present in blood and several chemical operations, often very complicated, are necessary to eliminate them. In the available literature we have found only one method for polarographic determination of uranium in serum[2], but the sensitivity of this method is not stated.

In a previous communication from our laboratory[3] a sensitive polarographic method for determining uranium has been proposed. The present paper reports an attempt to apply this method to the determination of small amounts of uranium in blood.

Direct polarographic determination in the solution remaining after the mineralisation of blood was first tried, but other ions, normally present in blood, made that impossible. It was decided, therefore, to separate uranium from other ions prior to polarographic analysis; the most convenient method seemed to be extraction with a suitable solvent. We found that extraction with tetrahydropyrane[5] — which is extensively used in chromatography of uranium[4] — is complete and fairly selective in the presence of nitric acid. However, this operation alone did not solve the problem completely. In the course of extraction a brown resinous product was formed (probably a tetrahydropyrane polymer) which interfered with polarographic determination. Further investigation showed that the interfering substance could be removed

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by heating with nitric acid and hydrogen peroxide. It is assumed that after these procedures all the uranium is present in the + 6 form.

APPARATUS AND REAGENTS

The extraction of uranium from mineralised blood was carried out by means of an automatic micro liquid-liquid extractor for continuous extraction designed and developed in our laboratory[5].

The dissolution of the residue remaining after extraction was carried out by means of a specially designed membrane micro-pipette, wich was used also as a micro volumetric flask of 200 or 500 microliters. This pipette is an improved modification of the micro-burette described by Gorbach[6]. The main advantage of this type of membrane arrangement is the possibility of changing the pipette. The rubber gasket *E* (Fig. 1) fastened by the nut *F* enables any pipette to be easily mounted to the membrane arrangement. The screw *A* which moves the rubber membrane has a very fine thread (the thread of the screw is 0.3 mm). Thus a fine, slow suction of even smallest amounts of liquid is possible and, consequently, a very precise dosage of the desired volume. The lock-nut *B* and the nail *C* protect the rubber membrane *D* from perforation by the screw *A*.

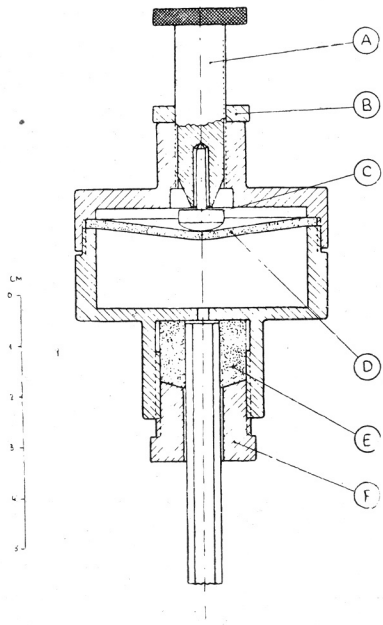

Fig. 1. Membrane pipette. *A* screw, *B* lock-nut, *C* nail, *D* rubber membrane, *E* rubber packing *F* nut

A Cambridge polarograph, Pen Recording Type was used for recording polarographic waves. Specially designed polarographic micro cells for a total volume of 200 or 500 microliters were used. During measurement the cells were kept in a constant temperature water bath at 25.0 ± 0.1°C.

Reagents: Nitric acid, reagent grade (Malincrodt, Saint-Louis), redistilled; hydrogen peroxide, reagent grade (Merck, Darmstadt); tetrahydropyrane (Eastman-Kodak, Rochester).

The preparation of the supporting electrolyte and the standard solution of uranium has been described elsewhere[3].

PROCEDURE

Mineralisation

Blood is mineralised by heating with nitric acid and hydrogen peroxide. 5 ml of blood is transferred to a 100 ml Kjehldahl flask, 15—20 ml of concentrated nitric acid added in portions of 5 ml and the mixture heated gently on an electrically heated air bath until the vigorous initial reaction subsides. The contents are evaporated to dryness. A further portion of nitric acid is added only when the preceding one has evaporated to dryness. When the liquid in the flask becomes clear and practically colourless, 10 ml of 30% hydrogen peroxide are added. The flask is put again on the air bath and heated to boiling. When the vigorous reaction starts, the flask is removed from the bath until the vigorous reaction ceases. The solution is next evaporated just to dryness, and then heated with a small quantity of redistilled water until all soluble material has dissolved. The contents of the flask are then transferred to the contact vessel of the micro-extractor.

Extraction of uranium

The solution obtained after mineralisation (about 10 ml) is extracted with about 8 ml of tetrahydropyrane for two hours in the micro-extractor as mentioned before.

The resinous product formed during extraction at elevated temperature is then destroyed with nitric acid and hydrogen peroxide in the same way as described before in the procedure for the mineralisation of blood. At this step great care should be taken to evaporate even the last traces of tetrahydropyrane before adding nitric acid, since tetrahydropyrane, similarly to diethylether[7], reacts with nitric acid (especially in the presence of uranium) very vigorously, sometimes even with violent explosions.

The residue is then transferred into a crucible, evaporated to dryness on a water bath and ignited at nearly red heat in an electrically heated oven.

Preparation of the solution for polarography

After mineralisation the residue is dissolved in 500 microliters of supporting electrolyte consisting of 0.16% salicylic acid, 0.009% thymol and 0.4% v/v sulphuric acid. Dissolution is carried out by adding a small amount of dilute sulphuric acid to the residue in the crucible and heating it over a water bath until all the water has evaporated and only the residue with some sulphuric acid is left in the crucible. Thereupon the residue is dissolved by successive addition of the remaining part of the electrolyte and by drawing the solution into the membrane micro-pipette. Finally the solution in the pipette is made up to volume (500 microliters). Attention should be paid to the fact that the amount of the sulphuric acid previously added should be sufficient to make the composition of the solution in which uranium is dissolved the same as mentioned when discussing the supporting electrolyte.

Oxygen is displaced by bubbling hydrogen through the solution for 10 minutes prior to the polarographic determination. The hydrogen is purified by being passed through a saturated solution of pyrogallol in sodium hydroxide.

The wave heights were measured according to the point method i. e. the increment in current between the extrapolation lines for the residual current and the diffusion current was measured at the half-wave potential.

ANALYSIS OF THE CALIBRATION CURVE

In order to establish the calibration line different quantities of standard solution of uranium were added to the same blood. Statistical analysis of the results gave the following regression equation (for the capillary and the apparatus used):

$$C = 7.71 \times h \ (\mu g \text{ U}/100 \text{ ml blood})$$

where h is the wave height in mm.

The standard error of the slope of the regression line amounted to ± 0.18. The regression of uranium concentration C upon wave height h and the 95% confidence limits for the prediction of C from the measured values of h are shown in Fig. 2. The standard errors and the relative standard errors for the concentration range up to 400 micrograms of uranium per 100 ml blood are given in Fig. 3.

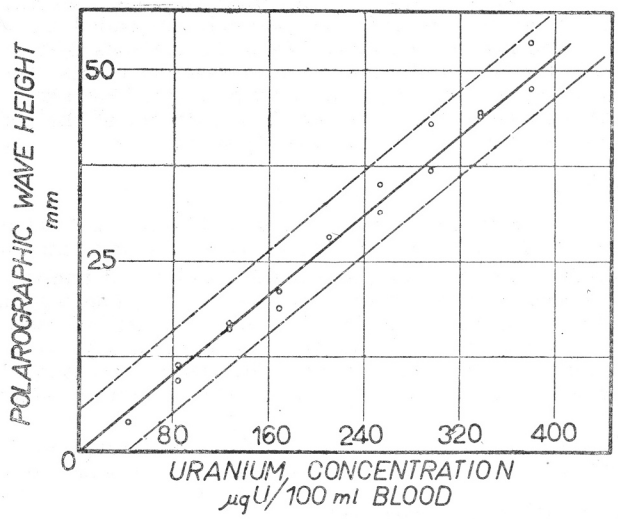

Fig. 2. Regression of C upon h and 95%-confidence limits for predictions of C from measured values of h. Uranium concentration C in µg/100 ml blood. Wawe-heights h in mm

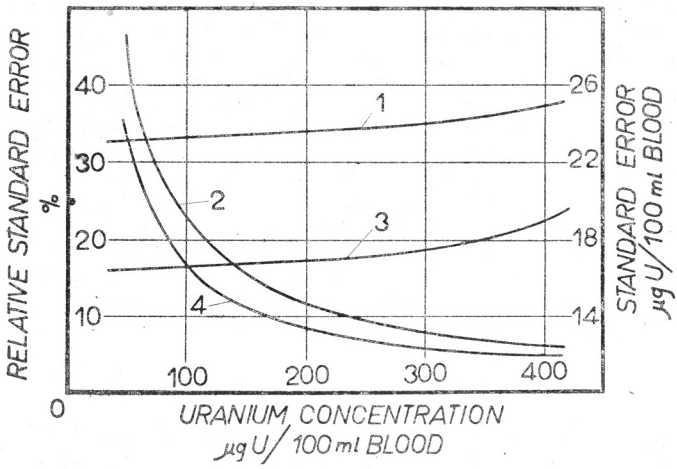

Fig. 3. Standard error (1, 3) and relative standard error (2, 4) of concentration v. uranium concentration in µg/100 ml blood. Curves (1) and (2) show the errors of a single determination. Curves (3) and (4) show the errors of two parallel determinations

These results show that the method described is sufficiently sensitive and precise even for low concentrations of uranium in blood. The standard error

for a single determination is not more than ± 25 μg U/100 ml blood. If two parallel determination for each sample are carried out, the standard error may be reduced to ± 19 μg U/100 ml blood.

The total amount of uranium in blood which can be determined by this method with sufficient precision need not be greater than 5 μg U in blood samples of 5 ml, corresponding to 100 μg U/100 ml blood. This sensitivity is satisfactory for the industrial-hygiene purposes and for toxicological research.

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IZVOD

Polarografsko određivanje urana u krvi

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Opisana je polarografska metoda za određivanje malih količina urana u krvi. Glavne točke opisane metode su ove: 1. Razaranje organske supstancije dušičnom kiselinom i vodikovim peroksidom; 2. Ekstrakcija urana iz mineralizirane krvi s pomoću tetrahidropirana u specijalnom mikroekstraktoru[5]; 3. Polarografsko određivanje u osnovnom elektrolitu, koji se sastoji od salicilne i sumporne kiseline, te timola.[3]

Statistička obradba dobivenih rezultata pokazala je, da je opisana metoda osjetljiva i dovoljno precizna za kliničke i toksikološke analize. Standardna pogreška kod pojedinačnog određivanja u području do 400 μg U/100 ml krvi iznosi manje od 25 μg U/100 ml krvi. Vrši li se određivanje s dva paralelna uzorka, standardna se pogreška u određivanju smanjuje na manje od 19 μg U/100 ml krvi.

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