CCA-747

541.138:612.1
Conference Paper

Electrocatalysis Applied to Electrochemical Investigation *in vivo**

J. Pradáč, N. Ossendorfová, and J. Koryta[a]

Institute of Biophysics, Faculty of General Medicine, Charles University, Prague 2, and [a]*J. Heyrovský Institute of Physical Chemistry and Electrochemistry, Czechoslovak Academy of Sciences, Prague 1, Czechoslovakia*

Received October 16, 1972

The potentialities of electrochemistry for *in vivo* measurements are discussed. The advantages of cyclic voltammetry at inert electrodes for this purpose are pointed out. The voltammetric response of blood serum is explained.Cysteine and ascorbic acid are shown as suitable electrochemical indicators for *in vivo* measurements. A method based on cyclic voltammetry of ascorbic acid as indicator is suggested for assessment of the blood circulation of kidney determined for transplantation.

Significance of Electrochemical Measurement in vivo

The introduction of an electrode into a living system with the aim to obtain relevant data about that system is connected with complications of two kinds, one connected with disturbing interference of the electrode with the system under investigation, the other one arising from the distortion of the sensor by the system under investigation. The former difficulty depends generally on the ratio of the size of the electrode to the size of the system under investigation, be it a cell, tissue, organ or cell culture. Thus, significant data on individual cell membrane potentials were not obtained before giant squid axons were not detected as an excellent object for electrophysiological measurements[1]. The refinement of the experimental technique afterwards made it possible to approach much smaller objects.

Traumatization of the object should be limited to the minimum. This is, of course, again a function of the ratio device — system dimension. With measurements in whole tissues where electrodes of 0.1 mm or larger diameter are used the local situation around the place of introduction of the electrode should be assessed histologically. Quite often, for example with measurements in kidney cortex or liver tissue, the cells retreat from the place of puncture so that the electrode is immersed in the extracellular liquid.

Static measurements such as potentiometry do not electrically or chemically disturb the system under investigation if inert electrodes are used. Different situation arises if electric current is generated by the measuring system as, for example, is the case of cell membrane inpedance measurement or of voltammetric methods. Here, at least in some cases, the threshold limit of stimulation of the object or of the mass transfer connected with the flow of electricity can be

* Based on a lecture presented at the *III International Summer School on the Chemistry of Solid/Liquid Interfaces*, Rovinj, Yugoslavia, July 1—5, 1972.

estimated. The value of electric current should be considerably smaller than 1 microampere (less than 1 nanoampere in neural tissues[2]).

The measured system has different distorting impact on the sensor when static or dynamic measurements are made. In potentiometric measurements with glass or ion selective electrodes in excitable tissues their continuous electrical activity gives rise to DC potentials which should be subtracted from the EMF of the cell indicator electrode — reference electrode. The simplest solution of this problem means to place the reference electrode as close as possible to the indicator electrode. However, even in this case the results are not completely accurate if the electrodes are situated in the direction of a propagating potential wave[3].

Generally, in measurements in media containing high concentrations of ionized biopolymers these may cause formation of Donnan type potentials at liquid junctions[4]. This is the case with isotonic saline bridges connecting the reference electrode to the system under investigation. These should be avoided in the presence of high concentration of proteins and other polyions where a saturated KCl bridge is quite satisfactory but precaution must be taken in order to prevent stimulation of adjacent excitable elements by K^+ ions diffusing from the bridge.

Quite often platinum or gold electrodes have been employed or are still being employed for determination of the so called oxidation — reduction potential of a biological system. This case is typical for the distorting influence of the system under investigation on the sensor. In the biological system, together with a number of oxidation — reduction couples, there are various surface active species present which are adsorbed at the surface of the electrode and inhibit the establishment of the redox equilibrium at the surface of the electrode. These potentials show no Nernstian response to the activities of the components of a supposed couple, depend often on stirring of the solution and show negligible stability towards low current impulses. Finally, the electrode potential is probably a kind of an adsorption potential[5] and has no bearing to oxidation — reduction condition of the system. Thus, practically all data found mainly in biological literature of experimental redox potential of living systems have no thermodynamic relevance[6]. This does not mean that these measurements cannot, like some other biomedical methods, give an empirical correlation to other properties of the system (for example, the change of electrode potential of an electrode immersed into a tissue after X-ray irradiation or after application of radioprotectants[7,8]).

From the standpoint of voltammetry the resting potential represents only one point of a current-voltage curve, *i. e.* the zero current value. It may be expected that in voltammetric measurements still more complications will arise due to the presence of the biological medium.

The application of the dropping mercury electrode for direct measurements *in vivo* is impossible, partly due to the adsorption effects (together with catalytic hydrogen evolution) caused by biopolymers, partly due to the poisoning of the system by dissolving ions of mercury. There may be a suspicion of the latter effect even in the case of polarographic measurement of oxygen consumption by yeast described by Baumberger[9] in his classical paper where the system was grossly simplified the yeast cells being suspended in a phosphate buffer containing sucrose.

The inhibiting effect of the important components of the serum, cysteine and glutathione, on the oxygen wave on Pt[10] is shown in Fig. 1. This shows that attempts to determine oxygen concentration by means of an electrode placed directly in the biological system are hopeless. However, if the electrode is shielded by a teflon or polyethylene foil, permeable to gases but not to the electrolyte, the measurement is guite satisfactory. The electrode assemblies are supplied, for example, by Beckman Instruments or by Radiometer. However, for low oxygen concentration the high residual current is the main draw-back of this method.

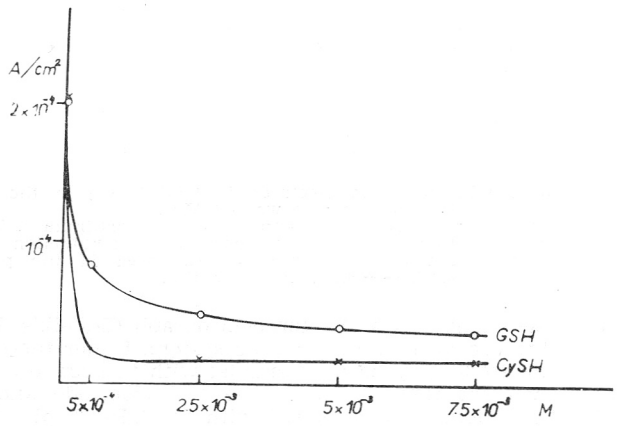

Fig. 1. Current density of oxygen reduction at 0.04 V (*vs.* N.H.E.) *vs.* concentration of cysteine (-x-) and glutathione (-o-) in phosphate buffer solution.

The simple amperometric method (at constant potential) for determination of substances present in or added to systems *in vivo* using stationary platinum or gold electrodes is affected both by depletion of these substances in the neighbourhood of the electrode and by the inhibiting action of the components of the system. This remark pertains also to the sometimes used »dilution curve« method based on amperometric measurement, for example, of oxidation of ascorbic acid[11,12]. On the other hand the cyclic voltammetry has shown itself, in several cases, as quite suitable for this purpose.

EXPERIMENTAL

As a test electrode a platinum wire (diameter of 0.1 mm) was used which was sealed in a glass capillary. The length of the electrode was 0.5 mm. A saturated calomel reference electrode and a platinum auxiliary electrode were used.

In order to bring the platinum electrode into the active condition it was first polarized in 0.5 M H_2SO_4 (without previous removal of dissolved oxygen) by cyclic triangular pulses (polarization rate of 0.1 Vs^{-1}). The range of polarization was 0.0 V to +1.6 V *versus* N.H.E. Simultaneously the I-E curves were recorded. On comparing them with a standard diagram (Fig. 2) we could also ascertain whether the electrode was in a satisfactory condition and exclude the electrodes with defects. In the initial part of the voltammetric curve in Fig. 2 corresponding to the increase of electrode potential a cathodic current due to reduction of molecular oxygen is first recorded (electrode potential range about 0.0 V to +0.7 V *versus* N.H.E.). Over this current two peaks a_1 and a_2 of oxidation of adsorbed hydrogen atoms are superimposed. The third more positive peak a_3 corresponds to the anodic process of formation of the oxide layer on the electrode. When the direction of the voltage sweep is reversed at +1.6 V, the surface oxide is reduced in a cathodic peak c_1 and finally hydrogen ions are discharged under formation of two peaks c_2 and c_3.

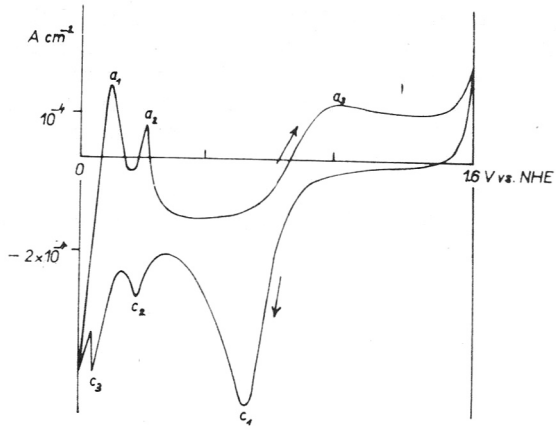

Fig. 2. Voltammetric curve with platinum electrode in 0.5M H_2SO_4 in the presence of air. Polarization rate was 0.1 Vs^{-1}.
a_1, a_2, anodic peaks of adsorbed hydrogen. a_3, anodic peak of platinum oxide formation, c_1, cathodic peak of platinum oxide reduction, c_2, c_3, cathodic peaks of reduction of hydrogen ions. The current of molecular oxygen reduction was superimposed in the polarization range 0.0 V — + 0.7 V *versus* N.H.E.

The recording instrument was a OH-102 polarograph (Radelkis, Budapest, Hungary) which enables the use of a three-electrode system. Under these conditions the potential of the test electrode may be programmed with respect to a reference electrode while electric current flows between the test electrode and an auxilliary electrode. The pH of solutions was measured with the OP-205 pH-meter (Radelkis, Budapest, Hungary).

All reagents used in the present experiments were analytical reagent grade. L-Cysteine-HCl and L-ascorbic acid (Lachema, Brno, Chechoslovakia), and BDH sulfuric acid were used.

Blood serum was obtained by centrifuging of blood at 3000 r. p. m. for 15 min.

In vivo experiments were carried out on isolated dog kidneys under perfusion with a 5% dextrane in BSS (basal salt solution after Manax). The activated test electrode was introduced into the renal cortex as shown in Fig. 3 and the reference

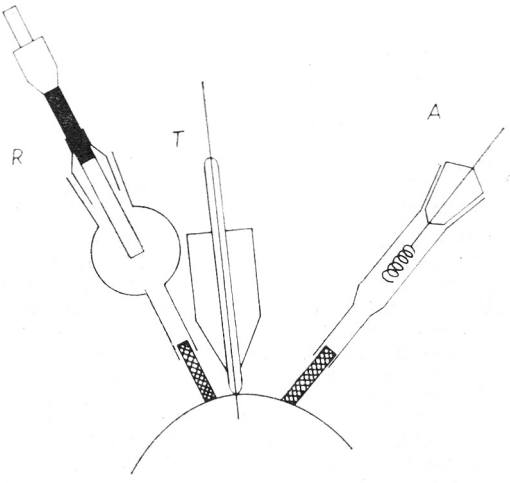

Fig. 3. The arrangement of electrodes for cyclic voltammetry in organs. T, platinum test electrode. R, A, reference and auxilliary electrodes connected to the tissue with a cotton wick wetted with saline.

and the auxilliary electrodes were connected with the kidney by means of a cotton wick saturated with saline.

In a given moment, the electrochemical indicator was added to the perfusion solution.

RESULTS AND DISCUSSION

In the preparatory stage of investigation of voltammetric measurements *in vivo* it was attempted to choose substances which are present in the living organism and which would show appreciable effects with the cyclic voltammetric method. The sulphur containing aminoacids are strongly adsorbed at platinum and gold electrodes. The electrocatalytical reaction mechanism of cystine oxidation[13,14] is

$$RSSR \longrightarrow 2\,RS_{ads}$$

$$RS_{ads} \longrightarrow RSO_3^- + 5\,e$$

The adsorption process is slow as shown by radiometric measurements using ^{35}S cystine. The oxidation occurs by reaction of the adsorbed radical RS with surface oxide. This process is independent of the rate of stirring the solution. The final oxidation product, cysteic acid, is immediately desorbed from the surface of the electrode. As a consequence of the adsorption nature of the process the concentration dependence of cystine wave shows a saturation.

Cysteine shows a much larger, partly transport controlled oxidation wave[15]. The supposed mechanisms is

$$RSH \longrightarrow RSH_{ads}$$

$$RSH_{ads} \longrightarrow RS_{ads} + H^+ + e$$

$$RS_{ads} \longrightarrow RSO_3^- + 5\,e$$

The adsorbed radical RS_{ads} is oxidized in the same way as in the case of cystine. The concentration dependence of the first wave of cysteine is almost directly proportional to concentration and increases somewhat with increasing pH, so that at neutral pH the second wave (oxidation of RS) completely merges with the first one.

Both the oxidized and the reduced form of glutathione show a single wave similar to cystine, only the saturation is reached at low concentrations[16].

Ascorbic acid is oxidized at the platinum electrode in a single two electron diffusion controlled wave[17]. In contradistinction from mercury electrodes, where ascorbic acid is oxidized reversibly with a subsequent rapid deactivation reaction of the intermediary product, at platinum electrodes the oxidation is irreversible. This is caused by strong adsorption of the substance itself which covers about 70% of the surface. The observed anodic irreversible wave corresponds to oxidation at remaining less active sites of the electrode. The adsorbed substance is oxidized in the potential range of surface oxide formation. This latter process can be detected only with high rates of polarization of the electrode.

Even in such a complicated system like blood serum a reproducible anodic wave is obtained (Fig. 4). The double wave observed depends slightly on stirring of solution. Among major components of the serum (see Table I) the following substances are oxidizable at Pt electrode: cystine, cysteine, glutathione, uric acid, glucose, pyruvic acid and lactic acid. In order to find out the substance

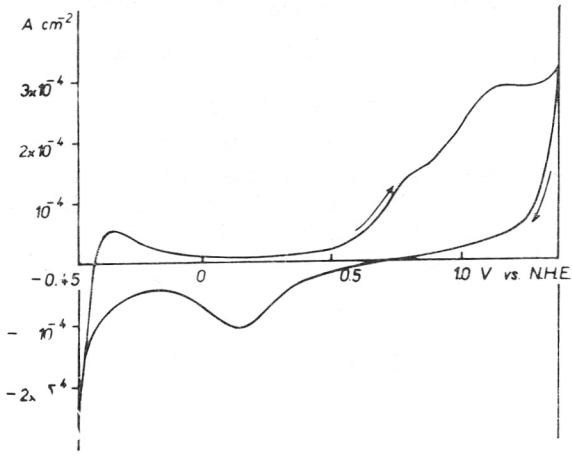

Fig. 4. Voltammetric curve of rat blood serum (full line) in the absence of air.

TABLE I

Major Components of Blood Plasma

Component	Concentration
Cl^-	95 —110 mM
K^+	4.1 — 5.6 mM
Na^+	121 —143 mM
Albumin	0.7 — 0.9 mM
Fibrinogen	0.005— 0.01 mM
Globulins (total)	1.5 — 3.5 g/100 ml
Glutathione* (total blood)	1.3 — 2.2 mM
Cystine*	0.05 mM
Cysteine*	0.5 — 0.58 mM
Uric acid*	0.15 — 0.39 mM
Glucose*	3.9 — 6.6 mM
Ascorbic acid*	0.028— 0.084 mM
Pyruvic acid*	0.09 — 0.14 mM
Lactic acid*	0.55 — 3.4 mM

* Electroactive at Pt electrode.

responsible for the voltammetric effect the rat blood serum was subjected to gel filtration on Sephadex G75. The anodic wave observed in the serum was found only in the low molecular fraction while the high molecular fraction gives no voltammetric effect. Among the substances suspected for the anodic effect only cysteine gave the anodic wave when added to the high-molecular fraction in the same concentration as in the original serum. This wave is almost identical with the wave of the serum. Obviously the presence of proteins inhibits partly the anodic process of cysteine so that somewhat distorted waves are observed (see Fig. 5). Similarly the wave of ascorbic acid is somewhat inhibited by the serum (see Fig. 6).

Thus either strongly adsorbable substances like cystine or cysteine or rapidly reacting species (*e. g.* ascorbic acid) only give a satisfactory electrochemical response under *in vivo* conditions.

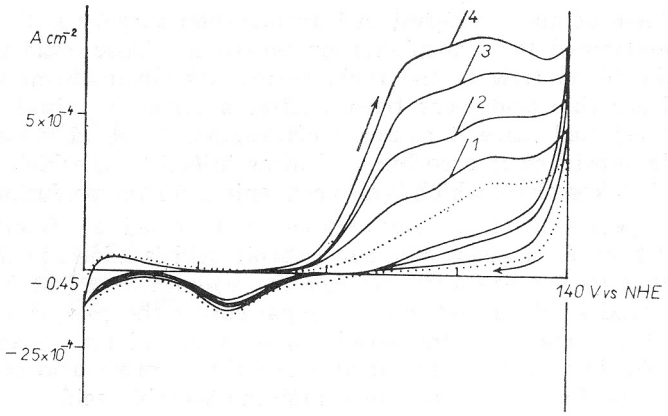

Fig. 5. Voltammetric curves with platinum electrode in rat blood serum without the addition of cysteine (. . .), and after addition of cysteine in absence of air. Concentrations of added cysteine: *1*, 5×10^{-4} M; *2*, 2.5×10^{-3} M; *3*, 5×10^{-3} M; *4*, 7.5×10^{-3} M.

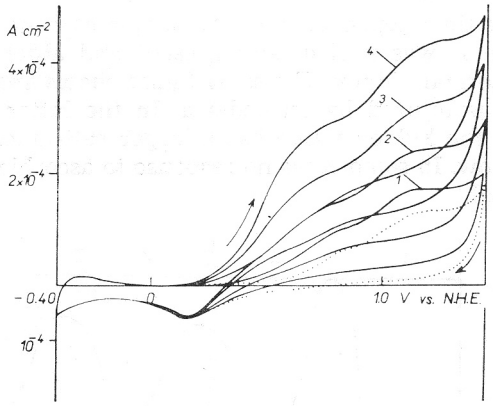

Fig. 6. Voltammetric curves with platinum electrode in rat blood serum without addition of ascorbic acid (. . . .) and after addition of ascorbic acid. Concentrations of added ascorbic acid: *1*, 5×10^{-4} M; *2*. 2.5×10^{-3} M; *3*, 5×10^{-3} M; *4*, 7.5×10^{-3} M.

The applications of the methodological approach described are in three fields:

i. Determination of an electroactive substance already present in the biological object.

ii. Determination of the concentration and of transformations of substances added to the organism.

iii. Monitoring of electrochemical indicators for estimating various functions of organs.

The examples for the first two items are the assessment of cysteine concentration in the organism after X-ray irradiation and the following of concentration and chemical changes of radioprotectants added to the organism[18]. Most of our investigations fall, however, under the heading *iii*.

The problem of major interest in transplatation surgery is the assessment of kidney determined for transplantation where the basic issue is the maintenance of the blood flow to the renal cortex. As electrochemical indicators cysteine and ascorbic acid were tried[19]. After a series of initial experiments cysteine was rejected since it causes a contraction of blood vessels. A much more suitable substance is ascorbic acid after injection of which a dilatation of bood vessels takes place, which is also convenient for the perfusion procedure.

In the experiment an isolated kidney is perfused as described in the experimental part. At a given moment ascorbic acid is added to the perfusion solution so that its concentration reaches the value 5×10^{-3} M. By means of platinum electrodes introduced into the renal cortex the rate of supply of the indicator to the cortex and the steady state value of its concentration are determined. Further data are obtained when the ascorbic acid is washed-out by means of a perfusion solution not containing ascorbic acid.

Usually three indicator electrodes are used at the same time, two attached to the poles of the kidney and one the centre of the lobe of the kidney. When the steady state is reached the current values at individual electrodes are also determined. The blood (and indicator) supply to the poles is lower than to the centre of the normal kidney.

Fig. 7 shows the time dependence of the anodic current density of ascorbic acid oxidation when it was periodically present and absent in the perfusion solution fed to the normal kidney. The same figure shows analogous dependence when the kidney was injured by an oedema. In the latter case the transient time is longer since the kidney tissue has a larger resistance towards blood or perfusion solution flow. In some cases no response to ascorbic acid in the kidney could be traced at all.

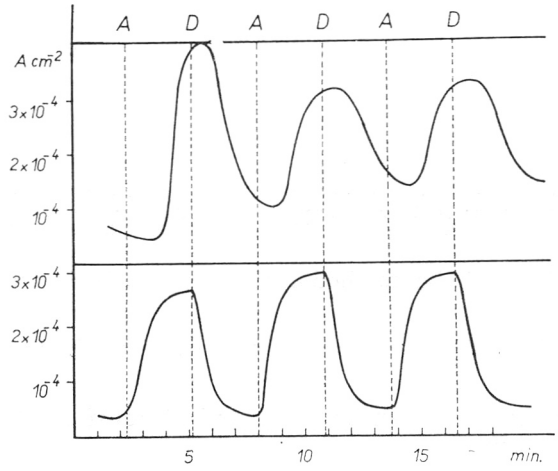

Fig. 7. Time dependence of the peak current of ascorbic acid in renal cortex. Bellow: Dependence in a normal kidney. Above: Dependence in an oedematous kidney. Time interval AD: ascorbic acid present in the perfusion solution. Time interval DA: supply of ascorbic acid discontinued.

The kidney kept for several hours in the absence of air (ischemia) showed a decrease of the steady state value of the anodic current of ascorbic acid while the transient was also longer.

Up to now this method has been applied only to experimental animals. It could be also used in other circulation problems of surgery and other fields of medicine. We should like to stress that a progress in this field could only be achieved by cooperation of electrochemists, biochemists and surgeons.

REFERENCES

1. K. S. Cole and H. J. Curtis, *J. Gen. Physiol.* **22** (1939) 649.
2. T. C. Ruch and H. D. Patton, *Physiology and Biophysics,* W. B. Saunders Co., Philadelphia, 1966.
3. S. M. Friedman, *H^+ and Cation Analysis of Biological Fluids in the Intact Animal,* Chapter 16 of *Glass Electrodes for Hydrogen and Other Cations,* G. Eisenman, (Editor), M. Dekker, New York, 1967, p. 443.
4. R. N. Khuri, *Glass Electrodes and their Uses in Biological Systems,* Chapter 18 of *Glass Electrode for Hydrogen and Other Cations,* G. Eisenman (Editor), M. Dekker, New York, 1967, p. 483—384.
5. A. N. Frumkin and O. A. Petry, *Electrochim. Acta* **15** (1970) 391.
6. J. Pradáč, J. Koryta, and V. Kalous, *Folia Biolog.* (Praha) **11** (1965) 311.
7. N. M. Dobrovolskii, *Radiologiya* **7** (1967) 240.
8. D. B. Cater, *Oxygen Tension and Oxidation — Reduction Potentials in Living Tissues.* Progress in Biophys. and Biophys. Chem., Vol. 10, Pergamon Press, New York, 1960.
9. J. P. Baumberger, *Cold Spring Harbor Symposia Quant. Biol.* **7** (1939) 195.
10. J. Pradáč and J. Koryta, *Anal. Lett.* **3** (1970) 335.
11. P. L. Fromer, W. W. Pfaff, and E. Brauwald, *Circulation* **24** (1961) 1227.
12. L. Seipel, N. Gleichman, H. Kreuzer, and F. Loogen, *Z. Kreislauf-forcsh.* **58** (1969) 946.
13. J. Pradáč and J. Koryta, *J. Electroanal. Chem.* **17** (1968) 167.
14. J Koryta and J. Pradáč, *J. Electroanal. Chem.* **17** (1968) 177.
15. J. Koryta and J. Pradáč, *J. Electroanal. Chem.* **17** (1968) 185.
16. N. Ossendorfová, J. Pradáč, and J. Koryta, *Electroanal. Chem.* **28** (1970) 311.
17. M. Březina, J. Koryta, T. Loučka, D. Maršíková, and J. Pradáč, *J. Electroanal. Chem.* **40** (1972) 13.
18. J. Pradáč, J. Pradáčová, and J. Koryta, *Proceedings of the VIIth Internat. Congres on Chemotherapy,* Prague, 1971, in press.
19. J. Pradáč, J Pradáčová, J. Koryta, and J. Vrubel, *Folia Biologica* (Praha) **17** (1971) 322.

IZVOD

Primjena elektrokatalize na elektrokemijska istraživanja *in vivo*

J. Pradáč, N. Ossendorfová i J. Koryta

Razmatrana je primjena indikatorskih elektroda od inertnog materijala za proučavanje nekih elektrokemijskih reakcija *in vivo*. Navedene su glavne komponente krvne plazme i pokazane one elektroaktivne. Od tih se cistein i askorbinska kiselina pokazuju pogodnim kao elektrokemijski indikatori *in vivo*. Askorbinska kiselina podesan je elektrokemijski indikator za procjenu cirkulacije krvi u bubrezima, koji se pripremaju za transplantaciju.

INSTITUT ZA BIOFIZIKU
KARLOV UNIVERZITET
I
J. HEYROVSKÝ INSTITUT ZA
FIZIKALNU KEMIJU I ELEKTROKEMIJU
ČEHOSLOVAČKA AKADEMIJA NAUKA
PRAG, ČSSR

Primljeno 16. listopada 1972.