



*Acceptor.* K.S., male, 61 years of age. Group T<sub>1</sub>, 14 days after grafting on the anterior surface of the leg. a, *Donor.* D.J., male, 42 years of age. Group T<sub>1</sub>. Completely necrotized; b, *Donor.* K.J., male, 46 years of age. Group T<sub>4</sub>. Implanted, alive.

tions were performed on individuals belonging to two extremes: on those denoted with T<sub>1</sub>, having a rapid transport capacity, and on those denoted with T<sub>4</sub> having the slowest transport capacity. Onto the tibial surface of the legs of 5 acceptors (two belonged to type T<sub>1</sub> and the others to T<sub>4</sub>) skin grafts were transplanted: onto the one grafts from T<sub>1</sub> and onto the other grafts from T<sub>4</sub> donors. Complete grafts were transplanted. The blood groups of the acceptors and donors were also taken into consideration. The transplantations were observed macroscopically.

It was revealed that the survival of the graft depends on the fact as to which T group the donor belongs; on the other hand, the circumstance as to which group the acceptor belongs is of no consequence. The grafts derived from individuals of group T<sub>4</sub> with the slowest transport capacity survived by 3–21 days those of individuals belonging to the T<sub>1</sub> group with the most rapid transport capacity (Figures 1a and 1b). This was the case in both grafts from

subjects belonging to the same blood group and those belonging to different blood groups.

The investigations performed so far suggest that the procedure is suitable for the selection of donors; and, on the other hand, the possibility arises that there is some correlation between the antigen of the graft and the peripheral lymph vessel system.

*Zusammenfassung.* In Homotransplantationsversuchen wird gezeigt, dass der neu verwendete Lappchentest zur Messung des makromolekulären Transports des Lymphapparates für die Auswahl des Spenders geeignet ist.

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### Amperometric Determination of $\text{UO}_2^{++}$ as Vanadate

A rapid amperometric method for the determination of  $\text{UO}_2^{++}$  ions has been described which consists of titrating against sodium *ortho*-vanadate solution at  $E_{d.e.} = -0.85$  v (vs. SCE). Uranyl contents down to 0.5 mM can be determined with an accuracy of 1%.

In an earlier publication<sup>1</sup> the authors have studied the compositions of uranyl vanadates formed by the interaction of  $\text{UO}_2(\text{NO}_3)_2$  and different sodium vanadates (*ortho*-, *pyro*-, *meta*-, and *poly*-) by electrometric techniques; and have concluded that out of three compounds formed,  $(2.5\text{UO}_2 \cdot \text{Na})\text{V}_2\text{O}_8$ ,  $(1.5\text{UO}_2 \cdot \text{Na})\text{V}_2\text{O}_7$ , and  $(0.5\text{UO}_2 \cdot \text{Na})\text{V}_2\text{O}_6$ , the precipitation of the first, i.e.  $(2.5\text{UO}_2 \cdot \text{Na})\text{V}_2\text{O}_8$ , is quantitative in a pH range of 5.6–6.5. The purpose of the present investigation is to study the possibility of determining  $\text{UO}_2^{++}$  ions as  $(2.5\text{UO}_2 \cdot \text{Na})\text{V}_2\text{O}_8$  amperometrically. There is, however, no reference in the literature to the amperometric study of this reaction.

Anal. R. (BDH) reagents,  $\text{UO}_2(\text{NO}_3)_2$ ,  $\text{KClO}_4$ , thymol and Merck's (GR) Vanadium pentoxide were used and

their solutions prepared in air-free conductivity water. A manual polarograph with scalamp galvanometer as current recorder was employed for amperometric work. A capillary having the following characteristics,  $m = 2.416$  mg/sec,  $t = 3.58$  sec, and  $m^{2/3}/t^{1/6} = 2.226$   $\text{mg}^{2/3}/\text{sec}^{1/2}$  was used in conjunction with SCE connected to the cell by a low resistance salt bridge; 20.0 ml of the test solution was taken into the cell each time, deaerated and stirred by bubbling hydrogen.

The standard solution of sodium *ortho*-vanadate was prepared by dissolving a weighed amount of vanadium pentoxide in a boiling solution of NaOH of the required strength.

A series of solutions containing different concentrations of  $\text{UO}_2(\text{NO}_3)_2$ , appropriate amounts of supporting electrolyte  $\text{KClO}_4$  (40–70 times more than  $\text{UO}_2^{++}$  ions) and  $2 \cdot 10^{-4}\%$  thymol were prepared and titrated with a standard solution of sodium *ortho*-vanadate at  $E_{d.e.} = -0.85$  v (vs. SCE), which is the limiting current plateau potential

<sup>1</sup> M. L. MITTAL and R. S. SAXENA, J. Nucl. inorg. Chem., in press (1964).

of the second step of the wave produced by  $\text{UO}_2^{++}$  ions in the presence of  $\text{KClO}_4$  and thymol. The amounts of standard solution consumed were determined from the equivalence points located graphically. The Table will illustrate the results and accuracy of the method.

It is evident from the Table that the amperometric titrations of sodium *ortho*-vanadate with solutions containing uranyl ions in the presence of  $\text{KClO}_4$  and thymol provide precise results and can be suitably employed for the quantitative determination of uranyl ions. The accuracy and reproducibility of the results have been found to be excellent and this reaction offers a simple and rapid method for the determination of uranyl ions in solutions.

Amperometric determination of  $\text{UO}_2^{++}$  as vanadate at  $E_{d.e.} = -0.85$  v (vs. SCE). Volume of the solution taken = 20.0 ml

	mg	mg	mg	mg
$\text{UO}_2^{++}$ present	21.606	6.752	4.501	2.701
$\text{UO}_2^{++}$ found	21.606	6.667	4.457	2.624
Error	0.000	0.085	0.044	0.077

The cations which yield precipitates with vanadate ion and anions such as molybdate, chromate, tungstate, etc., interfere and should be avoided. The pH of the solution containing uranyl ions should be in the range of 3–4.0<sup>2</sup>.

**Zusammenfassung.** Es wird eine schnellamperometrische Methode für die Bestimmung von  $\text{UO}_2^{++}$  Ionen beschrieben: Titrium gegen Natrium-Orthovanatlösung mit  $E_{d.e.} = -0.85$  v (vs. SCE). Bei einem Uranylinhalt bis 0.5 mM kann so noch eine Genauigkeit von 1% festgestellt werden.

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### Thin Layer Chromatography of Catecholamines and their Metabolites

The separation of catecholamines and their metabolites by means of organic solvent extraction, column or paper chromatography, followed by either biological or chemical quantitation, is not only a very time-consuming procedure but also presents various problems with regard to resolution, i.e. specificity, and recovery, i.e. sensitivity, both of which determine the accuracy of the method.

The method described in the present paper combines the use of thin layer chromatography, for the separation of catecholamines and their metabolites, with the use of fluorescence assay, for subsequent quantitation. It offers several advantages in the sense of simplicity, rapidity and accuracy.

**Technique.** Chromatoplates are made as follows: 20 × 20 cm glass plates are covered, by means of the STAHL<sup>1</sup> spreading device, with a 300  $\mu$  layer of cellulose powder, prepared by suspending 7.5 g of cellulose powder (Machery, Nagel & Co. MN-300) in 45 ml of methanol (Merck, for chromatography), the suspension being shaken mechanically for 5 min. The plates are dried for 10 min at 105°C and stored at room temperature over  $\text{CaCl}_2$ . Noradrenaline (NA), adrenaline (A), normetanephine (NMN), metanephine (MN), 3, 4-dihydroxymandelic acid (DOMA) and 3-methoxy-4-hydroxymandelic acid (vanillylmandelic acid, VMA), purchased commercially (Calbiochem, Los Angeles, California), are spotted in 0.2 to 1  $\mu$ g amounts, 1.5 cm from the bottom edge of the plate. The plate is run by ascending chromatography in a closed glass chamber, which is saturated with the solvent. The following solvent systems were used: *n*-butanol saturated with 1N HCl, *n*-butanol saturated with 3N HCl, *n*-butanol saturated with 4N HCl, *n*-butanol saturated with 6N HCl, *n*-butanol saturated with 1N acetic acid, *n*-butanol saturated with 3N acetic acid, *n*-butanol/3N

acetic acid/water (4:1:1), *n*-butanol/pyridine/water (46:31:23), isobutanol/acetic acid/cyclohexane (65:7:25), propanol/3N HCl (80:20), isopropanol/formic acid/water (70:6:24), amylalcohol saturated with 3N HCl, ethylmethylketone saturated with 1N HCl. *n*-Butanol saturated with 3N HCl proved to be the most suitable solvent. The solvent front is allowed to rise 15 cm, which point is reached in about 3 h at 20°C.

After development, the plates are dried with warm air and the substances separated are detected by spraying the plate with one of the following reagents: (1)  $\text{K}_2\text{Fe}(\text{CN})_6$  2 – 0.44 g/100 g of phosphate buffer pH 7.8; (2) ethylenediamine (Merck)<sup>3</sup> – predistilled and mixed with an equal volume of water, the sprayed plates are dried for 20 min at 50°–60°C and the spots located under UV-light (max. at 360 m $\mu$ ); (3) *p*-nitroaniline<sup>4</sup> – immediately before use a 1:1:2 mixture is made of the following solutions, kept at 2°C: (a) 0.1 g of *p*-nitroaniline (Merck) dissolved in 2 ml of HCl conc. and made up to 100 ml with distilled water, (b) 0.2 g of  $\text{NaNO}_2$  dissolved in 100 ml of water, (c) 10 g of  $\text{K}_2\text{CO}_3$  dissolved in 100 ml of water.

**Results.** Using *n*-butanol saturated with 3N HCl as solvent, the R<sub>f</sub> values for the substances studied were as follows: NA 0.31, A 0.38, NMN 0.48, MN 0.58, DOMA 0.80, and VMA 0.89, as illustrated in the Figure.

The sensitivity of the spray reagents used varied according to the compound being identified, as shown in the Table, being most constant for *p*-nitroaniline.

<sup>1</sup> E. STAHL, *Dünnschicht-Chromatographie* (Springer-Verlag, Berlin-Göttingen-Heidelberg 1962).

<sup>2</sup> W. O. JAMES, *Nature* 161, 851 (1948).

<sup>3</sup> R. SEGURA-CARDONA and K. SOEHRING, *Med. exp.* 10, 251 (1964).

<sup>4</sup> W. VON STUDNITZ, *Scand. J. clin. lab. Invest.* 12, Suppl. 48 (1960).