of the second step of the wave produced by UO_2^{++} ions in the presence of $KClO_4$ 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 $KClO_4$ 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 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
UO_2^{++} present	21.606	6.752	4.501	2.701
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[2].

Zusammenfassung. Es wird eine schnellamperometrische Methode für die Bestimmung von UO_2^{++} Ionen beschrieben: Titrium gegen Sodium-*Ortho*vanatlösung mit $E_{d.e.} = -0.85$ v (vs. SCE). Bei einem Uranylinhalt bis 0.5 m*M* 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[1] spreading device, with a 300 μ 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 $CaCl_2$. Noradrenaline (NA), adrenaline (A), normetanephrine (NMN), metanephrine (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 μg 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 1*N* HCl, *n*-butanol saturated with 3*N* HCl, *n*-butanol saturated with 4*N* HCl, *n*-butanol saturated with 6*N* HCl, *n*-butanol saturated with 1*N* acetic acid, *n*-butanol saturated with 3*N* acetic acid, *n*-butanol/3*N* acetic acid/water (4:1:1), *n*-butanol/pyridine/water (46:31:23), isobutanol/acetic acid/cyclohexane (65:7:25), propanol/3*N* HCl (80:20), isopropanol/formic acid/water (70:6:24), amylalcohol saturated with 3*N* HCl, ethylmethyl-keton saturated with 1*N* HCl. *n*-Butanol saturated with 3*N* 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) $K_3Fe(CN)_6$[2] – 0.44 g/100 g of phosphate buffer pH 7.8; (2) ethylenediamine (Merck)[3] – 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 mμ); (3) *p*-nitroaniline[4] – 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 $NaNO_2$ dissolved in 100 ml of water, (c) 10 g of K_2CO_3 dissolved in 100 ml of water.

Results. Using *n*-butanol saturated with 3*N* HCl as solvent, the Rf 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.

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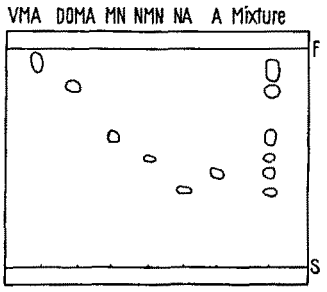
Quantitative estimations were performed by double spotting a known standard mixture of the substances studied. After development of the chromatogram one series of spots is sprayed for identification purposes whereas the corresponding spots of the other series are brought (1 cm² areas) into a centrifuge tube, to which are added 1 ml of 0.1 *N* HCl as well as the various reagents for the fluorimetric assay of NA and A at pH 5 according to SHORE and OLIN[5], and of NMN and MN according to BERTLER et al.[6]. After centrifugation for 15 min at 4000 rpm, the supernatant is removed for measurement of the fluorescence, the excitation and fluorescence wavelength maxima being respectively for NA and A 395 mμ and 525 mμ, for NMN 385 mμ and 490 mμ, and for MN 395 mμ and 515 mμ.

Reproducibility and linearity were found to be quite satisfactory in the 0.2–1 μg range, whereas the recovery from the chromatoplates was of 97–100% for NA, A and MN, and of 75% for NMN[7].



Thin layer chromatogram (cellulose powder) using *n*-butanol saturated with 3 *N* HCl as solvent. See text for abbreviations.

Substance (μg of pure base)	$K_3Fe(CN)_6$	Ethylene-diamine	*p*-Nitro-aniline
NA	0.008	0.008	0.050
A	0.010	0.008	0.050
NMN	–	0.416	0.080
MN	–	0.160	0.080
DOMA	–	0.080	0.100
VMA	–	0.500	0.080

Zusammenfassung. Eine einfache, schnelle und empfindliche Methode zur dünnschichtchromatographischen Trennung von 0,2–1 μg Menge von Noradrenalin, Adrenalin, Normetanephrin, Metanephrin, 3,4-Dihydroxymandelsäure und 3-Methoxy-4-hydroxymandelsäure wird beschrieben.

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An in vivo Method for Evaluating the Hypothalamic Follicle Stimulating Hormone Releasing Factor

It has recently been suggested that, in addition to the releasing factors for ACTH, TSH, LH and GH[1], the median eminence (ME) region of the hypothalamus might also contain a neurohumoral agent which specifically stimulates the release of follicle stimulating hormone (FSH). IGARASHI and MCCANN[2] have shown that crude acidic extracts of rat or bovine ME elevate plasma FSH after intravenous injection into ovariectomized rats, in which the secretion of FSH has been blocked by lesions in the ME, or by large doses of ovarian steroids. KUROSHIMA et al.[3], and MITTLER and MEITES[4] have confirmed these results and reported in addition that rat pituitaries cultured in vitro with hypothalamic exctracts (from sheep, beef or ovariectomized rats) release significant amounts of FSH.

The methods so far employed for evaluating the hypothalamic FSH-releasing activity do not, however, seem completely adequate for assessing the physiological role of this new releasing substance. As far as IGARASHI's[2] procedure is concerned, no data have been reported so far on the amounts of FSH available in the pituitary of the animals (brain-lesioned or treated with ovarian steroids) receiving the injections of the hypothalamic extracts: differences in the amounts of hormones stored in the pituitary may obviously greatly influence the reactivity of the gland. In addition to this, the specificity of the FSH assay method in the mouse used by IGARASHI et al.[2] also requires further study.

On the other hand, doubts have been raised as to the physiological significance of in vitro incubation methods as used by KUROSHIMA et al.[3] and by MITTLER and MEITES[4]; release of FSH in the medium might simply reflect passive leakage of preformed hormone rather than activation of true secretory processes[5].

A new procedure for the quantitative evaluation of the FSH-releasing factor (FSH-RF) has recently been developed in this Department as will be reported in this preliminary note.

This method, which is based on the ability of hypothalamic extracts to deplete pituitary FSH stores in vivo,

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