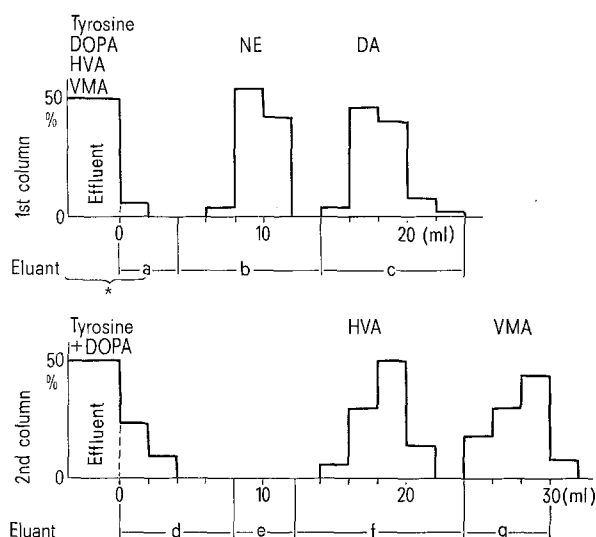


centrifugation, the pH of the supernatant fluid was adjusted to 6.1 with K_2CO_3 , and then the precipitated potassium perchlorate was removed by centrifugation. HVA was determined according to ANDÉN et al.³ and VMA to WEIL-MALHERBE⁴; 10 μ g of authentic HVA in 3 ml of the extract solution were applied to the column, washed with 8 ml of water and 4 ml of 0.02 M phosphate buffer, pH 6.1. HVA was then eluted with 12 ml of 0.4 N acetic acid-ethanol (50% v/v); 30 μ g of authentic VMA in 3 ml of the extract solution were eluted with 8 ml of 4.0 N acetic acid after the same procedures as HVA. HVA was eluted in 0.4 N acetic acid-ethanol fractions with $85.4 \pm 19.4\%$ (S.D.) recovery and then $98.2 \pm 5.7\%$ (S.D.) of



Elution Pattern of NE, DA, Tyrosine plus DOPA, HVA and VMA.

*; The mixture of the first effluent and the following 2 ml of water to be applied to the second column. 1st column, Dowex-50W-X8; 2nd column, Dowex-1-X2; a, d, water; b, 0.8 N HCl; c, 2.0 N HCl; e, 0.02 M phosphate buffer; f, 0.4 N acetic acid-ethanol (50% v/v); g, 4.0 N acetic acid.

added VMA was found in 4.0 N acetic acid fractions, while some attempts with other eluants resulted in no higher recoveries. Overlap of the acids into adjacent fractions did not occur at all.

In order to separate these acids from DA, NE and their precursors, another ion-exchange resin, Dowex-50W-X8, 200-400 mesh (hydrogen form) was used. DA and NE were separated by a slight modification of the method of BERTLER et al.⁵. The results obtained are shown in the Figure. NE adsorbed on the Dowex-50W column was eluted with 0.8 N HCl in a fraction volume of 2 ml after 4 ml of washing water, and 95-97% of added NE were recovered in the 3rd and the 4th HCl fractions. DA adsorbed on the Dowex-50W column was not eluted with 10 ml of 0.8 N HCl, but found in the following 10 ml of 2.0 N HCl elute with 89-92% recovery. Tyrosine was not adsorbed on the Dowex-50W column at all and DOPA, HVA and VMA behaved similarly on this column. The effluent off the column, together with 2 ml of washing water, was applied to the Dowex-1 column and then 4 ml of washing water followed. Tyrosine was found in these effluents off the second column with 93-100% recovery. DOPA was found in the same fractions as tyrosine with 99% recovery. A large portion of HVA and VMA passed through the Dowex-50W column and the rest was completely washed out with 4 ml of water.

The method described here may require some improvements in order to estimate the endogenous catecholamines and metabolites in the brain but it would be useful for study of the metabolic changes in the brain catecholamine system using an isotope-labelled amine precursor. The study of the separation of intermediate metabolites of catecholamines with these column system is in progress in our laboratory.

Zusammenfassung. Zugefügte exogene Homovanillinsäure und Vanillylmandelsäure im Extrakt aus Rattenhirn können durch Säulenchromatographie (Dowex-1-X2) abgetrennt werden. Die erstere wurde zu 85%, die letztere zu 98% wiedergewonnen.

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³ N. E. ANDÉN, B. E. ROOS and B. WERDINIUS, *Life Sci.* 7, 449 (1963).

⁴ H. WEIL-MALHERBE, *Analyt. Biochem.* 7, 485 (1964).

⁵ A. BERTLER, A. CARLSSON and E. ROSENGREN, *Acta physiol. scand.* 44, 273 (1958).

CONGRESSUS

Italy

The 3rd International Symposium on Mass Spectrometry in Biochemistry and Medicine

in Alghero (Sardinia), 16-18 June 1975

Topics: Gas chromatography, mass spectrometry, mass fragmentography, stable isotope measurements, field ionization, field desorption, chemical ionization. The areas of application will include: Biochemistry, medicine, toxicology, drug research, forensic science, clinical chemistry and pollution. Further information by Dr. A. Frigerio, Istituto di Ricerche Farmacologiche 'Mario Negri', Via Eritrea 62, I-20157 Milano, Italy.

Switzerland

International Symposium on Enzymes and Proteins from Thermophilic Microorganisms

in Zürich, 28 July-1 August 1975

Topics: Thermophilic enzymes (proteins): Isolation, characterization and general properties. Structural basis of 'thermophilic' properties of enzymes and proteins (thermostability, specific activity). Structure function relationships. Enzymes in thermophilic metabolism. Temperature adaptation. General aspects of the thermophily problem.

Information and registration: Prof. Dr. H. Zuber, Institut für Molekularbiologie und Biophysik, Eidgenössische Technische Hochschule, CH-8049 Zürich, Switzerland.