fibre bundles provided the necessary orientation so that, in these studies, wedge-shaped tissue samples of the DLGN could be taken (Figure 2). The wedge-shaped samples were placed in individual glass vials and further processed by post-fixation for 2 h in 1% osmium-tetroxide and 4% sucrose in 0.1 *M* phosphate buffer. The samples were rapidly dehydrated through a graded series of ethyl alcohols and propylene oxide then infiltrated in Epon. All wedges were carefully oriented as they were embedded in flat molds that contained Epon so that transverse semi-thin and thin sections could be cut.

Semi-thin sections of the entire block face, 1 to 3 µm thick, were cut with glass knives on a Sorvall Porter-Blum MT2 ultra-microtome. These sections were stained with Mallory's azure II-methylene blue[1] and examined with the light microscope. From the study of the semi-thin sections an appropriate area coud be chosen for subsequent thin sectioning and study.

This sampling technique provided for the precise localization within the DLGN of the thin sections used in electron microscopy. The localization and orientation of a DLGN tissue sample is illustrated in Figures 1 and 2. The curved side of the wedge-shaped sample (arrow in Figure 2) permitted the immediate identification of the optic tract surface. This, along with orientation drawings completed at the time of initial osmication, enabled one to correctly embed the wedge-shaped sample in a flat mold. By this method the thin sections could always be obtained in the desired plane of section. To date the samples were all embedded so that transverse sections were made through the nucleus from rostral to caudal. Since the thickness of each sample was known, it was possible to determine the rostral to caudal location of each of the semi-thin and thin sections within the DLGN.

The neuropil of the DLGN is packed between the myelinated axon bundles. Careful examination of such semi-thin sections enabled us to identify pockets of neuropil (dotted line in Figure 3) in the DLGN. In these neuropil pockets the reduced number of myelinated structures was noticeable. Such areas were usually surrounded by several neuronal perikarya. These areas of special interest were isolated into pyramid form by the 'mesa-pyramid' technique[2]. Thin sections of these neuropil pockets usually demonstrated many areas of complex synaptic contacts.

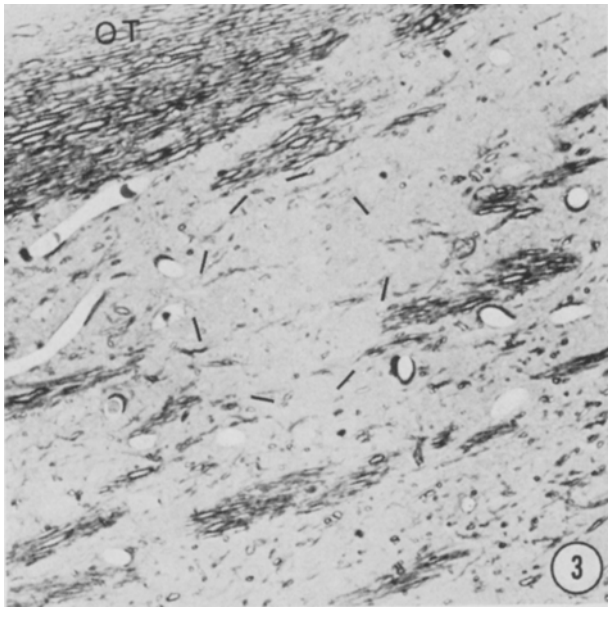

Fig. 3. Transverse 1 µm section through DLGN. Dashed line indicates pocket of neuropil. Note optic tract (OT) on surface of sample. ×600.

Zusammenfassung. Um eingehend elektronenmikroskopisch den Verlauf, die Endigungen und die Verteilung der Axone von der Retina des Auges im Nucleus geniculatus dorsalis lateralis untersuchen zu können, wurde die hier beschriebene sichere Methode für Übersicht und Lokalisierung ausgearbeitet. Mit dieser Methode können nun genau lokalisierte Stellen im Nucleus geniculatus dorsalis lateralis aufgesucht und anschliessend mit dem Elektronenmikroskop untersucht werden.

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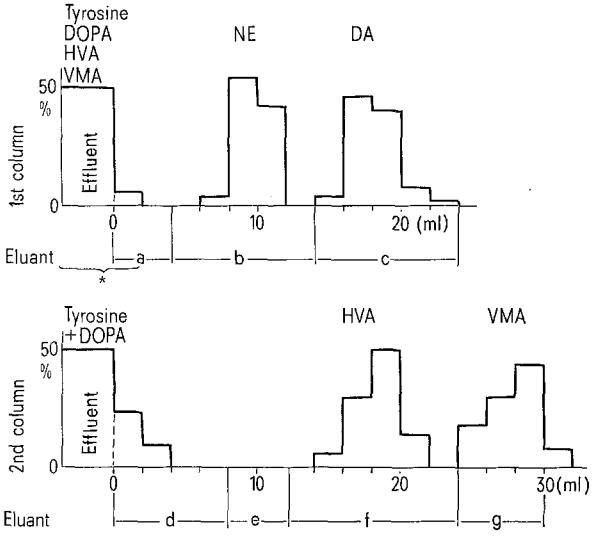
Separation of Homovanillic Acid and Vanillylmandelic Acid with Ion-Exchange Resin Column

In the study on the roles of dopamine (DA) and norepinephrine (NE) in brain functions, it is essential to examine the metabolic changes of these amines. Although various methods for the simultaneous estimation of catecholamines and their metabolites in the brain have been presented, they leave much to be improved with regard to the separation, the recovery rate and the practical simplicity of procedures. Above all, the separation of homovanillic acid (HVA) and vanillylmandelic acid (VMA) has been considered most difficult. There are two studies on the separation method of HVA and VMA by column chromatography. Taylor and Laverty[1] had separated these acids of the brain by elution of a Dowex-1-X2 anion exchange resin column with increasing concentrations of HCl. Under our experimental conditions, however, some overlap of the acids into adjacent fractions remained with their method. Messiha et al.[2] reported separation of urinary HVA and VMA with basic alumina column.

We have successfully separated HVA from VMA using the method here given. Ion-exchange resin employed was Dowex-1-X2 anion exchange resin, 200–400 mesh (chloride form) (Dow Chemical Co.). The brain extract of Wistar rats was obtained by homogenizing the brain, except for the olfactory bulb, pineal gland and cerebellum, in 5–6 volumes of ice-cold 0.4 *N* perchloric acid. After

[1] K. M. Taylor and R. Laverty, J. Neurochem. *16*, 1361 (1969).
[2] F. S. Messiha, E. Bakutis and V. Frankos, Clinica chim. Acta *45*, 159 (1973).

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-MALHERBE[4]; 10 µg 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 µg 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± 19.4% (S.D.) recovery and then 98.2 ± 5.7% (S.D.) of 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.



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.

In order to separate these acids from DA, NE and their precursors, another ion-exchange resin, Dowex-50W-×8, 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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1 October 1974.

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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.