electro-ejaculated 4 times at 2-day-intervals and the spermatozoa counted after liquefying the copulation clot in 1% α-chymotrypsin[4]. The scrotum containing the testes was then exposed to 41 °C for 27 min in a water bath[5]. Until the 97th day following the exposure spermatozoa output was evaluated in 2-to 3-day-intervals in the manner described above.

Spermatozoa output before heat exposure of the testes ($\bar{x} \pm s$ in million per total ejaculate) was 66.2 ± 16.4 33 days after heat exposure it was at its lowest point of 2.2 ± 2.1. On the 97th day spermatozoa ontput reached 65.7 ± 11.3: the counts before heat exposure (see diagram).

Neither the heat exposure of the scrotum nor the regular electroejaculations seemed to affect the well-being of the animals. Thus it seems possible to test with this method: 1. Protective effects of drugs on heat damage to the testes. 2. Accelerating or depressing effects of drugs on the spontaneous regeneration of spermatogenesis after heat damage to the testes bei der Ratte.

Zusammenfassung. Methode als Modell zur Prüfung der Wirkung von Pharmaka auf die Regeneration der Spermatogenese bei der Ratte.

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A Micro-Chromatographic Method for the Detection of Biologically Active Monoamines Isolated Neurons

Progress in neurochemical research is often restricted by the sensitivity and specificity of methods available for detecting and measuring small amounts of different substances. During the course of some studies concerning the uptake and conversion of certain monoamines (5-hydroxytryptamine, 5-hydroxytryptophan, adrenaline, dopamine and noradrenaline) in different neurons, it became necessary to have a simple and reliable method for separating and detecting minute quantities of monoamines in individually isolated neuron samples. It is known that microchromatography of monoamines which have been dansylated[1,2] allows picogram levels of 5-HT to be detected in individual snail neurons[3]. However, this method is not convenient for the localization of some other monoamines, e.g. dopamine, because the number of dansylated derivatives formed from the single substance makes it difficult to localize[1,2].

From the reaction between certain amines and formaldehyde vapour, BELL and SOMERVILLE[4] described a method for the detection of as little as 30 ng of substance, using conventional paper or thin layer chromatographic techniques. The following communication describes a micro-chromatographic procedure from the original BELL and SOMERVILLE's method which permits minute amounts (5–7 ng) of dopamine, noradrenaline and 5-hydroxytryptamine to be detected.

Materials. 3,4-dihydroxyphenylalanine (DOPA) and 5-hydroxytryptophan (5-HTP) were supplied from Sigma adrenaline (AD) and noradrenaline (NA) from British Drug Houses and dopamine (DA) and 5-hydroxytryptamine (5-HT) from Koch-Light Laboratories; 3,4-dimethoxyphenylamine, melatonin, α-methyl-m-tyrosine, bufotenine, 3-hydroxy-4-methoxy-phenylethylamine, kynurenine, 3-hydroxy-kynurenine, kynurenic acid, 3-methoxy-4-hydroxyphenylethylamine, octopamine, n,n-dimethyltryptamine, normetadrenaline, mescaline, metadrenaline and kynuramine were all kindly provided by courtesy of Dr. A. R. SOMERVILLE, Imperial Chemical Industries Ltd., Cheshire. Standard concentrations were made up in 50% acetone in 0.01 *N* HCl.

Chromatograms were developed on 3 × 3 cm polyamide layers (Carl Schleicher and Schüll, DC-Fertigfolie F1700 Muirpolyamide). All solvent systems were of analytical grade from May and Baker Chemical Company.

Methods. Standard (1–1000 ng) amounts of amine were each spotted 0.4 cm from the bottom edge of a 3 × 3 cm polyamide layer. This was achieved by sucking a known amount of amine (0.1 × 0.5 µl) out of a 5 µl capillary tube (Drummond Scientific Company) into the tip of an ultra thin capillary (diameter 0.5 mm) and then applying it to the chromatogram under a stereomicroscope (for details see NEUHOFF[1]). After drying with cool air, the chromatogram was developed in an ascending fashion in a 50 ml beaker, the bottom of which was just barely covered with either methyl acetate/isopropranol/ammonia 25% (9:7:5) or butanol/chloroform/acetic acid (4:1:1). The beaker was covered to prevent any evaporation of the solvent. When the solvent reached the upper edge of the chromatogram

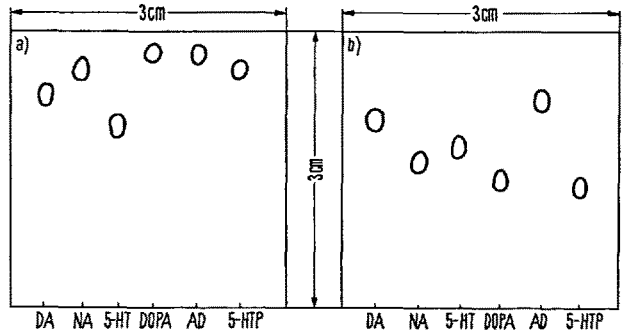

Diagram of micro-chromatograms (polyamide layers) using the solvents. a) Methyl acetate/isopropanol/ammonia and b) butanol/chloroform/acetic acid. Abbreviations as in text.

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after 3–6 min), it was removed, dried carefully with cold (ir from a hair dryer, and then placed in a sealed jar (500 ml) containing about 3 g of paraformaldehyde (the paraformaldehyde had been stored at a relative humidity of 60% for at least 5 days). After heating the jar in an oven for a period of 3 h at 80 °C, amines were located by viewing chromatograms under UV-light.

In order to demonstrate the applicability of the method, 6–10 giant neurons (each cell measures 120 µm across its major axis) from the metacerebral ganglion of *Helix pomatia* (Gastropod-Mollusca) which is known to contain only 5-HT[5] were dissected and placed in a 5 µl capillary containing snail saline[6]. The position of these cells has been described[7] and so has their dissection[8]. The neurons were then sedimented by centrifuging the 5 µl capillary in a MSE automatic superspeed 50, especially adapted to centrifuge capillaries[8] for 10 min at 30,000 *g*. After removing the saline and replacing it with approximately 1 µl of 70% acetone in 0.01 *N* HCl the cells were homogenized with a nerve canal drill which has a diameter of 0.03 mm[1,2].

The precipitation of neuronal proteins by the acetone was completed by placing the capillary in a freezer (–30 °C) for 30 min. The capillary and its contents were centrifuged again and the supernatant chromatographed. Standard amines were chromatographed separately or added to the extract to assist in their identification. The capillary was centrifuged again and the supernatant chromatographed.

Results and discussion. Minimum detectable amounts of DA, NA, 5-HT and related amines on 3 × 3 cm polyamide layers are shown on the Table where it is clear that this technique is specifically sensitive for the detection of NA, DA and 5-HT. In all instances less amine could be detected when compared with ordinary chromatography[4]. Unlike the method described by BELL and SOMERVILLE[4], spraying chromatograms with glycine before exposure to formaldehyde vapour was not found to be a prerequisite. It was, however, important to dry chromatograms thoroughly before formaldehyde treatment. Furthermore, paraformaldehyde must have been stored at a relative humidity of 60% to obtain optimum fluorescence.

In order to separate biologically active amines and their immediate precursors which are associated with some nervous tissues, the combined use of the solvent systems methylacetate/isopropranol/25% ammonia and butanol/chloroform/acetic acid is required. Diagrams of microchromatograms of amines using these two solvents are shown in the Figure. It can be seen that the solvent system methylacetate/isopropranol/25% ammonia is most suitable to separate 5-HT. Using this solvent a very clear spot corresponding both in position and colour to pure 5-HT was shown to occur in neuronal extracts of the snail brain. It was moreover proved with an Aminco-Bowman spectro-photofluorimeter that excitation and emission spectral characteristics of the fluorescent spots of 5-HT and the corresponding spot in the nerve extract were identical. Although extracts from 6 to 10 giant neurons revealed the presence of 5-HT, extracts from as little as 6 neurons still proved adequate.

Quantitative estimation of 5-HT in giant neurons was performed by visually comparing the intensity of fluorescence of standard amounts of 5-HT with that of cell extracts. From a combination of twelve different experiments it is estimated that a single neuron contains 0.9 ng of 5-HT. This figure is slightly higher than obtained by COTTRELL and OSBORNE[5] who showed by biological assay that the 5-HT content is 0.7 ng per cell.

This very simple procedure for the analysis of biological amines in very small quantities of tissue can be applied to many neurochemical problems. We can now analyze the amine content of neurons which are thought to contain more than one amine[9]. Furthermore this method can be adapted to measure the rate of incorporation of radioactivity into specific neurons, so enabling us to measure the biosynthesis and half-lives of certain monoamines. Recent studies in this laboratory have shown that the giant metacerebral cells of *Helix pomatia* can not only take-up radioactive 5-HTP but also convert it to 5-HT[10].

Minimum detectable amounts of biogenic amines and other compounds after formaldehyde treatment

Compound	Detectable amount (ng)
Adrenaline	100
Bufotenine	80
n, n-Dimethyltryptamine	1,000
3,4-Dimethoxyphenethylamine	15
3,4-Dihydroxyphenylalanine	50
Dopamine	6
5-Hydroxytryptophan	50
5-hydroxytryptamine	5
3-hydroxykynurenine	80
3-Hydroxy-4-Methoxyphenylethylamine	20
Kynurenine	100
Kynurenic acid	100
Kynuramine	1,000
Melatonin	90
Metadrenaline	1,000
Mescaline	90
α-Methyl-m-tyrosine	1,000
3-Methoxy-4-hydroxyphenylethylamine	50
Noradrenaline	7
Normetadrenaline	100
Octopamine	1,000

Zusammenfassung. Es wird eine mikrochromatische Methode an 3 × 3 cm Polyamidfolien beschrieben, welche den Nachweis geringer Mengen biologisch tätiger Monoamine erlaubt und für die Lokalisierung und Schätzung von 5 HT in isolierten einzelnen Neuronen wertvoll ist.

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