mμl of 0.5 *N* NaOH was added to each group of cells. Constriction micropipettes, measuring volumes of the order of 0.1 mμl with 2–3% accuracy, were used (Figure). The micropipettes were made in the de Fonbrune microforge, siliconized and then calibrated by measuring the diameter of the distilled water drops let into the paraffin oil. The cells were left for 20 h at 4°C in the NaOH solution. Then alkaline extract was removed, dried on another slide and rinsed with 80% acetic acid to neutralize NaOH. Surplus acetic acid was evaporated.

The extract was treated overnight at 37°C with 0.20 mμl of 0.5% papain solution in 0.1 *M* phosphate buffer, pH 6.8, containing 5 m*M* cysteine and 5 m*M* EDTA. The solution obtained was then evaporated on another slide and the residual dissolved in 0.13 mμl of HCl solution, pH 1.5. No precipitate appeared. The solution was transferred onto another place and the same volume of 1% rivanol (2:5-diamino-7-ethoxyacridine lactate) solution in HCl, pH 1.5, freshly prepared just before use, was added. After the addition of rivanol a complex of rivanol-SM precipitates. The concentration of sodium acetate in the reacting mixture was about 0.6 *M*. Four samples of precipitates, placed on the same slide, were washed twice with water (0.2mμl each), then covered with a collodion membrane and subjected to autoradiography (stripping film AR-10, Kodak). Radioactivity was found in preparations after 4 weeks of exposure. Counts of silver grains were performed in the autoradiographs of precipitates on the same slide, and the results were expressed in terms of grains per preparation.

Silver grain counts in autoradiographs of rivanol-treated preparations, obtained from different groups of cells, gave relatively uniform results (Table). The coefficient of variation of 12% is comparable with that for the individual cell's nucleic acid determination[4]. The evaluation of the method on a scale of small groups of cells is possible when there is no significant variation in: (1) the degree of ^{35}S incorporation into SM contained in individual alkaline extracts (this is assumed by the authors), and (2) the relation of ^{35}S–SM to the other ^{35}S components of the extract. From the results obtained, this seems to be the case. Thus, the rivanol precipitation of SM present in the extracts occurs quantitatively. However, as yet there is no way of estimating the amount of SM in the preparations obtained.

Number of silver grains in autoradiographs of total extracts and rivanol treated preparations. Each preparation obtained from 20 cells. Number of silver grains in total extract is the sum of grain number in autoradiographs of washings and precipitates of the respective alkaline extract. All washings and precipitates were placed and autoradiographed on the same slide. Background is already subtracted (0.5 silver grains per 29.2 μ^2).

Total extract, grain number $\cdot 10^3$	Rivanol treated preparation grain number per 29.2 μ^2	total $\cdot 10^3$	% of total extract
38.0	21.4	33.6	88.4
35.1	14.6	31.6	90.0
46.4	18.3	41.1	88.5
40.9	16.0	33.9	82.8

Résumé. Les petits groupes des chondrocytes étaient isolés du cartilage après incubation avec [^{35}S]Na_2SO_4. Les mucopolysaccharides étaient extractés de ces cellules par la technique de micromanipulation. Le rivanol permet la précipitation quantitative des S-mucopolysaccharides extractés de ces groupes des cellules.

J. KAWIAK and H. KOWALSKI

Department of Histology and Embryology and Department of Physics, Medical School, Warszawa (Poland), December 4, 1963.

[4] J. E. EDSTRÖM and J. KAWIAK, J. Biophys. Biochem. Cytol. *9*, 619 (1961).

Localization of Monoamines in the Lower Brain Stem

With the help of a new fluorescence method for the histochemical localization of monoamines, it has been shown that noradrenaline and 5-hydroxytryptamine, in, for example, the hypothalamus and spinal cord, are accumulated in very high concentrations in synaptic terminals[1,2]. The terminals originate from two special types of neurons, which contain in their cell bodies low concentrations of the respective amines and which may be characterized as adrenergic and 5-hydroxytryptaminergic respectively[3]. Evidence has now been obtained that also the dopamine, which is to be found in significant amounts in the lower brain stem[4], is localized to special neuron systems.

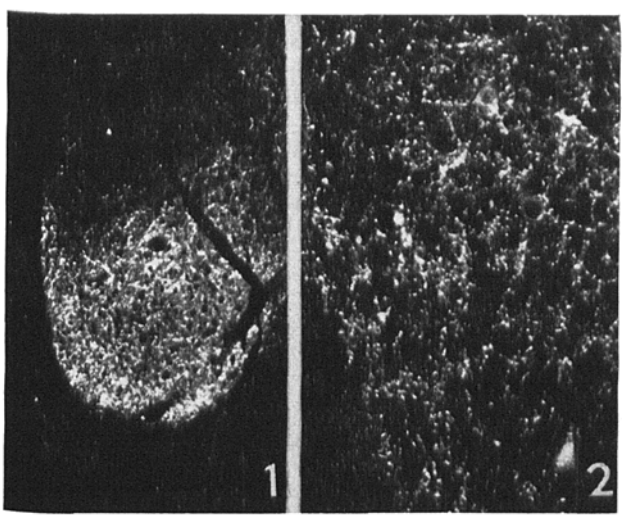
Mesencephalon, pons and medulla oblongata were examined in some 250 adult male albino rats, with the method of FALCK and HILLARP[5] as described in detail in another paper[3]. Histochemical and pharmacological experiments (reserpine, reserpine-nialamide, *m*-tyrosine, α-methyl-*m*-tyrosine) were used to check the specificity of the fluorescence reactions[1–3].

Following formaldehyde treatment, a specific fluorescence due to the presence of monoamines developed in the pericarya of several groups of nerve cells, and in typical nerve terminals present, in varying abundance, almost everywhere in the lower brain stem. The terminals were

[1] A. CARLSSON, B. FALCK, and N.-Å. HILLARP, Acta physiol. scand. *56*, Suppl. 196 (1962).
[2] A. CARLSSON, B. FALCK, K. FUXE, and N.-Å. HILLARP, Acta physiol. scand., in press (1964).
[3] A. DAHLSTRÖM and K. FUXE, Acta physiol. scand., in press (1964).
[4] Å. BERTLER, Acta physiol. scand. *51*, 97 (1961). – Å. BERTLER and E. ROSENGREN, Acta physiol. scand. *47*, 350 (1959). – A. CARLSSON, Pharm. Rev. *11*, 490 (1959).
[5] B. FALCK, N.-Å. HILLARP, G. THIEME, and A. TORP, J. Histochem. Cytochem. *10*, 348 (1962). – B. FALCK, Acta physiol. scand. *56*, Suppl. 197 (1962).

fine and had abundant varicosities, which showed an intense green or yellow fluorescence. Histochemically and pharmacologically, three types of terminals could be distinguished containing very high concentrations of – in all probability – dopamine, noradrenaline and 5-hydroxytryptamine respectively.

Noradrenaline and dopamine terminals. In agreement with the chemical determinations[4], most of the green fluorescent terminals were found to be of the noradrenaline type, which have a wide-spread distribution, i.a. in the entire reticular formation. More or less dense accumulations of such terminals exist in several nuclei of the lower brain stem (e.g. nucleus of Edinger-Westphal, motor nucleus of the trigeminal nerve). The nucleus tractus solitarius and the dorsal motor nucleus of vagus have a very high accumulation of green fluorescent terminals (Figures 1 and 2) but a large proportion of them seem to contain dopamine. Other nuclei of the cranial nerves receive a much sparser innervation (Nucleus n. VII, XII acusticus, and nucleus ambiguus) or none at all (Nucleus n. III, IV, VI and vestibularis).



The cranial part of the nucleus tractus solitarius (1) and the dorsal motor nucleus of the vagus (2) in the rat. A high accumulation of fine, varicose, green fluorescent fibres is present. Fluorescence microphotograph: ×75 and ×190.

5-Hydroxytryptamine terminals. These terminals were more difficult to observe. They are mostly very fine, and their yellow fluorescence disappears rapidly on irradiation with UV-light[3]. In contrast to that of the catecholamine terminals, the fluorescence of the yellow terminals reappears in reserpinized animals after nialamide treatment[3]. Many terminals of this type were found together with catecholamine terminals in the lower brain stem, e.g. in the motor nuclei of the vagus and trigeminal nerves and the nucleus tractus solitarius. A more dense accumulation of terminals, almost exclusively of this type, was detected in an area approximately corresponding to nucleus intercalatus. Some of the nuclei of the cranial nerves, such as nucleus cochlearis, Edinger-Westphal and ambiguus, which receive catecholamine terminals, seem to lack terminals of the 5-hydroxytryptamine type.

Several groups of nerve cells with low concentrations of either a primary catecholamine or 5-hydroxytryptamine in their cell bodies and processes were found in the lower brain stem. Of particular interest is the finding that a large group of nerve cells which seem to contain dopamine is present in the area of substantia nigra. This area, which is connected to the caudate nucleus, has been found to have a fairly high concentration of dopamine[4]. Since nerve terminals containing very high concentrations of dopamine have been identified in the caudate nucleus[6], it seems possible that the nerve cells detected in the substantia nigra area are dopaminergic neurons that send their axons to the caudate nucleus.

Zusammenfassung. Histochemische und pharmakologische Experimente sprechen stark dafür, dass Dopamin, Noradrenalin und 5-Hydroxitryptamin im Hirnstamm der Ratte in drei Typen von Nervenzellen und Endsynapsen gespeichert werden.

ANNICA DAHLSTRÖM and K. FUXE[7]

Department of Histology, Karolinska Institutet, Stockholm (Sweden), February 14, 1964.

[6] K. FUXE, T. HÖKFELT, and O. NILSSON, Z. Zellf., in press (1964).

[7] This work has been supported by research grants from the United States Public Health Service (NB 02854-04), the Knut and Alice Wallenberg Foundation, and the Swedish Medical Research Council.

The Importance of Sulfur and Iron in the Retina as Determined by Paramagnetic Resonance Studies

Several mechanisms have been suggested for the mode of action of light transfer in the retina[1]. The complete mechanism, however, is still unknown. Intermolecular energy transfer in the retina is considered to be the most probable process for nervous excitation in the rods and cones. It is the purpose of this communication to discuss another possible concept and to give some of the evidence which supports it.

Recently we have reported[2] the presence of sulfur in many biological systems as determined by means of electron paramagnetic resonance (EPR) studies. The importance of sulfur for charge transfer was pointed out.

A sulfur signal (characterized by a delocalization of odd electrons by the sulfur-containing group) was also found to be present in retinae which were obtained from deceased human beings within hours after death occurred. After air drying, the EPR spectrum of samples of the retinal tissue (a few mg) was measured at 295° K as well as at 77° K. Experimental details are given elsewhere[2].

[1] G. WALD, in *Proceedings of the Symposium on the Structure of the Eye* (Academic Press, New York 1961).

[2] W. LOHMANN, Biochim. biophys. Acta, in press.