Table II. Content ($\mu g/g$) of dopamine and noradrenaline in the rat brain excluding the cerebellum 0, 1, 12, and 24 h after death

h after death	No. of animals	Dopamine Mean ± S.E.M.	%[a]	P[b]	Noradrenaline Mean ± S.E.M.	%[a]	P[b]
0	14	0.61 ± 0.035			0.49 ± 0.015		
1	8	0.52 ± 0.054	85.2	> 0.05	0.36 ± 0.026	73.5	< 0.01
12	4	0.51 ± 0.049	83.6	> 0.05	0.32 ± 0.019	65.3	< 0.001
24	4	0.36 ± 0.033	59.0	< 0.001	0.32 ± 0.018	65.3	< 0.001

[a] Percentage of 0 h value. [b] Compared with 0 h value.

The results show that the areas including the tuberohypophysial system contain the highest concentration of dopamine and noradrenaline in the hypothalamus. According to the pharmacological experiments, the main monoamine in the fluorescent nerve fibres in the median eminence of the rat and mouse is dopamine, but noradrenaline is also present, although to a lesser degree[1]. In the species investigated in the present work, relatively high amounts of noradrenaline were present in the median eminence and infundibular stem region. This is presumably due to species differences as has been observed in cat, sheep and goat[7]. The highest value of noradrenaline was in the mediobasal hypothalamus which is obviously based on the existence of many noradrenaline-containing neurons in this area; these do not belong to the tuberohypophysial system and terminate elsewhere than in the median eminence.

It was found that the content of dopamine and noradrenaline in the rat brain decreased after death, as has been reported in rabbit[8] and man[9]. Thus the relatively long interval between death and autopsy in man has very probably affected on the values obtained.

Zusammenfassung. Mit chemischer Methode wurden bei grösseren Tierarten die Mengen von Dopamin und Noradrenalin im Eminentia-mediana-Infundibulum-Gebiet, im mediobasalen Hypothalamus und im Endteil des Hypothalamus bei Mensch, Schwein und Rind genau bestimmt. Die höchste Konzentration von Dopamin wurde in Eminentia mediana und die höchste Konzentration von Noradrenalin im mediobasalen Hypothalamus gefunden.

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[7] R. LAVERTY and D. F. SHARMAN, Br. J. Pharmac. Chemother. *24*, 538 (1965).
[8] Å. BERTLER and E. ROSENGREN, Acta physiol. scand. *47*, 350 (1959).
[9] P. O. GANROT, E. ROSENGREN and C. G. GOTTFRIES, Experientia *18*, 260 (1962).
[10] Aided by a grant from the Sigrid Jusélius Stiftelse.

Staining of the Reducing Material in the Tubero-Hypophysial System of the Rat with Osmium Tetroxide

Histological[1–4], histochemical[2,5] and, recently, electron microscopic[6–10] techniques have demonstrated that numerous hypothalamic nerve fibres terminate in the median eminence around the capillaries of the hypophysial portal vessels constituting a neurovascular link between the hypothalamus and the anterior pituitary gland. The exact site of origin of these nerve fibres is not completely clear. However, recent experimental studies[11,12] with the histochemical fluorescence method have indicated the arcuate and periventricular nuclei to be the main site of origin of the catecholamine containing nerve fibres. In the present work the existence of a similar tuberohypophysial system is demonstrated using a combination of glutaraldehyde and osmium tetroxide technique to visualize reducing material in its neurons.

Material and methods. The material consisted of adult female and male rats of Long-Evans strain. After rapid decapitation, the hypothalamus was removed and fixed for 4–6 h in $6^1/_2$% glutaraldehyde buffered to pH 7.3 with a phosphate buffer. Then the tissue blocks or 20 μ thick frozen sections were treated with cocodylate buffered 0.5% osmium tetroxide (pH 7.0) for 20–40 min in room temperature. Some of the sections were treated with 0.1-*N* NaOH for 20–30 min.

Results. The treatment in glutaraldehyde and osmium resulted in a black staining of the perikarya of the neurons in the arcuate (infundibular) and periventricular nuclei (Figure 1). The finely granular precipitate was evenly distributed in the cytoplasm of the perikaryon and extended often into the axon (Figure 2). The staining was restricted to the small neurons of the nuclei. They were, however, scattered throughout the above-mentioned nuclei (Figure 1).

[1] H. NOWAKOWSKI, Dt. Z. NervHeilk. *165*, 261 (1951).
[2] U. K. RINNE, Acta endocr., Copenh. *35* Suppl. 57 (1960).
[3] U. K. RINNE, Acta neuroveg. *25*, 310 (1963).
[4] J. SZENTÁGOTHAI, B. FLERKÓ, B. MESS and B. HALÁSZ, *Hypothalamic Control of the Anterior Pituitary* (Académiai Kiadó, Budapest 1962).
[5] K. FUXE, Z. Zellforsch. mikrosk. Anat. *61*, 710 (1964).
[6] J. BARRY and G. COTTE, Z. Zellforsch. mikrosk. Anat. *57*, 714 (1961).
[7] H. KOBAYSHI, H. A. BERN, R. S. NISHIOKA and Y. HYODO, Gen. comp. Endocr. *1*, 545 (1961).
[8] M. MAZZUCA, Annls Endocr. *25*, 643 (1964).
[9] U. K. RINNE and A. U. ARSTILA, Med. Pharmac. exp. *15*, 357 (1966).
[10] U. K. RINNE, Z. Zellforsch. mikrosk. Anat. *74*, 98 (1966).
[11] W. LICHTENSTEIGER and H. LANGEMANN, J. Pharmac. exp. Ther. *151*, 400 (1966).
[12] K. FUXE and T. HÖKFELT, Acta physiol. scand. *66*, 245 (1966).

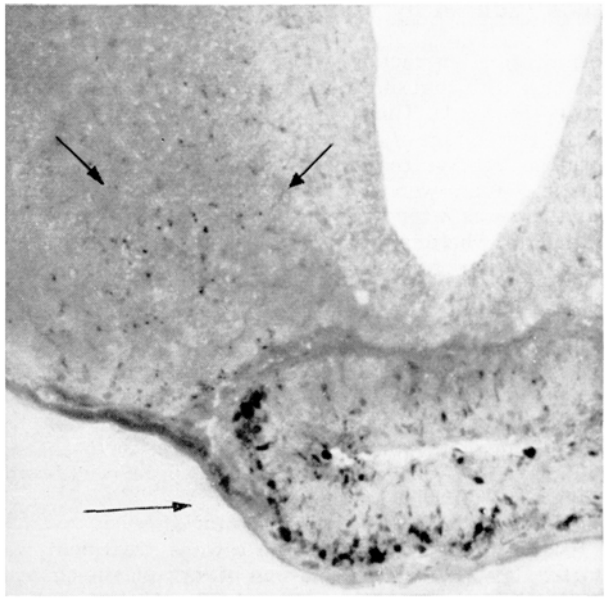

Fig. 1 Black stained reducing material in some cells of the arcuate nucleus (short arrows) and in the median eminence (long arrow). × 45.

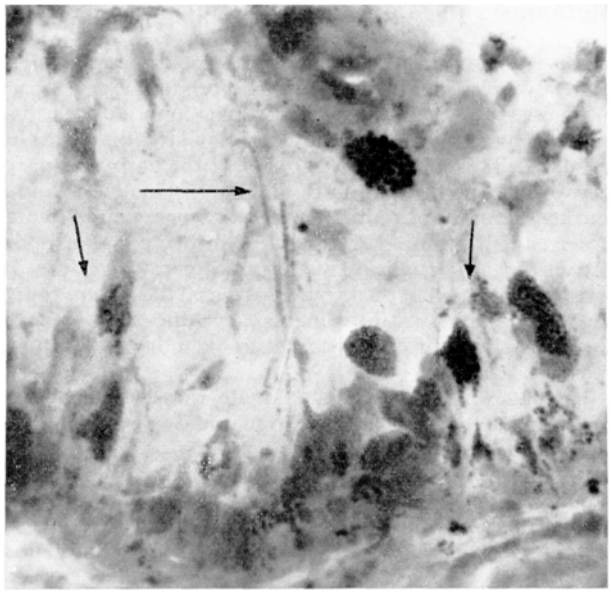

Fig. 3. A greater magnification of the median eminence. There are seen bulbous perivascular structures (short arrows) and beaded striae (long arrow). × 250.

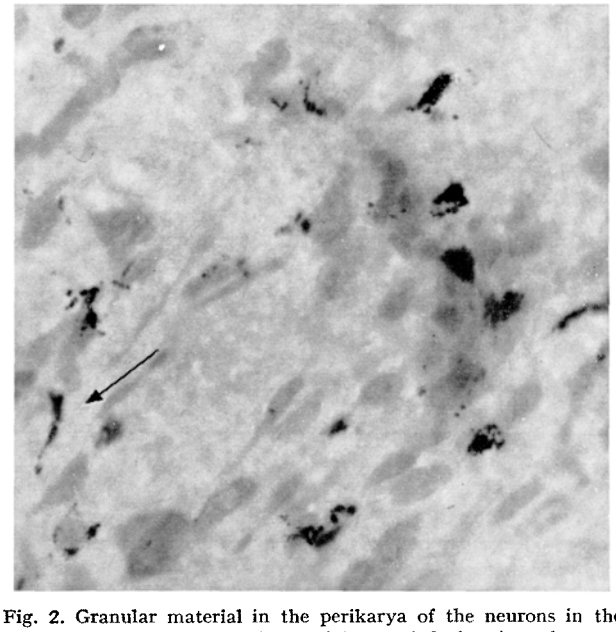

Fig. 2. Granular material in the perikarya of the neurons in the arcuate nucleus. The stained material extended often into the axon (arrow). Slightly counterstained with methylene blue. × 250.

The stained material was also seen in the median eminence as coarse granules of about 2–3 μ in diameter in bead-like striae terminating perivascularly (Figures 1 and 3). Bulbous perivascular structures resembling the perivascular nerve endings demonstrated[2] in this region were often seen as well.

Both in the perikarya and in the perivascular endings the material was dissolved or auto-oxidized in sodium hydroxide. In the median eminence, the stained material became gradually dissolved to the mounting medium in the course of several hours.

Discussion. The results show that, with glutaraldehyde and osmium tetroxide technique, reducing material can be demonstrated in the neurons of a few hypothalamic nuclei only. However, it seems likely that equal reducing material is present in the perivascular nerve terminals in the median eminence. These nerve endings may therefore belong to the stained neurons of the arcuate and periventricular nuclei. The final elucidation of this relationship calls, however, for more direct evidence. The neurons demonstrated with glutaraldehyde and osmium tetroxide apparently belong to the tubero-hypophysial system[4,11,12].

The main catecholamine of the fluorescent nerve fibres in the median eminence has been reported to be dopamine, although noradrenaline is also present[5]. One can think that the reducing material demonstrated with our glutaraldehyde-osmium technique could be catecholamines, especially as noradrenaline is histochemically demonstrable in the adrenal medulla with this method[13]. However, there was no depletion of the stained material after reserpine treatment[14], which speaks in favour of the fact that the reducing material in the tubero-hypophysial system is not catecholamines.

Zusammenfassung. Im Perikaryon der kleinen Neuronen des Nucleus arcuatus und periventricularis des Rattenhypothalamus kann man nach Fixation mit Glutardialdehyd und nach Oxydation durch Osmiumtetroxyd reduzierendes Material darstellen. Die gleichen reduzierenden Substanzen gibt es auch in der Eminentia mediana, wo sie perivasculär teilweise in Form kleiner Granula angehäuft sind oder eine bandartige Anordnung zeigen.

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Department of Anatomy and Department of Neurology, University of Turku (Finland), 11 September 1967.

[13] R. E. Coupland and D. Hopwood, J. Anat. *100*, 227 (1966).
[14] E. Mäkinen and U. K. Rinne, to be published.
[15] Aided by a grant from the Sigrid Jusélius Stiftelse.