technique has now been developed for the separation of relatively pure specific nerve terminals. Cerebellar mossy-fibre terminals are among the largest nerve terminals in the central nervous system. This anatomical feature was utilized in earlier attempts for their separation (Israel & Whittaker, 1965). Hajós *et al.* (1971) have since reported the isolation, in a relatively well-preserved state, of large glomerular structures from cerebellum consisting of central 'rosettes' of mossy terminals surrounded by synaptic digits of granule-cell dendrites. Contamination by structures of probable molecular-layer origin was still in evidence. The further purification of the 'mossy-terminal fraction' is now described.

Development of the fractionation procedure was guided by electron-microscopic examinations. In addition, the following enzyme activities were determined as markers of subcellular constituents: cytoplasm, lactate dehydrogenase; mitochondria, glutamate dehydrogenase and succinate dehydrogenase; nerve terminals, glutamate decarboxylase and choline acetyltransferase. Protein and DNA were also determined.

Crude 'mossy-terminal fraction' was obtained as described by Hajós *et al.* (1971). All sucrose solutions contained 1mM-MgSO₄. The cerebellum was dispersed in 0.3M-sucrose manually with a modified Dounce homogenizer, and the homogenate was centrifuged at 900g for 10min after filtration through four consecutive nylon sieves (1000, 120, 75 and 50µm). The resuspended pellet was resolved on a discontinuous sucrose density gradient by centrifugation in a Spinco SW25 rotor at 53000g for 2h. Mossy terminals were obtained at the 1.2M/1.4M-sucrose interphase. This fraction was further purified by centrifugation at 53000g for 2h on a continuous sucrose density gradient (1.2–1.35M) layered on top of 2M-sucrose. Relatively well-preserved mossy-terminal structures were concentrated in a narrow band above the 1.35M/2M-sucrose interphase (*M* fraction) and were clearly separated from a particulate layer in the upper half of the gradient. This contained mainly damaged mossy terminals in the lower region and large tissue fragments in the upper regions, which are the contaminants of the initial crude 'mossy-terminal fraction'. Significantly, in the *M* fraction only 1% of the total protein was recovered, in contrast with about 30% in the crude 'mossy-terminal preparation'. In five experiments the relative specific activities of the *M* fraction were 2.6 ± 0.35 for glutamate decarboxylase, 1.09 ± 0.135 for choline acetyltransferase, 2.1 ± 0.26 for succinate dehydrogenase, 1.2 ± 0.10 for glutamate dehydrogenase and 0.86 ± 0.17 for lactate dehydrogenase. Electron-microscopic examination showed that mossy-terminal-containing structures constituted the major part of the *M* fraction; very occasionally an endothelial-cell nucleus or molecular-layer fragment was seen.

Preparation time of the *M* fraction can be shortened by separating this fraction directly from the resuspended initial low-speed sediment on a linear sucrose density gradient described above. Both electron-microscopic and biochemical studies suggested that the *M* fraction so obtained is less pure than that prepared by the longer procedure described, but the morphological preservation of the mossy-terminal fragments is better.

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Use of Dansyl Chloride and Micro-Chromatography to Detect and Study the Metabolism of Suspected Transmitter Substances and Amino Acids in Single Neurons

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A micro biochemical method (Neuhoff, 1971; Osborne *et al.*, 1971) was used to study the amine and amino acid contents in two specific types of giant cells in the snail *Helix pomatia*. One type of cell (GSC) is known to contain 5-hydroxytryptamine (serotonin) (Cottrell & Osborne, 1970), whereas the other type of neuron lacks this amine (see Briel *et al.*, 1971). Cell extracts were treated with dansyl chloride, and the dansylated substances were separated on 3cm × 3cm polyamide layers. The patterns of amino acids in both were similar. However, the buccal neurons contained significantly more methionine and glycine but less ornithine than the GSC neurons, where the presence of 5-hydroxytryptamine was confirmed and the two additional substances 5-hydroxytryptophan and 5-hydroxyindole were detected. Analysis of the 5-hydroxytryptamine-containing Retzius cells (Rude *et al.*, 1969) of the leech *Hirudo medicinalis* showed that these cells, like the GSC neurons, contained large amounts of glycine and alanine, although they differ in having higher amounts of 5-hydroxytryptamine, tryptophan, 5-hydroxytryptophan and glutamate.

When 5-hydroxy[¹⁴C]tryptophan was perfused through the snail's central ganglia only the GSC

neurons (and not be buccal neurons) synthesized 5-hydroxy[^{14}C]tryptamine. It was estimated from a number of experiments that the GSC neurons *in vivo* form 0.8 ng of 5-hydroxytryptamine/2h at 20°C.

The capacity of the GSC neurons to metabolize [^{14}C]glucose and [^{14}C]glutamate *in vivo* was also investigated. Glucose was metabolized to form alanine, glutamine, glutamate and aspartate, but no γ -aminobutyrate. Histidine, arginine and five unknown substances were also formed. One of the unknown substances occurred in greater quantities than alanine, which is normally the predominant substance produced by the metabolism of glucose in snail nervous tissue *in vitro* (Osborne *et al.*, 1971). Electrical stimulation of the GSC neurons during perfusion with [^{14}C]glucose increased the production of some of the metabolites and also produced two further unidentified substances. Only three radioactive substances (glutamine, alanine and an unidentified compound), all in low concentrations, were detected in GSC neurons perfused with [^{14}C]glutamate. Electrical stimulation of the GSC neurons increased the concentrations of all these substances. These experiments demonstrated the chemical heterogeneity of neurons in the snail's central ganglia and also the chemical variation between the two 5-hydroxytryptamine-containing neurons in the snail *Helix pomatia* and the leech *Hirudo medicinalis*. Moreover, it was possible to assess the formation of 5-hydroxytryptamine *in vivo* and the effect of electrical stimulation on the metabolism of glucose and glutamate *in vivo* in a single neuron.

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Effect of Potassium Ion Concentration on Cultured Sympathetic Ganglia: Conclusions about Trans-synaptic Modulation

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Changes in trans-synaptic impulse traffic are capable of inducing long-term changes in the tyrosine hydroxylase activity of both sympathetic ganglia and adrenal medulla (Molinoff & Axelrod, 1971). However, the mechanism of such neurally mediated changes in enzyme activity is not yet understood.

We have examined the effect of raising the external K^+ concentration as a depolarizing stimulus on the tyrosine hydroxylase activity of adrenergic neurons of isolated mouse superior cervical ganglia maintained in organ culture. The ganglia were explanted from mice of from 2 to 28 days of age and maintained on membrane-filter rafts in modified Eagle's medium (see Mackay & Iversen, 1972) for up to 48 h. Choline acetyltransferase activity, a marker for presynaptic nerve remnants, had completely disappeared after this period in culture. Raising the K^+ concentration in the medium from 5.4 mM to 27, 54 or 81 mM for 48 h resulted in increases of up to 114% in tyrosine hydroxylase activity. Similar increases in the Na^+ concentration of the medium had no significant effect on this enzyme activity. The response was obtained both in the presence and in the absence of the nerve growth factor isolated from adult male mouse submaxillary glands. We have found the 7S fraction of nerve growth factor to be a potent stimulus for increasing tyrosine hydroxylase activity in neonatal superior cervical ganglia in culture, and when ganglia were stimulated maximally by 1.6 μg of nerve growth factor/ml they failed to respond further to increased K^+ . However, in the absence of added nerve growth factor K^+ evoked a 70% increase in tyrosine hydroxylase activity within 24 h.

The capacity of ganglia from very young animals to respond to a depolarizing stimulus may be important in the induction of enzyme activity in developing neurons. Black *et al.* (1971) observed a threefold increase in tyrosine hydroxylase activity in the mouse superior cervical ganglion between the 7th. and 13th. days of life. This increase correlated with the development of ganglionic synaptic junctions and could be prevented by decentralization.

Our present findings support the hypothesis that a trans-synaptic stimulus is responsible for the biochemical maturation of these ganglia, and furthermore suggest that increased depolarization of adrenergic neurons may be sufficient to account for the effects of presynaptic nerve traffic on post-synaptic activities. Work with cholinergic agonists