Log concentration-effect curves were nearly linear over a 10-fold range of concentration. For any one drug the line representing the denervated strips was not appreciably different from that representing innervated strips. EC50s were calculated; they are as follows: procaine, $4{\cdot}2\times10^{-4}$M innervated and $3{\cdot}6\times10^{-4}$M denervated; nupercaine, $3{\cdot}5\times10^{-6}$M innervated and $4{\cdot}0\times10^{-6}$M denervated; quinidine, $1{\cdot}9\times10^{-4}$M innervated and $1{\cdot}8\times10^{-4}$M denervated. In four experiments, the effect of procaine on denervated muscle was measured in the presence of $2{\cdot}5\times10^{-7}$ M TTX; the average maximum rate of rise in the TTX-resistant portion of the action potential was depressed to essentially the same relative degree as the normal action potential. The sensitivity to procaine thus seems to be the same in TTX-resistant and TTX-sensitive channels.

The results suggest that the change in the sodium channels following denervation is a selective one, resulting in a decrease in their sensitivity to TTX but not to the less specific local anaesthetics. It is possible that the changes are greater on the outer surface of the membrane, where TTX and acetylcholine are known to act, rather than the inner surface, where local anaesthetics are thought to act (e.g., see Frazier, Narahashi and Yamada, 1970).

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Application of the dansyl procedure to study the metabolism and accumulation of 5-hydroxytryptamine in characterized neurones of *Helix pomatia*

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A microbiochemical method involving the use of dansyl chloride and microchromatography (Osborne, Briel & Neuhoff, 1971; Briel, Neuhoff & Maier, 1972; Osborne, 1973) was used to analyse the metabolism and accumulation of 5-hydroxytryptamine (5-HT) in characterized snail neurones (GSCs) which contain the amine (Cottrell & Osborne, 1970; Osborne & Neuhoff, 1973). ^{14}C-Tryptophan (10^{-7}M radioactive tryptophan/ml over 2 h: 1 ml/snail) perfused through the central nervous system of the snail was taken up by the GSCs and also cells (buccal cells) which lack biogenic amines (Cottrell, 1970; Osborne, 1972). Only the GSCs however, have the capacity to metabolize ^{14}C-tryptophan to form some 5-hydroxytryptophan and slightly more 5-HT. Electrical stimulation of the GSCs, strong enough to elicit cell firing, resulted in very much more 5-HT being produced, though there was a slight increase in the amount of labelled tryptophan and 5-hydroxytryptophan. Doubling the duration of stimulation and the amount of ^{14}C-tryptophan perfused through the central nervous system had no great influence on the content of radioactive substances found in the GSC. Pretreatment of snails with p-chlorophenylalanine, an inhibitor of tryptophan-hydroxylase in the vertebrates (Koe & Weissman, 1966), though not interfering with the uptake of tryptophan into the GSCs, almost completely prevented the formation of 5-hydroxytryptophan. Perfusion of the central nervous system with ^{14}C-5-HT (10^{-7} mol radioactive 5-HT/ml: 1·5 ml/snail over a period of 4 h) showed that the GSCs accumulated amine, while the buccal cells lacked this ability. None of the ^{14}C-5-HT within the GSCs were metabolized. In contrast, the whole central nervous system not only accumulated radioactive 5-HT, but also metabolized part of it to form 5-hydroxyindoleacetic acid.

The effects of imipramine, desipramine and nialamide upon the accumulation of ^{14}C-5-HT into the GSCs were also studied, and the results are summarized in Table 1.

TABLE 1. *Effect of imipramine* (10^{-4}mol/*ml*), *desipramine* (10^{-4}mol/*ml*) *and nialamide* (10^{-3}mol/*ml*) *on the accumulation of* ^{14}*C-5-HT into the GSCs of* Helix pomatia. *The figure in brackets represents the number of experiments performed.*

	Accumulation of ^{14}C-5HT into GSCs	Accumulation of ^{14}C-5-HT into brain tissue
%Inhibition of ^{14}C-5-HT accumulation caused by imipramine	24±6% (5)	51±4% (5)
% Inhibition of ^{14}C-5-HT accumulation caused by desipramine	18±3% (4)	40±5% (4)
% Increase of ^{14}C-5-HT accumulation caused by nialamide	3±1% (5)	48±5% (5)

It is concluded from these studies that the tryptophan hydroxylating enzyme (tryptophan-hydroxylase) is present in 5-HT containing cells (GSCs) alone. From the specific accumulation of ^{14}C-5-HT by cell somata containing the amine (GSCs), and from the effects of imipramine, desipramine and nialamide upon the accumulation process and the metabolism of the amine by whole nervous tissue (which consists of many 5-HT containing synapses), it is deduced that 5-HT is probably inactivated in two ways: by enzymatic oxidation and by reuptake into synaptic terminals.

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Amino acids in the frog central nervous system

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In a preliminary attempt to characterize the optic nerve transmitter in the frog the concentrations of a number of amino acids and related compounds in the cerebral cortex, optic tectum and optic nerve of the frog were measured as their ^{3}H-dansyl derivatives by the modified method described by Roberts, Keen & Mitchell (1973). A unique, or high concentration of a compound in the optic nerve and/or tectum, compared with other parts of the nervous system, might suggest a transmitter role for that substance at optic nerve terminals. In these studies no compound was found which occurred uniquely in the optic nerve or tectum.

In a comparison of the levels of nine amino acids in the optic nerve, tectum and cerebral cortex, the relative concentration of GABA in the cortex, (3·1±0·4 μmol/g wet weight (mean±S.E.)) and in the tectum (2·2±0·3 μmol/g) when compared with the optic nerve (0·4 μmol±0·01 μmol/g), was the most outstanding feature. This finding, and the large body of evidence which indicates that GABA is likely to be an inhibitory transmitter in the central nervous system of mammals, suggested that GABA, while almost certainly not being the optic nerve transmitter, might nevertheless have a transmitter function in the frog cortex and tectum.