NIKIFOROWSKY[9] wies bereits darauf hin, dass die Eintrittsschwankung nach Einsetzen des Lichtreizes bei tiefen Temperaturen verschwinden kann, obgleich die Verdunklungsschwankung nach Beendigung des Lichtreizes weiterhin bestehen bleibt. Diesem Befund entspricht die Beobachtung, dass der verspätete Aus-Effekt noch bei 6 °C auslösbar ist, wenn die b-Welle bereits nicht mehr registrierbar ist.

Summary. In a range from 6 to 30 °C, the influence of temperature on the relation between the light intensity and the amplitude of the b-wave of the exposure potential has been investigated for the isolated frog retina. Between 10 and 25 °C, the gross activation energy of the response to light leads to a temperature coefficient which shows that the formation of the b-wave is mainly checked by diffusion processes. While at 6 °C the b-wave has vanished, a complete delayed off-response can still be registered.

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The Effect of Imipramine and Nialamide on the Accumulation of Serotonin in Snail (*Helix pomatia*) Nervous Tissue

Though there is evidence that serotonin is specifically taken up by serotonin-containing nerve terminals[1], which might be a possible way of inactivation, 5-hydroxyindole acetic acid (5-HIAA), a major breakdown product of serotonin in mammals[2] also occurs in molluscan nervous tissue[3]. In the light of these observations we decided to analyse the accumulation and catabolism of serotonin in the nervous tissue and identified serotonin-containing somata (GSCs)[4–6] of the snail *Helix pomatia*, using a sensitive microprocedure which detects picomole quantities of this amine[7,8]. Since imipramine potentiates transmission between the GSCs and other cells[9] and inhibits the uptake of serotonin into blood platelets[10], the role of this chemical in the uptake of serotonin was also studied. In addition, the effects of nialamide, a specific inhibitor of monoamine oxidase (MAO), on the catablism of ^{14}C-serotonin were analysed.

Materials and method. The anterior aorta was canulated just before it enters the central ganglia and perfused with snail saline[11] containing either ^{14}-C-serotonin (from Radiochemical Centre, Amersham; specific activity 57 mCi/m*M*: the concentration of the perfused ^{14}C-serotonin was always 5.4 µCi/ml of perfused saline; this corresponds to 10^{-7} *M* serotonin) alone, ^{14}C-serotonin plus imipramine (100 µg/ml) or ^{14}C-serotonin plus nialamide (1 mg/ml). The perfusion fluid was in a 1.5 ml syringe which could be adjusted so that the time and pressure of the perfused solution could be controlled. After perfusion with 1.5 ml radioactive substance (at a rate of 1.5 ml/4 h), the brain was perfused with snail saline for 5 min and then rapidly dissected. Individual GSCs were carefully hand-dissected[4–6,12]. The dissection of a single GSC took less than 4 min, so that any further metabolism or redistribution of the labelled substances was kept to the minimum. 6 neuron somata (GSCs) were transferred to a microtube containing 10 µl distilled water and frozen and thawed in liquid nitrogen to release the cell's contents. The sample was then frozen and freeze-dried, resuspended in 2 µl of 0.05 *M* sodium bicarbonate pH 9 and acetone (dilute 1:2 v/v) and dansylated with 1 µl dansyl chloride (1 mg/ml in acetone)[6,7,12,13]. This extract was then subjected to 2-dimensional microchromatography as described previously[6,7,12,13]. In other experiments,

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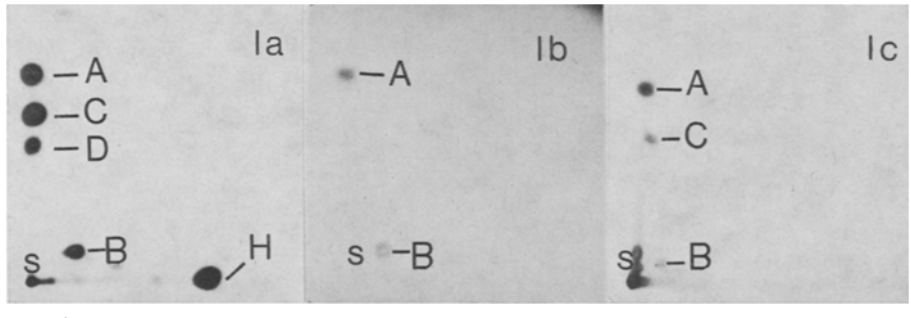

Fig. 1. Autoradiograms of microchromatograms from GSCs (Figure 1b) and brain tissue (Figure 1c) from snails perfused with ^{14}C-serotonin, and extracts reacted with unlabelled dansyl chloride. Figure 1a is an autoradiogram of pure radioactive dansyl substances to show the chromatographic positions of dansyl serotonin (A and B), dansyl 5-HIAA (C), and dansyl 5-hydroxytryptophol (D). It can be seen that both the GSCs and brain accumulate ^{14}C-serotonin, whilst only the former converts part of it into 5-HIAA. The original size of a single microchromatogram measured 3 × 3 cm, and was developed in the first dimension (horizontal direction) with water/formic acid (100:3 v/v) and in the second dimension (vertical direction) with benzene/acetic acid (9:1 v/v). s, starting point; H, dansyl-OH, the reactive product between ^{14}C-dansyl chloride and water.

Effect of imipramine (100 µg/ml) and nialamide (1 mg/ml) on the accumulation and metabolism of ^{14}C-serotonin in the GSCs and brain of *Helix pomatia*

	Accumulation of ^{14}C-serotonin into GSCs	Accumulation of ^{14}C-serotonin into brain tissue	Formation of ^{14}C-5HIAA by GSCs	Formation ^{14}C-5HIAA by brain
Snail saline	77 ± 12 ng (5)	73 ± 8 ng (10)	0 (5)	18 ± 7 ng (10)
Snail saline plus imipramine	59 ± 14 ng (5)	36 ± 9 ng (10)	0 (5)	16 ± 9 ng (10)
Inhibition of ^{14}C-serotonin accumulation caused by imipramine (%)	24	51	—	—
Snail saline plus nialamide	79 ± 11 ng (5)	108 ± 10 ng (10)	0 (5)	10 ± 4 ng (10)
Increase of ^{14}C-serotonin accumulation caused by nialamide (%)	3	48	—	—

The density of the GSCs was assumed to be that of water; a single GSC was hence calculated to weigh 1 ng[16]. From the measured counts and specific activity, the content of ^{14}C-serotonin and ^{14}C-5-HIAA in the samples (in terms of ng/g wet wt. of tissue) could be measured. The radioactivity associated with these 2 substances on 3 separate microchromatograms was pooled and considered as a single experiment. The mean value for a number of experiments (figure in brackets) and the mean deviation in each case is given below.

amines and amino acids were extracted from whole perfused brains (including connective tissues), reacted with unlabelled dansyl chloride and some of the dansyl substances chromatographed[6,7,12,13]. Autoradiograms of the microchromatograms were then prepared (exposure time 3 days). The radioactive spots on the chromatograms were subsequently localized, scraped from the plates and then suspended in scintillation liquid and counted in a Packard spectrometer[7,8].

Results and discussion. Figure 1a is an autoradiogram showing the position of pure dansylated ^{14}C-5-hydroxytryptophol and 5-HIAA, 2 metabilitis of ^{14}C-serotonin in vertebrate tissues[14]. It should be pointed out that substances like serotonin and 5-hydroxytryptophol, which contain more than one aliphatic amine and/or hydroxy group, form one or more dansyl derivatives depending on the experimental conditions[4,7,8,13]. Under the conditions used in this study, all substances with the exception of serotonin[13] occur as a single substance. By adding a minute but definite amount of radioactive serotonin to extracts before dansylation, the recovery of the amine was found to be 85%.

It is evident from the autoradiograms that, although both the GSCs (Figure 1b) and whole brain tissue (Figure 1c) accumulate ^{14}C-serotonin, a single metabolite is formed in the brain tissue. From a number of experiments in which authentic substances were added to the tissue, it was concluded that the metabolite is 5-HIAA. It can be seen from the Table that the radioactivity associated with ^{14}C-serotonin in the brain is more than 4 times greater than the ^{14}C-5-HIAA content. The data from the Table also indicate that the GSCs accumulate only slightly more ^{14}C-serotonin than the brain. This is surprising, especially since the brain contains only a small population of serotonin-containing nerve tissue. However, a number of explanations can be given. Firstly, some non-serotonin-containing tissue in the brain accumulates serotonin. Secondly, a loss of some ^{14}C-serotonin from the GSCs probably occurs during the dissection period. Thirdly, the error in calculating the net weight of single GSCs can be great, as one assumes that GSCs are of the same size (see Table). Fourthly, the neuron somata is not likely to be the most active part of the GSC involved in the accumulation process. With regard to the active uptake process, the fact that imipramine inhibits the serotonin accumulation to a greater extent in the brain than in the GSCs suggests that only a part of the neuron is involved in a specific uptake of the amine. This view is supported by the fact that the majority of synapses in molluscs are axo-axonic[15], and should the inactivation process of the released transmitter substance include a specific uptake mechanism, one would expect it to take place in the synaptic region.

Nialamide treatment results in the accumulation of very much more ^{14}C-serotonin in the brain than in the GSCs. This is associated with a decrease of 5-HIAA in the brain which indicates that a MAO-like enzyme is localized within the synaptic region. This, together with the fact that ^{14}C-5-HIAA occurs only in regions rich in synapses, i.e. the whole brain, suggests that breakdown of the serotonin may also be a way of inactivation.

It would thus appear from these studies that molluscan nervous tissue may, like mammalian nervous system[2], inactivate the released serotonin within the synapses in 2 ways, viz., enzymatic oxidation and re-uptake into the presynaptic terminals. The partial inhibition of the enzymatic oxidation process by nialamide also indicates that a MAO-like enzyme is responsible. These data also show that serotonin is partly metabolised in snail nervous tissue to form 5-HIAA.

Zusammenfassung. Sowohl Nervengewebe als auch definierte Nervenzellsomata (GSC) von *Helix pomatia* können zwar ^{14}C-Serotonin aufnehmen, aber nur im Nervengewebe wird es zu ^{14}C-Hydroxyindolessigsäure umgewandelt. Die Wirkung von Imipramin und Nialamid macht wahrscheinlich, dass Serotonin auf zwei Wegen inaktiviert wird, d.h. durch enzymatische Oxydation und durch Wiederaufnahme in die synaptischen Boutons.

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