observed in the eye structure of the shrimp[9] and bee[10]. It would appear possible that at least part of the UV-stimulus could have been detected by the receptors as secondary blue-green light.

The assumption of a direct interaction between ionizing radiation and rhodopsin can only be inferred from the knowledge that the energy delivered is more than adequate to produce stereoisomerization of retinal. There is the additional possibility that ionizing radiations may induce a visible fluorescence within the eye structures, which may serve as a source for receptor excitation. Rhodopsin solutions can fluoresce in vitro under relatively intense X-irradiation[11], but a contribution of fluorescence to visual stimulation through brief exposure has not been shown.

Assuming a direct action of ionizing radiation on the visual pigment, responses analogous to those elicited by visible light should be observed. This was the case with X-radiation in which 'on' and 'off' responses were recorded during abrupt changes of stimulus intensity, but not during gradual intensity changes. The amplitude of responses to a train of light stimuli declined rapidly to reach a nearly constant value, whereas the response to X-rays remained stable over a comparable train of exposures. Presumably, the migratory shielding pigment layer expanded between ommatidia so as to reduce the effectiveness of light stimulation during repeated exposures. This pigment barrier would be ineffective against penetrating X-rays so that the response amplitude remained constant.

Higher-energy β-radiation stimuli, on the other hand, produced a monophasic response. By inference from the responses to visible light and X-rays, the photoreceptors did not experience a rapid decline in stimulus intensity at cessation of β-exposure. High energy β-radiations have been shown to induce fluorescence in the eye structure of mammals[4]. There is also a unique effect, Cerenkov radiation, which occurs when highly energetic electrons exceed the velocity of light in a medium of relatively high refractive index. The Cerenkov radiation could become a source of visible light in transparent eye structures and appears relevant to this study. Cerenkov emission from a 1 MeV electron has been calculated to yield about 300 quanta between 400 and 800 nm[12]. For the rhabdom of the arthropod eye, the minimum electron energy required for this effect was calculated to be roughly 171 keV[13]. Primary and secondary electrons in this energy range were available in the present study with β-radiation, but not with X-radiation. Secondary sources of visual stimulation (strong fluorescence and Cerenkov radiation) could have produced a gradually decaying visible light output that may explain the absence of a detectable 'off' response. The response amplitude from repeated exposures to β-stimuli followed a curve similar to that for visible light (Figure 3). This finding is consistent with the hypothesis that secondary light production plays a significant role in the ERG response to energetic β-radiations. The shielding pigment would be expected to reduce the response as it did with repeated exposure to visible light.

Zusammenfassung. Im Krabbenauge von *Hemigrapsus nudus* entstehen elektroretinographische Reaktionen (ERG) mit sichtbarem Licht und Röntgenstrahlung, die andeuten, dass dieselben Rhodopsin-Erregungsprozesse mit beiden Strahlungsarten stattfinden. Nach ^{90}Sr-β-Strahlung wird angenommen, dass Cerenkov-Effekt- und induzierte Fluoreszenz-Lichterzeugung die β-ERG-Reaktion deutlich beeinflussen.

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[9] H. SCHIFF, Am. J. Physiol. *205*, 927 (1963).
[10] B. CHANCE, Proc. natn. Acad. Sci., USA *51*, 359 (1964).
[11] Ts. M. AVAKYAN, Biophysics *10*, 215 (1966).
[12] B. MARKUS, Strahlentherapie *125*, 51 (1964).
[13] N. M. JORDAN, Thesis on file, Oregon State University Library (1970), p. 75.
[14] Public Health Service Radiological Health Fellowship No. 97996-06-69.

Study of Neurosecretory Cells of *Helix pomatia* by Intracellular Dye Injection

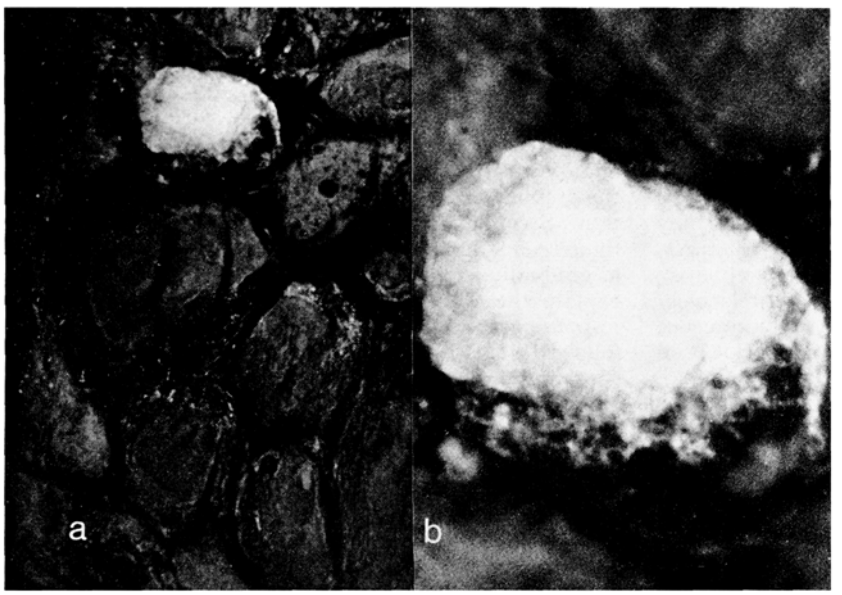
The central ganglia of the snail, *Helix pomatia*, offer a convenient preparation for studying individual nerve cells differing in their properties. Among identifiable neurones, cells designated previously[1] as cluster RPa-D are of special interest since they are neurosecretory cells. To investigate the pattern of their connections, we have applied the fluorescent dye injection technique of STRETTON and KRAVITZ[2].

Material and method. Active snails collected locally (Tihany, Hungary) were used. The ganglia were isolated as described previously[1]. The same intracellular electrode was used for both recording membrane and action potentials and dye injection. Unlike some other superficial neurones, neurosecretory cells are usually damaged during the removal of the sheath of the ganglion. It is therefore essential to drive the electrode through the intact inner layer of the sheath.

The resistance of microelectrodes filled with a nearly saturated aqueous solution of the dye, Procion Yellow M4RAN, was about 10–20 megohms. The negatively charged dye was injected by passing a steady current of about 10 nA for 40–60 min. During the subsequent 24–48 h at 4 °C the dye was allowed to become diffused. Then the ganglia were fixed in 4% formol-saline overnight, dehydrated in ethanol, cleared in methyl benzoate and embedded in paraffin. 10 μm sections mounted in Entellan-xylol were examined with a fluorescence microscope.

Results and discussion. Uniformly large neurosecretory cells constitute a compact group occupying a somewhat variable position on the dorsal surface of the right parietal ganglion. Cells of the cluster have remarkably similar morphological, physiological and pharmacological properties. They have no pigment inclusions characteristic for neighbouring neurones and are colourless, whitish or white in different specimens, due to the content of secretory material. The cells send their axons into the right pallial nerve, mainly into its external branch, where the secretory material seems to be collected under the perineurium.

Physiologically, RPa-D cells may be categorized as monomodal oscillators showing either both axonal and somatic action potentials or only the former. The duration of the somatic potential is remarkably long due to a delay in its falling phase. The cells receive fairly rich excitatory and inhibitory synaptic input. They give



a) Section through the cluster of neurosecretory cells of the right parietal ganglion. One of the cells is filled with the dye. ×202. b) The same cell at higher magnification. Fine somatic processes can be seen. ×607.

hyperpolarizing responses to acetylcholine and dopamine. Serotonin, in addition to its excitatory action, increases the duration of the delay in the somatic action potentials.

Like some identifiable neurones of *Helix aspersa* investigated by KERKUT et al.[3], RPa-D cells could produce action potentials after injecting the Procion dye, although the amplitude was somewhat smaller than before the injection. The dye did not influence resting and postsynaptic potentials.

Microscopic examination of cells filled with the dye in serial sections has shown that each cell of the cluster gives rise to one thick process (Figure). Just in the vicinity of the cell body the process divides into 2 branches, one running to the right pallial nerve and another which runs medially, passes through the cellular mass, then turns back and enters the fiber matter. Sometimes it can be followed through the connective up to the fused neuropile of the left parietal and visceral ganglia.

The cell body gives rise to multiple thin processes easily distinguishable from the thick one (Figure). These are especially numerous under the sheath of the ganglion and some of them can be followed to the sheath. The brightness of the fluorescence in thin processes is low as compared with that in the perinuclear cytoplasm, so that it is difficult to determine how far they spread within the sheath. Thin processes of the same type intermingle with similar processes of neighbouring RPa-D cells without obvious participation of satellite glia.

It seems of interest to compare cells examined in the present study with a cluster of neurosecretory cells of the abdominal ganglion of *Aplysia* ('rostral white cells', R3-R13)[4]. The 2 groups show striking similarities. Rostral white cells of *Aplysia* are reported to be distinctive because of 1. the white appearance due to the presence of secretory material, 2. the relative absence of pigment bodies, 3. the numerous deep infoldings of their plasma membrane, 4. the presence of somatic processes which end within the sheath of the ganglion, 5. the duration of their action potentials which are 2 or 3 times longer than that of most neighbouring cells, 6. the inhibitory response to acetylcholine[4,5]. Cells of the RPa-D cluster in *Helix* share all these characteristics.

It is noteworthy that R3-R13 cells of *Aplysia* are located in the right hemiganglion which is essentially the homologue of the right parietal ganglion of pulmonates. Thus, the homology of the two clusters seems quite possible. Our preliminary physiological experiments indicate that neurosecretory cells of the right parietal ganglion of *Lymnaea stagnalis*[6] have similar characteristics.

In general, the problem and criteria of neuronal homologies are discussed elsewhere[7], and some other examples of homologeous neurones in pulmonate and opisthobranch molluscs have recently been considered[8].

As far as synaptic input is concerened, the RPa-D cells are different from the rostral white cells: unlike the latter[4], the former have well-developed intraganglionic connections. This difference might be the result of the fact that *Helix* has no osphradium, the peripheral sensory organ which, in *Aplysia*, influences autogenic axonal pacemakers of white cells[9].

Выводы. Каждая клетка исследованной группы отсылает по аксонной ветви к периферическому депо и в синаптический нейропиль, а также имеет множество соматических отростков. Клетки получают богатый синаптический приток, тормозятся ацетилхолином и дофамином и стимулируются серотонином.

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1 D. A. SAKHAROV and J. SALÁNKI, Acta physiol. hung. *35*, 19 (1969).
2 A. O. W. STRETTON and E. A. KRAVITZ, Science *162*, 132 (1968).
3 G. A. KERKUT, M. C. FRENCH and R. J. WALKER, Comp. Biochem. Physiol. *32*, 681 (1970).
4 W. T. FRAZIER, E. R. KANDEL, I. KUPFERMANN, F. WAZIRI and R. E. COGGESHALL, J. Neurophysiol. *30*, 1298 (1967).
5 R. E. COGGESHALL, J. Neurophysiol. *30*, 1263 (1967).
6 S. E. WENDELAAR BONGA, Z. Zellforsch. mikrosk. Anat. *108*, 190 (1970).
7 D. A. SAKHAROV, Zh. obshch. Biol. *31*, 449 (1970).
8 D. A. SAKHAROV, A. Rev. Pharmac. *10*, 335 (1970).
9 B. JAHAN-PARWAR, M. SMITH and R. VON BAUMGARTEN, Am. J. Physiol. *216*, 1246 (1969).