Intracellular water content of the jejunum epithelial cells, mobility of acetamide (ω) and glucose transport activities across the intestinal wall (Sprague-Dawley Albino male rats)

Incubation fluid	Intracellular water content ml g^{-1}	ω μmoles g^{-1} h^{-1} Atm^{-1}	Glucose trans-epithelial transport μmoles g^{-1} h^{-1}	Fluid trans-epithelial transport ml g^{-1} h^{-1}
Basic perfusion fluid	5.56 ± 0.15 (9)	721 ± 55 (14)	280 ± 51 (14)	6.64 ± 1.16 (14)
Phlorhizin added $10^{-4}M$	4.50 ± 0.17 (8)	634 ± 57 (5)	-14 ± 12 (5)	4.22 ± 0.75 (5)
Tris Cl substituted isosmotically for NaCl	4.60 ± 0.14 (12)	354 ± 34 (9)	75 ± 17 (9)	2.51 ± 0.48 (9)

Mean values $\pm$ S.E. referred to 1 g of dry tissue and 1 h are reported. The numbers of experiments are in parentheses.

Tris Cl substitution for NaCl in the bathing fluid was paralleled by a significant shrinkage of the epithelial cells.

This observation suggests the hypothesis that cellular swelling under normal perfusing conditions may result in a mechanical increase of the cellular surface and, therefore, of passive flow across the cell membranes. No glucose accumulation and swelling are detectable by perfusing at a lower sodium concentration, and the permeability of the epithelium decreases.

In order to check this hypothesis, experiments after phlorhizin poisoning have been performed.

All the experiments were carried out between November and April. Sprague-Dawley Albino male rats, initially weighing about 250 g, semistarved over a 15-day-period (final percent weight decrease 15–25%) were used[12,13].

A tract of small intestine 15 cm long was removed from the animal at about 10 cm from the pylorus, under barbituric narcosis. Each tract was everted according to the WILSON and WISEMAN technique[14], and perfused at 28 °C as in previous experiments[11]. The basic perfusion fluid was Krebs-Henseleit bicarbonate solution added with acetamide 10 mM and glucose 13.89 mM.

At the end of the experiment, the emptied intestine was dried at 100 °C overnight. The transepithelial mobility coefficients calculated by disregarding the drag effect[11] and the net glucose transport per g dry weight (from mucosa to serosa) were at first calculated for all the 10 or 20 min periods and then the mean values between all the single data belonging to 1 experiment (1 h) were obtained. Only these mean values constitute the pool of data for statistical treatment in the Table.

In another set of experiments we tried to determine the water content of the mucosal epithelial cells. In this case polyethylenglycol ^{14}C (PEG) was added to the basic solution at the beginning of the experiment both to the serosal and mucosal fluids. The subsequent procedure was the same as in a previous paper[11].

These two kinds of experiments were repeated by replacing NaCl in the basic incubation fluid with *Tris* Cl in an isosmotic quantity, or by adding to the basic incubation fluid phlorhizin $10^{-4}M$.

The results obtained are reported in the Table. The permeability of the phlorhizin-poisoned epithelium, where glucose transport is absent and cellular swelling is reduced to the same degree as in the intestines perfused with a *Tris* substituted perfusing fluid, is as high as in the cases of glucose transporting intestines.

Therefore the conclusion can be drawn that presumably the degree of swelling of the epithelial cell is not critical for the passive permeability to acetamide of the intestinal barrier. If we take into consideration the influence of the solvent drag, the above conclusion does not change. The substitution of sodium ion with *Tris* ion could directly affect the physico-chemical properties of cell membrane due, for instance, to the varied Na:Ca ratio of the perfusion medium.

Riassunto. La permeabilità all'acetamide dell'epitelio intestinale di digiuno di ratto non sembra influenzata dal grado di rigonfiamento delle cellule epiteliali.

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The Visual Response of the Purple Shore Crab, *Hemigrapsus nudus*, to Ionizing and Non-Ionizing Radiations[1]

Physiological and behavioral responses to ionizing radiations, attributable to radiation effects on the visual system, have been known for over 70 years[2–4]. The nature of this action, however, is not completely understood, partly because of the difficulty in specifying the mechanisms through which high-energy quanta might act to stimulate the visual receptor system[5,6]. A comparative study of the response to visible and near-visible light in the compound eye, followed by stimulation under the same conditions with X-rays and β-radiation, has made possible some inferences concerning the reception of ionizing radiations.

Methods. The isolated compound eye of the crab was used for this study. Electroretinographic (ERG) responses were detected by platinum microelectrodes and recorded on a Grass model 7 oscillograph after appropriate preamplification.

Visual stimuli from 250 to 700 nm were supplied by high intensity lamps in conjunction with a Bausch and Lomb diffraction grating monochomator. Light exposures were controlled by a leaf-type camera shutter and monitored by a photocell. X-ray exposures from a diagnostic unit (70 kVp, 0.54 mm Al HVL) were controlled with a solenoid-actuated, lead focal-plane shutter and monitored

by a photocell that was covered with fluorescent screen. β-radiation was provided by compact sealed applicators containing either 100 mCi or 50 mCi of strontium-90 in equilibrium with yttrium-90. Details of construction and operation of the shielded shutter system for β-radiations have been published elsewhere[7]. For dosimetry, visible light intensities were measured independent of wavelength at the position of the eye with an YSI-Kettering radiometer. Calibrated lithium fluoride thermoluminescent dosimeters, having the dimensions of the eyestalk, permitted absorbed doses from ionizing radiations to be measured at the eye.

Results and discussion. With light exposures, peak spectral sensitivities were found at 350 and 500 nm, corresponding to near-ultraviolet and blue-green light. Single exposures to 500 nm resulted in a sharp cornea-negative 'on' response at the onset of illumination, and a positive 'off' response at the cessation (Figure 1, A). When the light stimulus intensity was gradually increased or decreased, neither response was observed (Figure 1, B). The responses to a series of stimuli exhibited a gradual decline in amplitude (Figure 1, C). The rate of the initial decrease and the relative depression of the asymptote were greater at higher intensities.

These 'on' and 'off' responses were also noted in the X-ray ERG (Figure 2, A) and, as with visible light, neither response was elicited by a gradual change in stimulus intensity (Figure 2, B). Series of X-ray stimuli caused the ERG amplitude to decrease only slightly relative to the effect of a comparable train of visible stimuli.

With β-radiation, a sharp 'on' response was noted, but the peak decayed with no 'off' response to the baseline. Responses to a series of β-stimuli (Figure 2, C) decreased in amplitude and reached an asymptote directly related to stimulus dose rate in the manner observed for light stimulation. The initial decline and asymptotic response levels under light and β-radiation stimulation are compared in Figure 3.

It appears that a wide range of energies may act as visual stimuli in the eye of the crab. The sensitivity peak at 500 nm is similar to that for other crustaceans and appears to correspond to the absorption peak of rhodopsin. The second peak at 350 nm may also reflect a small rhodopsin absorption peak in this region[8], but fluorescence produced by UV-light could also be postulated as a cause of the second peak. Absorption of UV-radiation with re-emission in the blue-green region has been

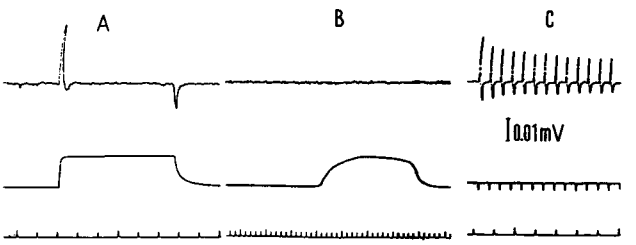

Fig. 1. A) The ERG, showing 'on' and 'off' response peaks. B) The absence of a response with a gradual change in light intensity. C) The responses to a series of $^1/_{40}$ sec stimuli. Upper tracing, ERG response; middle, stimulus monitor marker; lower, time record in sec. Light stimulus in A) and B), 500 nm, 5.78×10^8 ergs/cm^2-sec; C) 500 nm, 20.4 ergs/cm^2 per stimulus.

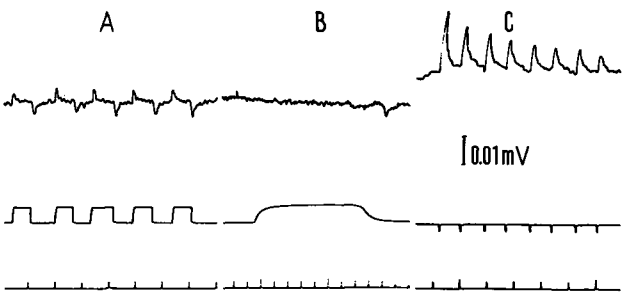

Fig. 2. A) ERG response from X-ray stimuli; B) absence of this response with gradual change in X-ray intensity; and C) ERG response to β-radiation stimuli. Tracing designations are as described in Figure 1. X-ray stimuli, 17.5 keV effective energy, 1.7 rads per sec; B-stimuli, 2.26 MeV$_{max}$, 11.6 mrads per $^1/_{20}$ sec flash.

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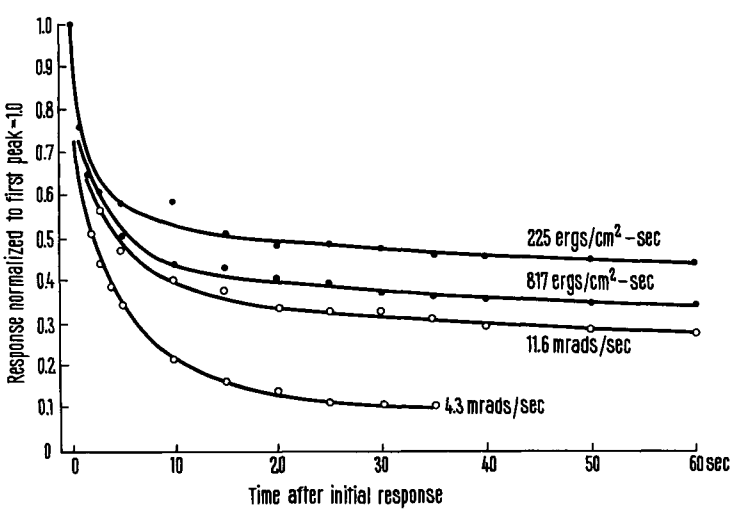

Fig. 3. The decrease in amplitude of response with repeated exposure to β- and light stimuli. Curves were derived from repeated esxposure at the rate of 1–3 flashes/sec over the time period indicated. Response amplitudes normalized to the initial response.

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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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