Zusammenfassung

Ein neues Cytochrom ist aus Schweineleber isoliert worden. Es wird durch DPNH-Cytochrom-c-Reduktase reduziert. Antimycin A hemmt diese Reaktion, die nur in Gegenwart des Slater-Faktors abläuft.

Es ist wahrscheinlich, dass dieses Cytochrom in die Elektronentransportreihe, und zwar zwischen Reduktase und Cytochrom c, eingeschaltet ist.

Effects of Ultraviolet Light on some of the Electrical Characteristics of Action Potentials of Single Unmyelinated Nerve Fibers of the Crab *Carcinus*[1]

As a corollary to photochemical work done in this laboratory with single myelinated nerve fibers[2], a number of experiments have been carried out to test the effects of monochromatic ultraviolet irradiation on resting single unmyelinated fibers from the walking leg of the crab, *Carcinus maenas*.

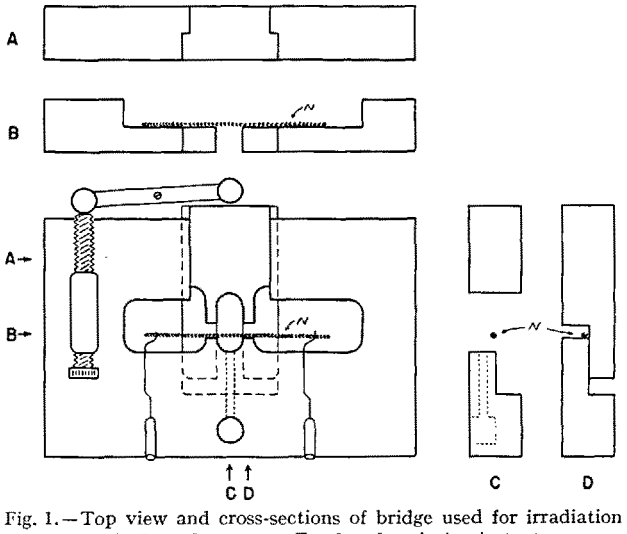

Fig. 1. – Top view and cross-sections of bridge used for irradiation of single crab neurons. Further description in text.

The nerve was obtained by the "pullout" technique of FURUSAWA[3], and a single large fiber (20–30 μ in diameter) was isolated, the dissection being carried out in artificial sea water[4]. After isolation, the nerve was mounted on the bridge diagrammed in Figure 1. This apparatus consisted basically of three pieces of perspex, one a large block, not shown in the Figure, which served as a base for the bridge and which held a quartz window directly under the region of the nerve to be irradiated. The upper part of this bridge, shown in the drawing, was screwed onto the base. It was so constructed that the fiber (*N*) lay across three wells containing sea water. The ends of the fiber were clamped to the floors of their respective wells by stainless steel electrodes. The center well was connected by a tunnel to a fourth well, in which was immersed a Ag–AgCl electrode in sea water agar. The channel in which the nerve lay could be altered in width by a screw-and-lever mechanism which allowed fine movement of a separate, close-fitting, perspex block from which the walls of one side of the channel had been machined. With the nerve in position, the width of these channels could be brought to within a few microns of the diameter of the fiber, providing a high electrical resistance between the three wells without mechanical damage to the fiber itself. It will be seen from cross sections *B* and *C* that the space under the nerve in the center well was open, that is, filled only with sea water. The floor of this space was formed by the quartz window in the base block, through which the entire length of the fiber in the center well (0·6 mm across) could be irradiated from underneath.

The circuit for stimulating and recording is that shown in Figure 2. A Grass stimulator provided monophasic square wave impulses of 0·5 ms duration at a frequency of 10/s. The intensity of the shock could be read, in relative values, from a graduated potentiometer. For protection of the nerve, a resistance of 470 kΩ and capacity of 0·01 μf were placed in series across the stimulating electrodes. Another capacity and resistance, 200 kΩ and 100 pf, (RC = 20 μs) could be switched into the recording circuit for obtaining "differentiated action potentials" on the oscillograph. Variables measured during any given experiment were threshold, height of the spike, and, from the "differentiated action potential", the rate of rise of the spike.

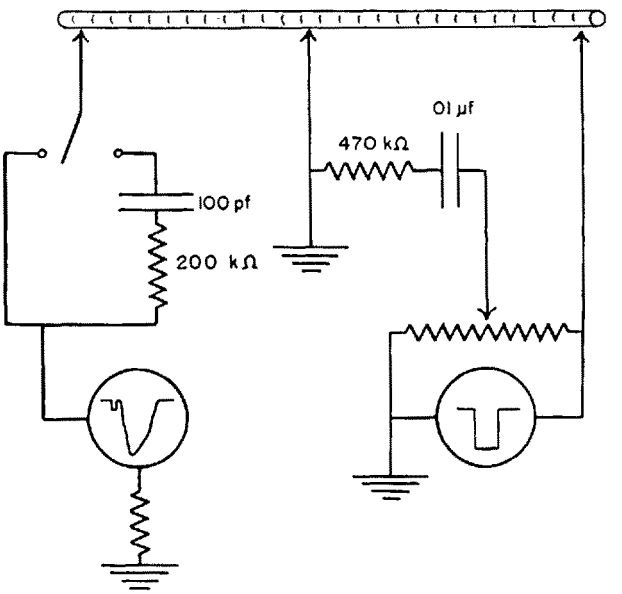

Fig. 2. – Diagram of stimulating and recording circuit. Square waves from Grass stimulator (right); recordings over de Gruyter pre-amplifier and Dumont double-beam cathode ray oscillograph (left).

Radiation of $\lambda = 265$ mμ was obtained by double monochromation of radiation from a Philips high pressure mercury arc. The monochromator used has been

[1] This work was carried out during the tenure of a Postdoctoral fellowship from the United States Public Health Service.

[2] M. HUTTON-RUDOLPH, Helv. physiol. Acta *1*, C15 (1943); Dissertation, Bern 1944. – J. BOOTH, A. v. MURALT, and R. STÄMPFLI, Helv. physiol. Acta *8*, 110 (1950). – A. v. MURALT and R. STÄMPFLI, Helv. physiol. Acta *11*, 182 (1953). – H.-C. LÜTTGAU, Helv. physiol. Acta *12*, C56 (1954); Pflügers Arch. ges. Physiol. *262*, 244 (1956).

[3] K. FURUSAWA, J. Physiol. *67*, 325 (1929).

[4] Based on an analysis of North Sea water, with the following ionic molarities (m*M*/*L*): Na^+ – 477·4; K^+ – 9·0; Mg^{++} – 55·0; Ca^{++} – 8·1; Cl^- – 552·2; $SO_2^=$ – 28·0; NO_3^- – 1·2; HCO_3^- – 2·6; $HPO_4^=$ – 0·3. Total ionic molarity – 1·1338.

described by v. MURALT and STÄMPFLI[5], and the technique for focussing the beam on the desired region of the nerve by HOPPE and v. MURALT[6].

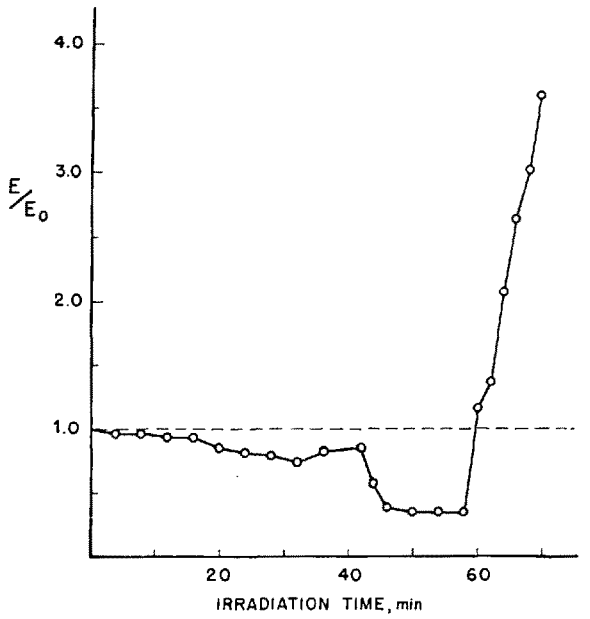

Fig. 3.—Changes in threshold of single crab neuron with irradiation. Nerve presumably stimulated in unirradiated zone.

After the nerve was mounted on the bridge in position to be irradiated, the variables to be measured were checked at intervals for 20–30 min to ensure that the fiber was in good condition, and then the irradiation was started and allowed to continue to the end of the experiment. The nerve was stimulated only for brief periods every 2 min when the readings were taken.

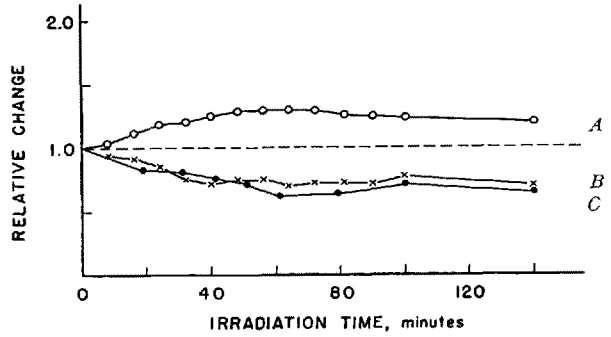

Fig. 4.—Changes in (*A*) threshold, (*B*) spike height, (*C*) rate-of-rise of action potential of single crab neuron with irradiation. Nerve presumably stimulated in irradiated zone.

Two types of results were encountered, one when using the bridge as described and the other after a slight modification of the technique. In the one case, illustrated in Figure 3, the threshold decreased slightly, then decreased markedly during a period of "over-excitability", and finally rose sharply until the fiber became inexitable. This type of curve is very similar to that found both by HUTTON-RUDOLPH and BOOTH *et al.*[2] when irradiating the internode of a myelinated fiber. Since the current density of the stimulus shock is greatest where the fiber enters the channel between wells, it is possible that in this type of experiment the fiber is not being stimulated in the irradiated region at all, but inside the channel where it is shielded by the perspex floor. The decrease in threshold can be ascribed to a partial depolarization due to injury potentials from the irradiated region. When the injury potentials become of sufficient magnitude, the resting potential is eventually reduced to the point where the fiber becomes inexitable. In these final stages the threshold rises steeply.

The second type of result encountered is shown in Figure 4. In these experiments, the walls of the channels were coated with a fairly thick insulating layer of vaseline, so the fiber would be stimulated in an irradiated region in the open center well rather than in the channels. That this vaseline coat effectively raised the resistance in the channels is evidenced by the fact that the output of the stimulator required to reach threshold was reduced by a factor of 20 from the preceding experiment. Here we see that, with the onset of irradiation, the threshold rises while the height and rate-of-rise of the action potential decreases all 3 parameters leveling off at new values and remaining fairly constant for several hours. It is probable in this case that the irradiated side of the fiber has been inactivated, while the unirradiated side has remained relatively intact, the results of the damage only partially affecting the electrical characteristics as measured. Unilateral damage of this type is not surprising in view of the fact that, at $\lambda = 265$ mμ, the extinction of this type of fiber is about 3[7]. In other words, the intensity at the surface away from the source is only $^1/_{10}$ of 1% of that impinging on the exposed surface. The possibility that the unexposed surface remains excitable would further suggest that, in the first type of result, the final steep rise in threshold was due to causes other than the irradiation.

It will be noted that these experiments ran for times of 1–3 h, whereas the work on myelinated fibers at this wavelength involves radiation times of the order of 10 min. Further experiments with unmyelinated fibers should include (1) an arrangement for exposing the surface to irradiation from all sides and (2) assurance that the stimulus is applied and the readings are made in the irradiated zone of the fiber.

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Physiologisches Institut, "Hallerianum", Bern, June 15, 1956.

Zusammenfassung

Die marklosen Nerven-Einzelfasern von *Carcinus maenas* wurden mit monochromatischem Ultraviolett bestrahlt. Zuerst tritt bei Bestrahlung ein langsamer Abfall der Reizschwelle, dann ein rascher Abfall, gefolgt von einem steilen Anstieg auf. Der Abfall rührt von einer Depolarisation her, deren Zunahme dann zu Unerregbarkeit führt. Durch Verbesserung der Ableitungstechnik konnten die elektrischen Veränderungen lokalisiert an der bestrahlten Stelle gemessen werden. Anstieg der Reizschwelle, Abfall der Steilheit und Abfall der Höhe des Spitzenpotentials wurden auch bei diesen marklosen Fasern beobachtet, aber nicht so ausgeprägt wie bei der Bestrahlung markhaltiger Fasern.

[5] A. v. MURALT and R. STÄMPFLI, Helv. physiol. Acta *11*, 182 (1953).

[6] W. HOPPE and A. v. MURALT, Helv. physiol. Acta *12*, C54 (1954).

[7] H. BAMMER (personal communication).

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