A HUNDRED YEARS AGO AND NOW: A SHORT ESSAY ON THE STUDY OF THE CRUSTACEAN HINDGUT

[Vor hundert Jahren und jetzt. Eine kurze Abhandlung über die Erforschung des Hinterdarmes der Crustaceen]

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The beginning of this story goes back to an outstanding Polish neuroanatomist, J. S. Alexandrowicz, who contributed to the initial discovery and description of the cardiac ganglion in the heart of different decapods, the description of the pericardial organs, and explanation of their function. In 1909, he published a paper (this might have been his first scientific publication) titled "Zur Kenntnis des sympathischen Nervensystems der Crustaceen". The paper was based on an investigation which he conducted under the guidance of Professor A. Lang. The work was conducted in Zürich, Switzerland and at the zoological station in Villefranche-sur-Mer, France. He investigated several crustacean species but his main focus was on the crayfish and spiny lobster.

The title of this paper may mislead a current reader: now the term 'sympathic, or sympathetic, nervous system' means a subdivision inside the autonomous nervous system. At that time, the same term meant a system that controls the vegetative organs including the hindgut. Thus, the aim of the investigation was, in the author's words, "die feineren histologischen Verhältnisse in der Innervation des Hinterdarmes zu prüfen" [Translation: . . . to determine the fine histological interrelations in the innervation of the hindgut.]

He demonstrated by histological techniques that there is a nerve plexus on the outer surface of the hindgut ("Grundplexus") formed by the nerve fibers leaving the terminal abdominal ganglion. The nerve fibers from this plexus reach single muscle bundles where they are seen as "Endplexuses". In addition, he revealed many (3-4 thousands) bipolar nerve cells that are distributed uniformly over the

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length of the hindgut. One process of such a cell goes to the hindgut's lumen and ends there between the epithelial cells, whereas the other is directed to its outer wall, "indem er sich unterwegs mit den gleichen Fortsätzen der anderen Zellen verbindet" [so that on its way it establishes contacts with similar processes of [the] other [nerve] cells]. Alexandrowicz (1909) noted that he failed to trace the way of these outside-directed processes in *Astacus*, therefore his conclusions were made from investigations of the spiny lobster, *Palinurus*. The processes form a net on the surface of the hindgut, "in dem die Fasern in zahlreichen Anastomosen nach allen Richtungen hin verlaufen ... Das ganze Geflecht tritt zu dem Grundplexus, der vom Nervus intest. Posterior gebildet wird, in engere Beziehungen, indem die Wege des letzteren durch die Fasern der ersteren benutzt werden und höchstwahrscheinlich ziehen die Nerven beider nebeneinander zu den Muskeln" [the fibers [of the net] run in all directions building numerous anastomoses with each other. ... The whole network has close connections with the "Grundplexus", which consists of the fibers belonging to the nervus intestinalis posterior, so that the ways of the latter are used by the fibers of the former; most likely, the fibers of both [origins] go to the muscles together].

The second part of the same paper contains results of the physiological experiments on the isolated hindgut of the crayfish. Alexandrowicz (1909) appears to have been the first one who conducted such experiments. It was shown that the hindgut, isolated from the abdominal nerve cord and placed into Ringer's solution, contracted spontaneously for a long time (up to 36 hours!). The contractions existed not only in the whole hindgut but also in its fragments. A problem was, however, in the direction of these contractions: they were directed forwards (antiperistaltic movements). The author tried to explain this fact but his explanation does not seem convincing.

The results of the physiological experiments were very important for Alexandrowicz because they confirmed his anatomical data. Taken altogether, they allowed him to conclude:

"1. Das Nervensystem des Krebsdarmes besteht aus autonomen nervösen Einrichtungen, die mit dem Zentralnervensystem in Verbindung stehen.

2. Das autonome Nervensystem ... besteht aus bipolaren Zellen, die den einen (rezeptorischen) Fortsatz zum Lumen des Darmes schicken, der zweite dagegen, effektorischer Natur, tritt mit den Fortsätzen anderer Zellen in ein Geflecht ein, von dem die Fasern zu den Muskeln ziehen ...

3. Der vom letzten Abdominalganglion kommende Nerv mit seinen zahlreichen Verästelungen in der Muskulatur, wo er sich mit den motorischen Fasern vereinigt, hat die Regulierung der automatischen Bewegung zu besorgen."

[(1) The nervous system of the crayfish hindgut consists of autonomous nervous devices, which are connected with the central nervous system. (2) The autonomous nervous system ... consists of bipolar cells whose sensory process goes to the lumen of the hindgut, whereas the second, effector process, together with similar processes of other nerve cells, forms a bundle whose fibers go to the muscles. (3) The nerve that leaves the last abdominal ganglion and that has numerous branches within the muscles, unites there with the motor fibers [from the autonomous nerve net]; [its function] is to control the automatic movements [of the hindgut].]

Thus, this start in studying the structure and function of the hindgut in the crayfish and spiny lobster seemed very promising. Moreover, the physiological data (existence of spontaneous contractions in the isolated hindgut) fit well to the anatomical observations (existence of its own nervous elements capable to drive these contractions). The author, however, noted that he could not fulfill his task in the whole volume. He wrote at the end of the paper: "Am Anfang dieser Arbeit schwebte mir als Ziel vor, durch die vollständige Kenntnisse des Verlaufes einer jeden Nervenfaser in den Mechanismus der Peristaltik einen klaren Einblick zu gewinnen. Es stellte sich bald heraus, dass das nicht in vollem Umfang sich erfüllen ließ" [At the beginning of this work I was hopeful to achieve a clear view concerning the mechanism of peristalsis by means of the complete description of the pathway of each nerve fiber. It turned out soon that the full extent of this task could not be fulfilled]. He also mentioned that he never could trace the way of the process going upwards after it left the cell body. Therefore, wrote he, "ist es mir nicht möglich, aus den anatomischen Daten ein Schema der Nervenverteilung zu konstruiren" [I failed to construct a pattern of nervous supply [of the hindgut] based on the anatomical data]. Nevertheless, he believed that the spontaneous activity in intestinal muscles obviously had a neurogenic origin.

For the current neurobiologist it may seem strange that any one neuron can have both sensory and motor functions ... but at the beginning of the last century when the neuron doctrine was so young there were probably different views. Today it seems unusual that the intestinal muscles of the crustaceans, which long ago (Lemoine, 1868) were known to be striated, can be spontaneously active after isolation from the CNS. This problem also appeared not to be of interest at that time and was not discussed by Alexandrowicz (1909).

It would be interesting to estimate the progress in this field during the last hundred years which passed since the publication of Alexandrowicz' seminal paper.

The hypothesis that the intrinsic nerve cells in the hindgut are responsible for its spontaneous peristaltic movements not only was not confirmed but probably never has been discussed. Though the nerve cells in crayfish hindgut were found by other neuroanatomists (Janish, 1923; Orlov, 1926), they were treated as sensory neurons (probably, chemosensory) (Orlov, 1926). It may be noted, however, that Orlov (1926) also could not trace the path of the upwards-directed process. There are no data concerning the structure and function of these nerve cells in the current publications. It is noteworthy, however, that Winlow & Laverack (1972) did not succeed in recording the electrical activity of sensory nervous elements in the lobster hindgut. No mention about these cells occurs in the neuroanatomical investigations performed with modern techniques (Elekes et al., 1988; Audehm et al., 1993).

However, the presence of the spontaneous activity in the isolated hindgut discovered by Alexandrowicz (1909) was repeatedly confirmed by many researchers. At the first sight, this may seem strange since: (1) Alexandrowicz was not a physiologist, he made these experiments just because he wanted to test his neuroanatomical results, and in the publications of that time he could not find the appropriate experimental data. (2) Now his experiments can be seriously criticized from a technical point of view, because he placed an isolated hindgut in an inadequate saline (the Van-Harreveld physiological solution for crustaceans is known to appear later, at the end of the 1930s), and oxygenation of the saline was not used. Nevertheless, the spontaneous contractions of the isolated hindgut were recorded in a number of modern works; therefore, the observations described by Alexandrowicz seem to be doubtless.

The most thorough investigations on the crustacean hindgut were made by Ebara (1969) and by Winlow & Laverack (1972). Ebara (1969) showed that an isolated intestine of the crayfish, *Procambarus clarkii* (Girard, 1852), continued its movements for several hours in a physiological salt solution. The contractions of the whole hindgut were more or less irregular, while a single strip isolated from the wall of the intestine demonstrated a regular rhythm of mechanical waves. In most cases, the contraction wave started from the anterior part of the hindgut but sometimes it originated in its posterior part and was then conducted anteriorly. It is noted that the contractions of the hindgut which received innervation from the tail ganglion did not differ of those in the isolated preparation. The author concluded: "It is conceivable that this striated intestinal muscle exhibits spontaneous activity *in situ,* similar to the case of visceral smooth muscles of the vertebrate, and cardiac muscle of the vertebrate, the mollusc, and the tunicate. The pacemaker seemed to be diffusely distributed over the whole muscle bundle ... It is not yet clear whether the slow depolarization in the crayfish intestine is the pacemaker potential or not. However, the shape of action potential at the pacemaker site of the present material closely resembled that of the pacemaker tissues of the vertebrate heart" (Ebara, 1969: 173).

Similar results were obtained on the isolated hindgut of the lobster, *Homarus gammarus* (L., 1758) (cf. Winlow & Laverack, 1972). These authors stated that the hindgut can show many forms of spontaneous activity. "Such spontaneous motility was not dependent on the presence of the sixth abdominal ganglion which seemed to exert little or no influence on the rectum when not actually driving it. Longitudinal and circular muscles were found to beat at their own rate ... In addition, the longitudinal muscle strips were also found to beat independently of one another ... The independent rhythmicity of longitudinal and circular muscles suggests that spontaneous hindgut motility is due to presence of numerous independent oscillators, situated within the longitudinal and circular muscles" (Winlow & Laverack, 1972: 22).

Thus, the above-mentioned authors (Winlow & Laverack, 1972) propose that the spontaneous activity of the crustacean hindgut has a myogenic nature. A recent investigation sheds some light on the peculiarities of the contractile physiology of the intestinal muscles in the crayfish (Brenner & Wilkens, 2001). The main conclusion from this study is that the muscles of the hindgut differ from skeletal muscles in the crustaceans. These muscles cannot be tetanized either by repetitive stimulation or by elevated potassium saline. They can work in trace amounts of external Ca^{2+}, and they have some differences in E-C [= excitation-contraction] coupling as compared to crustacean skeletal muscles. This may be a prerequisite for the intestinal muscles to be capable of generating peristaltic contractions via endogenous pacemakers without neural input.

Hence the most substantiated hypothesis is that spontaneous contractions in crustacean hindgut are of myogenic origin though the mechanism of this phenomenon is far from being elucidated. Does this mean that Alexandrowicz (1909) was absolutely wrong? Probably this is not the case. The results of comparative investigation made on different invertebrates including crabs and lobster by Prosser et al. (1965) are in favor of a nervous mechanism of spontaneous activity in crustacean intestinal muscles. A recent study of glutamate receptors associated with the crayfish hindgut (Wrong et al., 2003) showed unequivocally their role in contractions of both circular and longitudinal muscles. Three main effects of L-glutamate on the hindgut were observed: an increase in tonus, a transient increase in contraction frequency, and suppression of spontaneous rapid contractions. Considering the latter, the authors note: "Although the spontaneous hindgut contractions are thought to be myogenic ... no one has definitely ruled out the possibility that they result from spontaneous release of glutamate from nerve terminals or that the hindgut plexus is spontaneously active".

This assumption turns us back to the beginning of the story. Let us see what happens in the next hundred years ...

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