

Aerial and Aquatic Respiration of Certain Trochids

The two mid-littoral trochids *Monodonta lineata* and *G. umbilicalis*, the low-tide level species *G. cineraria*, and the virtually sublittoral *Calliostoma zizyphinum* experience at Plymouth the following maximum degrees of exposure to air at their respective intertidal levels^{1,2}: *M. lineata* 75%, *G. umbilicalis* 63%, *G. cineraria* 40%, and *C. zizyphinum* 10%. This seems to correlate with their ability to utilize atmospheric oxygen or oxygen dissolved in sea-water.

Determinations of the rates of oxygen uptake at selected temperatures ranging between 3.5 and 25.0°C, in air and in water, were carried out on adult snails of these species. The experimental animals were collected from Rum Bay, Plymouth, and kept under conditions of constant temperature (9–10°C) and humidity (95%) for at least 3 or 4 days before experimentation. A volumetric respirometer based on BLISS's³ simplified design of the plastic manometers devised by Scholander was used for all the measurements of oxygen uptake. For aquatic

determinations, a perforated shelf carrying a potassium hydroxide thimble and a guarded built-in magnetic stirrer was incorporated into the Perspex animal chamber.

The rate of oxygen uptake was expressed in $\mu\text{l/g}$ wet weight of flesh/h. The rate of oxygen uptake of the 4 species increased linearly with temperature. The increments observed are shown in the Table.

Monodonta has the peculiarity that its oxygen uptake in air continued to rise linearly with temperatures between 15.0 and 20.0°C, but it decreased in this temperature range when the snail was submerged in water. The uptake observed at 18.5°C was only 52% of that at 15.0°C in water. It increased again and approached the aerial oxygen uptake above 20°C.

The present findings seem to show that the ability of these species to utilize atmospheric oxygen, or oxygen dissolved in sea-water at a given temperature, is one of the factors which determine their position on the shore.

Résumé. La consommation d'oxygène de 4 espèces de Troques dans l'air et dans l'eau est en relation avec leur distribution dans la zone intercotidale.

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Oxygen uptake of 4 species of snails

Snails	Aerial oxygen uptake $\mu\text{l/g/h/}^\circ\text{C}$	Aquatic oxygen uptake $\mu\text{l/g/h/}^\circ\text{C}$
<i>C. zizyphinum</i>	1.8	10.6
<i>G. cineraria</i>	4.0	9.0
<i>G. umbilicalis</i>	5.0	5.5
<i>M. lineata</i>	5.5	5.0

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May 31, 1966.

¹ J. COLMAN J. mar. biol. Ass. U.K. 18, 435 (1933).

² R. G. EVANS, J. mar. biol. Ass. U.K. 27, 173 (1947).

³ D. E. BLISS, Biol. Bull. 104, 275 (1953).

⁴ I thank Professor G. E. NEWELL for his advice.

Neurone Number in the Myenteric Plexus in New-Born and Adult Rats

It is generally admitted that during post-natal growth the neurones of the Auerbach plexus (myenteric plexus) react to the remarkable volume increase of the smooth muscular tissue by increasing their volume considerably. Evidence is lacking as to whether an increase in the number of neurones also takes place during this tremendous augmentation of their innervation territory.

Material and methods. The nerve cells were stained according to the histochemical technique for the demonstration of the NADH diaphorase with nitro-BT as electron acceptor¹; a similar technique has been employed by DUPONT et al.² for studying the Auerbach plexus in the rat caecum. This enzymic activity is so intense in the nerve cell bodies that their staining occurs long before the staining of the other elements of the intestinal wall. This method allows us to detect also the small nervous elements with only a thin layer of cytoplasm around the nucleus. Another advantage of this technique is that it can be easily employed on the intestinal wall in toto; in small-sized animals, in fact, the external muscular layer being very thin, the Auerbach plexus is so superficial that it can be reached by the substrate solution.

The whole intestine, without previous fixation, was incubated in the substrate for 30 min at room temperature. Then, after formalin fixation, entire tracts of the canal were isolated and trimmed out; the muscular layer

with the interposed plexus was isolated and mounted in jelly. Employing this technique, some 250 laminae were prepared from 15 adult and 15 new-born rats. Some of these laminae, taken from a tract equally distant from the duodenal flexure and the ileo-colic valve, were systematically photographed, and photographic reconstructions, enlarged $\times 56$ and $\times 175$, were made. The countings of neuroblasts and neurones on the photographic plates were carried out, checking cell by cell on the original strip under the microscope (Figures 1 and 2). The countings were always carried out on entire rings of the intestinal wall, so that in a given tract of the small intestine the differences in neurone density in the mesenteral and anti-mesenteral zone³ could be overlooked. The intestine being a distensible organ, I tried to ensure a constant degree of distension before fixation; moreover, after the fixation, the total length of the intestine was measured.

The Nissl method and the silver impregnation method according to Bielschowsky (Figure 3) were also employed.

Results. In strip preparations stained by the Bielschowsky method the meshes of the plexus are much more tightly packed in the new-born than in the adult

¹ A. G. E. PEARSE, *Theoretical and Applied Histochemistry* (Churchill, London 1960).

² J.-R. DUPONT, H. R. JERVIS, and H. SPRINZ, J. comp. Neurol. 125, 11 (1965).

³ D. B. LEAMING and N. CAUNA, J. Anat. 95, 160 (1961).

rats. Furthermore, in the new-born rats, the neurones are fewer in comparison with the neuroblasts and the nerve fibres are scantier than in the adult.

In the histochemical preparations, the nerve cells are very crowded in the new-born rats; their density does not vary much in different tracts of the small intestine. From the countings carried out on photographic reconstructions on a total surface of about 1 cm², 64,900 nerve cells/cm² were calculated.

In adult specimens, the histochemically detected cells of nervous nature are much fewer. The countings carried out on a total surface of a few square centimeters give an average number of 9350 cells/cm². As regards the volume, the neuronal population is much less homogeneous than in the new-born rats.

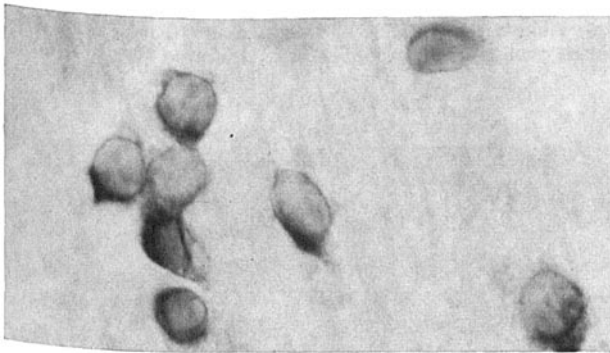


Fig. 1. Nerve cells of the myenteric plexus, stained by NADH diaphorase histochemical reaction, in the new-born rat, $\times 665$.

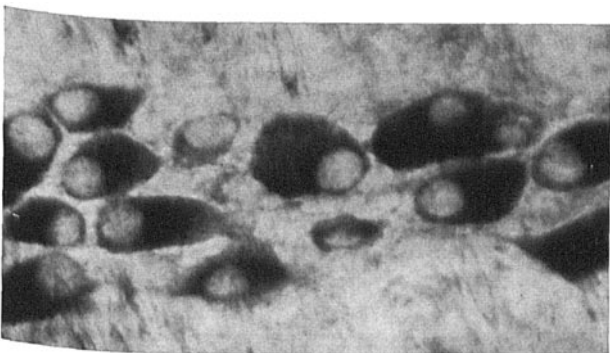


Fig. 2. Nerve cells of the myenteric plexus, stained by NADH diaphorase histochemical reaction, in the adult rat, $\times 420$.

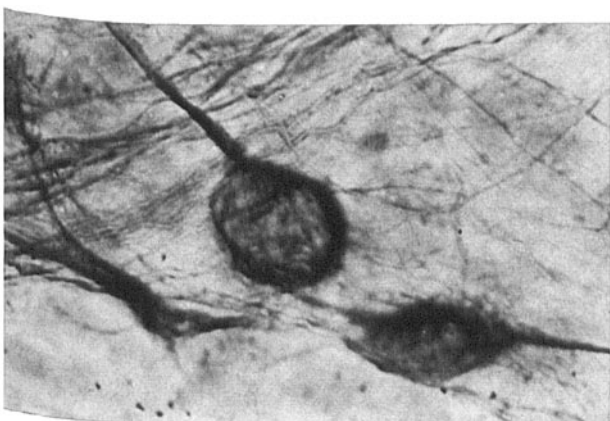


Fig. 3. Nerve cells of the myenteric plexus, silver impregnated by the Bielschowsky method, $\times 1070$.

The present results indicate that the new-born rat's Auerbach plexus shows some features of immaturity when compared with the adult one; in addition, the density of the nerve cells in the former is much higher. The 7-fold reduction of the number of nervous elements/cm² may be due to the large increase of the total surface of the intestine. Actually, in the specimens from which the photographic reconstructions were prepared, the total surface of the small intestine was 6.51 cm² in new-born animals and 183.20 cm² in adult ones respectively, with a total surface increase of about 28-fold. The number of nerve cells/cm² is more than 7 times smaller in adult than in new-born animals whereas the total surface is increased about 28-fold, so that the total number of nerve cells of the myenteric plexus in the small intestine may be presumed to be 4 times greater in adult than in new-born specimens.

The remarkable increase in the absolute number of nerve cells during post-natal growth is the main finding of the present investigation. The most likely explanation for this fact is that a large number of morphologically undifferentiated elements, whose potential nervous nature is already established when the animal is born, may function as a reserve pool and undergo progressive differentiation when the innervation territory of the myenteric plexus increases and its functional organization is defined. The occurrence of a clear-cut neurone number increase in the myenteric plexus of the experimentally hypertrophied intestine in adult animals⁴⁻¹⁰ suggests that the differentiation in this reserve pool is probably still incomplete in the adult animal.

The hypothesis of mitotic divisions in nervous elements, or of migration of new nervous elements from outside the intestine during post-natal growth, is not supported by this investigation; at present, however, these theories cannot be excluded.

Whatever the origin of these new elements, it has been ascertained by the present investigation that, during post-natal growth, together with neuronal hypertrophy, an important increase in the number of nerve cells is paralleled by the tremendous volume increase of the innervation territory.

Riassunto. Nel plesso di Auerbach dell'intestino tenue il numero di cellule nervose, colorate con una tecnica istochimica e contate su larghe lamine della parete, è risultato più di 7 volte minore per unità di superficie nel ratto adulto rispetto al neonato. Poiché nello stesso tempo si verifica un aumento di circa 28 volte della superficie totale dell'intestino, si può presumere che il numero complessivo delle cellule nervose del plesso di Auerbach nell'intestino tenue aumenti di circa 4 volte durante l'accrescimento post-natale.

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May 28, 1966.

⁴ M. MIURA, *Aus den Notizen meiner physiologischen und pathologischen Forschung. XXVI Muscularis mucosae et muscularis propriae gastrointestinalis* (Nankōdo Verlag, Tokio 1913).

⁵ S. AMBROSI, *Arch. De Vecchi* 5, 215 (1943).

⁶ A. BENNINGHOFF, *Z. Naturforsch.* 66, 38 (1951).

⁷ G. FILOGAMO and G. VIGLIANI, *Atti Soc. ital. Anat.*, Napoli, ottobre 1952.

⁸ G. FILOGAMO and G. VIGLIANI, *Riv. Patol. nerv. ment.* 75, 1 (1954).

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¹⁰ G. FILOGAMO, *G. Accad. Med. Torino* 7, 1 (1960).

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