Occurrence of Giant Nuclei and Pharyngeal Tumour in the Earthworm *Lumbricus terrestris*

Recently HANCOCK[1] described giant nuclei in the epithelial cells of the pharynx in the earthworm, *Lumbricus* sp., originating from Kansas City. As these nuclei were not observed in specimens obtained from other sources, this is certainly a particular case.

We observed these giant nuclei in the epithelial cells of the pharynx in *Lumbricus terrestris* and were also in a position to study a case of pharyngeal tumour, in which particularly the giant nuclei cells appeared to have grown.

In the pharynx the giant nuclei were observed in a fair number, which decreased towards the oesophagus. Cytometrically the greatest average diameter amounted to 21.2 μ. The greatest average diameter was 5.7 μ in the normal epithelial cells of the pharynx. On the strength of the significant increase in number of chromocentres and in amount of Feulgen-positive material, the giant nuclei have probably to be considered as polyploid. In general the large nucleoli were irregularly shaped, whereas the chromocentres were easily observed.

The pharyngeal tumour was largely composed of cells with giant nuclei showing a distinct polymorphy. Mitotic figures were frequent. In the nuclei of some tumour cells, two or more nucleoli appeared to be present. Also in the giant nuclei of the tumour cells, the chromocentres were much more clearly visible than in normal epithelial cells.

Endoploidy appears to be most highly expressed in gland cells, for instance in the cells of the pharyngeal gland in the worker bee[2]. There is possibly a close relationship between the protein formation of these gland cells and endopolyploidy[3]. In this respect, the increase in heterochromatic regions and in nucleolar organizers may be of fundamental importance. This relationship undoubtedly plays an important part in tumour genesis.

Zusammenfassung. Beschreibung von Riesenkernen und einer Geschwulst des Pharynx bei *Lumbricus terrestris*.

A. STOLK

Department of Histology, Free University, Amsterdam (The Netherlands), March 23, 1961.

[1] R. L. HANCOCK, Nature *189*, 685 (1961).
[2] M. J. D. WHITE, in *Cytology and Cell Physiology* (Ed. by G. H. BOURNE, second ed., Oxford Univ. Press 1951), p. 183.
[3] T. S. PAINTER, J. exp. Zool. *99*, 523 (1945).

Enzymatic Activity (three Dehydrogenase Systems) of the Pharyngeal Tumour in the Earthworm *Lumbricus terrestris*

Recently we studied a pharyngeal tumour in the earthworm, *Lumbricus terrestris*[1]. This tumour mainly consisted of cells with giant nuclei showing a distinct polymorphy and also occurring in the normal pharynx[1,2]. It seemed desirable to us to study this invertebrate tumour histochemically for the three dehydrogenase systems, viz. succinic diphosphopyridine nucleotide (DPN) and triphosphopyridine nucleotide (TPN) diaphorase and the DPN-dependent enzymes: lactic, malic, glutamic, β-hydroxybutyric, and ethanolic dehydrogenases, according to the methods of SELIGMAN, NACHLAS et al.[3–6]. The sections were incubated at 37°C for 15 min, some sections being also processed with Regaud's stain[7] to demonstrate the mitochondria.

The succinic dehydrogenase activity, which was presumably linked to the mitochondria and the cell membranes, appeared to be low in the pharyngeal tumour as compared to that in the normal tissues. On the other hand, the DPN diaphorase activity was high, similar to that in the most intensely stained normal cells. Although this enzyme and the DPN-linked dehydrogenases were linked to the mitochondria and some cell membranes, the TPN diaphorase activity appeared to be much lower than the DPN-diaphorase activity, showing a more finely and widely dispersed intracytoplasmic distribution, which is suggestive of the presence of microsomes, and displayed a high concentration in the cell membranes. The glutamic dehydrogenase activity was found to be only weak in the tumour cells, while the lactic, malic and β-hydroxybutyric dehydrogenase activity was quite high. The malic dehydrogenase activity was less intense than that of the lactic dehydrogenase. In contradistinction to most of the normal tissues, the activity of the ethanolic dehydrogenase could be demonstrated.

As these results, which are given in the Table, are in general in good agreement with those obtained with some human tumours[6] and the renal adenocarcinoma of the oviparous Toothcarp *Aplocheilus lineatus*[8], they are probably of more general importance.

Distribution of succinic dehydrogenase, DPN and TPN diaphorases and several DPN-linked dehydrogenases in the pharyngeal tumour of the earthworm. The enzymatic activity was graded microscopically on the basis of colour reaction in 0 to 4 by inspection of the tumour cells with giant nuclei and with normal nuclei.

Succinic dehydrogenase	1	Malic dehydrogenase[a]	2
DPN diaphorase	4	Glutamic dehydrogenase[a]	1
TPN diaphorase	2	β-hydroxybutyric dehydrogenase[a]	2
Lactic dehydrogenase[a]	4	Ethanolic dehydrogenase[a]	0

[a] DPN-linked dehydrogenases.

Zusammenfassung. Drei Dehydrogenase-Systeme (Succino-, DPN- und TPN-Dehydrogenase sowie einige DPN-ähnliche Dehydrogenasen, wie die auf Milch-, Malon-, Glutamin- und β-Hydroxybuttersäure sowie auf Äthanol wirkenden substrat-spezifischen Dehydrogenasen) wurden an einer Geschwulst des Pharynx des Regenwurmes *Lumbricus terrestris* untersucht.

Die Enzymaktivität war für die DPN- und die Milchsäure-Dehydrogenase sehr hoch; eine mittlere Aktivität zeigten die TPN-, die Malonsäure- und die β-Hydroxybuttersäure-Dehydrogenase. Sehr gering war die Reaktion für die Succino- sowie die Glutaminsäure-Dehydrogenase, während sie für die Äthanol-Dehydrogenase negativ ausfiel.

A. STOLK

Department of Histology, Free University, Amsterdam (The Netherlands), May 2, 1961.

[1] A. STOLK, Exper., in press (1961); Proc. Kon. Ned. Akad. Wet., in press (1961).
[2] R. L. HANCOCK, Nature *189*, 685 (1961).
[3] A. M. SELIGMAN and A. M. RUTENBURG, Science *113*, 317 (1951).
[4] K. C. TSOU, C. S. CHENG, M. M. NACHLAS, and A. M. SELIGMAN, J. Amer. chem. Soc. *78*, 6139 (1956).
[5] M. M. NACHLAS et al., J. Histochem. *5*, 420 (1957); J. biophys. biochem. Cytol. *4*, 467, pl. 223 (1958), 29, pl. 11 (1958).
[6] B. MONIS, M. M. NACHLAS, and A. M. SELIGMAN, Cancer *12*, 1238 (1959).
[7] R. D. LILLIE, *Histopathologic Technic and Practical Histochemistry*, 2nd ed. (The Blakiston Company Inc., New York 1954), p. 185.
[8] A. STOLK, Naturwiss. *47*, 188 (1960); Proc. Kon. Ned. Akad. Wet. *63*, 548, 567 (1960).