C. Netscher 1): A rapid technique for mass-killing of nematodes with hot fixative.

Though excellent results have been obtained with Seinhorst’s technique for killing nematodes with hot acetic or propionic acid (Seinhorst, 1966, 1962; Netscher & Seinhorst, 1969), this method may be too time consuming if many nematode samples have to be killed. This is mainly due to the fact that concentrating the nematodes in a small drop of water before killing them, by drawing off the excess water, is an operation that must be done very carefully.

Nematodes collected in large numbers can be concentrated for subsequent killing in bulk by vacuum-filtering the suspension. As soon as the filter appears dry, a hot f.p. 4 : 1 or f.a. 4 : 1 solution is poured on to it. After drawing off the liquid the nematodes are immediately washed from the filter with a little 4% formaldehyde into a small vessel. After fixation the nematodes may be processed to glycerin by the alcohol method (Seinhorst, 1959).

A very handy device for filtering the nematodes is the Millipore Pyrex filter holder equipped with a suitable filter (Ederol filters were used in this study).

The disadvantage of this method is that an important number of nematodes may be lost, especially small specimens, as shown in the following table.

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of nematodes killed</th>
<th>Number of nematodes recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meloidogyne sp. (larvae)</td>
<td>340</td>
<td>232</td>
</tr>
<tr>
<td>Scutellonema bradyi</td>
<td>614</td>
<td>540</td>
</tr>
</tbody>
</table>

Fixation has been excellent in Meloidogyne sp. (larvae and males), Heterodera oryzae (larvae), Scutellonema bradyi and Hemicicliophora paradoxa. Details usually difficult to observe, like the hemizonid and rectum of larvae of H. oryzae, were easily seen in specimens killed by this method.


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It is still not known whether striated muscle fibers occur in the pharynx of nematodes (De Coninck, 1965) hence some histological sections were made of the nematode Enoplus communis (Bastian, 1865) to see if there was any evidence of their existence.

The nematodes were fixed in Bouin-Hollande fixative and the paraffin sections stained with paraldehyde-fuchsin-orange G.

In some of the sections the muscles of one of the three sectors of the pharynx contracted violently, completely distorting the pharynx. This resulted in the elongation of the muscles 1 and 4 and in the shortening of the muscles 2 and 3 while the muscles 5 and 6 remained at rest length (Fig. 1 B). In the radial muscles of the pharynx we found that a central part ('A' in Fig. 1, C, D, E) which stained with orange G, had the same length in the three sectors, whereas the total length of the muscles was very different in the three sectors. Examination of the sections with the polarization microscope showed the central part 'A' to be birefringent, while the more extreme parts 'I' of the muscles were isotropic. This alternation of isotropic and anisotropic regions is a first indication of striation. A further criterion is the fact that the anisotropic region 'A' has a constant length.

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Fig. 1. Diagrammatic drawing of an undistorted (A) and a distorted (B) cross-section of the pharynx of *E. communis* and diagrams of the radial muscles of the pharynx in extended (C), resting (D) and contracted (E) condition. In these diagrams the following regions are indicated: A: anisotropic part of the muscle fiber, I: isotropic part of the muscle fiber, Z: outer limits of the muscle fiber.

Fig. 2. The relation between the sarcomere length (*Lₜ*) and the length of the anisotropic region (*Lₐ*) as indicated by the regression of *Lₐ* on *Lₜ*.