On sectioning lice

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The following method was found successful in cutting sections of various species of lice (both the Anoplura and the Mallophaga).

- Live specimens were killed and fixed in the following mixture:
  - Saturated solution of picric acid in 90% ethyl alcohol .... 70 parts by volume.
  - Formalin 40% ........................................... 15 " " "
  - Glacial acetic acid ....................................... 10 " " "
  - Saturated solution of mercuric chloride in 80% ethyl alcohol ... 5 " " "

This mixture assures the following advantages:
1. The penetrating power is greatly enhanced by the use of increased quantity of glacial acetic acid and by the addition of mercuric chloride.
2. The detrimental effects of nitric acid or of diaphanol (to be subsequently used as an agent for softening chitin) on soft tissues are avoided by the addition of mercuric chloride.

To ensure rapid penetration, the legs of the specimens were first cut off and the amputated body transferred to the above mixture which was heated previously to 50—55°C. The heat also drives away a greater quantity of air from the trachea.

The specimens were kept in the mixture for 12 hours, then were washed thoroughly first in 90% alcohol (to dissolve away the residual picric acid) and later on in 10% iodin solution in 90% alcohol (to remove mercuric chloride crystals).

For softening chitin, the fixed lice were kept for 5—7 days in a solution of 5 c. c. of 35% nitric acid in 100 c. c. of 90% ethyl alcohol. For obtaining the best result, the extent of softening of chitin was examined at the end of fifth day by pressing the insect body with the tip of a needle under a stereo-binocular.

Since prolonged emersion in ethyl alcohol hardens the material, dehydration was done by few rapid changes in absolute alcohol.

The material was then transferred to a mixture of absolute alcohol and ether (1 : 1) and then to a thin solution of colloidin (dissolved in absolute alcohol and ether in 1 : 1 proportion). The material was kept in the solution for a fortnight, gradually raising the concentration of solution from thin to very thick. THOUGHT SENNA (1953) claims good result by using colloidin dissolved in clove oil. I found it entirely unsatisfactory inasmuch as it not only hardened the material but also imparted a dark colour which seriously interfered with the proper orientation of the object in wax during block-making.

After satisfactory colloidin infiltration, a tiny block was made of colloidin.

The block was either stored for a long period in a mixture of chloroform and cedar-wood oil (1 : 1) or was directly transferred to paraffin bath for infiltration. Care should always be taken to ensure that the chloroform is moisture-free, otherwise paraffin infiltration will be difficult.

Contrary to the common practice, the colloidin block was kept in the heated paraffin (55°C, M. P.) for 8 to 12 hours without any damage to the tissues or any mechanical interference in sectioning.
The paraffin (65°C, M. P.) used for making the block was previously boiled to smoke until a yellow colour was obtained as suggested by Webb (1946, p. 118). Owing to this pretreatment, the paraffin becomes tough in consistency and readily supports the chitinous parts against the mechanical impact of the microline razor.

The sections were stretched in usual manner. The floating away of the sections from the slide or loosening of tissues caused by water-absorption of chitin in lower grades of alcohol was avoided by plunging the slide in a thin solution of celloidin, thus providing it with a thin film of celloidin all round.

The sections were stained in MALLORY'S triple stain, or in DELAFIELD'S haematoxylin counterstained with eosin, or in HEIDENHAIN's iron-alum haematoxylin counterstained with orange G.

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Literature
