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R. E., Parasitologický ústav ČSAV,
Flemingovo n. 2, 166 32 Praha 6,
ČSSR

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SELECTIVE STAINING OF OPISTHAPTOR SCLERITES OF SOME MONOGENEANS

The shape of monogenean opisthaptor sclerites (called "hard parts" in systematic papers) has been studied mostly by means of phase-contrast microscopy. For this purpose, either fresh or fixed material has been used. A mixture of ammonium picrate with glycerine or glycerin-gelatine has recently been used for fixation and embedding (Ergens R., Folia parasit. (Praha) 16: 320, 1969; Ergens R., Lom J., Původci parasitárních nemocí ryb. Publ. House Academia, Praha, 1970), but the contrast of opisthaptor sclerites is gradually reduced in these media.

Our method using peracetic acid-aldehyde fuchsin provides intensive staining of monogenean opisthaptor sclerites. The material embedded in Canada balsam is persistent and the intensity of staining does not change. We have verified this method on the whole mounts of some species of the genus *Gyrodactylus* (*G. macronychus* Malmberg, 1957 and *G. aphyae* Malmberg, 1957) and on the sections from the species of the genus *Tetraonchus* while studying the histochemistry of their sclerites (Ždárská Z., Folia parasit. (Praha) 21: 345—347, 1974).

The material was obtained by the courtesy of Dr. Ergens to whom my thanks are due. The monogeneans of the genus *Gyrodactylus* were collected from the skin of fishes and placed in a Petri dish with water. A large number of specimens were transferred by a pipette to a slide with a drop of water. A cover slip was placed over the drop and the excess water was sucked off with filter paper and replaced by Baker's fixative (Pearse A. G. E., Histochemistry: theoretical and applied. Vol. 1. J. and A. Churchill Ltd., London, 1968). After 10 min the cover slip was removed and the specimens sticking to the slide were further fixed for 24 h in Baker's fixative (may be fixed for a longer time) and then washed in water for 2 hours.

Staining procedure

1. Treat with peracetic acid prepared according to Pearse (1968) for 5 minutes (longer treatment with peracetic acid may damage the sclerites).
2. Wash in water for 2—5 minutes.
3. Stain with aldehyde fuchsin prepared according to Pearse (1968) for 30 minutes.
4. Wash in water for 10 minutes.
5. Dehydrate in alcohol, clear in xylen and mount in Canada balsam. The sclerites stain deep purple.

The entire marginal hooks of *Gyrodactylus macronychus* (Plate I, Figs. 1, 2; Plate II, Fig. 1) and *G. aphyae* (Plate II, Fig. 2) stain intensely. In the shaft of the anchors only the outer layer is stained, whereas the inner one remains unstained (Plate I, Fig. 2; Plate II, Figs. 1, 2). The connecting bars also remain unstained (Plate I, Fig. 2; Plate II, Figs. 1, 2).

The method using PAA aldehyde fuchsin is one of the methods applied for the detection of proteins with SS groups, which were demonstrated by Lyons (Parasitology 56: 63—100, 1966) in opisthaptor sclerites of different genera of Monogenea and by Ždárská (1974) in the sclerites of the members of the genus *Tetraonchus*. We assume therefore that this method may be applied for permanent slides of these parasites serving as document material.

Z. ŽĎÁRSKÁ,

Institute of Parasitology,
Czechoslovak Academy of Sciences,
Prague

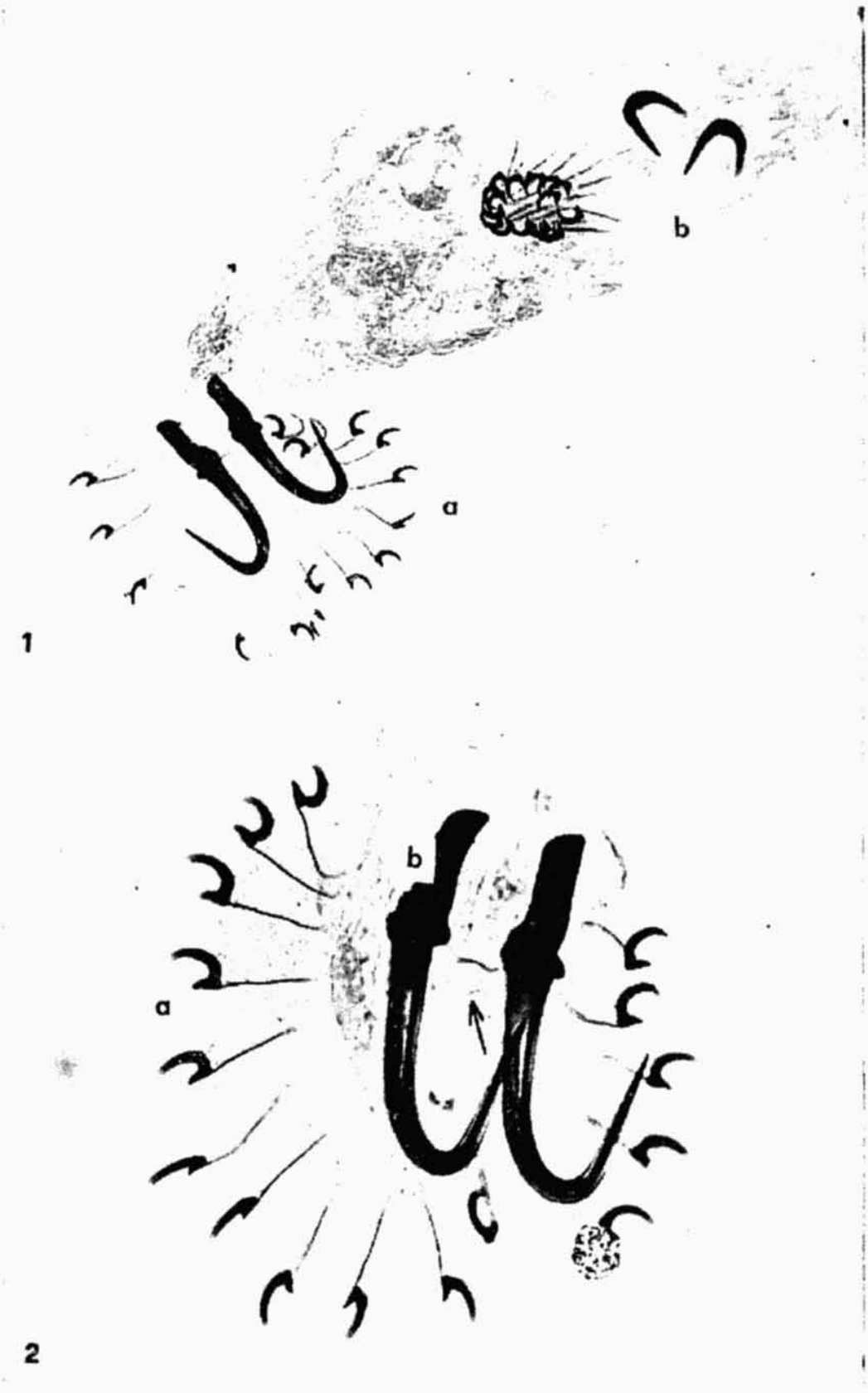

Fig. 1. *Gyrodactylus macronychus* with developing embryo. Not only the marginal hooks and anchors of the mature worm (a), but also the developing marginal hooks and anchors of the embryo (b) stain intensely. PAA — aldehyde fuchsin (140×).

Fig. 2. Detail of the opisthaptor of *G. macronychus*. The marginal hooks (a) and anchors (b) stain intensely, but the connecting bars (arrow) remain unstained. PAA — aldehyde fuchsin (300×).

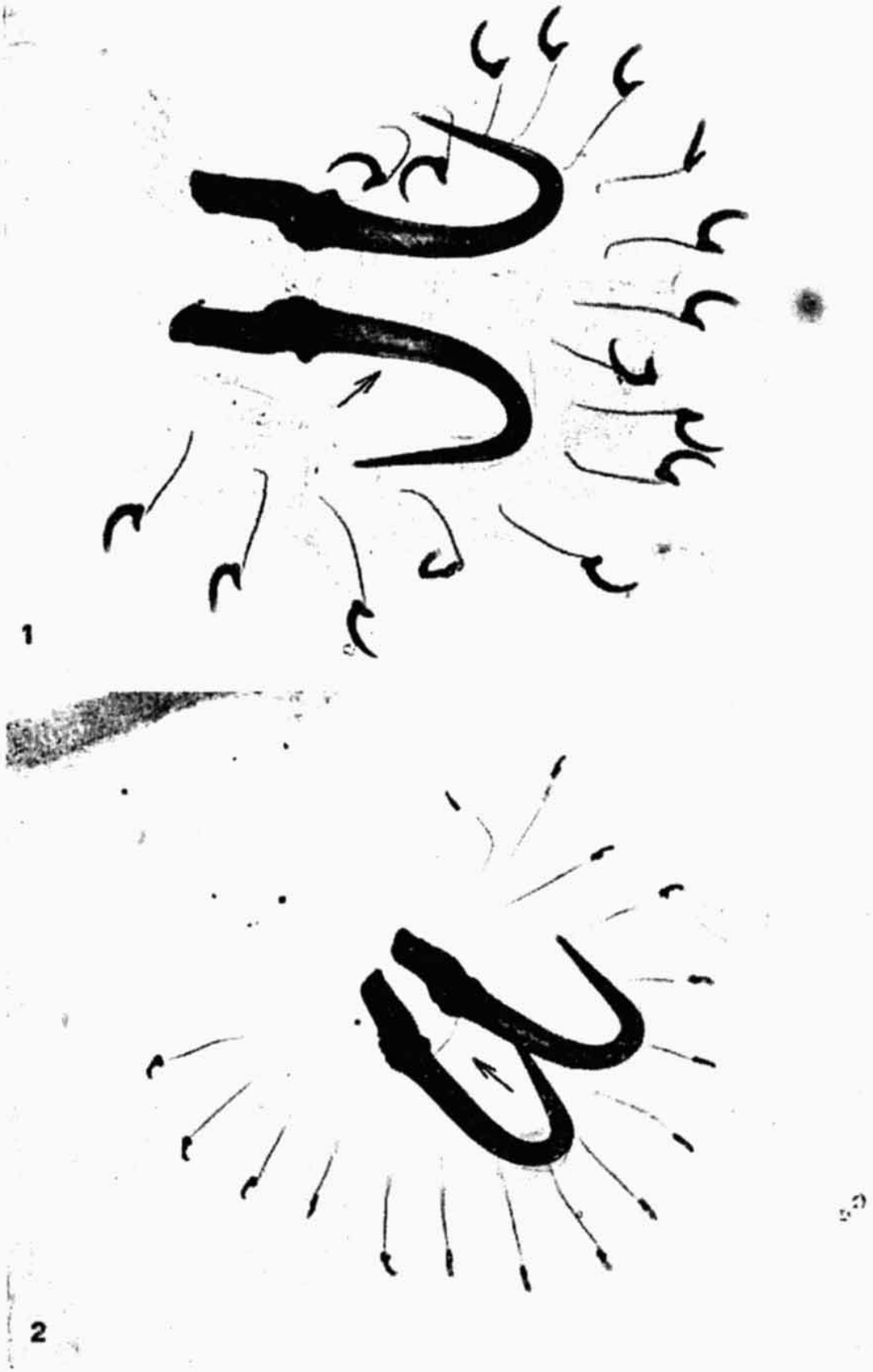

Fig. 1. Detail of the anchors and marginal hooks of *G. macronychus*. The point of anchors is stained entirely, whereas in the shaft of anchors only the outer layer is stained and the inner layer remains unstained (arrow). In the marginal hooks both the shaft and hook proper are stained intensely. PAA—aldehyde fuchsin (300×).

Fig. 2. Detail of opisthaptor of *Gyrodactylus aphyae*. Like in *G. macronychus*, the marginal hooks are stained entirely, whereas in the shaft of anchors only the outer layer is stained and the inner layer remains unstained. The connecting bars (arrow) also remain unstained. PAA — aldehyde fuchsin (315×).