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ON THE PRESENCE OF ELASTIC MICROFIBRILS IN LIVER GRANULOMA OF MURINE SCHISTOSOMIASIS MANSONI

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Swiss Webster mice, infected at 5 days old by transcutaneous route with 50 cercariae of B. H. strain, have been killed on the following days: 45, 75, 100, 160, 230, 320 and 425. The livers of all animals have been resected and divided into two parts: A and B. Part A has been processed for histological study by light microscopy and part B served for extracting granulomas which have been studied by electron microscopy.

Part A liver fragments, when being processed for light microscopical study, have been fixed for 3-5 days, at 4°C, in a solution containing 10% formaldehyde in PBS, dehydrated in ethanol at several concentrations, starting on 50% until the final treatment with absolute alcohol, and cleared in two changes of xylene, before being impregnated and embedded in melted paraffin at 58°C. Five micrometer sections have been stained either with hematoxylin & eosin for general morphology, or with resorcin-fuchsin (sections previously oxidized with oxone or not) for elastic tissue observations (L. G. Luna 1968, *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*, (3rd ed) McGraw-Hill Book Company, New York).

The fragments processed for obtaining granulomas (Part B) have been triturated for 2 min and the suspension has been washed trough grids in order to select granulomas of two different sizes: smaller and larger than 300 μm.

The granulomas have been washed in Millonig buffer and then fixed in a solution containing 3% glutaraldehyde and 0,25% tannic acid in Millonig buffer, 0.1 M, pH = 7.3, at 4°C, during 2 h (G. Cotta-Pereira et al., 1976, *Stain Technol., 51*: 7-11). After this period, the suspensions have been centrifuged in order to transfer the fragments for washing and postfixation in a solution containing 1% osmium tetroxide in Millonig buffer, 0.1M, pH = 7.3, during 1 h. The granulomas were washed again and then dehydrated in changes of ethanol and embedded in Epon. The polymerization time extended to 48 h at 60°C and ultrathin sections have been double stained with uranyl acetate and lead citrate prior to the observations in the transmission electron microscope.

The observation of the histological sections stained with resorcin-fuchsin revealed in the early granulomatous reaction the presence of elastic fibers in the wall of portal blood vessels and central lobule vein (Figs 1, 2). Such stainability enhances intermediate and full mature elastic system fibers (respectively elaunin and elastic fibers) as demonstrated by Z. Gawlik (1975, *Folia Histochem. Cytochem., 3*: 233-251)

After 75 days of infection, the granulomas already presented some fibrous structures intermingled with epithelioid cells and revealed by resorcin-fuchsin (previous oxidation with oxone) (Figs 3, 4). These tinctorial characteristics are consistent with a very primitive elastic fiber, which has been described by H. M. Fullmer & R. D. Lillie (1958, *J. Histochem. Cytochem., 6*: 425-430) and named oxytalan fiber. As a matter of fact, using isolated granulomas processed for electron microscopy including fixation with tannic acid-glutaraldehyde, it was possible to reveal bundles of tubular microfibrils, 10 to 12 nm in diameter. These bundles represent the ultrastructural pattern of oxytalan fibers, as demonstrated by G. Cotta-Pereira et al. (1976, *Invest. J. Dermatol., 66*: 143-148). Previously, the oxytalan fibers have been reported in dental granulomas, adventitia of blood vessels, epineurium (H. M. Fullmer & R. D. Lillie, 1958, *J. Histochem. Cytochem., 6* 425-430), dermo-epidermal junction (G. Cotta-Pereira et al., 1977, *Adv. Exptl Med. Biol., 79*: 19-30) and its presence in granulomatous reactions may be important not only because its probable structural role, but also it is an indicative of elastogenesis that was not yet reported in such condition. Also these fibers could interact with other extracellular matrix components in order to participate of the reactions subsequent to cell matrix interactions.

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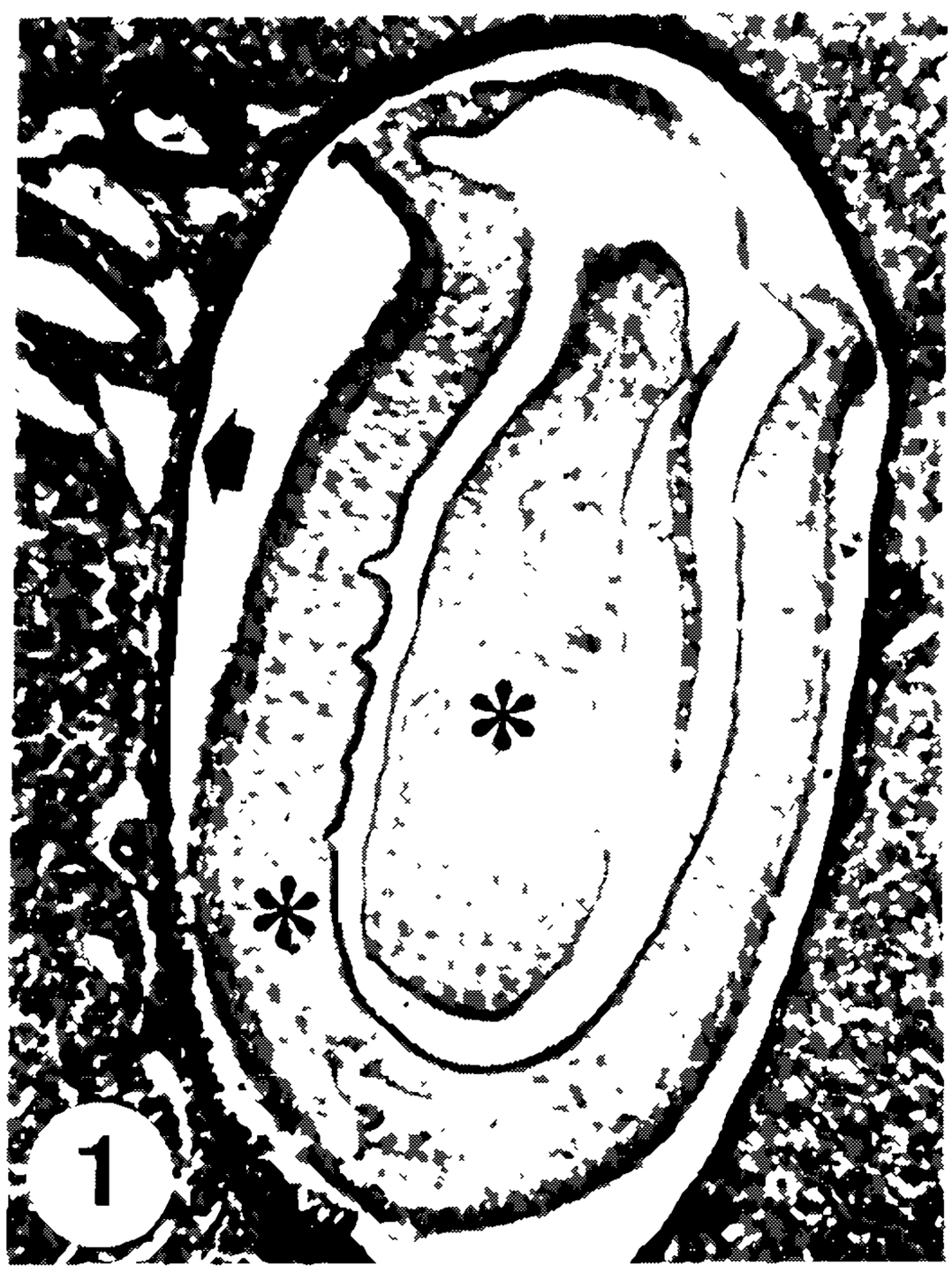

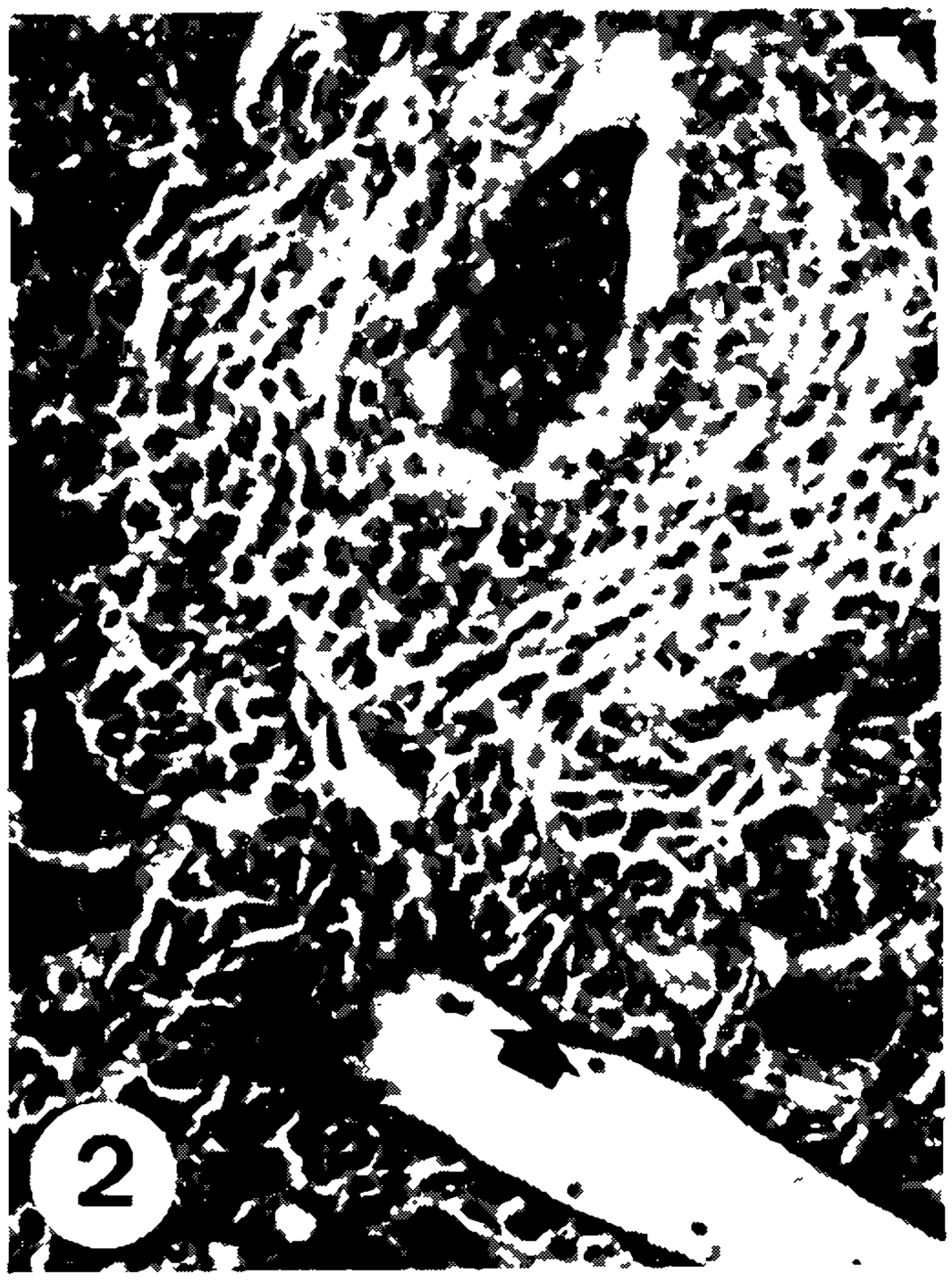

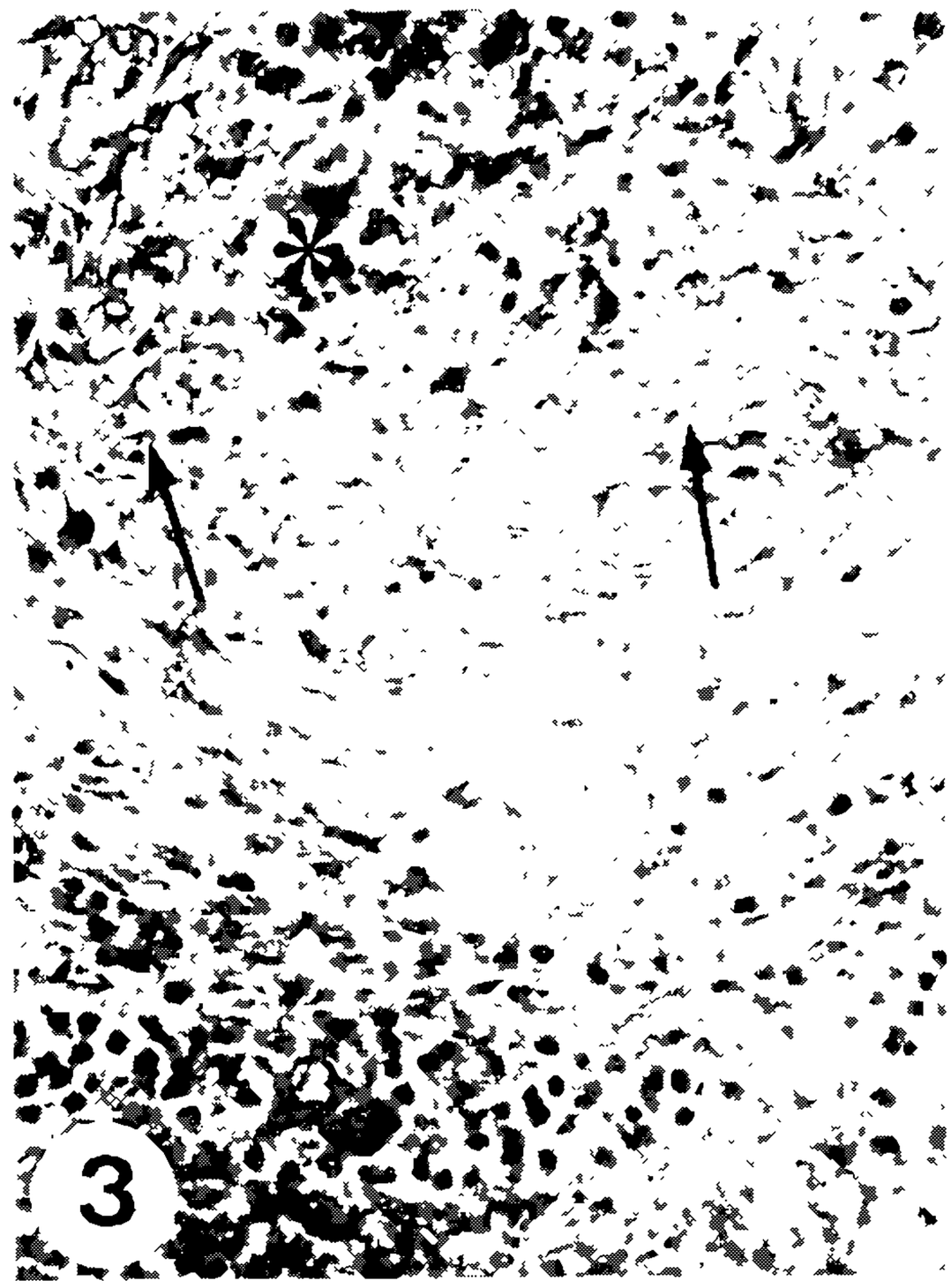

Fig 1: liver portal space of a 45 days infected mouse showing adult worms (asterisks) in a portal vein branch. Elastic fibers (arrow) are still preserved and appear as constituent of the blood vessel wall. Resorcin-fuchsin stain without previous oxidation. X250. Fig. 2: exsudative-productive hepatic granuloma in a 75 days infected mouse. Observe the *Schistosoma mansoni* egg (asterisk) centralizing a granulomatous reaction when blood vessel elastic fibers are no longer visualized. Such fibers are still noted in an intact vessel (arrow). Resorcin-fuchsin stain without previous oxidation. X310. Fig. 3: productive hepatic granuloma in a 100 days infected mouse. Note the *S. mansoni* egg (asterisk) in the middle of the granulomatous reaction which exhibits several layers. In the inner layer several oxytalan fibers (arrows) are observed. Resorcin-fuchsin stain after oxidation with oxone. X310. Fig. 4: higher magnification of the Fig. 3 showing the newly formed oxytalan fibers (arrows). Resorcin-fuchsin stain after oxidation with oxone. X540.