The supporting stroma of the lymph nodes consists of an envelope, the capsule, a variable quantity of hilar connective tissue, and a network of reticular fibers which passes from the hilum through the medullary and the cortical to join up with the capsule: this network is connected to the vasal walls and forms a framework supporting the lymph node cells.

Electron microscope studies (started with dissociation methods by v. Herrath and Dettmer (1951) and by Bairati and co-workers (1951, 1952) and then continued with the ultrathin section technique, have cleared up some points left unsolved by light microscope investigations on the reticular fibres, by showing that they always contain collagen fibrils; but none of the research so far conducted has provided an answer to the main problem, that is whether the lymph node stroma is, or is not, actually a specific form of connective tissue possessing structural characteristics that justify its being considered as a tissue in its own right.

Many workers, especially pathologists, were formerly, and indeed some still are, partial to the theory of the specificity of the reticular tissue, backing it up two arguments: a) the fibrillar stroma is always joined to specific cells called reticular cells; b) the stroma is of specific nature and structure because it is formed by a special protein called "reticulin".

Other workers have disputed the validity of the latter proof, because the data acquired so far on the ultrastructure and chemical nature of reticular stroma are definitely negative to the identification of a specific protein: full data on the literature and the preliminary results of personal experimental research are referred to the critical appraisal offered by one of us (Bairati 1958a): at all events, these first investigations called for further, more advanced research which will be reported here.

The other point in support of specificity, based on the fact that the stroma is joined to lymph node cells, still offers contrasting aspects. After the first data submitted by Bairati and de Petris (1959, 1960), some other contributions were published in the literature (Sorensen, 1960; Han, 1961; Clark, 1962; Moe, 1963; Pischinger, 1963); but the doubt and, in some measure, the discrepancy of the findings prompted us to embark upon a further, more advanced study.

* To Prof. F. E. Lehmann on his sixtieth birth day.
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On the ultrastructure of the lymph nodes

Materials and methods

The submicroscopic organisation and nature of the reticular stroma were studied on isolated fibres from the lymph node stroma. Further data were obtained by electron microscope studies using the technique of ultrathin sections from lymph nodes of various animals, which also provided information on the problem of how the stroma joins up with the cells.

I. Technical data of the electron microscope studies on lymph nodes

Axillary and cervical lymph nodes of cat, dog, rabbit and rat were studied. The best results are achieved by injecting the osmic fixation liquids into the cephalic vascular district of the animal, but this method can be replaced by immersion fixation using some technical expedients. The lymph nodes were prepared in the anesthetised animal, and covered with fixing agent, after which they were separated with a thin knife into the thinnest possible sections: these sections removed from the animal are immerged in osmic liquid at 0°C and subsequently divided into fairly large fragments: these were embedded both in methacrylic and in epoxy resins (araldite), the latter giving definitely better results. We adopted the expedient of embedding fairly large sections in wide, flat vessels for polymerisation, after which the small blocks were cut out from this section with a fretsaw.

A Porter-Blum and an LKB ultratome microtomes were used to obtain the ultrathin sections. The methacrylic sections were placed on a thin film of celloidin and protected by a layer of carbon; those obtained from the epoxy resins were examined without any support, often covered with a very thin layer of carbon. The preparations were examined under Siemens Elmiskop I and II electron microscopes.

II. Technical data of the studies on the nature of reticular network

A detailed description of the technical procedures employed for isolating the reticular stroma has to be given, since in order to give an interpretation it is essential to know the technical particulars. The lymph nodes were removed from the anesthetised dog and frozen at −20°C and preserved at this temperature until treated. The frozen lymph node was separated into slices a few millimetres thick and the capsule and hilar connective tissue removed, using a binocular to control these operations. The small blocks thus obtained are made up chiefly of cortical substance. These were sliced in the freezing microtome to 10 μ sections which, suspended in hypotonic liquid (Ringer's solution diluted 50%), were processed with low frequency ultrasonics in a 10 KHz Siemens sonic disintegrator, for two and a half minutes. This treatment is sufficient to swell and detach the cells which are broken up into minute fragments, while the stroma is separated into wafers and scraps some 0.1–0.5 mm large (Fig. 1).

This suspension is left to settle at 0°C for several minutes and the supernatant containing cell fragments is removed. The sediment is repeatedly washed in Ringer solution at 0°C and the fragments collected by low speed centrifugation, at not more than 1000 r.p.m. While this washing operation is going on, the suspension is controlled under the phase contrast microscope to identify fragments of reticular stroma and make sure there are no cells or parts thereof (Fig. 1). This procedure produces a small quantity of isolated material which is preserved at −20°C and the operation is repeated as many times as needed to provide a sufficient quantity of material for all the tests.

1. Morphological investigations. Besides phase contrast and dark field examination systematic polarised light investigations were also conducted. The apparatus employed must have excellent extinction and an extremely intense light source. We used a Leitz Ortholux binocular microscope equipped with a xenon lamp and excellent optical system virtually free of depolarising effects. The birefringence retardation values were measured with a Brace 1/30 λ compensator with which it is possible to make a reliable evaluation of retardation values in the area of about 1 Å.

A small quantity of the suspension can be processed by ultrasonics for a further short time to attain more advanced fragmentation of the reticular fibres and isolate the fibrils; after contrast staining with uranyl acetate or phosphotungstic acid, the suspension is spread on a thin film of carbon for examination under the electron microscope.