METHOD OF OBTAINING MICROPREPARATIONS OF INTESTINAL VILLI

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To obtain reliable microscopic pictures, a method of processing and embedding in paraffin wax, followed by cutting complete series of sections in assigned planes through individual intestinal villi, isolated under the control of a binocular loupe from biopsy specimens of the mucous membrane of the small intestine, fixed with 12% neutral formalin and not attached to any surface, was used. The method ensures adequate evaluation of villi of different shapes both as a whole and in micropreparations.

KEY WORDS: enterobiopsy; intestinal villi; histological technique.

The unreliability of estimates of the mucous membrane of the small intestine as a whole (under low power magnifications) and in microscopic sections increases the likelihood of obtaining arbitrary sections through structures even if the piece of tissue is cut in a definite plane. This was demonstrated conclusively when it proved impossible, in microscopic preparations, to distinguish villi in a state of detachment of their distal fragment, found in whole enterobiopsy specimens, from oblique sections through ordinary villi. Variations in the interpretation of microscopic pictures can be eliminated by complete series of sections through concrete forms of villi in an assigned plane. In enteromorphology, however, no description of any suitable technique could be found and the processing of small objects in protozoology and embryology, with the exception of some general methods, is specific and time-consuming.

The suggested method is based primarily on a method of preliminary microscopic study of free (i.e., not attached to any surface) enterobiopsy specimens fixed with 12% neutral formalin, preliminary straightening and fixation of which with one or two drops of fixative are carried out on slides under the control of a binocular loupe. After remaining in formalin for at least 24 h, the biopsy specimens are transferred to a Petri dish with fixative or water, and during their examination under the binocular loupe by means of fine instruments it is possible to distinguish individual forms of villi for subsequent production of micropreparations from them. Processing of the villi thus isolated and their embedding in paraffin wax are carried out in accordance with the technique for processing of biopsy material in [1], modified by the writer to suit the aims of histological processing of enterobiopsy material after microscopic examination in toto. Some ordinary methods of work with protozoan and embryonic material also were used: more gradual dehydration, staining the objects to make them visible after cleaning, the use of pipets and, of course, an optical system.

Individual villi were processed in Petri dishes 5-6 cm in diameter and in low, wide bottles. For 96° and 100° alcohols, Petri dishes 10 cm in diameter are used. The objects are transferred between media with ophthalmic pipets, and only from caster oil in a drop of the oil between the tips of forceps. Care must be taken to ensure that some of them are not left in the pipet, or that they do not float away along the inside of the tips of the forceps. All stages of histological treatment of the intestinal villi are carried out under the control of a forehead binocular loupe or binocular microscope, at low-power magnification, and at the following times: rinsing in three portions of tap water — 20 min, dehydration for 5 min each in 40° (I and II), 50, 60, 70, and 80° alcohols, and for 30 min each in 96° and 100° alcohols, colored with a few drops of an alcoholic solution of eosin. From the 100° alcohol the objects are transferred for a few minutes by a pipet with a small volume of alcohol into drops of castor oil, placed in wells on slides, where an intermediate medium is thus created (alcohol + oil), and then they are transferred on the tip of forceps into pure castor oil for

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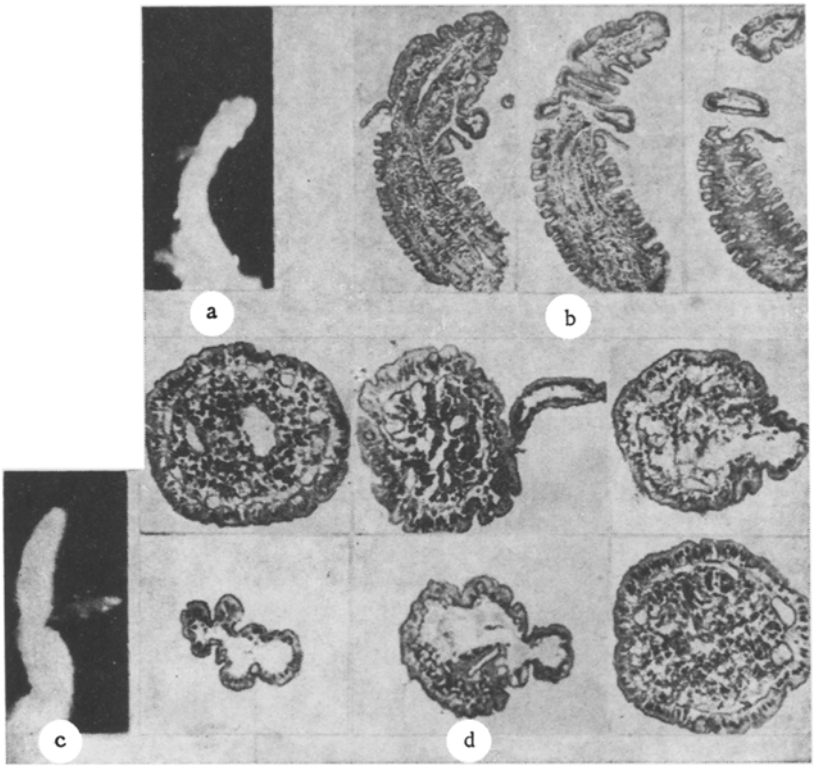

Fig. 1. Villi isolated from enterobiopsy material from a dog fixed with 12% neutral formalin. a, c) Whole villi (20×); b, d) examples of series of longitudinal and transverse sections through villi. In b: hematoxylin-eosin, 63×; in d: PAS reaction, hematoxylin-eosin, 140×.

30 min. The objects are freed from excess of oil in wells on slides with a small volume of xylene, from which they are transferred by pipet to pure xylene, and kept in two portions of it for 10 min. To prevent loss or confusion during simultaneous processing of 20-30 (or more) different forms of villi, arranged in separate groups and noticeably reduced in volume after dehydration, adequate illumination, a stable arrangement of the bottles with media convenient for transfer of the objects, and constancy of temperature despite the short stay of the objects in these media must be ensured. For soaking with paraffin and embedding, it was therefore found desirable to use two electric baths (sand and water), kept in a box with heat insulation (asbestos) and with controlled illumination from a table lamp, instead of a thermostat. During perfection of the technique it is recommended that the time taken to heat the sand bath to the necessary temperature be determined, after which it is disconnected from the supply system and the time during which the temperature in it remains at the assigned level is determined. This enables future work to be carried out without a thermometer, the sand bath to be switched on and off before the bottles containing media are placed in it, and some idea to be obtained of the duration of its use without additional reheating. In our own practice, with a box well insulated for heat, penetration of the paraffin wax and embedding are carried out in a sand bath which has been switched off after a single preliminary heating. Jars with paraffin wax for embedding and jars with ophthalmic pipets and dissection needles, partially immersed in paraffin wax, are placed in the water bath. By periodically switching on the power supply to the bath, the required degree of heating is maintained. The objects are kept in the mixture of paraffin and xylene for 30 min, in paraffin I for 5 min, and in paraffin II and a mixture of it with wax for 10 min each.

Directing the villi in the assigned plane is carried out under the control of a binocular microscope during embedding. To obtain a smooth surface of the blocks, a slide moistened with glycerol is placed on the bottom of the paper box. Paraffin wax is poured into a preheated box and the objects transferred into it with pipets. To obtain blocks with villi arranged longitudinally, the villi are placed on the bottom of the box. This procedure is technically simple, it allows many villi to be placed in the same box, and an appropriate number

of blocks can be obtained simultaneously. To prevent premature hardening of the paraffin the box is surrounded with sheets of asbestos and tied up with thread, which is cut after the villi have become properly oriented, so that the sheets of asbestos fall away to the side. To obtain blocks for preparation of transverse sections through villi, each must be placed in the vertical position in the box with paraffin. This is done by means of dissection needles and as quickly as possible, so that the paraffin, beginning to harden, can fix them in the required position. In such cases it is therefore more convenient to use small boxes, preheated, but not thermally insulated. The boxes are transferred to cold running water not before the surface of the paraffin in them has hardened sufficiently. The slide is removed from the hardened paraffin, freed from paper, by means of a scalpel. It is best to cut out blocks of separate villi, and also to prepare complete series of microtome sections from them under the control of a forehead binocular loupe. Preservation of the paraffin strips with sections 3-5 μ thick is best ensured if they are placed on dry, cold slides, and only then is the 50° alcohol, in which the paraffin sections straighten out during heating, applied under the control of a binocular microscope. After drying overnight on a thermostat, they are firmly held to the glass during dewaxing and various staining procedures.

By means of the suggested method reliable microscopic pictures can be obtained and unequivocally interpreted; this is of great importance for the investigation and clarification of various problems in enteromorphology.

LITERATURE CITED

1. P. A. Kanishev, Methods of Diagnosis of Diseases of the Stomach [in Russian], Leningrad (1964), p. 100.