SUBMICROSOCPIC STRUCTURE OF THE CHROMATOID BODY OF Entamoeba invadens AND Entamoeba moshkovskii

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The study of amebas with the optical microscope has shown that precystic forms of trophozoites and also the cysts of nearly all amebas contain cytoplasmic inclusions called "chromatoid bodies" [9] or "crystalloid formations" [4]. The chromatoid bodies vary widely in shape. The may be shaped like bars or rods with rounded (Entamoeba histolytica [4], Entamoeba moshkovskii [3]) or pointed (Entaboeba coli) ends, from 5 to 10 μ in length, and sometimes like fibrils (E. coli [4, 17]). In the case of Entamoeba invadens the chromatoid bodies are usually cylindrical or acicular in shape [10].

Statements concerning the nature and function of the chromatoid inclusions given in the literature are contradictory. The suggestion has been made [11, 12] that such inclusions are related to the nuclear chromatin. Other investigators [8] consider that the chromatoid bodies are formed in the cytoplasm as a result of pooling of the contents of the vacuoles. Some authors suggest [9] that the chromatoid body contains reserve nutrient matter.

Electron-microscopic investigations of entamebas have considerably modified our ideas of the nature of the chromatoid bodies. It has been shown [8] that particles of the chromatoid body are distributed throughout the cytoplasm of the trophozoite. Sometimes they may be joined to digestive vacuoles, forming elongated complex structures. Cytochemical investigations [5] have shown that the chromatoid bodies consist of RNA and protein. It was later found [6] that the chromatoid body of E. invadens in the initial stages of its development contains semicrystaline masses of ribonucleoprotein, formed by aggregation of particles measuring 200-300 A. On this basis a hypothesis has been put forward [6], according to which the chromatoid bodies are "crystalline ribosome reserves".

The study of the chromatoid bodies of the Entamoebae is of great importance to the taxonomy and identification of these microorganisms, for their shape, size, and number vary from one species to another. The information on the chromatoid bodies of the Entamoebae is incomplete in many respects, and the chemical nature of these formations still remains obscure.

The object of the present investigation was to study the submicroscopic structure of the chromatoid bodies of E. invadens and E. moshkovskii by the method of ultrathin sections. By electron-cytochemical methods an attempt was made to determine the chemical nature of these formations.

EXPERIMENTAL METHOD

The trophozoites of E. invadens and E. moshkovskii were cultivated on E. A. Pavlova's medium [2]. The trophozoites were separated by centrifugation, fixed by Sjöstrand's method [16], dehydrated in alcohols of increasing strength, and embedded in a 4:1 mixture of butyl and methyl methacrylates, with benzoyl peroxide as catalyst. Metal rings were used for flooding the material [1]. Polymerization took place at 56°. Ultrathin sections were cut to a thickness of about 250 A on a type LKB ultratome and studied by means of the JEM-6C electron microscope.

EXPERIMENTAL RESULTS

In the ultrathin sections of E. invadens the chromatoid body consisted of electron-dense helical strands arranged in a strictly definite order (see figure, a). The helical strands of the chromatoid body

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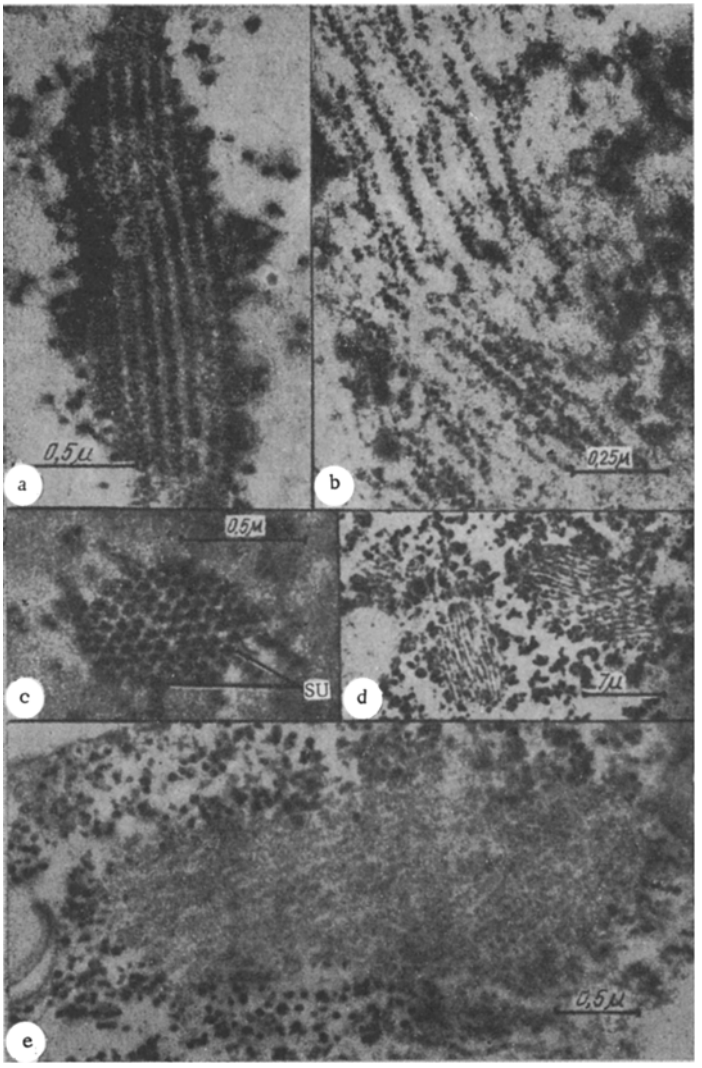

Chromatoid body of E. invadens and E. moshkovskii: a) longitudinal section through helical strands of the chromatid body of E. invadens; 45 000×; b) transverse section through the helical strands of the chromatoid body of E. invadens; SU-subunits; 45 000×; c) strands of the chromatoid body of E. moshkovskii; 71 000×; d) cluster of helical strands of the chromatoid body of E. moshkovskii; 14 000×; e) action of ribonuclease on the chromatoid body of E. invadens; 30 000×.

of E. moshkovskii, unlike those of E. invadens, were arranged singly or as clusters of various shapes in the cytoplasm (see figure, c, d). The thickness of the individual helices of E. invadens was 100-110 A, and of E. moshkovskii 80-100 A. In transverse sections through these helices of E. invadens, particles with blurred outlines and arranged as in a crystal were revealed (see figure, b). These particles were complex in structure and were composed in turn of subunits measuring 100-120 A. In transverse ultrathin sections through the chromatoid body of E. moshkovskii particles measuring 250-270 A were seen. Each such particle was formed of subunits measuring 70-90 A.

To study the chemical nature of the chromatoid inclusions, trophozoites of E. invadens and E. moshkovskii were treated with ribonuclease. After treatment with the enzyme the helical structure of the chromatoid body was destroyed and its electron-optical density was sharply reduced (see figure, e). X-ray structural analysis has shown that the RNA of yeasts, bacteria, and animals is helical in structure

[13, 14]. The ability of the chromatoid body to be broken up by ribonuclease, in conjunction with its helical structure, thus confirmed reports in the literature that the main component of this body is ribonucleoprotein (RNP).

The results obtained relative to the ultrastructure of the chromatoid inclusions of E. invadens confirmed those of other investigations [15] also showing having accepted that such a helical structure of the chromatoid bodies is possible, goes on to suggest that helices of this type may be formed by the folding of one layer of RNP particles on another. He considers that the structure of the chromatoid bodies changes depending on the physiological state of the cell, sometimes taking the form of particles in a compact cubic or hexagonal arrangement, sometimes the form of helical strands. In the present investigations only a helical structure of the chromatoid body was observed, both for E. invadens and for E. moshkovskii. RNP particles arranged in crystalline form were detected in transverse sections of the chromatoid inclusions.

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