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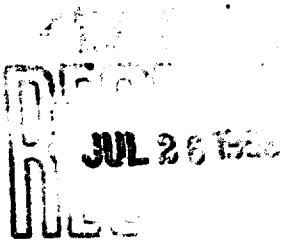

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Additional studies of microtomic sections of canary pox virus. Fourth report.

by K. Herzberg, A. Kleinschmidt and D. Lang.

Translated from Zbl. f. Bakt. I Orig. 179: 308-323 (1960) by the Technical Library, Technical Information Division.

It was demonstrated in our third report on the electron microscopy of canary pox virus that the sites of inception of elementary body (EB) development are scattered throughout the cytoplasm. We had designated Morgan's early forms as form 1 and form 2. Early forms consist only of viroplasm and a membrane (form 1) or of viroplasm, membrane and an undifferentiated core (form 2). The developed, mature form (form 4) of canary pox EB, on the other hand, was found almost exclusively near the virus vesicles, which may be considered as a later stage of Bollinger bodies. The wall of virus vesicles reveals only the terminal stage of EB development. An EB model was constructed from EB sections of these terminal stages. Numerous sections yielded an ellipsoid body. The form and internal structure of this EB were described and visualized in two model sections in perspective. Thus two areas in the plasma of infected histiocytes are relevant for future studies, depending on the point of attack: either the Bollinger bodies or virus vesicles, if the ultrastructure of mature EB is to be investigated, or the developmental sites of early forms scattered throughout the cytoplasm, if information is desired on chronological factors in the genesis of the first EB membranes or the finely granular densities enclosed by them.

The present study employed virus-infected histiocytes embedded in the polyester (= vestopal W) recommended by Kellenberger. This as well as other methacrylate-embedded preparations were suspended in agar after fixation and subjected to further treatment.

Method: Fixation in 1% buffered OsO_4 solution, either according to Ryter and Kellenberger at pH 6 or at pH 7.2. The fixed cellular sediment is heated to 45°C and blended on the water bath with liquid agar of identical temperature. The agar is allowed to solidify on a slide, and small bricks (maximum 2 x 2 x 4 mm) are cut and treated with 0.5% uranylacetate for 1-2 hours. Dehydration through an ascending acetone series (all at room temperature) and embedding in vestopal at 60°C in accordance with original instructions 1958.

Sections were prepared with an ultramicrotome with thermic couple after W. Vogell or with a Porter-Blum microtome, either singly or in series, transferred to grids or slit filters and exposed in the Elmiskop I (Siemens), usually at 20,000 magnification.

Results.

Plate 1a (exposure 4056). Dense, finely granular mass and just developing membrane (arrow). Differentiation of concentrated cytoplasmic areas into early forms.

Plate 1b (exposure 3979). Membrane development has progressed somewhat at the periphery.

Plate 1c (exposure 3584). The histiocytoplasm shows finely granular areas that assume a rosette shape due to still incomplete membranes forming at the periphery. Membrane development is seen only in places where such a finely granular substance is present. There are no membranes that enclose an electron-microscopically empty space. Observations similar to 1c were made by C. Morgan and coworkers in connection with fowl pox (1954), by Vallejo-Freire et al. (1957) and Peters (1959) in vaccinia virus, by Reczko in swinepox (1959), and by Higashi in ectromelia (1959). The elementary bodies have no core. The edge of the frame shows two drops of lipoid.

Plate 2a and 2b (exposures 3531 and 3530). Further developed stage than in Plate 1c, predominantly EB of form 1. Above (2a) and below (2b) the same locale in immediately following serial sections. Two indications of internal cores, i.e., transitions to form 2. The EB are widely oval. Corresponding EB were identified by same letters. It is evident that the surface area of the same EB changes with elevation. It is possible also that an EB with membrane on one section appears without membrane on the next, as in the case of b and k. This may be explained as follows. In one instance the cut has separated only a thin shell of the EB. Here the elementary body's surface area is smaller, the edge has been sliced at an angle, a membrane can no longer be discerned. In the next section the knife goes through the central part of the EB and the membrane is cut more vertically. The surface area is larger, the membrane becomes visible.

Plate 3 (exposure 2546). Section through a focus with many densely arranged early forms 1 and 2. One form 3 with a wide core. EB of form 2 have a core of variable size. These internal cores are so condensed that they appear as the darkest spots on the micrograph. They are surrounded by a light zone of variable width.

Plate 4a (exposure 2214). The micrograph shows early forms 1 without core and early forms 2 with cores of varying size and density. Membranes are widely oval.

Plate 4b and 4c (exposures 2491 and 2492). When we discovered that addition of agar to methacrylate produced a more regular form of EB when compared to earlier versions, we prepared serial sections through early forms 2 embedded in methacrylate (methacrylate / agar / uranylacetate). The exposures on the right and left show the same EB, but at different planes. Corresponding points are identified by the same letters. The size of the core and the surrounding light zone changes with every section. In some EB only this light area is cut, and not the core. This and other illustrations indicate that the light zone surrounds the entire core like a shell.

Plate 5a-5e (exposures 3609, 3608, 3611, 3612, 3615). The pictures show mature forms after embedding in vestopal and contrasting with uranyl.

a) The wall: The sections show two differentiable membranes. The outer one reveals three layers (dark-light-dark) of uniform thickness. This structure becomes visible when the mature EB are already situated outside of a Bollinger body (Fig. 5c-5e). The inner membrane shows the dark-light-dark alternation only in spots. Within (at?) this membrane are the two protruberances (bulges) directed toward the interior.

b) The intermediate layer: The light intermediate layer between the wall and the core has become more uniform and narrower in comparison to sections embedded in methacrylate. This is distinctly visible in mature EB still within the matrix of a Bollinger body (Fig. 5a).

c) The core: The core may be seen as a flat structure resembling an intact, non-nucleated erythrocyte with a bulge circling around its edge and an internal lumen. It was found that its rim consists of several layers: at least one central light layer and two adjoining dark ones. Another new factor was the discovery, after application of uranylacetate, of an electron-optically loose structure in the lumen of the core, clearly marked up to the enlargement in the rim's bulge (Fig. 5a and 5b). Thus the model of canary pox virus EB proposed in the 3rd report has gained a number of ultrastructural details based on the results obtained with vestopal.

Discussion.

Every kind of change in technique leads to thoughts about the extent to which deviations from physiological pH, precipitating effects of added contrasting agents, etc. could have influenced the structure artificially. Even when these factors are considered, we still deem the method of vestopal suspension introduced by Kellenberger et al. a step forward, based on our studies of canary pox virus. This progress is expressed by better stability in the electron beam (Kellenberger, Reimer) and by smaller volumetric changes during polymerization, allowing preservation of the form of intracellularly positioned EB as well as visualization of ultrastructure superior to methacrylate.

The micrographs of virus-infected histiocytes and EB have clearer markings than those of the same object embedded in methacrylate. This may be asserted after studying histiocyte sediments with both methods comparatively at pH 6 and pH 7.2 (fixing medium). It is true that the preservation of form is improved by addition of agar prior to suspension in both methacrylate and vestopal. However, the ultrastructure of viroplasm usually remains coarser in the methacrylate method, a circumstance that frequently differentiates the two techniques.

After embedding in vestopal, the intermediate layer (light zone between the wall and core) of mature EB is subject to smaller fluctuations than encountered in pure methacrylate suspensions. The mature EB is seen as a structure with three symmetrical planes vertical to each other (main sections); two of these cut it into ellipses, the third produces a surface whose outline is an intermediate form between ellipse and rectangle. The mature EB may be considered an approximate ellipsoid. Our sections only suggest the humping which is so clearly evident on EB that are dried and shadowed in a flat position, as shown first in 1948 by Dawson and McFarlane's well-known picture of vaccinia virus, or this property is absent altogether. These humps develop distinctly only when EB have been liberated from their intracellular environment. In their pronounced form they are a result of a partial contraction of the EB. For this reason our EB model has no humps, since it corresponds to an intracellularly positioned EB. Our studies with vestopal have revealed further that the outer and inner membranes of the EB each consist of three layers: two dark ones containing a light one. The edge of the core also disclosed stratification. Treatment with uranylacetate revealed a structure in the lumen of the core. This is assumed to be the elementary body's DNA. Assuming that about 6% of the EB consists of DNA (cf. Schramm), the volumetric factors of our sections would indicate that only part of the core consists of DNA, since the core's volume amounts to about 21% of the whole EB.

The question concerning the chronological relation in early forms of the first membrane and the granular substance enclosed by it may be answered in the sense that the fine granulation is already visible when membranes are still absent or just forming. This situation is shown by Plate 1a. Formation of the granular zone therefore precedes membrane development in time. Consequently there are no membranes which enclose an optically empty area that would fill subsequently. The development of the first membrane is probably governed by the finely granular zones of greater density. Creation of the second membrane, which for most EB occurs at the Bollinger body, is most likely due to a process of superimposition oriented on the preexisting first membrane. Just when and how this takes place is not clear. Should the discussed concept be correct, then the second, outer membrane may have a composition different from that of the first, inner one.

Illustrations

Plate I, Fig. 1a-1c: Differentiation of concentrated cytoplasmic zones into early forms of EB (47,000 x), vestopal.

Plate II. Fig. 2a and 2b. Two serial sections through early forms 1 (62,000 x), vestopal.

Plate III. Fig. 3. Focus of densely arranged early forms 1 and 2 (46,000 x).

Plate IV. Fig. 4a. Early forms 1 and 2 (47,000 x), vestopal. Fig. 4b and 4c. Two serial sections through early forms 2 (45,000 x), methacrylate.

Plate V. Fig. 5a - 5e. Mature EB form 4 (a-e 100,000 x), vestopal.

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