ULTRAVIOLET MICROSCOPY OF PANCREATIC ACINAR NUCLEI FIXED IN POTASSIUM PERMANGANATE

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The fixation of rat pancreas in potassium permanganate according to Luft (1) imparts a brown coloration and a strong ultraviolet absorption to the cytoplasm of the exocrine cells, but only a weak one to the nuclei. Within the latter an even paler zone surrounding the nucleoli can be observed. After the removal of the stain by means of oxalic acid, ultraviolet microscopy and the Feulgen technic reveal that the distribution of DNA within the nucleus is complementary to that of the staining caused by the $KMnO_4$ fixation.

Small pieces of rat pancreas were fixed, within a few minutes after the death of the animal, in 0.6 per cent potassium permanganate in veronal-acetate buffer at pH 7.2 for 1 hour at 0°C., washed in 30 per cent ethyl alcohol, dehydrated, and embedded in methacrylate, which was polymerized in ultraviolet light (2). Other specimens were prepared from the same material by freeze-drying and direct embedding in methacrylate (3). Sections were cut at 1 μ on a modified rocking microtome equipped with a glass knife and observed without the methacrylate having been removed (4). Ultraviolet microphotography was performed with "Old Delft" reflecting optics at condenser and objective numerical apertures of 0.65 and 0.85, respectively. The light was provided by a monochromator built for ultraviolet microscopy (5),

which was illuminated by a high pressure mercury arc (Philips HPK 125 W.). In the present investigation two spectral lines were selected, *viz.*, 302 mμ, at which wave length the brown stain evolved during $KMnO_4$ fixation, presumably manganese dioxide (6), absorbs strongly, and 265 mμ, which comes nearest to the absorption peak of the nucleic acids. At the latter wave length the manganese dioxide absorption is about as strong as at the former. Prolonged exposure to ultraviolet light causes a marked bleaching of the sections. The micrographs discussed below were made before this effect had become appreciable. In order to test the electron microscopic efficacy of the permanganate fixation and embedding technic, sections of similarly treated material were cut on a Sjöstrand microtome and studied with the Akashi electron microscope.[1]

Fig. 1 is a characteristic micrograph at 302 mμ of a pancreatic acinar cell fixed in $KMnO_4$. The weak absorption of the nucleus as compared to the cytoplasm is evident, as is the even more lightly colored halo of the prominent nucleolus. In the cytoplasm the swollen mitochondria are clearly outlined, but the zymogen granules are not visible.

At 265 mμ (Fig. 2), the ultraviolet absorption of the nucleic acids is added to that of manganese dioxide. Especially in the nucleus the structure is less distinct, presumably because at this wave length the lack of absorption of the parts weakly stained by the potassium permanganate fixation is made up by the extinction due to the nucleic acids.

In order to test the latter hypothesis, the section was photographed a second time at 265 mμ (Fig. 3) after it had been bleached by means of 0.2 N oxalic acid. (This procedure can be carried out within 5 minutes at room temperature in spite of the methacrylate remaining in the section.) Within the nucleus the absorption pattern has been completely reversed by the bleaching process: the parts darkened at 302 mμ by the potassium permanganate fixation are shown to contain the smallest amounts of substances absorbing at 265 mμ, and *vice versa*. Feulgen staining of similarly treated sections reveals a distribution of DNA virtually identical to the absorption pattern at 265 mμ.

[1] This part of the investigation was performed by one of the authors (I. M.) while working as a guest at Anatomiska Institutionen, Karolinska Institutet, Stockholm. We thank Dr. F. S. Sjöstrand for placing the facilities of his laboratory at our disposal.

Bleaching of the section with 2 per cent sodium bisulfite (10 minutes, room temperature) yields similar results as far as the nuclei are concerned.

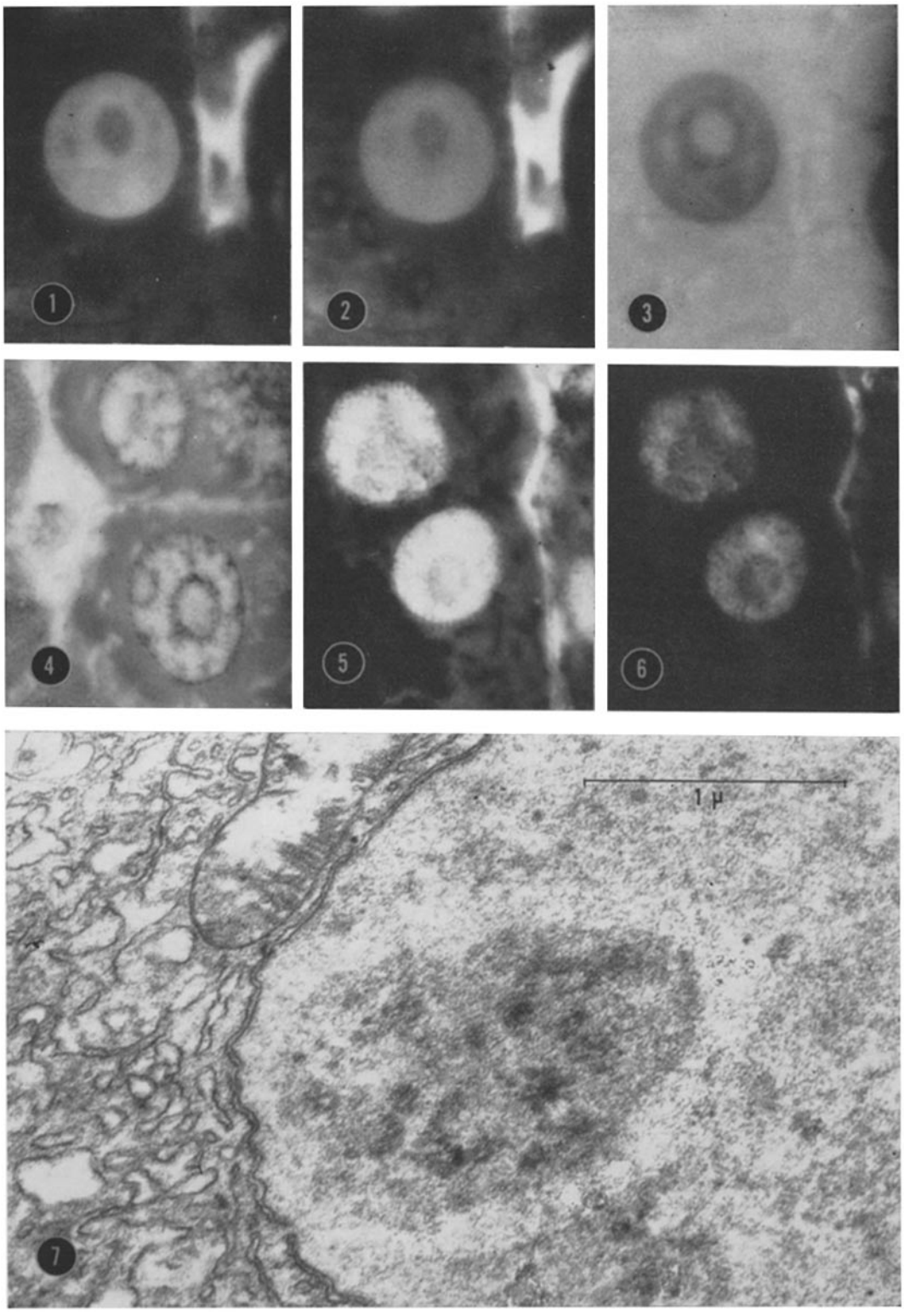
Within the cytoplasm a difference in the action of the two bleaching agents becomes apparent. In freeze-dried methacrylate-embedded pancreas (Fig. 4) there is a strong cytoplasmic absorption at 265 mμ, presumably due to RNA. This absorption is not found in oxalic acid–bleached sections of tissue fixed in $KMnO_4$ (Fig. 3) but it persists to some degree at least in sections bleached with sodium bisulfite. The former bleaching agent apparently dissolves whatever RNA is left in the cytoplasm during the fixation.

Sections of freeze-dried and directly embedded tissue have also been subjected to a potassium permanganate "fixation." Again, the methacrylate was not removed prior to this treatment. It is found (Fig. 5) that in this case, too, a striking difference in over-all stainability between nuclei and cytoplasm is obtained. In the cytoplasm the mitochondria display the same staining reaction, but the zymogen granules that do not show up after direct $KMnO_4$ fixation are very conspicuous in post-fixed freeze-dried sections.

A suitably exposed micrograph of the same section (Fig. 6) shows that the nucleus is not wholly unstained and that the nucleolus appears as a more darkly staining mass. No halo is seen, but the distribution of the nuclear constituents is not wholly comparable to that seen in permanganate-fixed cells. Micrographs at 265 mμ did not differ significantly from those taken at 302 mμ.

An electron micrograph of our permanganate-fixed material (Fig. 7) shows the well known enhancement of membranous structures and a distribution of electron-scattering material not unlike that of the brown stain as found at 302 mμ (Fig. 1). In Fig. 7, too, there is a faint indication of the perinucleolar halo.

Potassium permanganate is by no means a specific staining agent. Apart from proteins (1), lipoids have been stated to be stained or fixed by it (6, 7). Though accordingly the weak staining of the nuclei may in part be due to their low lipoid content, there remains the remarkable lack of staining of the nuclear proteins. The assumption that nuclear proteins may be dissolved during permanganate fixation, as apparently is the case with the zymogen granules, is rendered less probable by the outcome of the experiment with freeze-dried directly embedded tissue: the zymogen


1
2
3
4
5
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1 μ
7

granules are retained and amenable to permanganate staining, but the nuclear staining is, if anything, even weaker. The coincidence of regions of high nucleic acid concentrations with those of low reactivity towards $KMnO_4$ suggests that the proteins associated with DNA (and possibly RNA) are unable to react with potassium permanganate. This supposition is in keeping with the conclusion of Amelunxen and Themann (8) to the effect that potassium permanganate is a poor fixative of nucleoproteins.

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FIGURE 1

Micrograph taken at 302 mμ of pancreatic acinar cell fixed in $KMnO_4$. Note darkly stained cytoplasm and pale zone surrounding nucleolus. Zymogen granules not stained. Magnification, 2900.

FIGURE 2

Same cell at 265 mμ. Less nuclear detail. Magnification, 2900.

FIGURE 3

Same cell also at 265 mμ after bleaching with oxalic acid. Nuclear picture as seen in Fig. 1 reversed, negligible absorption within the cytoplasm. Magnification, 2900.

FIGURE 4

Freeze-dried pancreas, directly embedded in methacrylate, photographed at 265 mμ. Compare cytoplasmic absorption to Fig. 3. Magnification, 2900.

FIGURE 5

Section of freeze-dried methacrylate-embedded pancreas treated with $KMnO_4$, methacrylate not removed: 302 mμ. Heavily stained zymogen granules in lower left corner. Magnification, 2900.

FIGURE 6

Same as Fig. 5, but exposed for a shorter time so as to bring out nuclear detail. Magnification, 2900.

FIGURE 7

Electron micrograph of a pancreatic acinar cell fixed in $KMnO_4$, showing part of nucleus with nucleolus and faintly indicated perinucleolar halo. Magnification, 40,000.