Histochemical Determination of Ribose Nucleic Acid in Vertebrate Tissues Following Extraction with Perchloric Acid.* (17699)

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The standard procedure for determining histochemically the presence of ribonucleic acid in cells consists of treating sections with a buffered solution of ribonuclease prior to staining. The basophilic staining property of the ribonucleic acid is lost following digestion with the enzyme. The preparation of ribonuclease is beyond the scope of most biological laboratories and the cost of the prepared enzyme is prohibitive for routine investigation. Since the determination of the ribonucleic acid content of cells has become an important adjunct in histochemical and pathological studies, a more convenient substitute for ribonuclease is desirable. Using photocolorimetric estimation, Ogur and Rosen(1) obtained data which indicate that prolonged contact in the cold with perchloric acid extracts pentose nucleic acid from plant tissue residues. Seshachar and Flick(2), studying *Chilodonella,* report that prolonged treatment with cold perchloric acid extracts PNA from this protozoan. This investigation deals with the adaptation of a method for substituting cold perchloric acid for ribonuclease in the determination of ribonucleic acid in vertebrate tissues.

Methods. Treatment of deparaffinized sections on slides with cold perchloric acid showed no significant effects when compared with control sections. The following methods were then used:

A. Tissues from rats and cats (liver, autonomic ganglia, sensory ganglia, pancreas, thyroid and parotid glands) were fixed in Zenker's Fluid for 8-15 hours and washed in running water for 2 hours. One-half of each block of tissue was placed in 10% perchloric acid from 5 to 24 hours at 5°C. Following preliminary study, the 18 hour time interval was used exclusively. The other half of each block of tissue was placed in water for a similar length of time at the same temperature to serve as controls. The tissues were again washed in running water for 2 hours, dehydrated, imbedded and sectioned in the usual manner. Sections from both perchloric acid treated tissues and from controls were placed on each slide and stained with Mallory's phloxine-methylene blue.

B. Tissues similar to those in section A were fixed in Zenker's Fluid for 8-15 hours, washed in running water for 2 hours and sectioned at 15 microns with the freezing microtome. Half the sections from each block of tissue were placed in 10% perchloric acid for 18 hours at 5°C, the other half in water at 5°C for the same length of time. The sections were rinsed in water, stained with 1% toluidine blue for 5 minutes, counterstained with 0.5% alcoholic eosin for 15 seconds, differentiated in 95% alcohol and mounted in glycerin.

Autonomic and Sensory Ganglia. In the control sections of autonomic and sensory ganglia, the chromidial bodies and nucleoli are stained with the basophilic stains (Fig. 1 & 3). The changes induced by treatment with cold perchloric acid were very striking. The chromidial bodies and the nucleoli were completely unstained by the toluidine blue or methylene blue, whereas the chromatin material of the neurological cells was unaffected. (Fig. 2 & 4). The nucleolus and the nucleolar membrane in the treated sections appeared acidophilic. Similar results were obtained in ventral root cells by Gersh and Bodian(3) after treating sections of the spinal cord of rhesus monkeys with a buffered solution of ribonuclease.

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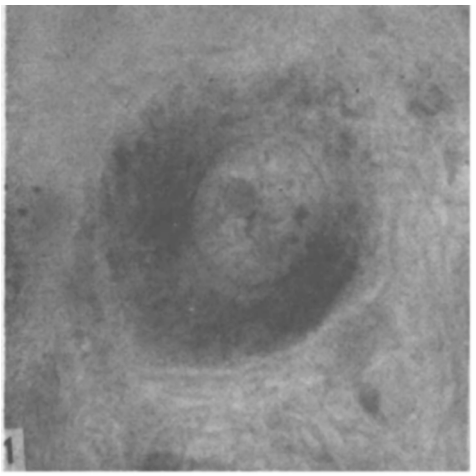
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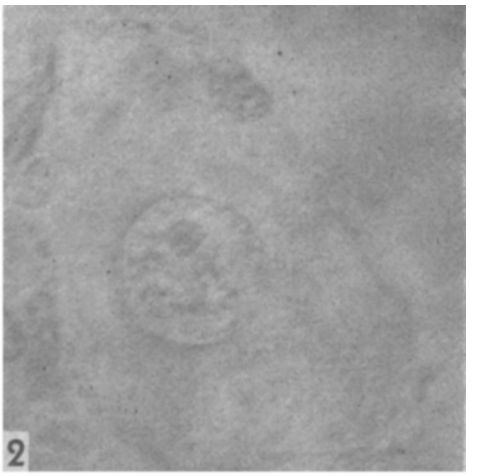
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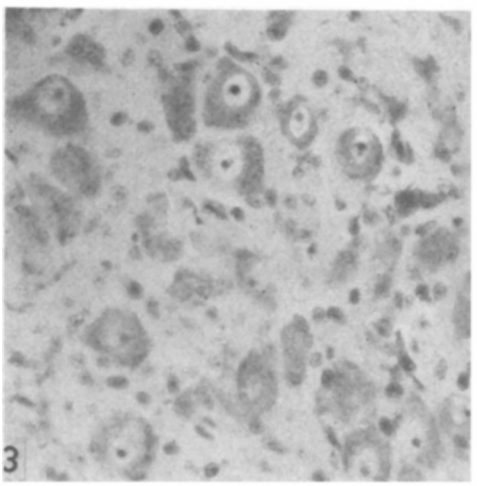
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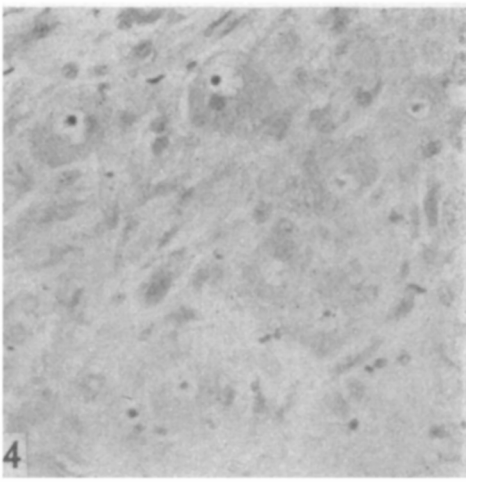
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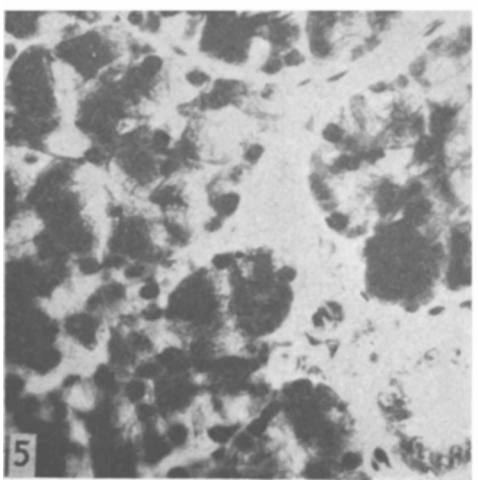
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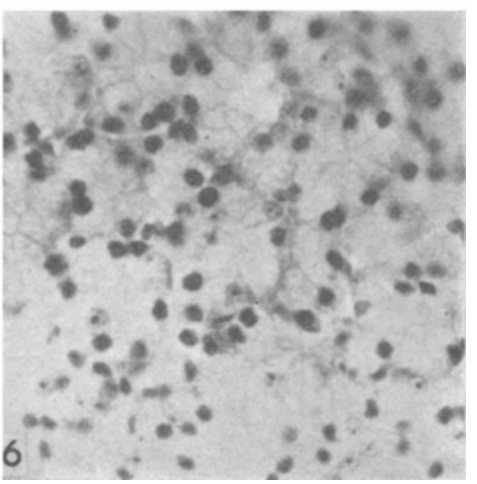
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FIG. 1. Frozen section of superior cervical ganglion stained with toluidine blue and eosin. Ocular 10×, obj. 2 mm.

FIG. 2. Frozen section of superior cervical ganglion treated with cold 10% perchloric acid and stained with toluidine blue and eosin. Ocular 10×, obj. 2 mm.

FIG. 3. Paraffin section of superior cervical ganglion stained with Mallory's phloxine-methylene blue stain. Ocular 10×, obj. 8 mm.

FIG. 4. Paraffin section from same slide as above of superior cervical ganglion which had been pre-treated with cold 10% perchloric acid and stained with Mallory's phloxine-methylene blue stain. Ocular 10×, obj. 8 mm.

FIG. 5. Paraffin section of parotid gland stained with Mallory's phloxine-methylene blue stain. Ocular 10×, obj. 8 mm.

FIG. 6. Paraffin section from same slide as above of parotid gland which had been pre-treated with cold 10% perchloric acid and stained with Mallory's phloxine-methylene blue stain. Ocular 10×, obj. 8 mm.

Pancreas. The cells of the pancreatic acini have a characteristic two-toned appearance when stained with basophilic and acidophilic stains. The basal zones of the cells, in which the nuclei are located, appear striated or homogenous and stain basophilically. The apical zones are acidophilic. Following treatment with cold perchloric acid the basal portions of the cells lose their basophilia, except for the chromatin material of the nucleus. Similar results are obtained by treating sections with ribonuclease.

Thyroid Gland. Dempsey and Singer(4) reported that the basophilia of the follicular thyroid epithelium with the eosin-methylene blue stain is an indicator of the presence of ribose-nucleoprotein. In their preparations, the basophilia of the follicular epithelium was lost following treatment of the sections with ribonuclease. Prolonged treatment with cold perchloric acid produces identical results. The cytoplasm of the follicular cells becomes slightly acidophilic although the chromatin material retains its basophilic staining properties.

Liver. The intensity and the frequency of the basophilic staining of the cytoplasm in hepatic cells is extremely variable, due to dietary, metabolic and other factors. In the normal healthy animal, some hepatic cells always show a basophilic staining reaction which disappears following treatment of the sections with ribonuclease. Similar loss of basophilia has also been induced in the liver of the rat and the cat following the described treatment with perchloric acid. Here, also, the nuclear basophilia remains unchanged.

The Parotid Gland. The cytoplasm of the albuminous secreting cells of the parotid gland stains lightly basophilic, apparently due to ribose-nucleoprotein. The basophilia is usually most intense in the basal portions of the cells (Fig. 5). Following extraction with cold perchloric acid, the basophilic staining reaction of these cells is completely lost (Fig. 6).

Conclusions. The data obtained in these experiments indicate that perchloric acid may be substituted for ribonuclease in the histochemical determination of ribonucleic acid. The results obtained by the use of perchloric acid were identical to those obtained by the use of ribonuclease as the digestive agent. The most consistent results were obtained with frozen sections, apparently because of better diffusibility.

4. Dempsey, E. W., and Singer, M., *Endocrin.*, 1946, v38, 270.

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