

tional picture of columnar liver structure. The bile capillaries run, in the majority of cases, between the cells and form hexagonal, intercellular, and pericellular networks within the liver plates.

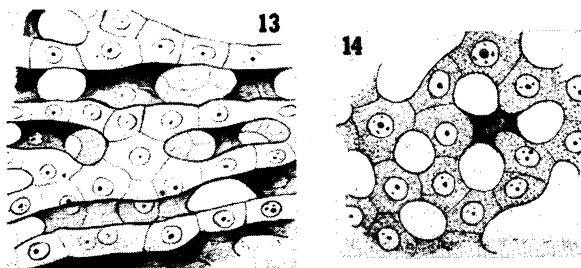


FIG. 13. Liver of horse, showing the tubular character of the lacunae, technique as in Figs. 7 and 8, perpendicular to central vein.

FIG. 14. Same as Fig. 13, but cut parallel to central vein.

From a historical point of view these observations are very interesting. The earliest account of the intimate structure of the liver was given by Gerlach in 1849 and upheld by Beale in 1856 (1). Beale described the liver as consisting of membranous tubes which contained rows of liver cells. Basically, this is the conception of modern textbooks. A cell cord surrounded by a network of lattice fibers is essentially the same as a row of cells contained in a noncellular tube.

In 1859, Budge (4) raised doubts. He objected to the inadequate methods Beale had used (maceration for days in water, not even in saline, at room temperature; teasing; no use of sections), but he did not find the correct solution.

In 1866, Hering (6) analyzed and described, in all correctness and exactly as outlined in this paper, the tubular type of liver (not using this expression) in the rabbit.

In 1867, Kölliker (8) described the human liver as a network of tender leaves and strings. Thus, he took a position intermediate between Beale and the view presented in this paper.

In 1869, Pflüger (9) ridiculed Hering and upheld the opinion of Gerlach and Beale. Both Beale and Pflüger were prominent men; hence their view was almost universally accepted. Pflüger ignored Kölliker's findings.

Once more, in 1872, Hering (7) asserted his viewpoint, bringing more evidence and considering very carefully the geometrical, three-dimensional aspect of the subject. However, his opinion was not accepted.

As late as 1889, Beale (2) restated and tried to prove his original concept by obsolete methods; washing the liver through the portal vein with water (not saline), then letting it soak for some time in soda water before beginning to inject his material and observe it with the teasing method. Inadequate as his methods were, Beale nevertheless prevailed, and the most modern textbooks presented the mammalian liver as made of cell columns ensheathed in an argyrophil network.

There are two exceptions to the majority of opinions in textbooks: Braus (3) found plates in the liver of man.

These are, however, according to him, two cells thick and occur simultaneously with cords which are also two cells thick. The other exception is Sharpey-Schafer (10), who described the liver as a parenchyma pervaded by cylindrical sinusoids. This is almost in accordance with Hering, from whom a figure is reproduced. But Sharpey-Schafer does not mention the fact that the walls separating the sinusoids are only one cell thick.

All other textbooks have accepted without question the statements of Beale and Pflüger and perpetuated for 99 years a fundamental misconception of this important organ.

It was the problem of stereographic screen projection of liver anatomy which stimulated a re-examination of the architecture of this organ.

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Perchloric Acid in the Cytochemistry of Pentose Nucleic Acid¹

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Cytochemical methods for demonstrating pentose nucleic acid (PNA) in tissue sections are based on the removal by ribonuclease of the ability of certain cell constituents to be stained by basic dyes, such as pyronin in Unna's pyronin-methyl green mixture (1), methylene blue (3), and toluidine blue (4). Parallel sections, treated and not treated with ribonuclease, are stained simultaneously and compared. The specificity of the demonstration, of course, depends upon the freedom of the enzyme preparation from proteolytic activity.

Analytical work in this laboratory (5, 6) on root tip homogenates has led to a method for the differential acid extraction of the constituents of PNA and desoxypentose nucleic acid (DNA) into separate fractions, which may

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then be assayed by spectrophotometric or other means. In the procedure used, the homogenate, after preliminary extraction with acidified alcohol, alcohol-ether, and cold 2% perchloric acid, is treated with 10% perchloric acid

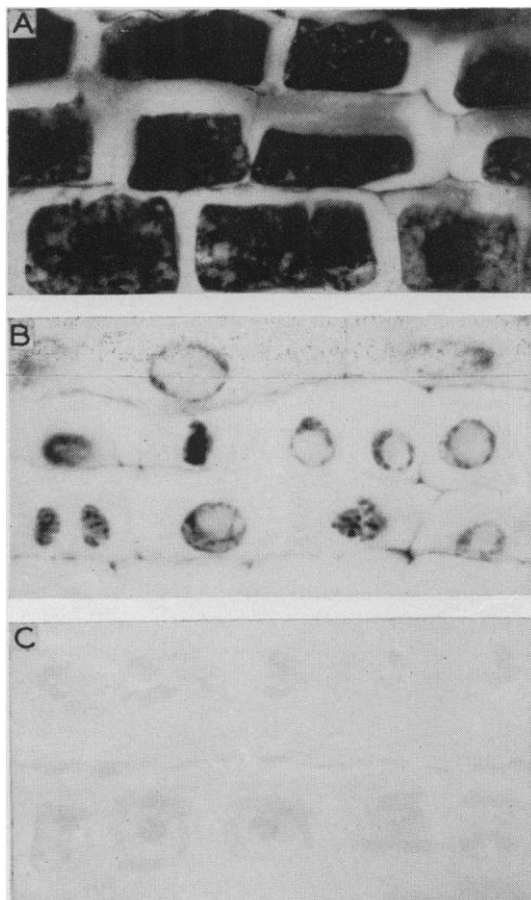


FIG. 1. Meristematic cells from nearly adjacent sections of a single primary root tip of *Zea mays*, fixed in ethyl alcohol, stained simultaneously in toluidine blue, and photographed identically, $\times 1,000$. A—Untreated cells. B—Cells treated with 10% perchloric acid for 18 hr at 4° C. C—Cells treated with 5% perchloric acid for 20 min at 70° C.

for 4–18 hr at 4° C. This treatment removes PNA but not DNA from the residue, and appears to have practically no proteolytic effect. Subsequent treatment with perchloric acid at an elevated temperature removes DNA from the residue.

Similar treatment of sectioned material might be expected to have similar effects. In particular, it should be possible to use cold perchloric acid, instead of ribonuclease, together with a basic dye to demonstrate PNA in sections. We have attempted such a demonstration in root tip sections. Although several problems, mainly of fixation, require more study, the results to date appear to be of sufficient interest to warrant publication.

In the experiments reported here, primary root tips of *Zea mays* seedlings have been fixed in 75% alcohol for 48 hr, dehydrated, embedded in paraffin, and sectioned longi-

tudinally at 8 μ . The paraffin ribbon obtained from each root tip was cut into segments of two sections. Segments from a single root tip were attached alternately to each of a set of slides, which were then passed through xylene and alcohols to water. This treatment undoubtedly removes water and fat-soluble constituents from the sections, as does the preliminary extraction of the homogenate in the analytical procedure. One or more slides from a root tip were then placed in 10% perchloric acid for 18 hr at 4° C, while control slides were kept in water at the same temperature. Other slides were placed in 5% perchloric acid for 20 min at 70° C, with control slides being given similar exposure to water. All the slides of a set were then washed in water, fastened into a wire clip (2), dehydrated together in 70% alcohol, and stained for 3 hr in a 1% solution of toluidine blue O in 75% alcohol. They were destained and dehydrated on the clip, by passing quickly through normal butyl alcohol to xylene, and mounted. Similarly treated slides, which have been passed through the solutions on the same clip, appear to be quite uniformly stained. Sections treated in this way were photographed with a red filter (Corning 2018) at the same exposure and printing times, and at a relatively low degree of contrast (Eastman Panatomic-X film and D-76 developer, No. 1 bromide paper).

Fig. 1, of meristematic cells of a single root tip, illustrates the results of this procedure. Ethyl alcohol is admittedly not a good fixative for plant material. It produces a great deal of shrinkage of the cell as a whole, and does not yield a good image of either cytoplasmic or nuclear structures. From a cytochemical point of view, however, the fixation is simple, and the effect of the perchloric acid treatment is clear. In Fig. 1A, of control cells treated with cold water, the cytoplasm, nucleoli, and chromosomes are strongly stained. In Fig. 1B, of cells treated with cold perchloric acid, neither cytoplasm nor nucleoli stain appreciably, while the chromosomes stain well. In agreement with the analytical findings, treatment of sections with 5% perchloric acid at 70° C for 20 min leaves the cells practically unstainable with toluidine blue (Fig. 1C), whereas the hot water control is indistinguishable from Fig. 1A. Similar sections have been stained by the Feulgen reaction. The chromosomes are Feulgen-positive before and after treatment with cold perchloric acid. After treatment with hot perchloric acid, no staining whatever occurs.

It appears from these results that treatment of sectioned material with cold and hot perchloric acid should be useful in the cytochemistry of the nucleic acids. The cold perchloric acid treatment, which appears to be quite comparable to that by ribonuclease, is of particular interest in the demonstration of PNA.

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