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Abstracts

protein with a unique structure the chemical behaviour of which is not yet predictable in detail there is ample room for trial and error experiments in this field. A lead to more rational fixation procedures can be found in the principle of substrate protection in which an enzyme is fixed in the presence of one of its substrates or of a reversible inhibitor. This principle proved to be successful in some instances(1) and probably can find further application.

The quantitative effect of the action of fixatives on enzyme activities can be studied in a system highly analogous to the actual cytochemical situation by the use of polyacrylamide films in which enzymes are immobilized by occlusion in cross-linked polymeric matrices (such as polyacrylamide). In combination with a simple film colorimeter(2) it is then possible to correlate biochemistry with cytochemistry, using films in which either purified (iso)enzymes, tissue homogenates, organelle suspensions (to study the influence of fixation on latency) or total cell suspensions are incorporated(3, 4).

Proportionality between enzymatic activity and amount of primary product in a cytochemical system can be expected to be influenced by diffusion coefficients of substrates and trapping agents, by the dimensions of the enzymatic sites, by the turnover number of the enzyme and by its local activity(5, 6). Empirical data on these factors are scarce. For the enzyme alkaline phosphatase in leucocytes several parameters of the cytochemical procedure important for adequate reflection of local enzyme activity as compared with biochemical activity determinations have been studied, using a film system(7). With the film system quantitative studies also have been carried out on horseradish peroxidase with DAB as substrate(8). Similar studies were carried out with the substrate 5,6-dihydroxyindole, which also has attractive properties for electronmicroscopic cytochemistry of peroxidases(9, 10).

Accurate localization of the enzymic sites depends on the efficiency of the trapping reaction. This in turn is determined by the competition between the tendency for diffusion of the primary product away from the site and the rate of the trapping reaction. On this aspect also few empirical data are available so far. Data obtained from model studies as factors determining the rate of diffusion of phosphate ions and their trapping by lead-ion containing media for acid phosphatase, will be discussed(11).

11. Cornelisse, C. J. and van Duijn, P., J. Histochem. Cytochem. 21 (1973) in press.

The membranes of the basal labyrinth in kidney cells of a teleost fish studied in thin sections and freeze-etch replicas after several preparatory procedures

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The structure of the basal labyrinth, a membrane system involved in transport of ions, was studied in kidney cells of the stickleback Gasterosteus aculeatus by thin sectioning and freeze-etching.

The structural organization of the basal labyrinth differs essentially from the type of
labyrinth found in mammals. In kidney cells of rats the labyrinth is constituted by inter-
digitating cytoplasmic processes(i). In sticklebacks, and in some other teleosts studied, it
consists of an intracellular system of branched membranes limiting narrow saccular
spaces. These spaces communicate with the extracellular space by means of rows of small
pores. The reconstruction of the labyrinth was based on tissue sections fixed with
potassium permanganate and on freeze-etch replicas.

After fixation involving osmium tetroxide serious structural changes were observed in
thin sections. In material fixed for 10 min in 0.5% osmium tetroxide, cell structure was
rather similar as after permanganate fixation. However, after prolonged fixation (1 hr)
loss of contact between the membranes of the labyrinth and the outer cell membranes
was common. The membranes were interrupted in many places, probably as a con-
sequence of diffusion of membranous material.

These changes could not be prevented by pre-fixation in 3% glutaraldehyde. The
trilaminar ‘unit membrane’ structure was absent after most fixation procedures involving
osmium tetroxide. The osmiophilia of the membranes was high after short fixation, but
diminished when fixation was prolonged. Unit membranes were invariably found after
fixation in Dalton’s mixture of osmium tetroxide and bichromate, in permanganate, or in
 glutaraldehyde poststained with uranyl acetate. The destructive effects of osmium
tetroxide on fish membranes indicate that the composition of these membranes is different
from those of e.g. mammals.

In freeze-etch replicas the particles were studied which cover both surfaces and both
fracture faces of each membrane. These particles have been supposed to represent
protein units involved in physiological membrane functions(2, 3). The numbers found
per μm² proved to be markedly constant and specific for each of the four faces of a
membrane. Marked differences were found between the particle densities of the outer
cell membranes and those of the basal labyrinth. These findings point to functional
differences between these types of membranes.

Particle densities were not influenced by immersion in glycerol, but these were
changed by prefixation in glutaraldehyde.


Ethanolic phosphotungstic acid staining and the quantitative stereology of
synapses

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Quantitative stereology of synapses in the visual cortex is of particular value for the
understanding of the central processing of the visual input in normal and experimental
conditions. The use of specific staining techniques greatly facilitates quantification with
points or test lines and is a necessary prerequisite for automated analysis with e.g. the
Quantimet.

Ethanolic phosphotungstic acid (E-PTA) stains specifically the synaptic contact zones
in the nervous system(1). Application at room temperature to about 1 mm blocks,
however, gives rise to great zonal variations in contrast between the synaptic contacts and
the rest of the neuropil. Incubation at 60° C of 50–100 μm vibratome sections results in
highly opaque synaptic junctions in an almost transparent background all over the tissue
slice.

Before using this method in experimental brain research it was necessary to verify that
the E-PTA stained junctions are quantitatively equivalent to the synaptic junctions in
OsO₄ fixed material. Using a system of test lines(2, 3), we have compared the number of
synapses per area (Nₐ), the surface of the contact zones per volume (Sᵥ) and the mean
length of the contact zones (L) in E-PTA and OsO₄ treated adjacent slices of the visual
cortex of the rabbit (Table).