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 polyacryl-
amide 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 cyto-
chemical 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-dihy-
droxyindole, 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(i1).

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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
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labyrinth found in mammals. In kidney cells of rats the labyrinth is constituted by inter-digitating cytoplasmic processes(1). 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 consequence 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 \( \mu m^2 \) 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 \( \mu m \) 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 OsO\(_4\) fixed material. Using a system of test lines(2, 3), we have compared the number of synapses per area \( (N_a) \), the surface of the contact zones per volume \( (S_v) \) and the mean length of the contact zones \( (L) \) in E-PTA and OsO\(_4\) treated adjacent slices of the visual cortex of the rabbit (Table).