different metabolites, such that one metabolite reacts with cell protein and causes cellular death (or lethal cell injury), while the other metabolite reacts with DNA and sets in motion a process of cell proliferation (acute sublethal cell injury). During the acute phase and also in the chronic phase of cellular injury, there is a sublethal phase during which the cell is unable to withstand any further metabolic loading.

Morphometry permits us to obtain exact measurements on the structure of the organisn, from which we may draw conclusions as to its functional status. The multiplicity of morphometric investigations has led to an explosive growth in data.

Using symbolic logic, we developed a quantitative organelle pathology in which nine pathologic stages (=diagnoses) suffice to describe the entire range of cellular injury. These nine pathologic states in turn correspond to five morphologic states (diagnoses) which give the mean change in organelle size. These nine pathologic and five morphologic states make it possible to express the average ultrastructural change in a cell during cellular injury which is both easy to visualize and unambiguous. Symbolic logic analysis by computer identifies incomplete and/or inconsistent statements both in the individual data sets and in the conditional logic. Our system of nine pathologic states and five morphologic states then forms an alphabet, from which we can construct reaction patterns of cellular organelles in the course of cellular injury. Using this quantitative organelle pathology and morphometric data from more than 150 experiments, we constructed a system of non-contradictory, mutually exclusive, reaction patterns for the cell and the organelles. We demonstrated that 85% of the experiments showed monotonous patterns which could be classified by symbolic logic as having a cytoplasmic adaptive reaction in the alarm phase, resistance phase, or exhaustion phase. The rule of homology has proved to be very useful in helping to elucidate difficult relationships in pathology. We attempted to show that this also holds true of quantitative organelle pathology. The types of homology occurring in the ergastoplasmic-mitochondrial-peroxisomal system reveal the evidence of existing interactions that will only be determined by means of morphometry and symbolic logic.

These homology types allow us to draw conclusions regarding the capacity of the cell to adapt itself and the extent of cellular injury.

IMAGE ANALYSIS AND PROCESSING AS A BASIS FOR THE CLASSIFICATION OF SKELETAL MUSCLE FIBRE TYPES

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Skeletal muscle fibres usually are classified on the basis of activities of enzymes that represent glycolytic and oxidative metabolism and speed of contraction. Enzyme activities can be estimated histochemically. Fibre staining with hematoxylineosin or delimitation of the fibres using connective tissue staining enables one to determine the position, form and size of fibres in cross-section.

At several levels 10 μm thick transverse serial sections of the muscle to be analyzed are cut. Using these sections, above mentioned staining procedures are successively performed and they are analyzed light microscopically. Enzyme activity within a fibre is measured as the mean absorption of monochromatic light of a certain wavelength.

The subject of this presentation is the possibility of overcoming the limitations of the classical methods of fibre typing and morphometry by using automated image analysis. The methods are well applicable to electron microscopic images.

First, images of the stained sections need to be digitized (image acquisition). This can be done with a scanning device or with a video camera in combination with a light microscope. The image acquisition stage may produce some deformations which one can try to compensate (image restoration). Finding the position, form and size of the fibres (image analysis) can be done automatically or interactively. For this purpose it might be helpful to emphasize structures in the image that one wishes to detect (image enhancement). When the contours of the fibres are found they can be "projected" on the digitized

enzyme stained images and the mean absorption of each fibre can be established by calculating the mean grey value within each fibre (also image analysis). Furthermore, texture analysis within the fibres may give additional information.

MORPHOMETRY OF FLAT CIRCULAR DISCS

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In addition to the numerical density, location along the neuronal surface and the excitatory vs. inhibitory character, the actual size of synaptic junctions is a relevant parameter determining the integrative power of a single neuron. Recently we observed in semithin E-PTA sections of the cerebellar cortex that synapses of different origin probably have a specific size. The functional importance of this observation led us to reinvestigate this in ultrathin OsO_4 sections.

In random ultrathin sections of the cerebellar cortex we measured the trace length and chord length of all synapses. Assuming that synapses are flat discs, we calculated the mean diameter and the frequency distribution using an unfolding procedure. From chord and trace length we also calculated the angle of curvature. The calculated parameters were compared with measurements of the maximal trace and chord length of the same synapses in consecutive serial sections. The observations revealed that the synapses in the molecular layer of the cerebellar cortex can be considered as flat discs. The observations also stressed the problems of unfolding procedures.

IMMUNOCYTOCHEMISTRY AND CYTOCHEMISTRY

Ia-ANTIGEN ON CELLS AS DETECTED BY ROZETTING AND IMMUNOCYTOCHEMISTRY IS DESTROYED BY SOME FIXATION REAGENTS

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Ia-antigen on peritoneal macrophages was assessed in vivo and during the course of in vitro cultures. Four assays were used to detect Ia: (a) binding of ^{125}I-anti Ia, (b) rozetting of SRBC conjugated with anti-Ia by $CrCl_3$, (c) ability of cultured Mø to present a copolymer to primed T-cells and (d) immunocytochemistry in a three-step procedure.

Three major findings are reported here. Firstly, the rozetting assay detected a higher proportion of Ia+Mø than other commonly used assays including immunocytochemistry. Secondly, the rozetting assay failed to reveal the loss of Ia in vitro, a finding supported by the fact that cultured Mø still were functionally capable of presentation of a copolymer in an Ia-restricted response. Finally, we could demonstrate that the apparent enhanced sensitivity over other assays as immunocytochemistry can be attributed--in part--to the lack of fixation step.

The effect of the fixation with paraformaldehyde and glutaraldehyde on the detectability of Ia-antigens was explored further for rat, mouse and human cells. The results indicated that the commonly used fixatives indeed affect dramatically the detectability of Ia on a variety of cells and results obtained with their use therefore must be interpreted with caution.

AN IMPROVED METHOD FOR PRODUCING GOLD PARTICLES OF PARTICULAR SIZES

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During the last decade colloidal gold has become very important as a marker in immunoelectronmicroscopy. Application of protein A-gold complexes combines electron density and specificity with the possibility of a double labeling using different sized particles.[1] A rarely mentioned limitation, however, is the difference in labeling efficiency between particles with var-