

molecules and epithelial cell markers have been used (Surh et al. 1994; Wack et al. 1996; Douek et al. 1996). In the course of an investigation on deletion of tumor-specific TCR-transgenic T-cells in the thymus (Lauritzsen et al.), TUNEL has been combined with commercially available monoclonal antibodies, that are monospecific for thymic epithelial cells, to unambiguously localize T-cell deletion. During the course of these studies, we established fixating conditions, that gave us superior results.

2.2 Metabolic assays

2.2.1 Biochemical and cellular basis of cell proliferation assays that use tetrazolium salts

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Summary: Tetrazolium salts (such as MTT, XTT, and WST-1) are used extensively in cell proliferation and cytotoxicity assays, enzyme assays, histochemical procedures, and bacteriological screening. In each, these tetrazolium salts are metabolically reduced to highly colored end products called formazans. Yet, the nature of their cellular bioreduction is poorly understood despite their long-time use (Stoward and Pearse, 1991).

In our laboratory, we demonstrated that most cellular reduction of MTT was dependent on the reduced pyridine nucleotides NADH and NADPH, not on succinate as had been previously believed (Berridge et al., 1993, 1994; Berridge and Tan, 1993). Cellular reduction of MTT was associated with enzymes of the endoplasmic reticulum and was more related to NADH production through glycolysis than to respiration.

Recently, assays have been introduced based on tetrazolium salts (such as XTT and WST-1) that are reduced to soluble formazans. These assays depend on intermediate electron acceptors such as phenazine methosulfate (PMS).

The question arises: Is the cellular reduction of these new salts similar to that of MTT? In this article, the answer to that question is attempted.

In summary, it could be shown that, unlike MTT, XTT and WST-1 are efficiently reduced by NADH and NADPH in the absence of cells or enzymes, and their reduction involves superoxide. Cellular reduction of WST-1 occurs at the cell surface and also involves superoxide.

2.3 Annexin-V assays

2.3.1 The use of annexin for concomitant detection of apoptosis and cellular phenotype

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Summary: Two distinct modes of cell death, apoptosis and necrosis, can be distinguished on the basis of differences in morphological and biochemical characteristics. Under the electron microscope, cells undergoing apoptosis display cell shrinkage, apoptotic body formation, and chromatin condensation. Biochemically, the apoptotic process is characterized by fragmentation of DNA into oligonucleosomal fragments. Furthermore, during

