Histochemical Studies on Nonspecific Reduction of Tetrazolium Salts in Tissue Sections

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The nature of the so-called endogenous dehydrogenase activity was studied with several techniques from the standpoint of the specificity of dehydrogenases. By use of the highly sensitive tetrazolium salts, as Nitro-BT or Tetranitro-BT the endogenous dehydrogenase activity is demonstrable even in fresh frozen sections.

In fresh frozen sections a slight positive reaction was observed even at pH 7.0. However, the reaction was negative in paraffin sections, probably because of washing away of the endogenous substrates during dehydration, clearing and embedding procedures.

A very weak reaction was obtained in the fresh frozen liver sections of 10μ in thickness, which had been incubated in the solution of Nitro-BT at pH 7.0, although the deposition of blue diformazan yielded in 30μ thickness. This is mostly due to the large amount of endogenous substrates in tissue sections.

The endogenous activity in the same thickness of various tissues and organs was quite different, the strong reaction was observed in the kidney and liver of rats and rabbits.

In general the addition of pyridine nucleotides or menadione to the reaction mixture resulted in a considerably greater enhancement of the endogenous dehydrogenase activity. However, phenazine methosulfate had little influence on the deposition of formazan.

The activity of endogenous dehydrogenase was completely abolished when sections were preincubated in the solution containing 0.1 M N-ethyl maleimide or p-chloromercuribenzenate. Pretreatment with these sulfhydryl reagents at 0.1 M prior to incubation in specific substrate solutions resulted in total inhibition of oxidative enzymes. It was shown that active sulfhydryl groups were essential for the reduction of tetrazolium salts in tissue sections. However, DPNH- or TPNH- diaphorase activity remained completely positive.

By the use of paraffin sections, the staining reactions for various dehydrogenases seemed to be specific by the absence of formazan formation when substrate or coenzyme was omitted from the incubation media.

Detailed results will be published elsewhere (Acta Tub. Jap. 13, 54, 1964).