

or sublimate, granules are stained with the dyes of large molecular weight, e. g. alcian blue and Janus green, but not with the dyes of small molecular weight. Fixation with osmic acid-containing fluid reversed these results. The granules become stainable with the dyes of small molecular weight, e. g. acridine red or thionin, while their affinity to alcian blue and Janus green is markedly lowered. Such difference of stainability is quite remarkable when the specimens are stained in the mixture of two dyes of different molecular weights. But, when the different molecular weights of the dyes in a mixture is very close, the preference and competition occurs quite at random. These results suggest that the apparent basophilia of the granules based upon rather physical, but not chemical, mechanisms. Selective stainability to certain dyes is seemingly due to the density of substances constructing the granules. Osmic acid seems to result in a higher structural density of the granules than alcohol or sublimate does.

**The Improved Techniques for the Histochemical Detections of
Several Mitochondrial Dehydrogenase Activities with
Acetone-fixed-paraffin-embedded Tissue Sections**

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The techniques to be discussed use 5—6 μ thick tissue sections prepared from tissue blocks fixed in ice cold absolute acetone and embedded in soft paraffin (melting point 52°C). With these techniques lipids which dissolve the reaction products to give artificial staining were removed. Artifacts due to phenazine methosulfate were eliminated by using Co Q₁₀ in place of phenazine methosulfate. Artifacts due to mitochondrial dislocation during fixation were eliminated by fasting animals overnight before sacrifice.

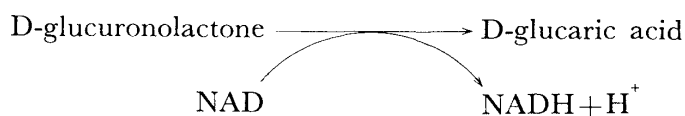
The histochemical findings will be presented and discussed in terms of the staining procedure.

**A New Method for the Histochemical Demonstration of
D-glucuronolactone Dehydrogenase.**

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The glucuronolactone dehydrogenase (α -D-glucuronolactone-NAD-oxidoreductase) catalyzes the following reaction,



and the glucaro (1→4) lactone one of the intermediates of the reaction is known as a strong inhibitor of β -glucuronidase. Hence, it is to be noteworthy that the glucarolactone dehydrogenase play a role of negative feedback control against the β -glucuronidase systems such as Tousters uronate cycle.

Now, we reported here a new histochemical method in order to detect the enzyme activity adopting nitro BT method with NAD.

By our method the enzyme activity was demonstrated positively on the human skin as following areas : the Malpighian layer, outer hair sheaths, eccrine sweat glands, vascular walls and cytoplasm of the fibroblasts. The similar findings were obtained on the rat skin but the reaction was somewhat weak compairing to human skin.

Histochemical Localization of Creatine Kinase in the Tissues

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The localization of the enzyme activity was made visible through reduction of nitro-blue tetrazolium in the presence of NADPH₂, which was generated essentially as in Oliver's biochemical method of creatine kinase measurement where creatine phosphate is used as the specific substrate. The specificity of the reaction and other factors affecting this histochemical reaction were studied in detail and finally optimal incubating conditions for their visualization on tissue slices were discussed in this report. By using this method, the adult human skeletal muscle revealed extremely high activity of creatine kinase in sarcoplasmic reticulum of the fibers. The heart muscle and the cortical layers of the brain also showed a weak reaction. Some organs like the liver or the kidney showed little or no enzyme activity. The histochemical localization of the enzyme activity in various organs was almost similar to the biochemical data already reported in the literatures.

On the Specificity of Histochemical Demonstration for Glucosephosphate Isomerase

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On the basis of biochemical knowledge (Noltmann et al. 1959, Baich et al. 1960 and Topper 1957), a method for histochemical demonstration of D-glucose-6-phosphate ketol-isomerase (glucosephosphate isomerase) was investigated, according to Kornberg's principle of biochemical assay(1950). Subsequently, a new method was reported by Yano (J. Kumamoto Med. Soc. 43(8) 1969). The same method in which the histochemical system of the enzyme reaction is similar was separately reported by Meijer and Bloem (1969). The authors had investigated