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New Histochemical Demonstration of Lipase for Saturated and Unsaturated Fatty Acid Ester

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Even after staining by some dyes, certain substrates of some enzymes still reserve the substrate property of the enzyme, the matter was proved in the experiment of proteinase and amylase. On the other hand, some elements like barium and calcium which can precipitate certain substances in the solution show same property even after staining by dyes. In these cases, stained precipitates will be yielded instantly. This phenomenon was applied for demonstration of sulfatase and phosphatase activities. Systematic studies of application of dye in enzyme histochemistry are being tried by the present authors.

If an unsaturated fatty acid of long carbon chain, for example oleic acid, splits out from the ester compound by catalytic action of enzyme, the unsaturated fatty acid can be precipitated from slightly alkaline solution by co-existing cadmium or calcium ion and in case of co-existence of Nile blue sulfate, a blue stained precipitate will appear in one step. Nile blue is characteristic and is useful for this experiment. Experiments with other dyes were unsuccessful. As substrate of the enzyme, synthetic compounds such as Tween 80, 85 and natural phospholipids such as lecithine are applicable.

On the other hand it was proved that the staining reaction was not occurred with synthetic ester of saturated fatty acids such as Tween 40 and 60. This result means probably the best control experiment. With this method chief cell of cardiac gland stained intensively and pancreatic cell showed less intensively and some other cells were more slightly.

For the demonstration of saturated fatty acid lipase, the authors sought suitable dyes and found Sudan Black BN to be adequate. All other dyes tested were failed in these experiments. The authors dissolved Sudan Black BN in Tween 60 water solution which corrected at pH7.4 by this buffer solution and

also dissolved cadmium or calcium ion. Adequately prepared paraffin sections were incubated in this mixture solutions at 37°C for several hours. When palmitic acid appears, as the reaction product in this experiment a stained precipitate can be seen on the site of enzyme activity of tissue sections in one step. As the result of the experiment, the site of the enzyme activities well stain blue black.

For these experiments, formol fixation and freeze sectioning method are not suitable. Lipids and lipid like substances of tissue sections should be removed by organic solvents. Because these substances may stain by the dye in the solution. The stained site of tissue elements is distributed and presumably coincides with results of our modified of Gomori's. New dye methods for demonstration of unsaturated fatty acid lipase and of saturated fatty acid lipase are not always superior than our other methods. Nevertheless, we believe that the finding in this study is very valuable, we must study the characteristics of individual dyes earnestly. Then we will find the usefulness of dye in enzyme histochemistry.

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Studies of Oxidative Enzymes for Phenolic Substances

—A Histochemical Approach for Catecholase—

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Regarding to oxidative enzymes for phenolic substances, the authors wished to present some important problems which have not been cared by other researcher. Takamatsu and Kitani concluded in their earlier studies that the factor of leucocytes responsible for Nadi reaction is thermostable and not an enzyme and the chemical mechanism of Nadi staining is not oxidation but polymerization of partly oxidized reagents. Many histochemists did not know the real meaning of so-called naphthol reaction. So-called DOPA staining can be seen in granules of myeloid leucocytes. Role of hydrogen peroxide for those oxidative reaction are obscure. We can put out some more important problems of oxidative enzymes for phenolic substances. Present studies were carried out to resolve those problems from stand-point of histochemistry.