Legends for figures

- Fig. 1 Dog pancreas. Activity without sodium taurocholate. Only a few widely dispersed blue granules are seen. This indicates how little hydrolytic activity there is in pancreas for naphthol-AS nonanoate, when unactivated. 300×
- Fig. 2 Dog pancreas. Lipase activity with sodium taurocholate. Compare with unactivated section in Fig. 1. Marked activation is demonstrated in the acinar cells. The reaction in the Golgi region is especially intense as well as the secretion product in a ductule. 300×
- Fig. 3 Same as fig. 2. 650×

Study on the Final Product and Inhibitors in Oxidative Enzyme Reactions (II Report)

Tadao MITSUI and Toshio SHINODA

Department of Anatomy, Keio University, Tokyo

M-Nadi reaction is widely used in the study of the morphology of leukocytes. The final product of this reaction is regarded as indophenol blue which distinctly appears within the cytoplasm of the myeloid leukocytes. In cytochemical reaction, indophenol blue is insoluble in acetone and Cargille's immersion oil. However, it is soluble in water and many chemical substances such as ethanol, methanol, glycerin, xylol, balsam, bioleite (resin for mounting), cedar oil, and apathy gum syrup. It was found by the present study that the indophenol blue produced by M-Nadi reaction became insoluble in many reagents mentioned above when the stained blood smear was heated for 5 to 30 minutes in an oven at 150°C as demonstrated in the annexed Table.

The indophenol blue is probably oxidized by this heating procedure, and changes into brown substance which is stable to various solvents. This experimental result should always be considered when determining whether human myeloblast is Nadi positive or negative, because Nadi-stained blood smear should not be observed using immersion oil or balsam before indophenol blue is stabilized to these organic solvents.

The final product of peroxidase reaction in blood cells showed different solubility from that of Nadi reaction. The final products of Patent blue V-H₂O₂ reaction, benzidine-H₂O₂ reaction, orthophenylenediamine-H₂O₂ reaction, and of paraphenylenediamine-H₂O₂ reaction, were more stable than indophenol blue in Nadi reaction. However, the final product in paraphenylenediamine-H₂O₂ reaction was soluble in methacrylate resin and epon, while not soluble

in polyester and QX 533 (epoxy resin after Kushida). This character should be emphasized when tissue is embedded in various resins in electron microscopy.

Aqueous solution of phenylhydrazine hydrochloride intensely inhibited benzidine- H_2O_2 , orthophenylenediamine- H_2O_2 , and paraphenylenediamine- H_2O_2 reactions at the concentration of pH 3.0 or less. The solution of pH 3.0 corresponded to 10^{-3} molar solution of phenylhydrazine hydrochloride.

The inhibitory action of monoaldehyde and dialdehyde to benzidine peroxidase reaction was compared. Molar as well as one half molar solutions of formaldehyde, glutaraldehyde and hydroxyadipic aldehyde were prepared respectively.

Solubility of the final product of Nadi reaction

	simple Nadi	ordinary Nadi	after heating the final product at 150°C 5-30 minutes	α -naphthol and thionin reaction
water	s	ps	i	i
ethanol	s	ps	i	i
methanol	s	s	i	i
xylol	s	s	i	i
balsam	s	s	i	i
bioleit	s	s	i	i
ceder oil	s	s	i	i
glycerin	s	ps	i	i
Apathy gum syrup	ps	ps	i	i
acetone	i	i	i	i
Cargille's immersion oil	i	i	i	i

Notes 1) s (soluble), ps (partly soluble), i (insoluble).

2) simple Nadi : Only α -naphthol and dimethyl-paraphenylenediamine were used.

3) ordinary Nadi : original method of Winkler-Schultze, namely simple Nadi plus Lugol solution and lithium carbonate.

4) α -naphthol and thionin reaction : α naphthol and 0.01% aqueous solution of thionin were used. Dimethyl-paraphenylenediamine was replaced by thionin in the simple Nadi reaction.

Each solution was adjusted to pH 7.2 to 7.4 with phosphate buffer, and human blood smears were exposed to these aldehyde solutions prior to benzidine peroxidase reaction. Glutaraldehyde was proved to have the strongest inhibitory action although tissue was well fixed with this. Hydroxyadipic aldehyde showed the next strongest inhibitory action, in which case tissue was not fixed very well. Formaldehyde (monoaldehyde) showed no inhibitory action under the same condition as glutaraldehyde.