Methods of histochemical demonstration of the presence of proteolytic enzymes locating in the tissue, such as pepsin, cathepsin and tripsin, had not been known until the authors’ methods\textsuperscript{1,2,3} were devised. Takamatsu and Wada reached a conclusion that specially treated proteins must be used instead of natural proteins as a substrate for the histochemical study. These investi-
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Gators published the "stained protein method" as one of these methods in 1954, which has been prevalently used in this country since then. The outline of this method is as follows: One ml. of 0.1% methylene blue solution is added to 200 ml. of 0.5% gelatin solution in water, and the pH of this mixture is adjusted to 6.5 or 8.6 with 0.1N HCl or 0.1N NaOH. Crystal violet may be used in place of methylene blue when pH is set at 2.7 in this case. A piece of tissue is fixed with acetone-alcohol, embedded in paraffin, and then microtomized. Following removal of paraffin and soaking in water in the ordinary way, the specimen is incubated in the stained gelatin solution at the temperature of 37°C for several hours. After washed with water, it is immersed in 1-2% phosphomolybdenic acid solution for 5-10 minutes. Then the nuclei are stained with nuclear-fast red or safranine. The positive sites in the tissue which contains pepsin, cathepsin or trypsin become stained with the dye. This reaction has been confirmed, as reported before, to be parallel with enzymic reaction in vitro. The nuclei are not stained until the specimen is fixed by heating. Findings in normal and pathological tissues, for example findings in leucocytes which digest coagulated protein, suggest that this reaction is due to proteolytic enzymes. However, the granules in mast cell are stained non-enzymically. It has been found with electrophotospectrometry that methylene blue is first combined with the gelatin in the solution and a part of the dye reveals the leuco-type in the bond but recovers the colour if the stained protein digested by the action of the enzymes.

Takamatsu and Wada are investigating also the "silver protein method". This method utilizes the phenomenon that an aqueous solution of the so-called mild silver protein, which usually does not contain ionized silver, liberates silver ions when digested with pepsin or trypsin. Turbidity and then precipitation occur in the solution containing with chlorine ions or cromic acid on the acid side and with phosphate on the alkaline side. This phenomenon may be used for nephelometry or quantitative analysis of enzymatic reaction.

The authors have been experimenting on the use of the liberation of ionized silver in the enzymatic reaction for histochemical demonstration of the enzymes, but no practical method has been found so far.

References