correlated with none of these phenomena. Silver grain counts were made on the "B" type inclusion bodies of cowpox virus. All "B" type inclusions, no matter how small, were labeled after exposure to H^3 -thymidine. The most intensive DNA synthesis occurred in "B" type inclusions with a diameter of more than 5μ . The intensity of DNA synthesis of the "B" type inclusions was as high as that of nuclei of noninclusion bearing cells. However, the degree of cytoplasmic DNA synthesis decreased in diffuse "B" type inclusions which were mostly accompanied by "A" type inclusions. Immediately cytoplasmic DNA synthesis begins, nuclear DNA synthesis is suppressed.

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Explanations of Plates

Plates 1-8. FL cells infected with cowpox virus, were exposed to 2ml of $5\mu\text{C}/\text{ml}$ of H^3 -thymidine for 2 hours. The preparations were stained with Giemsa solution after dipping autoradiography. The "B" type inclusions stained reddish purple and the "A" type inclusions showed pale blue colour.

Plate 1. Several "B" type inclusions with a diameter less than 2μ .

Plates 2, 3 & 4. Several "B" type inclusions with a diameter between 2 and 5μ .

Plates 5 & 6. "B" type inclusions with a diameter more than 5μ .

Plates 7 & 8. Diffuse "B" type inclusions accompanied by "A" type inclusions.

A Histochemical Study on The Specificity of Pyridoxal-phosphatase.

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Phosphatases hydrolyse the phosphate bond of pyridoxal phosphate. It was histochemically investigated in this paper whether a specific phosphatase for releasing this substrate is present or not in animal tissues. Glycerophosphate was compared with pyridoxal phosphate as the substrate for the specificity of enzyme.

The histochemical techniques for demonstration of phosphatases were

Fig. 1-2 Alkaline pyridoxal-phosphatase is demonstrable in the rat adrenal cortex (Fig. 1), while alkaline glycerophosphatase is detected only in capillary walls of the adrenal gland (Fig. 2).

Fig. 3-4 Acid pyridoxal-phosphatase is detected in glomeruli of the rat kidney (Fig. 3), while acid glycerophosphatase is demonstrable in the convoluted tubules of the same serial section (Fig. 4).

utilized according to Takamatsu-Gomori's metal-salt procedure. The rat tissues were used as the enzyme material.

The distribution pattern between pyridoxal-phosphatase and glycerophosphatase was almost similar in various tissues with the exception of kidney, adrenal gland and blood vessels of the rat. The pyridoxal-phosphatase at the acid range was demonstrated in the glomerulus of rat kidney, inspite of negative staining by acid glycerophosphatase. On the contrary, the former was not demonstrable in convoluted tubules of the kidney and the latter was intensely demonstrable in them. Cortical cells of the rat adrenal gland contained alkaline pyridoxal-phosphatase, but they did not reveal the glycerophosphatase reaction. On the other hand, the enzymatic reaction for glycerophosphate at the alkaline range was strongly demonstrable in capillary walls of the heart, liver, spleen, and adrenal cortex in the rat, but the alkaline enzymic reaction for pyridoxal-phosphate was not detected in them. The specific reaction for pyridoxal phosphate in glomeruli of the kidney appeared to occur most intensely at pH 6.0 in the medium. The specific reaction of alkaline pyridoxal-phosphatase in adrenal cortex occurred intensely at pH 9.0. Specific inhibitors and activators were not found in this study.

It was considered from this experiment that the phosphatase for pyridoxal phosphate may be present in certain tissue cells from a viewpoint of the

different distribution pattern. The true specificity for pyridoxal-phosphatase should be biochemically investigated in the future. (The detailed report will be published in the Kumamoto Igakkai Zasshi, Vol. 37, 1963).

Discussion

- Dr. Okawa: Do you mean the existence of a specific enzyme "pyridoxal phosphatase"? I think further studies are necessary to assert its specificity.
- Dr. Shiraishi: I agree with you. The specificity of "pyridoxal phosphatase" is not concluded from the present studies and it should be investigated by the further studies such as the action of inhibitors and activators including NaF.

On the Acid Phosphatase Activity of Kupffer Cells

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This investigation was performed to observe the functions of reticuloendothelial cells (RE cells) by means of enzymohistochemical method. It has been well reported by many workers that RE cells were rich in acid phosphatase (ac-ph.) activity^{1,2,3,4)}. The enzyme, moreover, has been considered to have some relation to pinocytosis and phagocytosis of RE cells^{5,6,7,8)}. From these points of view, we made an observation how the ac-ph. activity of the Kupffer cells would be changed when they were activated or inhibited on phagocytic activity. To determine the phagocytic activity of the Kupffer cells the carbon black clearance test was applied.

At the same time the ac-ph. activities of subcutaneous histiocytes were observed.

Procedures: All the experiments were carried out with male rabbits weighing 2.0 to 2.5kg. For the activation of phagocytic activity of RE cells, 5cc/kg of typhoid vaccine (T.V.) was once injected into the peritoneal cavity, and for the inhibition, 7.5 mg/kg of prednisolone (Pr.) into the femoral muscle. Liver pieces and subcutaneous connective tissue were biopsied 4 days after these procedures. The former was immediately immersed into fixative for 18 hours and the latter was soon stretched on object glasses and dried without further fixation⁹⁾. The fixative for the liver pieces was the cold 10% formalin, adjusted to pH 7.2 by 0.33M veronal buffer and added 5% sucrose after Holt¹⁰⁾. From these pieces ordinary frozen sections (10 μ) were made.

The substrate medium was prepared after Gomori¹¹⁾; 50ml. of 3% Na-glycerophosphate, 0.6 gm lead nitrate and 500ml. of 0.05 M acetate buffer with