Histochemical Studies on Lipid Metabolism in Liver of Rats with Experimental Diabetes

(1) Changes of Compound Lipids in the Liver of Diabetic Rats.

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The purpose of this study is to elucidate the chemical component and distribution of lipid droplets or granules in the liver of diabetic rats. Three kinds of diabetes mellitus were induced in rats as follows:

The first group of rats was treated 10mg of hydrocortisone acetate daily for one week; the second was injected with the same dose of hydrocortisone daily for two weeks; the third was injected with a single of 30mg alloxan two weeks before the experiment. Diabetic rats, whose blood sugar levels were ascertained to be over 300mg/dl, were sacrificed after fasting for seventeen hours. Their livers were resected for lipid staining. The following techniques were used: Sudan Black B (SBB) for total lipid, Sudan III (S-III) for neutral lipids, chromate reagent method of Baker for compound lipids and phosphatide staining method of Okamoto for ester phosphatides. The results were as follows:

(1) The cortisone one week group revealed the SBB stainable fine to medium sized granules distributing mainly in the peripheral part of liver lobules, both extra- and intrahepatocellularly. The S-III stainable granules were found in a similar degree and showed a similar distribution to those of SBB granules, but fine and smaller granules were not detected. And with Baker's method a few extracellularly scattered small granules were found in the peripheral parts of liver lobules. The slight increase of intracytoplasmic granules of smaller size and the decreasing tendency of fine granules were notable findings. The Okamoto's method presented the same extracellular and intracytoplasmic lipid granules as those that with Baker's method, but the intracytoplasmic smaller granules were not found. (2) In the cortisone two weeks group, a remarkable increase of the number and enlarged size of lipid granules with the spreading tendency were found by SBB and S-III stainings. By Baker's and Okamoto's staining extracellularly scattered small granules increased in their numbers and distribution, and intracytoplasmic fine granules were enlarged but decreased in number in comparison with the first group. Intracytoplasmic smaller granules also increased by Baker's method. (3) In the alloxan group, the remarkable increase of the SBB and S-III stainable lipid granules were observed, and the granules developed in the almost entire lobule, particularly densely in the peripheral portion. By Baker's and Okamoto's stainings almost similar extent of the distribution of extracytoplasmic smaller granules was found as in
the cortisone one week group. Intracytoplasmic fine granules were enlarged, but their number decreased remarkably in comparison with the second group. In proportion to the frequency of cortisone administration, the number of extrahepatocellular phosphatide granules increased. And in the liver of alloxan rats, such granules decreased but their distribution in the lobule extended. It is considered that these granules indicated phosphatides partially in Kupffer cells and their number increased in proportion to the accelerated transportation of fat into the liver. It was assumed that the intracytoplasmic fine phosphatide granules enlarged in size and decreased in number in the all diabetic groups, and these granules originated from phosphatides, which are in close contract with swelled mitochondria in liver cells of diabetic animals. The cause of the increase of extracellular phosphatides and the decrease of intrahepatocellular phosphatides observed in the diabetic liver demands further study.

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(2) Effects of Oral Administration of Fats.

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Experimental diabetes in male Wistar rats were induced by the administration of hydrocortisone or alloxan as the previous report. Soybean oil or cod liver oil were administered to diabetic animals in the emulsified form (3m./100gm. body weight) through the gastric tube after seventeen hours fasting. In three, six and eight hours after the administration of the oil the animals were sacrificed and their livers were resected and the sections were determined by Sudan Black B (SBB) fat staining. Fresh unfixed sections from another piece of the liver were examined histoenzymologically about the activities of \( \beta \)-hydroxy butyric acid dehydrogenase (\( \beta \)BDH), succinic dehydrogenase (SDH) and adenosine triphosphatase (ATPase). By the SBB staining it was revealed that lipid granules in the liver of normal and diabetic rats increased once in three to six hours after the administration of soybean oil, but after eight hours more or less decreased. By the SBB staining the retardation of emerging and disappearance of liver lipids was found in the diabetic animals especially in the alloxan treated group. In eight hours after the administration of soybean oil, remarkable elevation of activities of \( \beta \)BDH was exhibited especially in the midzone of lobule, corresponding to the localization of the eminent decrease of