ELECTRON MICROSCOPIC OBSERVATION OF THE INTRANUCLEAR GLYCOCEN OF THE LIVER IN DIABETES MELLITUS

KYUICHI TANIKAWA

The Second Department of Medicine, Kurume University School of Medicine, Kurume, Japan.

(Received for publication December 20, 1964)

The changes of the liver in diabetes mellitus have been paid much attention because of the role of this organ in carbohydrate metabolism.

The histological changes of the liver in this disease have been reported by Chipps (1), Bogoch (2), Zimmerman (3) and others, and it has been noted that intranuclear glycogen accumulation and fat metamorphosis are the characteristic findings under light microscope.

The increasing use of liver biopsy technique has made it possible to study such changes more easily in a number of diabetics under electron microscope.

This report is devoted to the electron microscopic observation of the intranuclear glycogen of the liver cell in diabetes mellitus and a discussion concerning the mechanism of glycogen accumulation in the nucleus.

MATERIALS AND METHODS

Liver biopsy specimens were taken from ten patients, diagnosed as uncontrolled diabetes mellitus of insular origin.

For light microscopy, specimens were fixed in formalin or alcohol-formalin solutions, embedded in paraffin, and stained with haematoxylin and eosin, and for glycogen by the periodic acid-Schiff (PAS) reaction with and without previous diastase digestion.

For electron microscopy, the specimens were fixed in a cold 1 per cent osmium tetroxide solution, buffered to pH 7.4 with sodium Veronal acetate. The specimens were then dehydrated in graded alcohols and embedded in butyl methacrylate.

Ultra-thin sections were cut with a glass knife on Porter-Blum microtome, and stained with lead hydroxide or uranyl acetate, and were examined in a JEM-4C electron microscope.

RESULTS

Light Microscopic Observation (Fig. 1 and 2)

In the sections stained with haematoxylin and eosin, the liver cells showed to be not markedly changed. However, many nuclei of the liver cells appear to be vacuolated. These vacuolated nuclei were stained by PAS reaction, but not after digestion with diastase.
It was also noted that the cytoplasms of the liver cells with such PAS positive nuclei were more markedly stained by this reaction than the one with normal nuclei.

These findings suggest that vacuolated nuclei in haematoxylin and eosin stain were actually due to glycogen accumulation, and the cytoplasms with glycogen-filled nuclei had greater contents of glycogen compared with normal nuclei.

Electron Microscopic Observation (Fig. 3 and 4)

The nucleus occasionally showed to be vacuolated and clearly demarcated by a band of nucleoplasm in the section, unstained or stained with uranyl acetate. In the sections stained with lead hydroxide, numerous small dense particles were seen to be centrally accumulated in the nucleus. They conform to the descriptions of glycogen particles, by Drochman (4) and Karrer (5), who identified the glycogen with lead staining in electron microscopy.

These stained intranuclear glycogen particles measured about 300 Å in diameter and seemed to be attached one another like a chain; they appeared to be an aggregate of smaller units of the size of 50 Å. Occasionally several units of glycogen particles were seen in close proximity to the pore of the nuclear membrane suggesting that they had passed through it.

The cytoplasm of the liver cell also contained numerous dense particles of glycogen.
INTRANUCLEAR GLYCOGEN IN DIABETES MELLITUS

Fig. 3

Electron micrograph of a section, stained with lead hydroxide. Numerous small particles of glycogen (i. G) are seen in the nucleus. They appear smaller than cytoplasmic glycogen particles (C. G). 18000.

They measured about 600 Å in diameter and some appeared to be less dense or less well defined; they also showed to be an aggregate of smaller units of the size as the ones making up the intranuclear glycogen particles.

These cytoplasmic glycogen particles were more numerously seen in the liver cell with intranuclear glycogen than with normal nuclei.

DISCUSSION

Glycogen is not normally seen in the nucleus of the liver cell. It has long been noted that glycogen-filled liver cell nuclei are occasionally seen in diabetes mellitus since Erlich (6) first described this finding in 1883. Recently the same finding has also been described in several other pathological conditions such as Wilson's disease (7) and glycogen storage disease (8). However, the mechanism for this accumulation is still unknown. Warren and Le Compte (9) suggested that the nuclear accumulation of glycogen may be evidence of altered permeability of the nuclear membrane to glycogen. Zimmerman (3) reported that such nuclear accumulation was closely associated with hyperglycemia, while other data (2) revealed no significant relationship between the degree of hyperglycemia and nuclear glycogen accumulation.

Our observations showed that cytoplasmic glycogen are more plentiful in the liver cells with glycogen-filled nuclei.
Electron micrograph of a section, stained with lead hydroxid. Glycogen particles.
both in the nucleus and cytoplasm, show to be an aggregate of smaller units. Several
smaller units of glycogen particles (at arrow) are observed near the pore of the nuclear
membrane. 40000.

Under electron microscope, glycogen particles, both in the nucleus and the cyto-
plasm, represent an aggregate of smaller units, and the cytoplasmic glycogen particles are
larger, consisting of more numerous smaller units, than the intranuclear ones, and also
appear larger than the size of the pores of the nuclear membrane. However, the smaller
units are small enough to pass the pore.
INTRANUCLEAR GLYCOGEN IN DIABETES MELLITUS

Under high power magnification, these smaller units of glycogen particles were seen very close to the nuclear pore. These findings might suggest that the smaller units of glycogen particle in the cytoplasm may go through the nuclear pore to accumulate in the nucleus as a result of increased amounts of glycogen in the cytoplasm and altered permeability of the nuclear pore to glycogen.

SUMMARY

The liver biopsy specimens taken from the patients with diabetes mellitus were studied under electron microscope. Numerous glycogen particles, measuring about 300 Å in diameter, were demonstrated in the nucleus of the liver cell, and a few smaller units of glycogen particles were also observed near the pore of the nuclear membrane. Cytoplasmic glycogen particles were more plentifully seen in the liver cells with glycogen-contained nuclei than without nuclear glycogen. A discussion has been made on the mechanism for intranuclear glycogen accumulation in this disease.

(Grateful acknowledgement is made to Prof. Okuda for his careful review of manuscript)

REFERENCES