HISTOCHEMICAL OBSERVATION OF GLYCOGEN AND LIPID IN THE CHICK LIVER CELLS IN VITRO

BY

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It is a well known fact that the liver cells keep glycogen in themselves after transforming glucose to it. During the development of the liver of the chick, glycogen begins to appear in the liver cells of embryo after 7 or 8 days of incubation (reported by A. Dalton, 1937), after 8 days of incubation (W.H. Lee, 1951) or after 6.9 days incubation (J.R. O'Connor, 1953). Such data were obtained by means of the histochemical or biochemical methods.

What is the behavior of glycogen in liver cells cultured in vitro, and whether glycogen appears in the liver cells cultured in vitro before it does in vivo, is one of the interesting problems concerning the cell differentiation of the liver and at the same time from the standpoint of tissue differentiation in general, and is worthy to be studied. By this study, one of the liver function might be clarified in vitro.

MATERIALS AND METHODS

White Leghorn's eggs were incubated at 100°F., and every embryo of 4~10-day-old and every other embryo of 11~20-day-old were used to get the liver tissue as the material.

Each liver tissue was divided into fragments of $2 \times 2 \times 1$ mm³ in size and grafted on the slide glass with the plasm clot made of a drop of cock plasm and a drop of 8-day-old embryonic extract (EEI). Then they were cultured by roller tube method with the nutrient composed of 45 parts of horse serum, 50 parts of Gey's solution, 100 parts of T.C. medium 199, 5 parts of EEI, and Penicillin was added (1,000 units per cc.). Nutrient was renewed every 5 days. After the next day of explantation the grafts were fixed in Carnoy's solution at 5°C. and was tested by Lillie's PAS reaction of glycogen using light-green as a after-staining. A salivary test was also applied. Lipid was stained by Sudan III with Daddi's method after 10% formalin fixation.

FINDINGS

The 4~5-day-old embryo: The epithelial cells grew out in a form of a sheet after two days when liver tissue of 4~5-day-old chick embryo was cultured in vitro. The nuclei of the epithelial cells have rich chromatin diffusely. Al-
most all the epithelial cells contain PAS positive substance in their cytoplasm evenly (Fig. 1). Such substance was digested by the saliva.

Salivary test proved it to be glycogen.

A few of the epithelial cells which have small granules stained by Sudan III were situated in the peripheral region of the sheet. During the cultivation of the liver tissue, glycogen which was contained diffusely in the cytoplasm of the epithelial cells began to aggregate in the peripheral cells of the sheet (Fig. 2), and soon it began to disappear. Then glycogen in the cells nearer to the explant also diminishes gradually. On the other hand, the small granules stained with Sudan III (lipid) came into existance increasing in number and size in the cells nearer to the explant. In 10 days of cultivation, therefore, glycogen existed only in the cells nearer to the explant, while lipid existed in the cells all over the epithelial sheet (Fig. 3).

As to fibroblasts, outgrowth took place on the second day of cultivation and they never contained glycogen in themselves for 10 days of cultivation. However, the fibroblasts with the granules stained with Sudan III (lipid) were seen in the periphery of the outgrowth like the epithelial cells.

In the older culture the lipid droplets in the fibroblasts did not increase in number and size in contrast to the epithelial cells, in which they grew gradually.

6, 7- and 8-day-old embryo: The state of outgrowth at these stages did not vary from each another. The number of cells containing glycogen was less than that of cells in 4~5-day-old embryo. This glycogen began to disappear from the periphery of the sheet (Fig. 4) and was lost later completely. While glycogen in the explant cells from 7- and 8-day-old embryo disappeared in 5~7 days of cultivation, that in the cells from 6-day-old embryo disappeared in 2 or 3 days.

As to lipid granules, those in cells from 6~8-day-old embryo increased in number and size compared with those in the cells from 4-day-old embryo as cultivation continued. Lipid granules in the cells from 7-day-old embryo increased in number before the fourth day of cultivation and did not increase anymore later. The chromatin disappeared in most of the nuclei after 7 days cultivation in 8-day-old material.

9-day-old embryo: The cells containing glycogen in the epithelial sheet from the explant of 9-day-old embryo were very numerous, but the glycogen disappeared in 6 or 7 days of cultivation. The lipid granules increased in number remarkably as cultivation continued, joined each other and changed into a large mass, which came to press the nucleus against the cell wall (Fig. 5). Chromatin could be found for a few day cultivation.

Embryos incubated more than 10 days: Cells containing glycogen in the epithelial sheet were rare at first. Their glycogen began to disappear in 3 to 4 days cultivation. But the epithelial cells from the explants of later stages than 18-day incubation contained as much glycogen when they grew out as those from 4-day-old embryo. Glycogen of these cells except for of those from 20-day-old embryo may decrease but not disappear during cultivation (Fig. 6). Lipid granules could be seen from the beginning of cultivation in most of the cells in the
epithelial sheet derived from the explants of 9-to 12-day-old embryo.

The lipid granules existing in the cells which were situated in the peripheral zone of the epithelial sheet, increased in number and size, but the granules in the cells nearer to the explant did not show any change. Appearance of lipid granules in the cells of the epithelial sheet from the explants derived from 15- to 20-day-old embryo was the same to that of the 8-day-old embryo.

DISCUSSION

The first appearance of glycogen in the liver of the chick embryo in vivo was reported by Dalton (1937), Lee (1951) and O'Connor (1953); their periods of incubation were 7 or 8 days, 8 days and 5.9 days respectively. In author's investigation, it was 8 days and agreed with that of Lee. In the explantation of the liver tissue of 4-day-old embryo, glycogen appeared in the epithelial sheet. In the cultivation of the liver tissue by the roller tube method, it was very curious that the material derived from 4- to 20-day-old embryo showed glycogen in the immediately outgrown cells, because up to 6th day of incubation the liver cells have no glycogen in vivo. O'Connor (1953), who studied "metabolism and glycogen formation in the liver of the chicken embryo" biochemically or histochemically, stated that "before the appearance of glycogen the isolated liver consumes glucose by respiratory mechanism, on the other hand, ....... after the appearance of glycogen, the isolated liver no longer utilizes the oxygen in the catabolism of glucose". So, in author's explantation experiment, it is possible that the appearance of glycogen in liver cells of 4-to 7-day-old embryo is due to the utilization of glucose in the nutrient, but the glycogen in the cultivated liver cells of 8- to 20-day-old embryo originates from the glycogen already existed in the liver in vivo.

Disappearance of glycogen in vitro during cultivation may be partly due to a consumptive function of the liver cell itself, and partly to an inadequate condition in vitro in synthesizing glycogen.

As to lipid, Kingsbury et al. (1956) reported that "the increasingly yellow liver towards the end of the incubation period may be attributable to yellow pigments carried from the yolk to the hepatic cells with the lipid infiltration." But in author's explantation experiment, appearance of lipid granules in the liver cells in vitro was independent of the appearance of yellow pigment throughout all the period of cultivation. This did not coincide with his explanation. At the beginning of outgrowth of the hepatic cells the lipid granules were small in number as well as in size, but they increased gradually as cultivation went on. So, in regard to the relationship between the fate of glycogen and lipid, it would be reasonable to consider that lipid might be produced at the expense of glycogen in vitro.

CONCLUSION

1. The liver tissue of chick embryo ranging from 4th to 20th day of incu-
bation was cultured and appearance of glycogen and lipid in the liver cells was
tested histochemically.

2. Glycogen could be found in the outgrown cells from the explants covering all the developmental stages of the chick embryo used, though the liver cells of 4-to 7-day-old embryo do not contain glycogen in vivo.

3. Appearance of glycogen in vitro in the liver cells originated 4-to 7-day-old chick embryo might be due to an acceleration of cell differentiation through an enviromental change in vitro such as administration of the horse serum and cock's blood plasm.

4. Appearance of lipid in the liver cells in vitro should not be related to the so called yellow liver but might be a degenerating process of the cells.

References


Explanation of Figures

Fig. 1. Liver cells of 4-day-old embryo. Second day of cultivation, showing glycogen droplets (black). PAS-reaction. 1,700×

Fig. 2. Liver cells of 5-day-old embryo. Third day of cultivation, showing aggregated glycogen droplets. PAS-reaction. 1,700×

Fig. 3. Liver cells of 5-day-old embryo. 6th day of cultivation, showing lipid granules (black). Sudan III staining. 660×

Fig. 4. Epithelial sheet of 8-day-old embryo. 7th day of cultivation, showing cells which have glycogen in the middle part (left) of the sheet and not in the peripheral zone (right). PAS-reaction. 660×

Fig. 5. Liver cells of 9-day-old embryo. Third day of cultivation, showing cells filled with aggregated lipid granules. Sudan III staining. 660×

Fig. 6. Epithelial sheet outgrown from the explant of the liver of 16-day-old embryo. 5th day of cultivation, showing co-existence of lipid (vacuole) and glycogen (black). PAS-reaction. 170×