SOME CYTOCHEMICAL STUDIES ON CHANGES IN MOUSE LIVER FOLLOWING THE IMPLANTATION OF CARCINOMA 51

(Plate VI)

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One of the previous papers dealt with the changes occurring in livers of rats bearing the ascites sarcoma and presented information on the marked depletion of glycogen, the increase of RNA- and DNA-contents, the frequent occurrence of mitosis and the sinusoidal dilatation (Hori et al. 1958). The present investigation was undertaken to examine cytochemical changes of livers following the subcutaneous transplantation of a tumor of a different type. In the former study the ascites sarcoma growing in the peritoneal cavity of the host was used, while in this study use was made of carcinoma 51 which develops in the subcutaneous tissue.

Material and Method: Carcinoma 51 was transplanted subcutaneously in dd-strain mice weighing 17 to 20 grams. The survival time of mice bearing the tumor was about 11 days. Tumor mice were sacrificed 3, 7 and 10 days after transplantation; they were conveniently referred to in description as follows:

- Group A: three mice killed 3 days after transplantation
- Group B: three mice killed 7 days after transplantation
- Group C: three mice killed 10 days after transplantation

The livers from normal and tumor-bearing mice were fixed with formal-calcium, subzero Gendre’s fluid and acetic alcohol, and subjected to a series of staining methods, as described in the previous reports (Hori 1958, Hori et al. 1958).

Observations

Glycogen (PAS positive material digestable with saliva): In well-nourished normal mice, the liver glycogen was evenly distributed in the hepatic lobules. The cytoplasm of liver cells shows generally a rich content of the PAS positive substance, the amount of which was more affected by alimentary conditions than in rats (Fig. 2). The saliva extraction test of slides showed that the PAS positive substance was glycogen in nature.

The mice bearing the tumor showed decrease of glycogen content in livers without exception. In group A mice, a slight depletion of glycogen content was observed in the region around the central hepatic veins (Fig. 3), and the depletion increased...
with the passage of days. Hepatic cells in the periportal area showed a striking reduction of glycogen in mice of group B (Fig. 4).

Further, almost complete or complete depletion of glycogen occurred in livers of mice carrying the tumor for 10 days or more (Fig. 5).

RNA (basophilla digestable with ribonuclease): By staining the slides with a 0.1% toluidine blue solution, liver cells of normal mice showed blue coloration in both cytoplasm and nucleoli, while their nuclear chromatin stained heterochromatically blue-violet (Fig. 13). Based on the digestion of the above slide with a 0.02% ribonuclease solution, it was found that the blue coloration after toluidine blue staining was a result of the existence of RNA in cell components. The cellular basophilia which increased in liver cells of tumor mice showed a fair degree of increase as compared with the control livers, though not so pronounced as in rat livers. In addition, a considerably strong tissue reaction seemed to occur in livers of tumor-bearing mice (Fig. 14), since many leucocytes, mostly polymorphic ones, appeared in the hepatic sinusoid and around the connective tissue. As a result, the structure of the liver was partially distorted.

Nucleoli positive to PAS reaction were found by Leuchtenberger et al. (1958) to occur in liver cells of mice which received intraperitoneal injection of the DNA which was extracted from the C3H carcinoma. No such elements were detected in the present case.

DNA (microspectrophotometric determination): By means of microspectrophotometry the DNA-contents in liver nuclei of tumor-bearing mice was measured in formal-calcium fixed and Feulgen stained slides.

The results are summarized in Figure 1, with the histograms showing frequency distributions of relative amounts of DNA-contents in livers of normal mice and those of groups A, B and C. The livers of groups B and C mice showed a slight increase of DNA-contents with wider scattering of values than those of normal mice. This increment of DNA-content occurred in concomitance with an increase in volume of cell nuclei, in a manner exactly similar to

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<thead>
<tr>
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<th>NUMBER OF NUCLEI</th>
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<tr>
<td>CONTROL</td>
<td>4.46 ± 0.15</td>
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<tr>
<td>A</td>
<td>4.35 ± 0.15</td>
</tr>
<tr>
<td>B</td>
<td>5.00 ± 0.24</td>
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<tr>
<td>C</td>
<td>4.85 ± 0.12</td>
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Fig. 1. Four histograms indicate relative amount of DNA in the liver cells of normal and tumor-bearing mice sacrificed on 3, 7 and 10 days after tumor transplantation and were referred to as A, B and C in the figure, respectively.
that encountered in rats.

Mitotic frequency in the livers of rats bearing MTK-sarcoma III was relatively high (Hori et al. 1958), while mitosis was almost completely absent in the livers of mice bearing carcinoma 51. The results seem to suggest that the DNA-content of liver nuclei might increase independently of the mitotic activity of cells.

Further, the evidence seems to indicate that the tumor transplanted into the subcutaneous tissue of mice exerts some influence upon the host liver to raise its DNA-content.

Mitochondria: In order to detect the mitochondria, the livers from both normal and tumor-bearing animals were chromated with 3% potassium bichromate for 6 to 8 days after fixation with formal-calcium, and then stained with Regaud’s iron-hematoxylin. In the livers of healthy control animals mitochondria were almost evenly distributed in the cytoplasm of liver parenchymal cells. Cells in the periportal area of hepatic lobules showed a rich content of mitochondria of voluminous rod-shape, while cells lying in the centrolobular region had filamentous mitochondria (Fig. 10). Livers from mice of groups B and C showed mitochondria, more abundantly in cells of the periportal area than in those of the centrolobular area. They were round and small granules in outline, showing less stainability than that of control cells (Figs. 11 and 12).

Acid hematein test: The cells of normal livers generally contained many cytoplasmic granules, probably mitochondria; they stained with acid hematein blue or blue black against a yellowish background (Fig. 6).

This staining situation suggests the existence of phospholipids in the granules. Cells of the periportal region of hepatic lobules contained mitochondria which showed slightly stronger affinity to acid hematein than did those of the centrolobular region. Such difference in stainability of mitochondria was probably due to differences in their morphology.

In liver cells of group A mice, decrease in stainability of mitochondria occurred in the centrolobular area, and then extended with the passage of days toward the peripheral portion of lobules (Fig. 7). With the decrease in stainability the granules became slender in shape (Fig. 8). In the livers of group C mice, cells of the centrolobular portion contained only a small amount of granules staining faint blue in the periphery of the cytoplasm (Fig. 9).

DISCUSSION AND CONCLUSION

Baldwin and Haries (1958) and Shetler et al. (1952) reported that polysaccharide-containing proteins of serum and serum polysaccharides associated with the albumin fraction were abundantly found in tumor-bearing animals and in cancer patients. An increase of glycolysis has also been reported in livers of leukemic mice by Hall
(1944) and Burk et al. (1942). Comparing the glucose and lactic acid contents of the blood from the axillary veins of a chicken bearing Rous sarcoma, Cori and Cori (1925) have found less glucose and more lactic acid in the vein draining the tumor. Warburg et al. (1926) also observed a great difference in lactic acid and glucose contents between arterial and venous blood of rats bearing the Jensen sarcoma; these findings indicate an active utilization of glucose by the tumor, with production of lactic acid. The present study has shown that all the mice carrying the tumor for 10 days or more showed a complete depletion of glycogen in their livers. On the basis of the above findings it is possible to speculate that liver glycogen is taken into the blood stream in order to meet the enormous requirement of energy by tumor cells. Goranson et al. (1954) have obtained results contradictory the above view in that the levels of liver glycogen were essentially the same between fasted control and tumor-bearing rats and that there was no real difference in the phospholylase activity of liver between the control and tumor rats. In the present study, the authors examined the stomachs of tumor mice when sacrificed and found that they were filled with food. It is therefore not likely that the depletion of liver glycogen in tumor mice was primarily caused by anorexia which was often said to be due to the presence of the tumor.

Chemical analysis using labelled precursors have revealed evidence which indicates an increased rate of nucleic acid synthesis in the tissue of tumor-bearing mice (Kelly and Jones 1950, Payne et al. 1952 a and b, Anderson et al. 1955). Khouvine and Mortreni (1954) have pointed out the possible existence of a substance which was derived from the tumor and exerted influence upon the nucleic acid synthesis of the liver. A significant increase of DNA-content was also observed by the present study in livers of mice bearing tumor transplants, exactly the same as is seen also in those of rats bearing tumors. There was, however, little mitosis in livers of tumor-bearing mice, in striking contrast to the frequent occurrence of mitosis in livers of tumor-bearing rats. Then, it seems probable that the increase of DNA-content might have taken place, independently of the mitotic events, due probably to the abnormal metabolic activity of liver cells induced by the presence of tumors.

It has been shown that the morphology of the mitochondria undergoes alterations under various physiological, pathological and experimental conditions (Lewis and Lewis 1945, Smith 1931, Weathereford 1933, Gey et al. 1955, Hori 1958). Hori (1958) has found in rats that mitochondria of hepatic cells of the centrolobular area transform in shape from filament into fine-spun threads and give up affinity to acid hematein, following cortisone administration. Similar observations were made during the present study in livers of tumor-bearing mice. Then it is likely that filamentous mitochondria of liver cells of the centrolobular area tend to loose affinity to acid hematein under certain conditions.
SUMMARY

Carcinoma 51 was transplanted subcutaneously into mice of dd-strain. The changes in content of glycogen, RNA, DNA and phospholipids in the livers, as well as alteration in mitochondrial morphology, were studied by a series of cytochemical staining and cytophotometric methods.

The results obtained indicate that: 1) glycogen is gradually depleted in livers of tumor-bearing mice and becomes completely absent when mice were moribund; 2) the DNA-content in liver cells of tumor-mice shows an increase 7 and 10 days after tumor-transplantation, in concomitance with the increase of nuclear volume of liver cells; 3) RNA was found to be rather rich in livers of some tumor-mice; 4) the phospholipid-content of liver cells as revealed by the acid hematein test shows a decrease specially in the centrolobular area of the liver, and 5) filamentous mitochondria in cells of the centrolobular area tend to break down into granules, and loose phospholipids.

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REFERENCES

EXPLANATION of PLATE VI


Fig. 2. Control mouse liver.

Figs. 3-5. Livers of tumor-bearing mice sacrificed on 3, 7 and 10 days after transplantation, respectively. Note the gradual diminution of PAS positive substance in the liver parenchymal cells.

Figs. 6-9. Fixative: formal-calcium. Stain: acid hematein. Fig. 6. Control mouse liver, showing strongly stained granules, probably mitochondria. 400×. Figs. 7-9. Livers from tumor-bearing mice killed 3, 7 and 10 days after transplantation, respectively. Magnif. 100×, except 400× in Fig. 9.


Fig. 10. Normal liver cells. Figs. 11 and 12. Liver cells of tumor-bearing mice killed 7 and 10 days after tumor transplantation, respectively. Mitochondria of cells in centrolobular area show marked morphological alteration and decrease in stainability.

Figs. 13-14. Fixative: formal-calcium. Stain: toluidine blue. Magnif. 100×. Fig. 13. Normal mouse liver. Fig. 14. Tumor liver from the mice killed 10 days after tumor transplantation. Note the increase in cellular basophilia and many leucocytes around the interlobular vein.