Cytological Studies of Tumors, XII. Cell Division in the Ascites Hepatoma of Rats Studied by Phase Microscopy[1)]

By

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(With 2 Plates)

Recent interest in modern oncology has arisen around the ascites tumors. Attempts have been made to transform a malignant tumor into a fluid from with variable results (Hesse 1927, Loewenthal 1932, Goldie and Felix 1950. Klein and Klein 1951, Hauschka and Levan 1952). It has been found that S–37, malignant lymphoma, S–180, melanoma, Ehrlich adenocarcinoma and Krebs–2, *etc.*, can be freely grown in the peritoneal cavities of tumor-bearing animals by successive transmissions. In these ascites tumors, the individual tumor cells are usually able to maintain themselves in an isolate form as autonomous units, or they are provided with a sufficient amount of nutritive material from ascites fluid in the isolated state. In striking contrast to them, the ascites hepatoma as established by Tanaka and Kanô (1952) shows a symbiotic relationship occurring between the hepatic parenchymal cells and endothelial cells in order to maintain its growth. The details of this character will be described in another paper (Tanaka and Kanô 1953). The present study deals with mitotic phenomena taking place in the tumor cells in the hepatoma island during the course of its growth.

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Material and Methods

The ascites hepatoma of rats here concerned was produced by the injection of hashed tissues of hepatoma induced by p-Dimethylaminoazobenzene (cf. Tanaka and Kanô 1952). In the case of this tumor, the neoplastic exudates can

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be obtained simply by puncturing the abdominal cavity with glass pipettes as in other ascites tumors. The samples for observations were obtained from the tumor-bearing animal on the 2nd to 4th day after the transplantation of the ascites hepatoma. During such an interval, tumor cells multiply very actively providing numerous mitoitc figures. The preparations were made with the tumor ascites thus obtained according to the new hanging-drop method after Makino and Nakahara (1952). The behavior and mitotic activity of hepatoma cells in the islands were followed with the aid of a phase-contrast microscope (Olympus), using the optical combination of a periplan 10 × ocular and a bright-medium contrast 90 × objective. The microscope was set in a chamber equipped with an electric warmer at a temperature of 35°C.

The successive mitotic figures, followed through the same cells, ranging from interphase to telophase were recorded in serial photomicrographs taken at adequate intervals with the aid of a Leitz-Mikas camera.

Observations

There are a number of hepatoma islands suspended in the peritoneal fluid of the tumor-bearing rat. Structurally, the hepatoma island is a mass of a certain number of hepatoma cells (cancerous hepatic cells) of varying sizes and shapes, in association with some endothelial cells. That is to say, a hepatoma island consists of two cellular elements, hepatic cells and endothelial cells, which are different in origin and nature. A few endothelial cells are found adhering the surface of the hepatoma-cell mass. The hepatoma (cancerous) cells seem to be hepatic parenchymal in origin. The resting hepatoma cell shows a defined, spherical nucleus, located in the central part of the cytoplasm. The nucleus contains two or three nucleolus-like bodies, small in size but clear-cut in appearance. There are a number of granules distributed throughout the cytoplasm. Some of them are characterized by a highly refractive nature, while others are less distinct in appearance. There is a remarkable Brownian movement of these granules in the cytoplasm. The endothelial cell is characterized by a small amount of cytoplasm carrying a kidney- or horseshoe-shaped nucleus. The cytolasm of the endothelial cell displays a characteristic amoeboid movement. It occurs tightly adhering the one side of the hepatoma cell. Sometimes, the cytoplasm of the endothelial cell extends to cover the surface of the hepatic cell body. The cytoplasmic granules are less in number in the endothelial cell than in the hepatoma cell. By its characteristic feature, the endothelial cell is clearly distinguishable from the hepatoma cell. Generally, the hepatoma island is, under a living condition, of a spherical shape.

The mitotic figures are usually observable in the tumor cells, and rarely in the endothelial cells. Because of ease of technique the study of mitosis can be readily made in the small hepatoma island consisting of two to five tumor cells.

The observations of the mitotic behavior were firstly carried out on the hepatoma island consisting of two cells, a cancerous hepatic cell (hepatoma cell) and an endothelial cell. The course of mitosis is shown in series in Figures 1 to 12.

The interphase of the hepatoma cell is followed by prophase in which the nucleus seems to swell up into a well-expanded body. In the early prophase, the chromatin threads are less distinct in the nucleus. The cytoplasmic granules decrease in number. By this time, the chromatin threads become distinct, and the nucleoli fade from view. Shortly before the end of prophase, the nuclear membrane becomes irregular and wrinked, then disappears. Within a few minutes after the disappearance of the membrane, the chromosomes have completed their arrangement on the equatorial plate.

During these changes occurring in the hepatoma cell, the endothelial cell seems to shrink but it is tightly adhesive to the hepatoma cell. At late metaphase it assumes a spherical form.

At anaphase, the chromosomes of the hepatic cell become double. The anaphase splitting of the chromosomes occurred very rapidly. The separation generally takes place synchronously in all chromosomes. At this stage, the endothelial cell spreads its surface over the hepatoma cells.

In the early telophase daughter chromosomes come closely together into masses. Around the chromosome masses, the nuclear membrane is formed. Meanwhile, all visible traces of the chromosome disappear from view, and the daughter nucleus is thus reconstructed. It continues to enlarge into a spherical form. The nucleus at this stage is reticular in structure. By that time, the nucleolar bodies appear again in the nucleus ; they are fairly similar in number, size, shape and location in the two daughter nuclei. The cleavage furrow begins to appear at the equator of the cell, and cuts the cell body rapidly across the equator, dividing the mother cell into two daughter cells.

Further observations were made on a large hepatoma island consisting of about two endothelial cells and three hepatoma cells. Serial figures of a mitotic tumor cell are given in Figures 13 to 22. The mitotic events, are strikingly similar to those described above. But the behavior of the cells after division is more remarkable in this case than in the former.

In the late anaphase, the characteristic behavior of the cytoplasm is found remarkably in the daughter cells after division. From the cell surface several processes like bubbles appear. They terminate in knob-like thickening. It is highly probable that the cytoplasmic bubbles are the result of hydration and dehydration phenomena of the cytoplasm. By this time, the interesting behavior is observable in the endothelial cells. They begin to move on the surface of the hepatoma-cell mass with the amoeboid pseudopodia of the cytoplasm. Soon, they migrate toward the cleavage furrow. After a short pause, a spherical hepatoma island is formed. Then the interphase nucleus begins to prepare for the succeeding division.

In addition to the hepatoma islands as described above, there are found a considerable number of free cells suspended in the peritoneal fluid of the ascites hepatoma-bearing rat. Two kinds of cells are distinguishable by their morphological characteristics. The one is the parenchymal cell and the other the endothelial cell. Mitotic figures of these free cells are seen in the pertioneal fluid, but the cells undergoing regular mitosis are rather few in number. Interestingly, the parenchymal cells tend to show the degenerative features more frequently rather than the endothelial cells. It is probable that the parenchymal cells are viable in association with the endothelial cells, and further that these free cells are not primarily important in the growth of the ascites hepatoma.

Remarks

As stated above, it is apparent that the hepatoma cells of the ascites hepatoma proliferate with the regular mitotic behavior under the association with the endothelial cells in the hepatoma island. There are free cells suspended in the peritoneal fluid of the ascites hepatoma rat. Though their exact origin is unknown, they are not of primary importance in the growth of the ascites hepatoma. These free cells comprise two cell types. From their morphological features, one is the parenchymal cell and the other the endothelial cell. Generally the parenchymal cells are inactive in behavior and degenerative in appearance. It is probable that the parenchymal cells are actively viable in symbiotic association with the endothelial cells.

Several kinds of ascites tumors have been found to occur in mice and rats. In these tumors, the tumor cells can be freely grown in the peritoneal fluid of the tumor-bearing animal. But in the ascites hepatoma, the symbiotic association of hepatic parenchymal cells and endothelial cells seems necessary for maintenance of their activity. Yoshida (1952) reported that the ultimate unit of the hepatoma island is a pair of cells, the one being the hepatic cell and the other the endothelial cell. Further, Hosokawa (1952) reported that in the experiments of a single cell inoculation in the ascites hepatoma, the hepatomal island can be grown only with the inoculation of symbiotic cells. Evidence seems to suggest that the hepatoma islands can accomplish their characteristic growth and function under the symbiotic association of the hepatoma cells with the endothelial cells.

Summary

The structural units of the ascites hepatoma are the hepatoma islands, which consist of two cellular elements, cancerous hepatic cells and endothelial cells, living in a symbiotic association in the peritoneal fluid. The process of the division was followed in living cells of the ascites hepatoma during its growth with the aid of a phase-contrast microscope. The mitotic events were continuously observed

from interphase to telophase in the same cells, together with the characteristic amoeboid behavior of the endothelial cells.

The cells of the ascites hepatoma proliferate with a regular mitotic behavior in the presence of the endothelial cells forming the hepatoma islands. Evidence presented seems to indicate that the hepatoma islands can accomplish their characteristic growth and function under the symbiotic association between the two component elements, the hepatoma cells and the endothelial cells.

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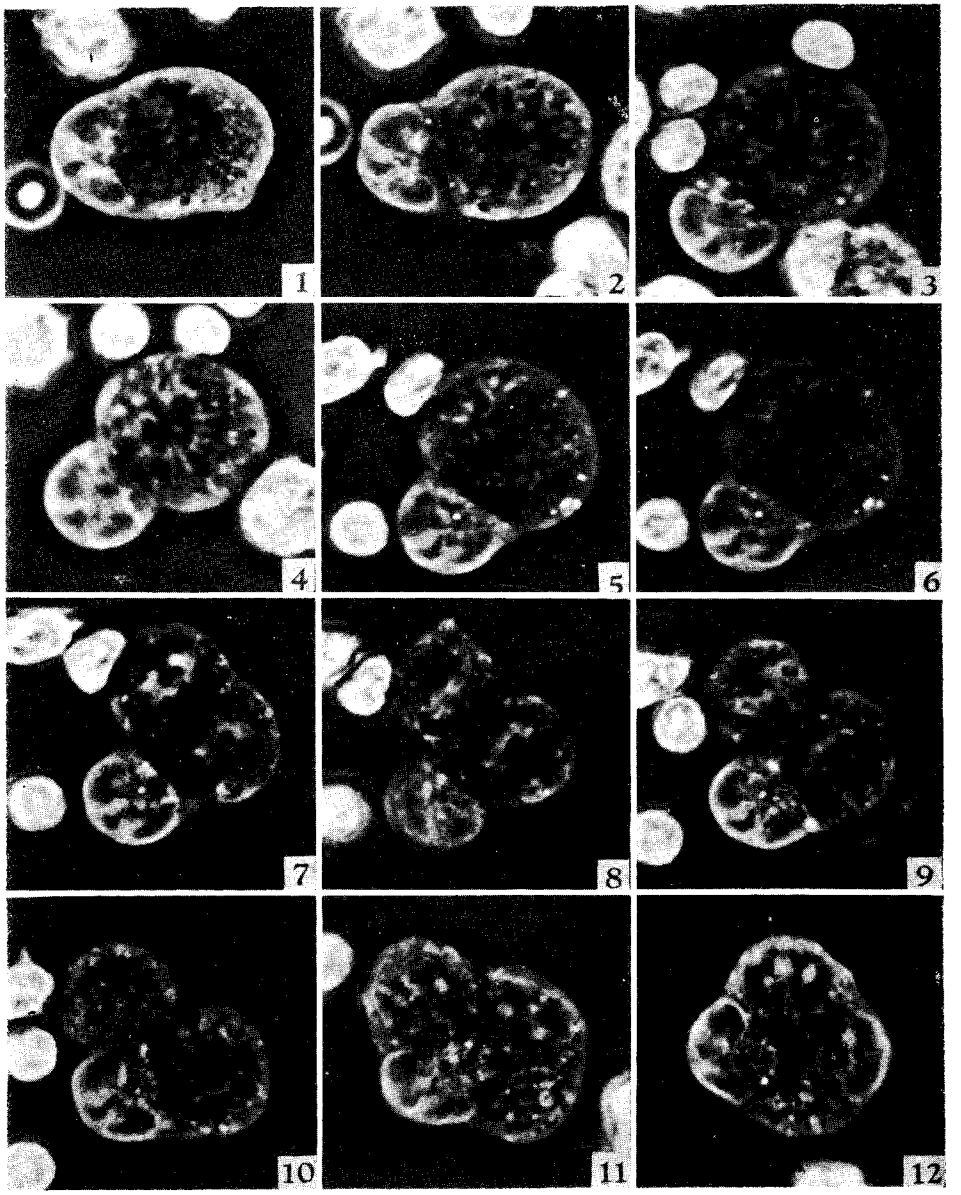
Explanation of Plate XI

Photomicrographs of the ascites hepatoma cells taken with the aid of the Olympus phase-contrast microscope. Successive series of a division process followed through a single cancerous hepatic cell, × 1200.

1, early prophase. 2, early metaphase. 3–4, metaphases containing shrunken endothelial cell. 5, late metaphase. 6–7, successive stages of anaphase, showing the separation of chromosomes. 8–9, successive stages of telophase, showing cytoplasmic bubbles. 10, two daughter cells, showing the reconstruction of daughter nuclei in each. 11–12, the formation of hepatoma island showing the characteristic amoeboid behavior of endothelial cell.

Explanation of Plate XII

Photomicrographs of a large hepatoma island. Successive stages of mitosis in cancerous hepatic cell, × 1200. 13–14, metaphases. 15, late metaphase. 16, early anaphase, showing the splitting of chromosomes. 17, anaphase. 18–20, successive stages of telophases. 21–22, the formation of hepatoma island showing the characteristic amoeboid behavior of endothelia cell.



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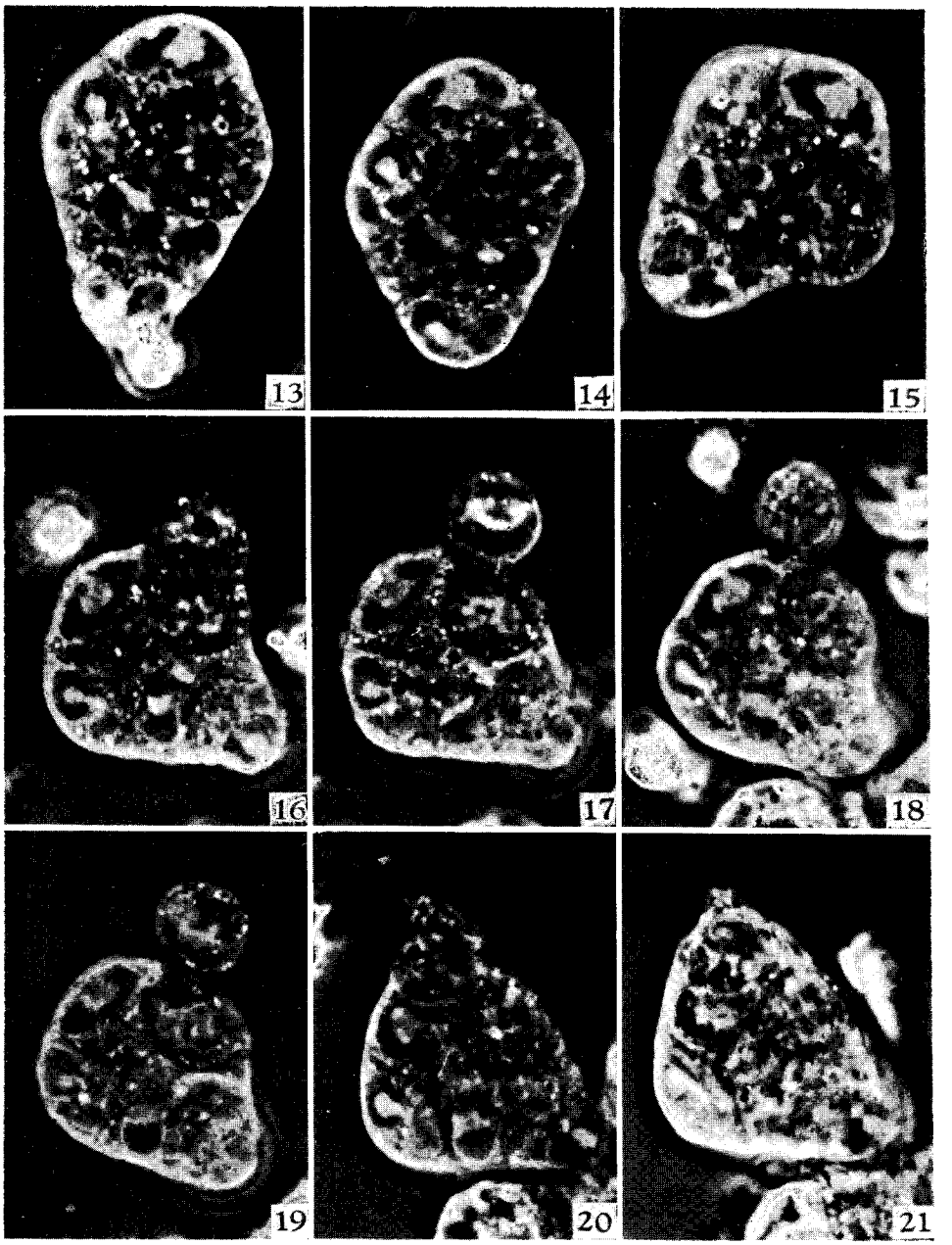

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