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Cyto-Histological Observations on Solid Tumors of Rat Ascites Sarcomas, I. On the Course of the Solid Tumor Formation

By

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(With 16 Figures in 2 Plates)

The MTK-sarcoma III is an ascites tumor of rats which was produced artificially as the result of prolonged administration of an azo dye (Tanaka and Kanô, unpubl.). Its general character is similar to that of the Yoshida sarcoma (Umetani 1953). The average life span is about 9 days ; the highest mitotic rate of tumor cells is found on the 3rd day of transplantation in the Wistar rats. Autopsies of rats which have died of the tumor have revealed without exception the formation of large masses of the solid tumor which seems to be due to the infiltration of tumor cells into the omentum.

A series of cytological studies on several kinds of ascites tumors of rats has been published by Makino and his collaborators (Makino 1952 a, b, 1956 ; Makino & Kanô, 1951, 1953, 1955 ; Makino & Tanaka, 1953a,b ; Makino & Tonomura, 1955 ; Tanaka & Kanô, 1951 ; Watanabe & Tonomura, 1956). However, cytological studies of the tumors in the solid phase or in infiltrative condition have remained without attention. Baillif (1954) and Yoshida (1949) studied the solid phase of the Ehrlich ascites tumor in mice and of the Yoshida sarcoma in rats respectively, though their studies were not extended to the daily change in the course of solid tumor formation. The present investigation was undertaken to clarify the course of solid tumor formation in the MTK-sarcoma III and to examine the histological features of the tumor in its solid phase.

Material and methods

The animals used were inbred Wistar albinos weighing 60 to 90 gm : they received intraperitoneal inoculation with about 60 million tumor cells in a volume of about 0.03 cc. Calculation of the cell number was made in the fluid by means of a blood cell counting chamber. Rats bearing tumors were sacrificed every day after the inoculation of the tumor. *Omentum gastrolienis* and omentum around the pancreas were removed from each animal and fixed in Zenker-formol or Bouin's fluid for histological studies. The fixed material was sectioned, subjected to the ordinary paraffin method of preparation and the sections were stained with hematoxylin and eosin.

Observations

The omentum is composed of loose connective tissue (Fig. 1). Parts of this

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membrane contain vessels accompanied by fatty tissue. In other parts there is present either no vessels or only a very few small ones ; here the membranes are pierced with numerous holes, showing a fine network. The threads of the net are formed by collageneous bundles which are covered with flat mesothelial cells (Maximow & Bloom, 1943). Parts of the omentum are rich in leucocytes, while other parts are poor in these cells. The former sections are called milky spots ; there are many histiocytes and lymphocytes occupying a large portion. The division of histiocytes is not rare. Cells in the membranous network are mainly macrophages, fibroblasts, mast cells, fat cells, eosinophile leucocytes and lymphocytes. There are also many mesothelial cell nuclei.

After inoculation of the ascites tumor into the peritoneal cavity, the tumor cells multiply rapidly. The active invasion of tumor cells into the omentum seems to take place sooner or later. The invading tumor cells divide actively in the nets of omentum. Thus the omentum becomes laden with a number of tumor cells in several places (Fig. 2). Histological examinations of such an omentum on the lst day after inoculation have revealed that the invasion of tumor cells into the omentum is carried out not through blood or lymphatic vessels, but by the direct attack of tumor cells on the tissue surface (Fig. 3). This is probably due to the direct movement of the tumor cells. This assumption was confirmed further by the fact that no tumor cells could be found in the blood vessels in the omentum.

The tissue reaction is usually observable in the ascites of rats after the tumor injection. It is specially remarkable during the first 24 hours after injection. The same feature was seen in the omentum undergoing the tumor invasion ; many leucocytes were found gathered about the invaded tumor cells (Fig. 4), resulting in the formation of large masses of cells in several places. Then leucocytes were found interspersed with the actively dividing tumor cells. However, the larger part of the omentum remained uninfected with tumor cells. Apart from the inside of the tissue, there were many fibrinous clots attached to the mesothelium and ladened with tumor cells, especially in the places showing the tumor invasion. This suggests that such fibrinous clots may facilitate the invasion of tumor cells into the omentum.

The tumor cells at the time of invading the omentum are characterized by their round shape, by the deep basophily of their cytoplasm and by conspicuous nucleoli (Fig. 3). The tumor cells after invading the omentum and starting to divide become larger in size, especially in nuclei and nucleoli, showing slightly faint basophilic cytoplasm.

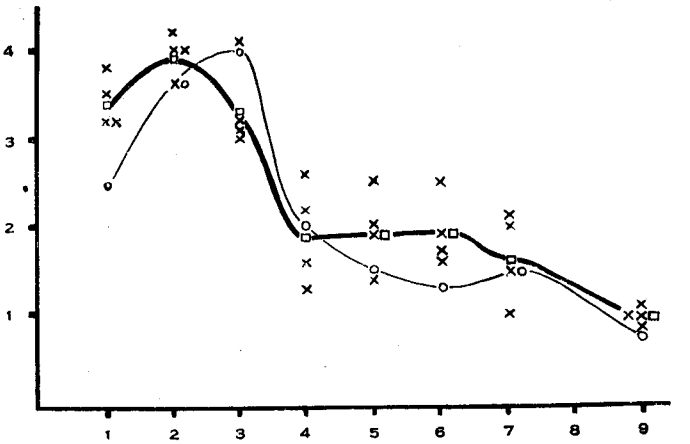
The most active proliferation of the invading tumor cells can be seen on the 2nd day (Fig. 6) : the nets of the omentum become filled with tumor cells, resulting in the formation of expansions in the tissue in several places (Fig. 5). The expansions contained many leucocytes together with tumor cells.

The tumor cells occurring around the omentum multiply independently in a matrix consisting of collagenous fibres which are to be derived from red corpus-

cles and/or fibrinous components of the ascites. As a result, several masses of tumor cells are formed and become attached to the nets. These are designated as "independent areas" of tumors (Figs. 5, 7 & 8). The areas become more marked and larger with time.

On the 3rd day the neighboring omentum nets expanding with a number of tumor cells show an inclination to associate with each other. It was found by histological study that such association of the net is due to the elaboration of certain tumor cells. The mesothelium of the omentum under expansion with many tumor cells is gradually destroyed. Such destroyed mesothelial tissue comes to join with the independent areas. In this state, the independent areas come together with other part of nets, resulting in the association of nets. The association of the nets proceed rapidly with time. On the 4th day *Omentum gastrolienis* appears as a very large tumor mass lying between the stomach and the spleen, weighing approximately 0.5 to 1.0 gm, and being 2 cm×2cm×0.5 cm in size.

Another remarkable feature on the 4th day is the appearance of necrotic areas in addition to the active invasion of tumor cells into the pancreas. Necrosis begins to occur in the independent areas lying between nets and in the outer regions surrounding the omentum; the necrotic areas consist of fibrinous clots loaded with tumor cells. In other words, necrosis begins to occur in the independent areas existing in several places. Besides the formation of these foci, an important change occurs in the inner portion of the omentum: a sinus begins to appear in some places after rearrangement of tumor cells. The tumor cells occurr-



Text-Figure 1. A graphical representation of the daily frequencies of dividing tumor cells in the solid phase (×, □ indicates mean) and in the ascitic phase (○). *Abscissa*: days after inoculation. *Ordinate*: mitotic frequencies in per cent.

ing in the omentum closely associate with one another leaving slight intercellular spaces. On the 4th day those intercellular spaces become larger and larger : such an enlargement of intercellular space is referred to as a sinus formation.

The tumor cells surrounding the pancreas gradually invade the interlobular connective tissue of the pancreas and come to infiltrate among the acinar cells. This causes a partial destruction of the pancreas (Fig. 14).

Passage of tumor cells into blood vessels also occurs on the 4th day after transplantation : some tumor cells can be seen floating in blood. Some individuals are observable in process of entering into blood vessels through the damaged portions of the endothelium (Fig. 10).

Two remarkable events appear simultaneously 6 to 7 days after inoculation of the tumor : the one is the active invasion of tumor cells into the neighboring nets and their continued multiplication ; the other is the increase of necrotic tumor cells and the remarkable evidence of coagulative necrosis (Figs. 12 a, b). On the ninth day, the rat shows a solid tumor formed on the base of the omentum, 3 to 4 gm in weight and 2.5 cm×2.5 cm ×1.0 cm in size, a portion larger than half the solid tumor being comprised of a number of necrotic cells.

The pancreas suffers heavy destruction through invasion of tumor cells on the 6th to 9th day after transplantation of tumor, resulting in disorganization of the acinar tubules. Thus each aciner cell loses its original appearance and takes a spindle shape. Its cytoplasm gradually shrinks and finally disappears (Fig.

Table I. The percentages of the dividing tumor cells in four regions in the solid tumor and in the ascites fluid.

Days after inoculation	The percentages of mitotic cells					
	Four regions in the solid tumor					ascites
	I	II	III	IV	average	
1st day	3.2% (20/633) §	3.2% (20/620)	3.5% (25/713)	3.8% (22/576)	3.4% (87/2542)	2.8% (56/2000)
2nd day	3.6% (81/2284)	4.0% (43/1091)	4.0% (69/1715)	4.2% (25/587)	3.9% (218/5677)	3.6% (72/2000)
3rd day	3.0% (60/2085)	3.1% (183/5810)	3.2% (102/3176)	4.1% (101/2451)	3.3% (446/13522)	4.0% (80/2000)
4th day	1.3% (57/4445)	1.6% (80/5011)	2.2% (120/5500)	2.6% (45/1730)	1.9% (302/16686)	2.0% (40/2000)
5th day	1.4% (60/4297)	1.9% (87/4602)	2.0% (94/4715)	2.5% (39/1560)	1.9% (380/15174)	1.5% (30/2000)
6th day	1.6% (30/1928)	1.7% (69/3959)	1.9% (41/2121)	2.5% (140/5619)	1.9% (280/13627)	1.3% (26/2000)
7th day	1.0% (16/1545)	1.5% (57/3800)	2.0% (78/3975)	2.1% (156/7383)	1.6% (307/16703)	1.5% (30/2000)
9th day	0.9% (19/2105)	1.0% (34/3320)	1.0% (16/1545)	1.1% (12/1123)	1.0% (81/8093)	0.8% (16/2000)

§ (Numerators denote the numbers of dividing cells ; denominators indicate the total cell numbers counted.)

15). The daily mitotic rates of tumor cells within the solid tumor were measured and were compared with those in the ascites. The results are shown in Text-Figure 1 and Table 1.

Discussion

Willis (1953) stated that the property of the tumor cell itself regarding the significance in its invasive capacity involves : (1) its power of progressive multiplication, (2) its mobility, (3) its possible phagocytic action and (4) its possible elaboration of toxic or lytic substances. He thought, however, that the invasiveness does not depend on any one of these properties, but is an important property of the tumor cell itself.

The results of the present observations seem to indicate that the invasion of tumor cells into the omentum depends first on the mobility of the tumor cells. This assumption seems to be supported by the fact that invasion of tumor cells into the omentum took place on the lst day after inoculation ; at that time a relatively small number of tumor cells are to be found in the peritoneal cavity of the rat. Recently the amoeboid movement of tumor cells of rat ascites tumors was demonstrated in living condition by means of phase cinematography by Makino and Nakanishi (1956, unpubl.).

Mutual adhesiveness of tumor cells also seems to be a significant factor in the solid tumor formation. This is shown by some of the tumor cells which play an important role in the fusion of omentous nets: they join with the nets by their adhesiveness. Further, some fibrinous materials seem also to play a part in the fusion of nets. These fibrinous materials are possibly regarded as derivatives from red corpuscles, or as derivatives from the ascites.

It is remarkable that tumor cells invading the omentum undergo rapid proliferation. Their mitotic rates often show a maximum similar to that found in the ascitic phase, showing a corresponding feature between the solid tumor and the ascitic tumor. The proliferation of tumor cells in the pancreas and their gradual destruction of the acinar cells are also of interest. With the multiplication of tumor cells in the pancreas, the gradual disorganization of the pancreatic tubules occurs, and this is followed by the complete disappearance of the acinar cells. However, no degradation product appears. The fate of the acinar cells in this case is left as a subject for further investigation.

Summary

The processes of the solid-tumor formation were studied in the omentum of the rat with intraperitoneal inoculation of the MTK-sarcoma III, an ascites tumor. The results are summarized as follows :

1) The metastatic invasion of tumor cells into the omentum from the ascites takes place directly through the mesothelium of the omentum, dependent on the mobility of tumor cells.

2) Then the tumor cells undergo a repeated multiplication. The mitotic frequency of tumor cells was found closely identical to that observable in the ascitic phase. Thus the omentum is expanded through the accumulation of a large number of tumor cells.

3) The omentum nets containing tumor cells come together in several places, resulting in the formation of large masses of solid tumor. Certain tumor cells and fibrins seem to take part in this fusion.

4) Then there often occurs the formation of a sinus which is formed after the rearrangement of tumor cells.

5) Almost simultaneously, tumor cells invade the blood vessels and the pancreas.

6) Following the development of solid tumors, necrotic tumor cells appear in the inner and outer parts of the mass. The larger part of the solid tumor, at the death of the host, comes to be made up of necrotic tumor cells.

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Explanation of Plate XXIV

Fig. 1. *Omentum gastrolienis*, from a normal rat. A large milky spot is shown by an arrow. ×40.

Fig. 2. *Omentum gastrolienis*, on the 1st day after transplantation of the tumor into the peritoneal cavity of rat. ×40.

Fig. 3. An invading tumor cell. ×40.

Fig. 4. Many leucocytes crowding around the invaded tumor cells ×400.

Fig. 5. Omentum expanded with tumor cells and the independent area, on the 2nd day after inoculation. ×40.

Fig. 6. Rapid multiplication of tumor cells invaded into omentum, on the 2nd day after inoculation. Six dividing cells are seen in this focus. ×800.

Fig. 7. A magnified view of the region outlined by the upper square in figure 5. Notice the fibrins between the tumor cells. ×800.

Fig. 8. A magnified view of the region outlined by the lower square in figure 5. The transformation of red corpuslces into fibrins and their possible role in the formation of the solid tumor. ×800.

Explanation of Plate XXV

Fig. 9. Some nets fused with each other, forming large solid tumor. ×40.

Fig. 10. Passage of some tumor cells into a blood vessel. ×800.

Fig. 11. The fusion of omentum nets. ×100.

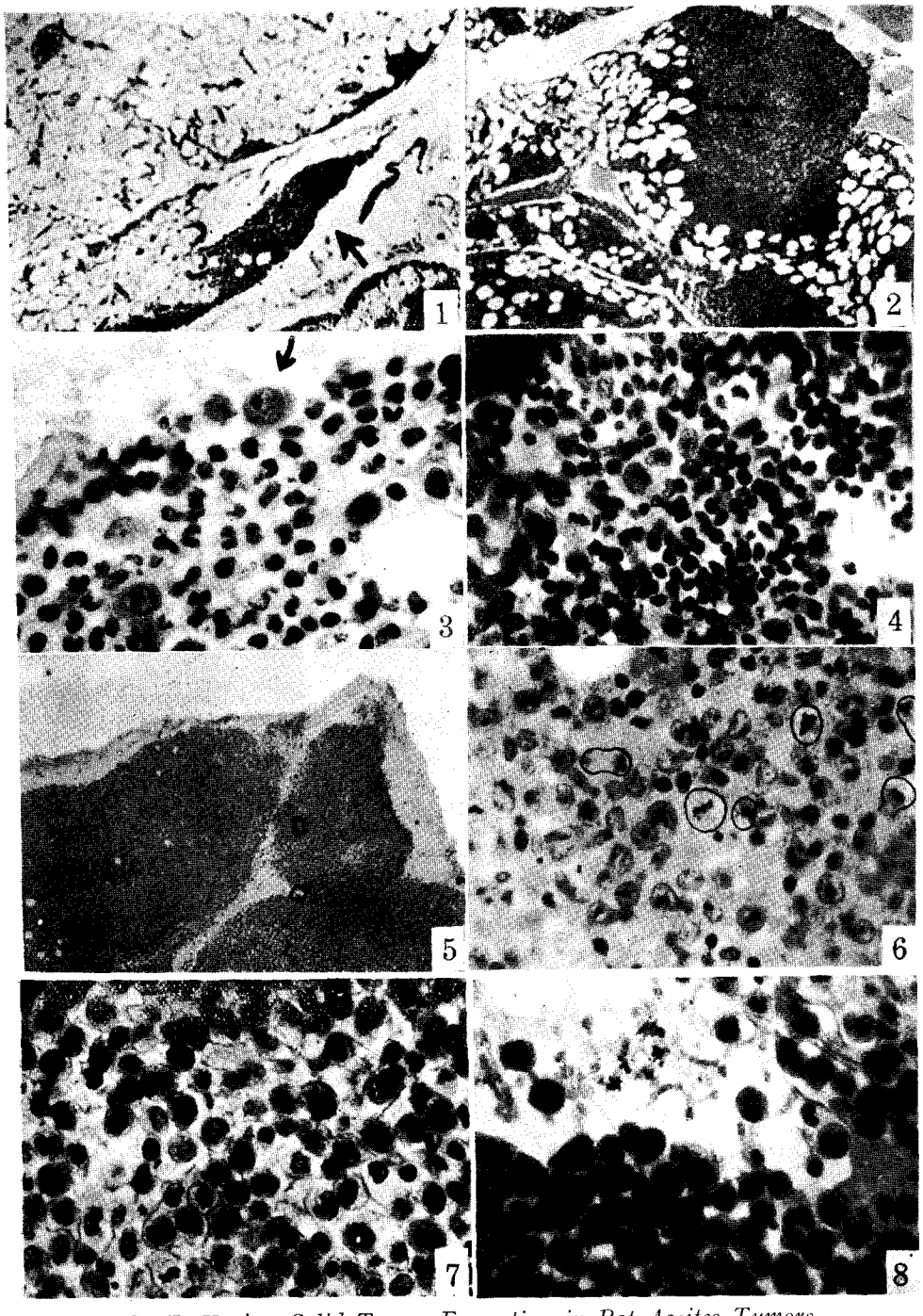
Fig. 12a. Regions contained developing necrotic areas. ×40.

Fig. 12b. Higher magnification of the area outlined in figure 12a, showing a boundary between the necrotic and the non-necrotic areas. ×400.

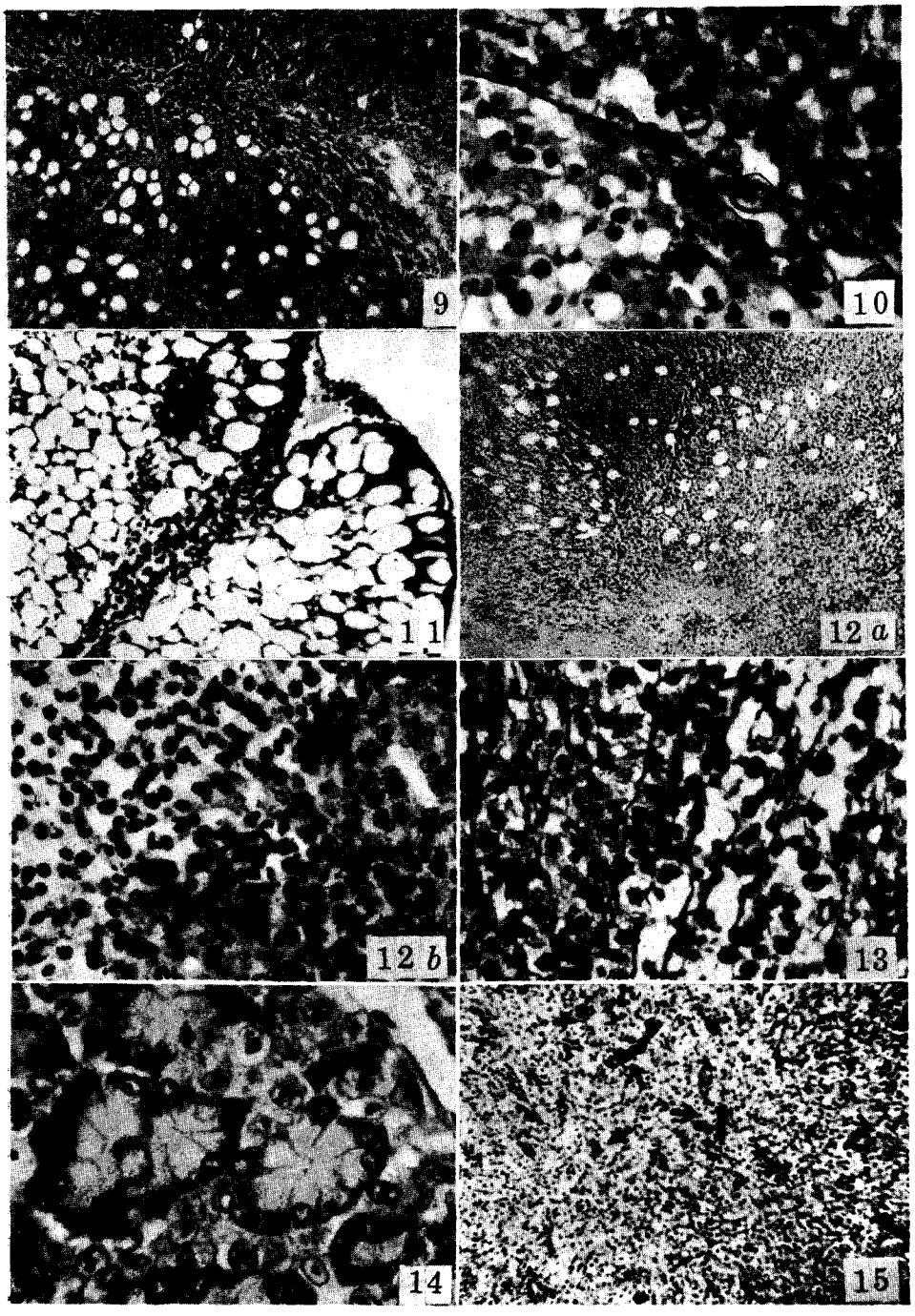
Fig. 13. Outer necrotic layer of the solid tumor. ×100.

Fig. 14. Invasion of the tumor cells into the pancreas, on the 4th day after inoculation. ×800.

Fig. 15. Disorganization of the pancreas caused by tumor proliferation. Damaged acini are shown by arrows. ×100.



S. H. Hori : Solid Tumor Formation in Rat Ascites Tumors



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