The Solid Phase of the Ehrlich Ascites Tumor in Mice

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The Ehrlich mouse ascites tumor has become one of the widely used experimental cancers, particularly, as pointed out by Klein (6), in those studies dealing with quantitative aspects of tumor biology. This tumor has been studied extensively by a number of previous investigators. Loewenthal and Jahn (11) found that intraperitoneal inoculation with organ brei containing the Ehrlich carcinoma produced not only a carcinomatous peritonitis, but also an ascitic fluid rich in free neoplastic cells. These remained infective despite dilution and various types of deleterious treatment. The cytology of this fluid cancer has been described in Seeger’s monograph (17), by Love, Koprowski, and Cox (12), and by Sugiura (19).

Formation of an ascitic fluid rich in neoplastic cells is not the specific property of the so-called ascites tumors. Warren and Gates (20) were able to produce this condition in a variety of tumors by grinding the solid form, resuspending the fragments, and injecting intraperitoneally the cell-containing fraction. The cell-containing ascitic fluid which resulted from this type of injection was then given intravenously to new hosts, and the fate of the tumor cells followed. More recently, Klein and Klein (7) were similarly able to convert the Krebs carcinoma into an ascites tumor.

Under favorable conditions the Ehrlich tumor readily forms metastases. Schairer (15), for example, has observed metastasis formation in the lungs, and Schmidt (16) has found them in regional lymph nodes after subcutaneous injection.

This earlier work, as well as that of Lettré (9) and Klein, Kurnick, and Klein (5) indicates that, after intraperitoneal inoculation, the Ehrlich ascites tumor grows rapidly and produces an ascitic suspension rich in malignant cells. The tumor is relatively nonspecific and can be maintained in most strains of Swiss mice. It also grows in rats and can be serially transplanted as long as the cell samples are taken before progressively increasing host resistance causes their deterioration (10).

The rate of accumulation of both cells and fluid is directly related to the number of inoculated cells (5, 19). When the size of the inoculum is kept constant, it is possible to predict with considerable accuracy the length of time which mice of a given strain can be expected to survive (9). There are several reports concerning the effects of various substances on tumor growth rate. Koprowska and Koprowski (8) have used viruses as test materials; Lewis and Goland (10) have employed dyes; and Sugiura (19), chemicals from a wide variety of classes.

Although the Ehrlich ascites tumor grows primarily as a cell suspension in ascitic fluid, solid tumors also appear in the same animals. Owing, perhaps, to a preoccupation with quantitative studies on the tumor suspension, the solid phase of the neoplasm has not been thoroughly investigated. An attempt has been made in the present study to supply some of the missing facets in its biology, including a possible relationship between solid and fluid tumors in the same animal.

MATERIALS AND METHODS

One hundred and forty CFW mice were each inoculated intraperitoneally with 10-15 million tumor cells in a volume of 0.1-0.15 cc. The number of cells used in each case was determined by counts made on the fluid in a blood cell counting chamber. Of these mice, 43 were inoculated with the tumor alone, while 85 were given both tumor and varying amounts of colloidal thorium dioxide (Thorotrast, Heyden), to determine if it would alter the pattern of solid tumor formation. Of those receiving tumor alone, 38 survived 10 days or more and were used in subsequent histological studies. Sixty-four of the second group survived 10 days or more and were used for microscopic studies.

After inoculation, ascites became apparent in most cases within 5-7 days, and the animals exhibited marked ascites by 10-12 days. In four of the mice no ascites developed. In each case, howe counter, wet smears obtained by imprint of the omentum show the presence of typical tumor cells, indicating that inoculation of tumor cells was made and that the cells persisted throughout the experimental period.

Approximately one-half of the mice were allowed to die spontaneously; the other half were sacrificed on the 10th postinoculation day. In both cases, the ascitic fluid was drained as completely as possible with the aid of a hypodermic needle, and each animal subjected to detailed autopsy. Special search was made for solid tumors, especially those appearing in structures related to peritoneum. The volume of the ascitic suspension was measured, and a sample was used for making cell counts. A portion of the fluid was centrifuged, and the supernate discarded, the cell mass was resuspended in several
volumes of Bouin's fluid and recentrifuged. The resulting concentrate was treated as an ordinary tissue specimen. Observations were also made on cells in the fresh ascitic fluid suspension with the phase microscope.

The principal organs were examined grossly and samples removed for histologic study. After fixation in Bouin's fluid or 10 per cent formalin, sections were stained in hematoxylin and eosin; check slides were also prepared using Mallory's triple connective tissue stain. Studies on stromal pattern were made according to Snook's modification of the Bielschowsky technic (18).

Toxic symptoms in the animals. Of the mice inoculated with the tumor alone, some died as early as the 9th day, while others lived as long as 92 days. The average survival time was 15.0 days. In the series which received both tumor and colloid, the range of life-span was 8-21 days, with an average of 13.2 days.

Of the 102 animals which survived 10 days or more after inoculation, 95 developed at least one solid tumor; most were small in size and frequently hidden by abdominal organs. Of the mice inoculated with tumor alone, 92 per cent developed solid tumors; 91 per cent of those with both tumor and colloid developed solid tumors. Intraperitoneal fibrin clots permeated with tumor cells were invariably found in mice with solid tumors and in one instance where no solid tumor could be found. Previous studies on survival times and on percentages of solid tumors have been carried out by Loewenthal and Jahn (11) and by Lettré (9). Particularly in the latter work, it has been shown that survival time, changes in total body weight, and volume of ascitic fluid are accurate indicators of cell growth rate.

The basic type of free cell characteristic of the Ehrlich ascites tumor has been carefully studied and described by Seeger (17). It suffices here to note that these cells are essentially spherical and contain relatively large, highly chromatophilic nuclei with one or more prominent nucleoli. The nuclear form is variable, ranging from a sphere to a lobulate condition simulating that seen in the megakaryocyte. The cytoplasm stains lightly and may contain achromatophilic vacuoles. Signet cells were frequently encountered.

The cytologic features of tumor cells studied in fresh state with the phase microscope were compared with those in stained preparations from cell concentrates and solid tumors. Such studies revealed no essential differences between cells secured from the three sources mentioned above. The characteristics of solid tumor cells varied somewhat depending on the region in which they grew, but still resembled those cells seen in the free state. Large solid tumors had regions of internal necrosis where cell study was impossible. The remaining parts, however, were composed of cells of a single fundamental type similar to the free tumor cells. Cells lying deeply within solid tumors were smaller than those more peripherally placed counterparts; the cytoplasm of the former element was more acidophilic and less burdened with vacuoles than the latter (Figs. 2, 4-7).

The more superficially placed regions of solid tumors were typically composed of cells that were intermediate in form between deeply lying tumor cells and those in ascitic suspension. There was, however, great variation in diameter, even within the same tissue region (e.g., Figs. 2, 8).

By selecting suitable regions of the peritoneum for microscopic study, it was possible to follow all phases of solid tumor development. In most cases, the center of a developing solid tumor was marked by small, highly chromatophilic tumor cells of variable shape, collected in the perivascular connective tissues (Fig. 2). At some distance from this focus, the tumor cells tended to be larger in diameter and scattered through the loose connective tissue (Fig. 1). When such tumor cells came into relationship with the peritoneal mesothelium, the latter became perforated and later disappeared over considerable areas (Figs. 3, 7). It was in such denuded areas that fibrin clots invariably appeared.

It soon became apparent from these observations that the solid phase of this tumor was represented by two rather dissimilar types of growing substance. In one instance the tumor developed within the stroma of various organs, while in the other it carried out the same activities while imbedded in a fibrinous feltwork formed by a localized intraperitoneal clot. The first type might be termed the true solid tumor, while the second was the semisolid or gelatinous tumor.

Semisolid tumors were most frequently encountered in the angles of peritoneal attachment, folds of the mesentery, and in the greater and lesser omenta. They were invariably attached to and imbedded in the peritoneum. Such tumors were plastic in character but of sufficient solidity that with care they could be handled in the same manner as ordinary tissue specimens. Histologically, such tumors were rich in cells identical with those in the ascitic suspension.

Solid tumors were encountered subcutaneously, intramuscularly, and subperitoneally in the anterior abdominal wall. In these locations inception was probably due to direct transfer of tumor cells from the inoculating needle, or to cell reflux through the wound. Structurally similar
tumors appeared within the pelvic fat mass, mesenteries, omenta, and inferior surface of the diaphragm. To some degree, these tumors assumed the pattern of the tissue in which they appeared, but the invading cells tended to develop as solid masses. These were occasionally tunneled by pseudotubules which simulated glands (Fig. 8).

Microscopic studies were made on the principal organs. This was done not only to locate metastases but also to determine any histologic change which might appear consistently during development of the fluid tumor.

Spleen.—No metastases were found in the pulp. Solid tumors were common, however, in the capsule and its peritoneal ligaments (Fig. 4). During the postinoculation period, the red pulp became progressively congested by an accumulation of hematopoietic and free tumor cells. The white pulp gradually decreased in volume owing to loss in thickness of the lymphatic sheaths which surrounded its blood vessels.

Liver.—The liver parenchyma became progressively shrunken during the postinoculation period. In occasional cases, these cells developed vacuoles of various sizes. Isolated tumor cells almost invariably appeared within the sinusoids, and emboli were often seen (Figs. 9, 10). Some of these emboli were of considerable size, but they never developed into growing metastases, even in animals which survived as long as 20 days after inoculation.

Peritoneum, mesenteries, and omenta.—As indicated above, these regions were commonly involved in solid tumor formation. It was not clear whether the origin of such tumors was invariably from free cells brought in via the blood vessels or whether occasionally there was direct invasion through the mesothelial covering. The mesentery, in particular, could develop large (up to 7 mm. diam.) solid tumors within 15-20 days after inoculation.

Lymph nodes.—Lymph nodes of the superior mediastinum and mesenteric root enlarged grossly during growth of the ascites tumor. Histologically, these nodes contained free tumor cells which were particularly prominent in the medullary regions (Figs. 11, 12). Early metastases were occasionally seen. These findings are in accord with those of Schmidt (16), who studied this reaction in regional nodes after subcutaneous inoculation of the tumor substance. There was a marked reticulo-endothelial reaction in nodes; this was indicated by hypertrophy and vacuolation of the sinusoidal lining elements.

Lungs.—The lung was little altered within 10 days after intraperitoneal inoculation of the tumor. There was a small amount of perivascular infiltration which may indicate initiation of metastasis formation, but even in mice which succumbed after 20 days no frank metastases were seen (cf. 15).

Diaphragm.—This organ almost invariably had at least a few small, nodular solid tumors on its peritoneal surface. The connective tissue was typically infiltrated so as to produce localized compression of the muscle fibers, sometimes converting them into homogeneous, acidophilic strands.

The red bone marrow and adrenal glands were searched for solid tumors with no success. Occasional free tumor cells were encountered within their sinusoids, but the nuclei were pyknotic, and the cells appeared to be disintegrating.

DISCUSSION

It is apparent that solid tumors develop in a high percentage of cases after routine intraperitoneal inoculations of ascitic fluid. This observation has been made previously by a number of workers, including Loewenthal and Jahn (11) and Lettré (9), but much still remains to be learned concerning the mechanism of solid tumor formation in this cancer and the relationships between the solid and suspended phases of the neoplasm.

Jones and Rous (4) found that various types of physical injury to the peritoneum made this membrane more susceptible to implantation with tumor. It has been previously shown by Baillif (1) that, after intraperitoneal injections of colloidal thorium dioxide, the salt is in part collected as nodular masses within the submesothelial tissues of the peritoneum. The histologic reaction to the thorium salt is similar to that to the lycopodium spores used by Jones and Rous. However, solid tumor formation in these mice did not appear to be enhanced by the presence of the thorium-containing nodules. Regional survey of the peritoneum indicated rather that implantation was most prone to occur in regions where fluid stagnation can take place, i.e., where tumor cells might remain in contact with peritoneum for considerable periods of time.

Sampson (14) has described in some detail the reaction of the peritoneum to implantation of tumor cells. It is his belief that the development of such secondary tumors is associated with and dependent on formation of a stroma by the host. The neoplastic elements are able to use this stroma as a substrate for growth and invasion of the host organism. Sampson also described the formation of new lymph vessels within the affected areas of peritoneum. He believes that such ves-
sels facilitate the escape of tumor cells into the lymphatic system of the host.

In the same manner, the development of ascites tumor is associated with production of inflammation and development of granulation tissue in the peritoneum. Invading tumor cells advance through the submesothelial connective tissue spaces to extend the solid tumor (Fig. 1). In regions where this is taking place, there is gradual loss of the mesothelial covering, allowing direct continuity to be established between the tissue spaces and peritoneal cavity.

These denuded regions of peritoneum, however, do not typically remain naked; a precipitate of fibrin appears and in some instances, at least, builds up a bulky tumor of semisolid consistency. This meshwork of fibrin threads becomes permeated with tumor cells. Enterline and Comans (2) have shown that a wide variety of tumor cells are motile in tissue culture, this movement taking place by ameboid activity. They have also found that small groups of cells are able to move as single physical units. While no direct analogy can be made between physiological conditions of in vivo and in vitro experimentation, the histologic pattern of tumor cells found in both solid and semisolid tumors indicates that migration may take place. Solid tumors are composed primarily of relatively small cells whose contours are molded by contiguity with their neighbors. Within semisolid tumors, the cells are larger, adspherical, and less crowded. Free cells in suspension are still larger and essentially spherical in form.

As indicated above, these experiments offer no direct proof of cell migration from solid or semisolid tumors into ascitic fluid. That some such process may take place, however, is indicated by Goldie and Felix (3), who state: "It is common knowledge that the presence of abdominal tumors in cancer patients and the growth of peritoneal implants in laboratory animals may be associated with the presence of free tumor cells in the peritoneal fluid" (p. 73).

Histologic examination of various organs in these tumor-bearing mice indicated that metastasis occurs, often within 10 days after inoculation. Formation of metastases has been studied previously by Schmidt (16), who centered his attention on lymph nodes, and by Schairer (15), who studied the lung. The mechanism of transport of such cells by the lymphatic system is well known. These observations, as well as those of Schmidt (16), indicate that the lymphatic reticuloendothelium is unable to effect complete destruction of the tumor cells and that some escape into the blood-vascular system for further distribution.

Ascites tumors have been rather extensively used as a quantitative tool in estimating the rate of tumor cell growth under various conditions, for example by such workers as Klein (6), Lettré (9), and Patt, Blackford, and Drellmeier (13). In making such estimates the experimental method is subject to a number of variables which may affect the totals obtained. In addition to the inherent technical errors, evidence has been presented above which indicates that growth of tumor cells in ascitic fluid does not take place within a closed system. At present we have no usable quantitative method for determining the number or percentage of cells added to ascitic fluid by escape from solid and semisolid tumors. Similarly, it is impossible to quantitate the degree of cell loss via lymphatic channels which drain the peritoneal cavity. It would be still more important if a reliable method could be worked out to determine whether there is a marked individual variability in the types of cell transfer just mentioned. Notwithstanding these difficulties, the workers in quantitative tumor biology should be aware of these variables as well as the physiologic factors concerned in their production.

SUMMARY

After the inoculation of 10–15 million Ehrlich ascites tumor cells, over 90 per cent of the mice developed solid tumors. Whenever these tumors lay in tissues adjacent to the peritoneum, there was established a region of direct continuity between these tissues and the peritoneal cavity. Particularly in regions of fluid stagnation within the peritoneum and of mesothelial denudation, semisolid tumors appeared. These masses were rich in tumor cells imbedded in a fibrin stroma. It is at least possible that the solid and semisolid phases of this tumor contribute cells which augment the number which appear in the ascitic suspension. The histologic identification of tumor cells in various viscera distant from the peritoneum indicates that tumor cells escape by way of the lymphatic drainage from this chamber and, in some locations, initiate metastases.

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REFERENCES


The specimens were obtained from mice at autopsy 10 days after intraperitoneal inoculation of the Ehrlich ascites tumor; they were fixed in Bouin's fluid and stained with hematoxylin and eosin.

Fig. 1.—A fold of tissue from the pelvic fat mass where the solid tumor is becoming established. At the right, the mesothelium has been destroyed and the denuded area is covered with fibrin clot which is richly infiltrated with tumor cells. ×102.

Fig. 2.—Region near the surface of the greater omentum showing a developing solid tumor. Small tumor cells abound in tissues adjacent to the dilated blood vessels. There is variation in the size of tumor cells within the small area of tissues seen here. ×375.

Fig. 3.—Surface of the greater omentum in a region where there is localized destruction of the mesothelium. The arrows point toward the omental surface. The upper right portion of the figure is composed of fibrin impregnated with tumor cells, while other cells lie on the surface of the clot. Note the variation in size of the tumor cells within the omentum proper (i.e., in solid tumor), in the meshes of the clot (semisolid tumor) and on the surface of the clot. ×375.

Fig. 4.—Solid tumor attached to the capsule of the spleen. Splenic red pulp comprises the left half of the figure and solid tumor the right half, while the fibrous capsule runs diagonally downward and toward the right between the two regions just delineated. ×375.

Fig. 5.—Solid tumor in fat of the mesentery. Note the density and pattern of the tumor cells as they invade the connective tissue between fat cells. ×281.

Fig. 6.—Solid tumor which has invaded the pancreas and largely destroyed its acini. ×281.
Fig. 7.—Solid tumor in a fold of the gastro-hepatic omentum, a region where there is destruction of the covering mesothelium. Solid tumor comprises the dense tissue at the lower side of the figure; most of this region is covered with a fibrin meshwork. The upper and right regions show tumor cells which are either entirely free or partially imbedded in fragments of the fibrin net. \( \times 375 \).

Fig. 8.—Solid tumor beneath the parietal peritoneum of the anterior abdominal wall. Note the density of the cells and variety in size. Near the top of the figure is a cell mass suggestive of pseudotubule formation. \( \times 1,086 \).

Fig. 9.—Liver with tumor cells lying free within the sinusoids. A mass of these cells has collected in one of these dilated spaces; this region is outlined with black lines. Elsewhere, the tumor cells appear to be traversing the channels more or less singly. \( \times 210 \).

Fig. 10.—Higher magnification of the area outlined in the preceding figure, to show relationships of tumor cells in the hepatic sinusoids. \( \times 411 \).

Fig. 11.—Lymph node medulla with free tumor cells. The lighter regions represent the nodal lymph sinusoids, variably filled with tumor cells. The darker regions are medullary lymphocytic cords, considerably fragmented by collections of tumor cells within the sinusoids. \( \times 210 \).

Fig. 12.—Lymph node medulla from a specimen similar to that shown in the preceding figure. The higher magnification clarifies the relationships between tumor cells and medullary cords. \( \times 411 \).

Fig. 13.—Lymph node medulla. A dense tumor mass is seen occupying part of the cortex. The medullary sinusoids are less prominent. \( \times 411 \).
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