A Spontaneous Ascites Tumor Originating and Transplantable in the Wistar Rat*

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In an attempt to find a spontaneous tumor which would be transplantable in a non-inbred strain of rats as widely used as the Wistar strain, the authors have, in the course of the past several years, transplanted a number of spontaneous tumors originating in Wistar rats. None of these transplants, however, survived the first passage.

Recently, an adult Wistar rat with a spontaneous ascites tumor was brought to the attention of the authors. Studies on the transplantation and characteristics of this tumor form the basis for this report.

In studies of problems of aging in the Wistar Institute colony, Farris and Yeakel (2) found several spontaneous tumors which were diagnosed as reticulum-cell sarcomas, accompanied by an accumulation of fluid in the peritoneal cavity. With the exception of the well known Yoshida sarcoma (9, 10) which may have been of spontaneous origin, a search of the literature has not revealed the existence of a spontaneous ascites tumor originating in a rat that has been successfully transmitted serially from the original host as an ascites tumor. The possibility that the Yoshida sarcoma may have resulted from feeding with o-amidoazotoluol followed by cutaneous application of potassium arsenite solution (9) must be considered. It is stressed, therefore, that the rats in the Wistar colony are not subjected to treatment with chemicals or carcinogens. Their normal diet consists of the standard Purina Dog Chow, ad libitum, with no supplements.

**MATERIALS AND METHODS**

An adult female Wistar rat, 7 months and 11 days of age, with a distended abdomen was etherized, and approximately 50 ml. of ascitic fluid was withdrawn by puncturing the abdominal wall with a 13-gauge trocar. One ml. of this fluid was inject-
ed intraperitoneally into each of two 30-day-old rats (taken at random) from the Wistar colony.

Exposure of the abdominal cavity revealed that the mesentery, omentum, and fatty tissue surrounding the ovaries and uterine horns, as well as other fatty tissues in the pelvic cavity, were studded with nodular tumors. Several of the nodules were excised, minced with a scissors, and then transplanted subcutaneously into three Wistar rats.

In the serial passages, a standard inoculum of 1 ml. of the ascitic fluid and a full 13-gauge trocar of the solid tumor tissue was used. All rats used in this study were from 30 to 32 days old. To determine whether sex would subsequently influence susceptibility to the ascites tumor, two parallel passages were carried out: one in which ascitic fluid was transmitted from male to male, and the other from female to female, rats. This method of transmission is being continued and may further elucidate the role of sex in the transplantability of the ascites tumor. On the other hand, the solid tumor tissue was and still is being transplanted subcutaneously without taking into consideration the sex of the animal.

**RESULTS**

Two hundred and thirty rats of the Wistar strain were used in the ten serial passages of the ascites tumor. As is indicated in Chart 1, while the percentage of successive intraperitoneal transmissions gradually rose from 50 per cent (one of two rats) in the first passage to 82–95 per cent in the fifth to the tenth passages, the survival time following implantation of the ascites tumor declined from a mean of 80 days in the second passage to 18.5 days in the tenth passage. Survival time in the first passage could not be determined, since the animal was sacrificed for transmission of the ascitic fluid. This trend toward the higher percentage of lethal takes and decrease in survival time may be considered an indication of increased viability or adaptability of the tumor. Once tumor growth was evident, no spontaneous regression of this tumor has been observed.
Solid tumor tissue from the original rat transplanted subcutaneously into three rats grew in only one animal in the first passage. In subsequent passages, successful transplants increased gradually from 33 to 80 per cent in the sixth passage. Sixty-seven rats were inoculated during the six passages.

When the ascitic fluid from a tumor-bearing rat in the sixth passage was injected subcutaneously into fifteen 30-day-old Wistar rats, fourteen rats (93 per cent) developed lethal subcutaneous tumors. This constituted the seventh transplant generation and can be compared with the same intraperitoneal passage, which gave 90 per cent lethal takes; there appears to be no significant difference in the growth potential between subcutaneous and intraperitoneal implantation of the ascites.

When solid tumor tissue from a rat in the third subcutaneous passage was inoculated into the peritoneal cavity of fifteen 30-day-old Wistar rats, twelve rats (80 per cent) developed lethal subcutaneous tumors with formation of ascites. This constituted the fourth transplant generation of the solid tumor, and, when compared with the same subcutaneous passage which gave 80 per cent lethal takes, there appears to be no significant difference in the growth potential between intraperitoneal and subcutaneous implantation of the solid, subcutaneously carried tumor.

Comparing the lethal takes of the ascites with the solid subcutaneous tumor in the first seven passages, we find that the mean percentage of lethal takes of the ascites tumor is 83 (125 rats), as compared with 67 per cent (67 rats) of the solid, subcutaneously carried tumor.

The results obtained in experiments to determine the effect of sex on susceptibility to ascites tumor transmission in the first ten serial intraperitoneal passages (Table 1) reveal no significant variations in lethal takes in the male and female lines, as well as in males and females within the individual lines. Survival times of males and females within the individual lines also show no significant variations. On the other hand, while the mean survival time of the male line is only 11.7 days, the mean survival time of the female line is 17.7 days. Using the t test for statistical analysis, it is evident that the 6 days' difference in survival time between the male and female lines is highly significant.

To determine the optimal time for transmission of the ascites, a study of the ratio of malignant cells to normal cells at various intervals following injection of ascitic fluid was made in the fifth passage, and it was found that the highest ratio of malignant to normal cells was 2.0. This occurred at about 10 days following implantation of the ascitic fluid. However, the mean of the highest ratios from the sixth to the twentieth transplant generation was 13, occurring at a mean of 7.6 days after implantation of the ascites. Microscopic examination of ascitic fluid from rats bearing 10-day-old ascites tumors, fixed, stained, and dehydrated according to the DeLamater technic (1), indicated that approximately 10–12 per cent of the tumor cells were in mitosis (Figs. 1 and 2).

Microscopic examination of the ascitic fluid and some of the abdominal organs of a rat in the fourth passage, and of the solid subcutaneous tumor of a rat in the third passage, revealed the following:

**Smear of ascitic fluid (Giemsa stain).—**There were numerous cells ranging in size roughly from 10 to 25 μ in diameter. They were intensely basophilic, with a relatively scanty cytoplasm. The nuclei were round or oval, with an occasional indented nucleus. There were many mitotic figures, with
mitosis taking place in cells of various sizes. Monocytes and lymphocytes were also present.

Solid tumor (H & E stain).—The tumor was made up of densely packed cells without any apparent structural arrangement, contained in a scanty fibrovascular stroma. The individual cells appeared polygonal in shape, with large nuclei and a scanty cytoplasm. The nuclei were round or oval, with an occasional indented nucleus. There were many mitotic figures and occasional areas of lymphatic infiltration as well as areas of necrosis. Scattered throughout the tumor mass were cells in various degrees of degeneration, most of them showing pyknosis or karyorrhexis. It was practically a pure culture of tumor cells. The over-all appearance of the tumor is suggestive of a sarcoma (Fig. 8).

Liver, spleen, lung (H & E stain).—There was no evidence of tumor cell infiltration or metastasis.

DISCUSSION

Flexner and Jobling (3) were among the first to produce abdominal tumors by injecting ascitic fluid from a rat bearing an induced abdominal carcinoma. Hesse (3), in 1927, produced tumors in the peritoneal cavity of rats with the formation of ascitic fluid by the injection of a saline suspension of Flexner-Jobling rat carcinoma tissue, and called these induced tumors "experimentelle Peritonealkarzinose." Recently, Tanaka and Kanô (8), following the method of Susaki and Yoshida of producing hepatomas in rats by the use of azo dyes, obtained two strains of ascites sarcoma in white rats, which they claim were essentially similar in nature to the Yoshida sarcoma.

In view of the rare occurrence of spontaneous tumors in rats, and recognizing the many advantages an ascites tumor presents over solid tumors for certain phases of cancer research, Goldie and Felix (4) and Klein and Klein (6, 7) have made many successful attempts to transform solid tumor tissue to the ascites growth form. Goldie and Felix produced growth of free tumor cells in the peritoneal fluid with Sarcoma 37 and a malignant lymphoma in mice. They made a thorough investigation of the specific biological characteristics of the free tumor cells. Klein and Klein transformed solid Krebs-2 carcinoma, after serial intraperitoneal passages, to an ascites tumor in mice, and studied the growth characteristics of the free cells in comparison with the Ehrlich ascites tumor cells, as well as the mechanism of the transformation from the solid to the ascites growth form.

At the time that this study was being elaborated, the authors were fortunate to have Dr. Tomizo Yoshida and Dr. Kyo Kaziwara visit their laboratories. After autopsy of one of the tumor-bearing Wistar rats and microscopic examination of smears of the ascites and solid tumor tissue, they identified the neoplasm as a typical ascites tumor, as differentiated from carcinomatosis, i.e., a condition wherein exfoliated cancer cells are present in pleural and peritoneal exudates in advanced malignancies.

It is interesting to report that every normal Wistar rat given intraperitoneal inoculations of ascitic fluid, and which has subsequently developed an ascites tumor, shows the same abdominal involvement with nodulous tumor growth as observed in the animal bearing the original ascites tumor.

SUMMARY

1. A spontaneous ascites tumor which originated in a Wistar rat is transplantable in the same strain of rats. The rate of lethal takes of the ascites increased from 50 per cent in the first passage to 90 per cent in the tenth serial intraperitoneal transfer, and the survival time of the rats decreased from a mean of 30 days in the second passage to 13.5 days in the tenth passage.

2. There appears to be no significant difference in the growth potential, based upon the ability to produce lethal takes, between subcutaneous and intraperitoneal implantations of the ascites or the solid tumor.

3. Two parallel passages of the ascites tumor are being carried out: one in which the ascitic fluid is transmitted from male to male, and the other from female to female rats. In the first ten serial intraperitoneal passages there were no significant variations in lethal takes, but there was a significant prolongation of 6 days in the mean survival time in rats of the female line as compared with those of the male line.

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REFERENCES


Fig. 1.—General view of the ascites from a rat bearing a 10-day ascites tumor, showing many mitotic figures. Variability in the size of the tumor cells is illustrated. DeLamater, ×600.

Fig. 2.—High-power magnification of Figure 1. DeLamater, ×1,500.

Fig. 3.—Histological section of solid, subcutaneous tumor, from the third passage. H & E ×800.

Fig. 4.—Abdominal organs of an ascites tumor-bearing rat exposed to show the involvement with nodular growths. The solid tumor on a flap of the abdominal wall resulted from seepage of ascitic fluid at the point of injection.
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