TRANSPLANTABLE STRAIN OF RAT SARCOMA TW-2357

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A new strain of rat sarcoma (TW-2357) is described. This strain was obtained for the first time in a Wistar rat infected in the neonatal state with virus of fowl erythroblastosis. The strain consists of a spindle-cell sarcoma with polymorphism of its cellular elements. Attempts to detect the genome of fowl erythroblastosis virus in the tumors were unsuccessful. The role of fowl leukosis virus in the origin of the rat tumor is discussed.

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Among viruses which, under natural conditions, induce tumor development in animals, the fowl leukosis viruses are particularly widespread. The great variety of methods of transmission of these viruses in nature, and their frequent occurrence in material used in virologic practice, especially in vaccines prepared in chick embryos or chick embryonic tissue, make the study of the interaction between these viruses and animals of heterologous species a matter of necessity.

In experiments in which newborn Wistar rats were inoculated with fowl erythroblastosis virus, the development of tumors was observed in some of the rats in the second year of life [1, 2]. Since the origin of these tumors required explanation, attempts were made to obtain transplantable strains from them for further study.

The object of this investigation was to isolate and study a strain from the first tumor appearing in this experiment.

EXPERIMENTAL METHOD

The original material for transplantation consisted of a primary tumor in a rat (No. 21681) from experiment No. 2357, aged 1 year 4 months 24 days. On the 1st, 3rd, and 4th days of its life this rat was injected intraperitoneally with fowl erythroblastosis virus (strain R) in a total dose of 0.8×10^5 infectious units of virus [2]. At autopsy on this rat a huge tumor was found in the abdomen (15×11×6 cm), invading stomach and liver, and fairly soft in consistency. A small and separate tumor nodule was also present on the diaphragm. A considerable volume of hemorrhagic ascites fluid was present in the abdomen. The mesenteric lymph glands were considerably enlarged. A 20% suspension was made of the tissue of the principal tumor, freed from areas of necrosis, in physiological saline, and this was injected into a month-old Wistar rat, which developed tumors 137 days later, and these were subsequently transplanted into similar rats. In addition, tumor material from the different passages was injected into noninbred rats, F_1 hybrid rats from crosses between Wistar and noninbred rats, into $C^{57}BL$, $CC^{57}W$, and $CC^{57}BR$ mice, and into day-old chicks. Immediately after hatching out, these chicks were injected intraperitoneally with 0.5 ml of the tumor suspension, or intravenously with 0.5 ml of blood plasma from rats with tumors.

Tumors for histological investigation were fixed in 10% formalin, and sections were stained with hematoxylin and eosin.

Rat sera for immunologic investigation were first absorbed with a powder from rat and hamster livers and then tested in the complement fixation test (CFT) in the cold in a volume of 0.2 ml, and in the micromodification described by Huebner and co-workers [6]. The following were used as antigens: 1)

Laboratory of Oncogenic Viruses, Institute of Experimental and Clinical Oncology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Timofeevskii). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 69, No. 2, pp. 81-84, February, 1970. Original article submitted July 17, 1969.

TABLE 1. Intraperitoneal and Subcutaneous Inoculation of Wistar Rats with Strain TW-2357

Passage No.	Age of inoculated animals	Number of rats		Incubation period (in days)	Localization of tumor
		total number inoculated	number with tumors		
1	3 weeks	1	1	135	At site of injection
2	Newborn	4	4	45-60	The same
3	3 weeks	3	2	60 and 1001*	" "
4	Newborn	6	5	20-27	" "
5-7	3 weeks	6	6	13-15	" "
8-9	The same	4	4	13-15	" "

*Tumor in lung, probably spontaneous.

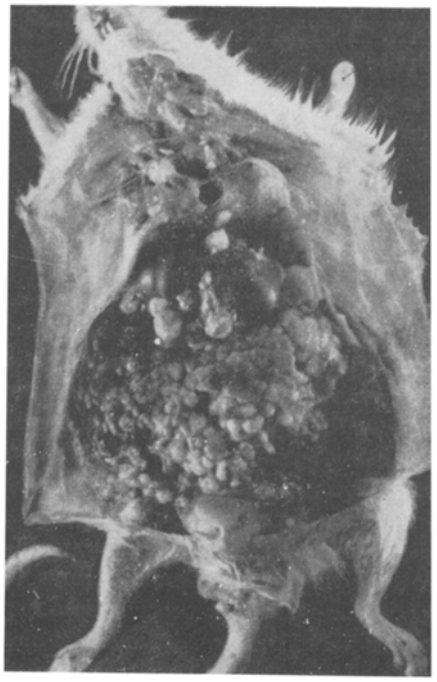

Fig. 1. Wistar rat inoculated intraperitoneally with strain TW-2357. Multiple tumor nodules in the abdomen can be seen.

transplantable tumors and ascites cells of rats; 2) tumors induced in hens by the Schmidt-Ruppin strain of Rous virus; 3) tumors induced in hamsters by Rous virus; type 12 adenovirus, polyoma virus, and OB40.* The antigens were prepared by homogenization of the solid and ascites tumors in physiological saline in proportions of 1:5 and 1:10, followed by clarification by centrifugation. Control antigens were prepared from the corresponding normal tissues and cells of aseptic ascites artificially produced by intraperitoneal injection of turpentine. The antigens were also tested in the hemagglutination reaction, and all sera of rats with tumors were tested in the delay of hemagglutination reaction with polyoma virus.

EXPERIMENTAL RESULTS

In a series of successive passages of tumor tissue a strain, subsequently called TW-2357, was obtained. This strain has now undergone twelve passages. Starting from the 5th passage the strain was stabilized, and the mean incubation period of tumor development was 13-15 days. Tumors began to develop in virtually all inoculated Wistar rats (Table 1, Fig. 1). In their histological structure, tumors of strain TW-2357 were indistinguishable from the original tumor, and consisted of spindle-cell sarcomas with a varied degree of polymorphism of their cells (Figs. 2 and 3). Strain TW-2357 was transplanted into F_1 hybrid, but further transplantation of tumors from these rats into noninbred rats, and also into mice of three different lines and newly hatched chicks, was unsuccessful. After observations for 38 days, none of the experimental birds showed signs of tumor development at autopsy.

The CFT was carried out with 71 samples of serum from Wistar rats from 9 passages of strain TW-2357. The overwhelming majority of sera were obtained from inoculated rats with large tumors and with ascites. No definitely positive result was obtained: in 62 tumors the reaction was negative and in 9 it was doubtful.

*These tumors were kindly presented by A. D. Al'tshtein and G. I. Deichman.

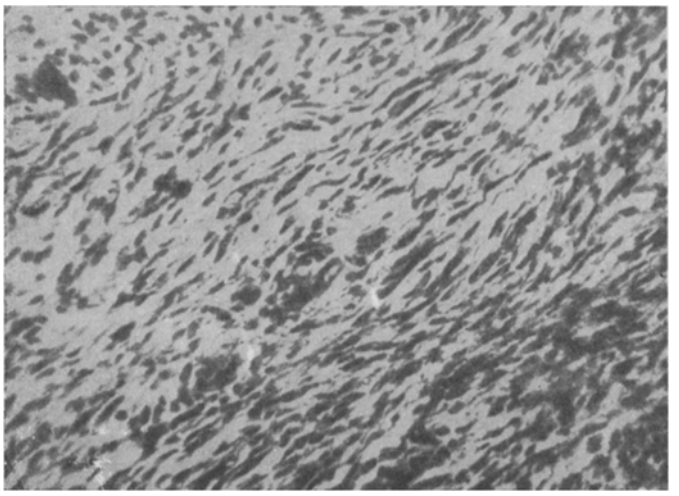

Fig. 2. Intraperitoneal tumor of strain TW-2357. Tumor consists of bundles of elongated cells with hyperchromic nuclei. Hematoxylin-eosin, 160×.

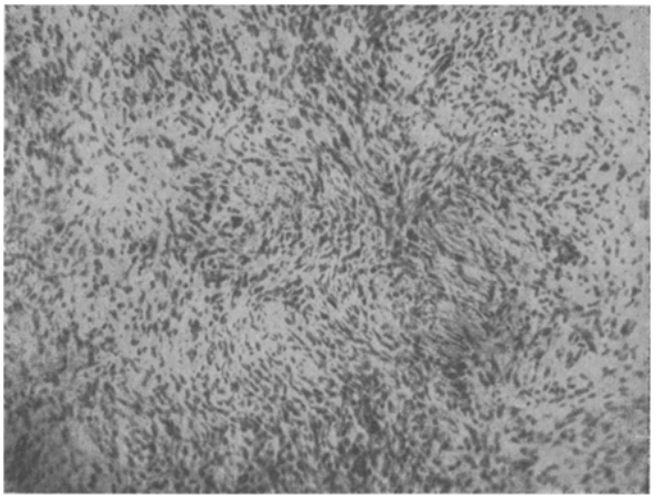

Fig. 3. Spindle-cell sarcoma in a Wistar rat inoculated with strain TW-2357. Hematoxylin-eosin, 340×.

The virus genome persists for a long time in transplantable strains derived from mammalian tumors induced with Rous virus [4, 9, 10]. These strains are very convenient for the detailed study of relationships between an oncogenic virus and cells of the heterologous organism. Accordingly, in the experiments described above, attempts were also made to obtain transplantable strains from tumors arising in rats.

An attempt to detect infectious leukemia virus or its genome in tumors of strain TW-2357 by injection of cell-free extracts from tumors, the plasma of rats with tumors, and a suspension of tumor cells into recently hatched chicks was unsuccessful. The content of virus in tumors induced in mammals by Rous virus is low and inconstant [8], and even inoculation of living tumor cells or combined cultivation of such cells with cells sensitive to the virus does not always lead to the successful detection of virus in them [3, 5].

During growth of a tumor in a heterologous organism, when synthesis of the mature virus does not take place, conditions are created for immunization of the host by the intrinsic antigenic components of the virus. For instance, antibodies against group-specific antigen of Rous virus can be found in hamsters and guinea pigs with large tumors induced with Rous virus [7]. Attempts in the present investigation to detect

complement-fixing antibodies in the sera of rats with large tumors of strain TW-2357 by the use of antigens from sarcomas of fowls and hamsters induced by the Schmidt-Ruppin strain of Rous virus, and from sarcomas of strain TW-2357 were unsuccessful. The negative results of these experiments could be attributed to the low content of these antibodies or to their complete absence. It is known that complement-fixing antibodies are formed in a sufficiently high titer in sera of hamsters with Rous sarcomas only in a certain proportion of cases [7].

It cannot therefore be concluded from these results that tumors of strain TW-2357 are connected with erythroblastosis virus. Before this problem can be finally settled, further experiments must be carried out by the immunofluorescence method and for the induction of resistance.

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