Cytological Studies on Cancer, VI. Spontaneous Tumors occurring in Inbred Mice, with Notes on the Establishment of Two New Strains of Transplantable Tumor[1)]

By

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(*With 6 Text-figures and 2 Plates*)

The transplantable nature of cancer is one of the most interesting and important problems in cancer research but it is left not fruitfully interpreted at present. Research by means of transplantation of the tumor spontaneously caused to occur is one of the avenues to approach its elucidation. Thus several investigations have been made along this line from both histological and pathological standpoints. Since 1944, the author has engaged in inbreeding experiments of mice with the support and direction of Professor Makino, in order to establish pure strains. During the course of the work the author has had several opportunities to meet with the occurrence of spontaneous tumors in mice of a certain strain which had been established and purely bred in this laboratory. This paper deals with the histological characters of these spontaneous tumors and their transplantability, with some notes on two strains of transplantable tumors newly established.

Here, the author would express his gratitude to Professor Sajiro Makino for his valuable advice and for going through the manuscript. Further thanks must be extended to Professor Katsuo Takeda of the Pathological Institute of the Medical Faculty for his important criticism.

1. Strains of tumor mice aud methods of transplantation

The number of individual mice in which tumors have developed was 11 in total; they are derivatives from three strains called D, B and So. They may be

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1) Contribution No. 275 from the Zoological Institute, Faculty of Science, Hokkaido University, Sapporo, Japan.

This article constitutes one of a series of studies carried through by S. Makino and his co-workers (S. M.).

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described briefly as follows:

D-strain : This strain was established under careful inbreeding in our laboratory, initiated in 1944. Genetical examinations revealed that the mice of this strain are practically pure in many genetical characters. One individual carrying a tumor was found in this strain.

B-strain : This started with hybrids between common albino and wild mice of agouti color. The mice have been inbred for ten generations to date. The mice of this strain are to be subdivided into several races according to their coat colors, such as agouti, black, dilute brown, spotted and albino. Five specimens carrying tumors were found in agouti mice only.

So-strain : This strain consists of the mice derived from the hybrids between the pure albinos called S-strain which were inbred pure in our laboratory and cross-bred albinos commonly found in Japan. Inbreeding of this strain has been continued for only a few generations. The spontaneous tumors occurred in five individuals of this strain.

The transplantation of the tumor as mentioned above was tried. At operation, disinfected instruments were used. The tumor was removed from the tumor-bearing animal, and cut into small pieces with fine scissors on a clean glass slide. One or two pieces of the tumor tissue were inserted into the epidermis of the dorsal side of the healthy mouse, with the aid of a transplantation-needle. The wound of operation was not closed in most cases. At two or three days after transplantation, the growth of inserted tumor tissue was externally visible when successfully implanted. But if not implanted successfully, the bit of inserted tumor became gradually small in size with time and finally disintegrated entirely.

2. Descriptions of tumors and histological observations

As mentioned above, eleven mice carrying spontaneous tumors were found in three different strains. They are designated by numbering from No. 1 to No. 11 for convenience sake.

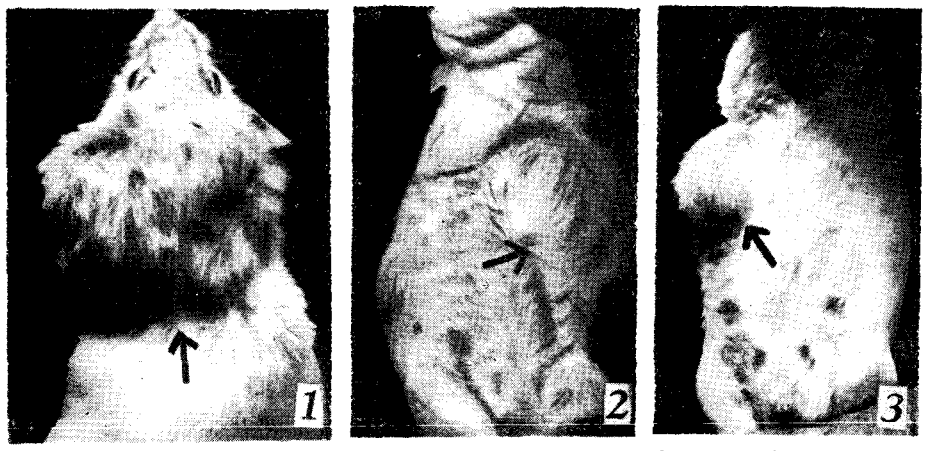
No. 1: In this case the tumor developed at the root region of left axillary of a female mouse derived from the So-strain (Text-fig. 2). The transplantations were undertaken with seven mice, but the inserted tumor pieces began to degenerate soon after transpltation in every case tried. This tumor was not microscopical observed.

No. 2: In a female mouse of the So-strain a tumor arose in the right axillary region (Text-fig. 3). The transplantation of this tumor likewise showed a negative result. This tumor also was not microscopically observed.

No. 3: A female mose of the So-strain developed a tumor on the dorsal side of her neck (Text-fig. 1). From histological observation it was classified as a gland cell carcinoma having its origin in a certain glandular tissue. An important characteristic of this tumor is that the glandular structure still remains in this neoplast (Figs. 1–2). The transplantation of this tumor was also unsuccessful as

in the above two cases.

No. 4: In this case the tumor developed at the root of the right thigh in a female mouse belonging to the So-strain. The histological study revealed that this is a sort of spindle cell sarcoma (Figs. 14–16). But it is noteworthy that there is a neoplastic configuration of gland cells in a certain region of this tumor (Fig. 16). This tumor seems to originate in the mammary gland considered from both anatomical and histological bases. Probably this tumor is initiated by the neoplastic growth simultaneously taking place in the mammary gland cells and the connective tissue cells of spindle shape. In the course of development of this particular tumor, however, the glandular cells become inactive admitting very active proliferation of spindle cells along. As the result, this tumor developed as a spindle cell sarcoma.



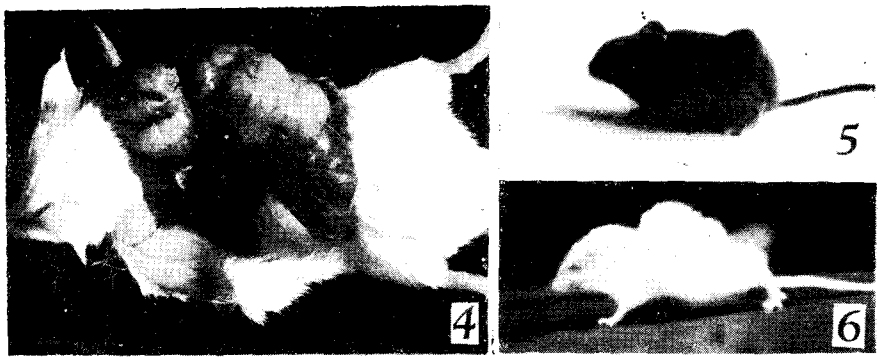
Text-figs. 1–3. 1, original tumor animal (No. 3). 2, original tumor animal (No. 1). 3, original tumor animal (No. 2). Arrow indicates tumor in each.

This tumor was transplanted into five other mice. Three cases of them showed positive transplantation; the inserted tumor began to grow soon after transplantation, leading to the death of the hosts of after about one month. By repeated experiments, this tumor was established as a transplantable strain of the spindle cell sarcoma. Two tumor-bearing mice at the 25th day after transplantation in the 5th transplant generation and at the 30th day after transplantation in the 7th transplant generation were shown in figures (Text-figs. 5 and 6).

No. 5: This tumor arose in the right axillary region of a female mouse derived from the B-strain. From histological observations it was found that this is a kind of gland cell carcinoma having the similar characters to those described in No. 3. As seen in Figures 3–4, the glandular structure of this tumor is more anaplastic in configuration than that of No. 3. Several attempts at trans-

plantation revealed that this tumor is not transplantable.

No. 6: By histological observations this tumor was proved to be a sort of gland cell carcinoma (Fig. 5). It developed in the region near the right thigh of a female mouse belonging to the B-strain. Histologically this tumor exhibits somewhat similar configuration to No. 5. Transplantation tried with several mice met with negative results in every case.



Text-figs. 4-6. 4. tumor animal (No. 8), at the 5th transplant generation. 5, tumor animal (No. 4), at the 5th transplant generation. 6, tumor animal (No. 4.), at the 8th transplant generation.

No. 7: In a female mouse of the B-strain a small tumor developed in the adjustment part of the anus. No histological diagnosis of the tumor was made. Attempts at transplantation proved negative.

No. 8: This is the only case of tumor found in the D-strain mouse. It developed in the right axillary region of a female in this strain. Histologically it proved to be a gland cell carcinoma, which is characterized by having a structure of a carcinoma simplex (Figs. 6–7). Nine attempted transplantations of this tumor proved positive in only three. In two other mice, however, the inserted tumor tissue showed a slight growth for the first several days, and afterward degenerated with time. The remaining four mice were absolutely resistant to transplantation. From the three mice to which transplantations were successfully made, successive transplantations of the tumor have been continued for over nine generations to date. Text-fig. 4 shows a tumor-bearing mouse which was opperated on 25th day after transplantation in 5th transplant generation.

No. 9: The histological features of this tumor proved it to be a kind of gland cell carcinoma similar to the other cases (Fig. 8). It was found at the right of the axillary of a female mouse coming from the B-strain. In general features this tumor was similar to No. 3. The transplantation of this tumor was negative.

No. 10: As was the case in most spontaneous tumors, this is a sort of gland cell carcinoma. It was similar in structure to No. 3; the original glandular

nature is still apparent in the tumor tissue (Figs. 9–10). Attempted transplantation of this tumor negative.

No. 11: In histological configuration this tumor was a kind of gland cell carcinoma somewhat similar to No. 6. It arose in the right axillary part of a female mouse from the B-strain. Transplantation of this tumor also proved unsuccessful. Here noticeable is the fact that neoplastic growth of connective tissue cells with spindl shape was found in a certain region of this tumor (Fig. 13).

3. Observations on transplantability

1). In the first place, transplantability of the spontaneous tumors is to be considered, especially concerning the above No. 4 and No. 8.

No. 4, the spindle cell sarcoma: The results of transplant experiments are shown in Table 1. The number of mice used in this experiment totaled 196. The mice which received a successful transplantation of the tumor (indicated by + in the table) were 91 in number, being 46.4 per cent. The number of mice in which the transplantation was unsuccessful (± in the table) were 11 (5.6 per cent); those which were completely resistant to transplantation (− in the table) were 84 (42.8 per cent). As to the other 10 indivuidals, no remarks could be made due to the accidental death or other causes; they are indicated by the question mark in the table.

Table 1. Results of transplant experiments in the tumor No. 4.

Transplant generations	No. of mice used in transpl.	Results of transpl.				% of positive results
		+	±	−	?	
1	5	2	1	2	0	40.0
2	11	3	0	3	5	27.2
3	12	6	0	6	0	50.0
4	27	14	4	8	1	51.8
5	8	5	2	1	0	62.5
6	42	13	0	28	1	30.9
7	53	25	1	26	1	46.2
8	38	23	3	10	2	60.5
Total	196	91	11	84	10	
%		46.4	5.6	42.8	5.1	

Remarks: + = positive transplantation, ± = unsuccessful transplantation, − = negative transplantation, ? = unknown results.

No. 8, the gland cell carcinoma: Based on the results from seven transplant generations, 67.6 per cent of the experimental animals were positively receptive to transplantation. The transplantability of the present tumor was higher than that of No. 4. From the data obtained in this case (Table 2), it is seen that cases of unsuccessful transplantation (±) were 8.5 per cent, and that those giving negative results (−) were 18.0 per cent.

Table 2. Results of transplant experiments in the tumor No. 8.

Transplant generations	No.of mice used in transpl.	Results of transpl.				% of positive results
		+	±	−	?	
1	9	3	2	4	0	33.3
3	7	5	0	2	0	71.4
4	20	16	0	3	1	80.0
5	22	16	0	6	0	72.7
6	26	16	3	3	4	61.5
7	21	15	4	1	1	71.4
Total	105	71	9	19	6	
%		67.6	8.5	18.0	5.7	

Remarks: The results in 2nd transplant generation was unknown.

ii) Next, sexual difference of transplantability of tumors is to be considered. It is interesting to learn whether or not the sex of the host has influence upon the transplantability of the tumor. Table 3 gives data concerning this point; it appear that in the spindle cell sarcoma the susceptibility of the female is slightly higher than that of the male, while in the case of No. 8 it is somewhat higher in the male. But, generally it can be said that the transplantability of tumors showed no remarkable difference by sex.

Table 3. Sexual difference in transplantability of tumors

Kind of tumor	Sex	No. of mice used	Results of transpl.				% of positive results
			+	±	−	?	
No. 4	♀	54	26	4	21	3	48.1
	♂	64	25	4	34	1	39.0
No. 8	♀	40	25	4	7	4	62.5
	♂	43	34	2	6	1	79.0

iii) Of importance is the difference of transplantability of tumors by strain of mice. It is generally recognized that the transplantability of tumors shows a remarkable difference by strain of mice, further that the mice of any certain strain show particularly a high susceptibility to its original tumor. In two strains of the tumor here established, the transplantability was examined regarding racial difference.

No. 4: As is clear by referring to Table 4, the tumor, No. 4, showed no remarkable difference by strain. However, the mice from the S-strain showed somewhat lower susceptibility to transplantations than did other strains. The S-strain is characterized by having pure genetical characters. No spontaneous

tumor has ever occurred in this strain. The lower transplantability in the S-strain seems to be due to the extreme purity of genetical characters.

Here noteworthy is the fact that the transplantability of the tumor was not high at all in the So-strain in which this tumor originated. As already mentioned, the So-strain is not pure. Thus, the reason for the low transplantability in this strain may be the impurity of genetical characters in this mice. On the other hand, the mice of B-strain with agouti color showed a considerably high transplantability (74.4 per cent), while in the albino mice of the same strain the transplantability was lower as compared with agouti mice.

Table 4. Transplantability of tumors by strain of mice

Kind of tumor	Strains of mice	No. of mice used	Results of transpl.				% of positive results
			+	±	−	?	
No. 4	S	71	23	6	39	3	32.4
	D	1	0	0	1	0	0
	B (ag.)	47	35	1	11	0	74.4
	B (al.)	8	4	0	3	1	50.0
	So	34	15	0	18	0	44.1
No. 8	S	46	28	4	12	2	60.8
	D	12	12	0	0	0	100.0
	B (ag.)	18	14	0	1	3	77.7
	B (al.)	4	2	0	2	0	50.0
	So	25	15	5	4	1	60.0

No. 8: The tumor, No. 8, originally developed in a mouse of the D-strain. It must be remembered that this tumor showed 100 per cent transplantability into animals of the D-strain. Probably this is due to the purity of the strain. In the mice of S-strain, however, the transplantability was evry low. The agouti mice of the B-strain showed a considerably high rate of transplantability, as was the case with tumor No. 4.

Remarks

Genetical studies on spontaneous tumors in mice have been carried out by several workers, such as Andervont, Bittner, Heston, Little, Lynch, MacDowell, Mercier, Murray, Pubus, Simonds, Slye, Tyzzer and Woolley (cited from Little, 1947). According to Bittner (1940), the following factors are to be considered in connection with incidence of mammary tumor in mice, viz., 1) Milk influence or mammary tumor inciter, 2) genetical influence, and 3) hormonal influence. Slye & Holmes (1931) expressed the view that the incidence of tumor in mice was due to a single recessive gene. On the other hand, Bittner (1940) was of the opinion that a single dominant genetic factor was involved in the incidence of mammary

tumor, and that the factor was also concerned with susceptibility to mammary tumor. However, Heston (1942), depending upon his detailed genetical analysis on lung tumor incidence in mice, demonstrated that neither a single dominant or single recessive factor is involved, but that incidence depends upon multiple genes with some evidence of linkage with known genes. Recently Bittner (1944) has reported that the incidence of mammary tumor in mice is concerned with certain genetic factors.

'High' tumor strain in mice, such as A, C_3H, and dba were established by Bittner (1935), Strong (1926) and Little (1939) as the result of inbredding for many generations. In the light of the above fact it is very probable that there is a repeated accumulation of genes concerning the tumor incidence due to inbreeding. The considerably high occurrences of spontaneous tumors in mice found in our laboratory can be considereded a result of inbreeding.

As described above, amongst spontaneous tumors found in eleven mice of three strains, seven are found to be a gland cell carcinoma, one is a spindle cell sarcoma, and the remaining three remain unknown in nature due to failure in their histological observation. The gland cell carcinomas found in seven specimens are to be classified into three tpyes in respect to their structure as follows: Type I) in which the tumor still remained in its original glandular structure; the tumors indicated as No. 3, No. 9 and No. 10 in the present paper are to be included in this type. Type II) in which the tumor tissue transforms into more anaplastic configuration than that of Type I. The tumors No. 5, No. 6 and No. 11 belong to this type. Type III) is a carcinoma simplex which shows more simple structures; tumor No. 8 corresponds to this type. Transplantation experiments reveal that the tumors belonging to Type I and Type II cannot be transplanted, while that of Type III posseses transplantability. From the fact that the tumor of Type III posseses no original histological structure and posseses transplantability, it may be that the tumor of Type III is more malignant than those of Types 1 and II. Relation between the strain of mice and the type of tumor is given in Table 5.

Table 5. Relation between the type of the tumor and the strain of mice. Numerals indicate the number of tumor bearing animals

Strain \ Type	I	II	III
So	1	1	–
B	2	2	–
D	–	–	1

Referring to the table it is clear that the tumors developed in the So-strain and the B-strain belong to both Type I and Type II, whereas that which occurred in the D-strain is of Type III. Here is an evidence worthy of attention that the tumors

occurring in a definite strain have similar histological characters. This is probably due to the influence of genetical factors regarding tumor incidence.

It has generally been recognized that the success or failure of the transplantation of tumor is dependent on the genetical factors of the host. The tumor which was developed in a pure strain of mice is generally very highly transplantable in the mouse of the strain where it originated, but transplantability is very low or entirely deficient in mice of different strains. Mice of D-strain show a high susceptibility to tumor No. 8 which originated in that strain.

The genetical relationship between the susceptibility of tumor and coat color genes has been discussed by some workers (Little, Strong, Bittner, Spangler and Murray). Little and Strong (1924), and Bittner (1933, 1934) have shown that one of the genes for susceptibility to adenocarcinoma in mice appearently links with the gene relating to dilute coat color. Later, from the study on the transplantation of melanoma, Spangler, Murray and Little (1942) have found the difference in tumor susceptibility between albino and colored mice ; in colored mice the susceptibility was 72.4 per cent, while in albinos it was 59.8 per cent. In the two strains of tumors, No. 4 and No. 8, the author has found that colored mice show higher susceptibility than albinos. This evidence seems to suggest that the genes for coat color have a genetic linkage with tumor susceptibility.

In one of the adenocarcinomas, Strong (1929) has shown that a few genes are required for successful growth of this tumor, and that one of these genes is sex-linked. Bittner (1932) has recorded an association between susceptibility to tumor 13714 and sex. So far as the author's observations have shown, there is no significant association between transplantability and sex.

Summary

Spontaneous tumors developed in 11 specimens of inbred mice were investigated in this study, with special attention to the histological structure and transplantability. After many times trials of transplantation, two strains of transplantable tumors were newly established. The one is a spindle cell sarcoma described as No. 4, and the other a gland cell carcinoma described herein as No. 8. Transplantability is 46.4 and 67.6 per cent for No. 4 and No. 8, respectively.

Sexual difference as well as racial difference of transplantability was investigated in these two strains of mouse tumor.

Hereafter, it is proposed to use the terms "MY mouse sarcoma" and, "MY mouse carcinoma", respectively for the tumors, No. 4 and No. 8.

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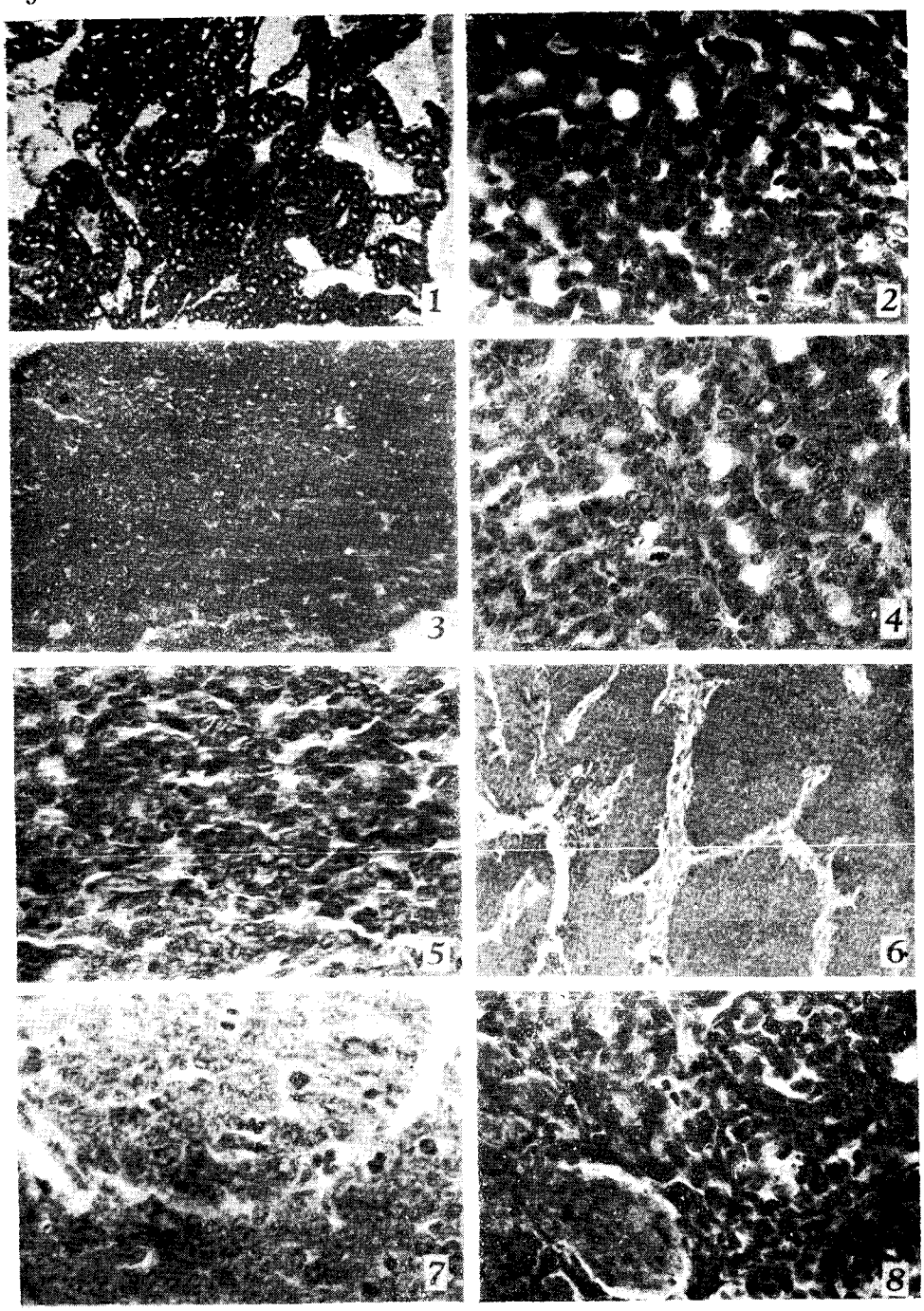
Explanation of Plate III

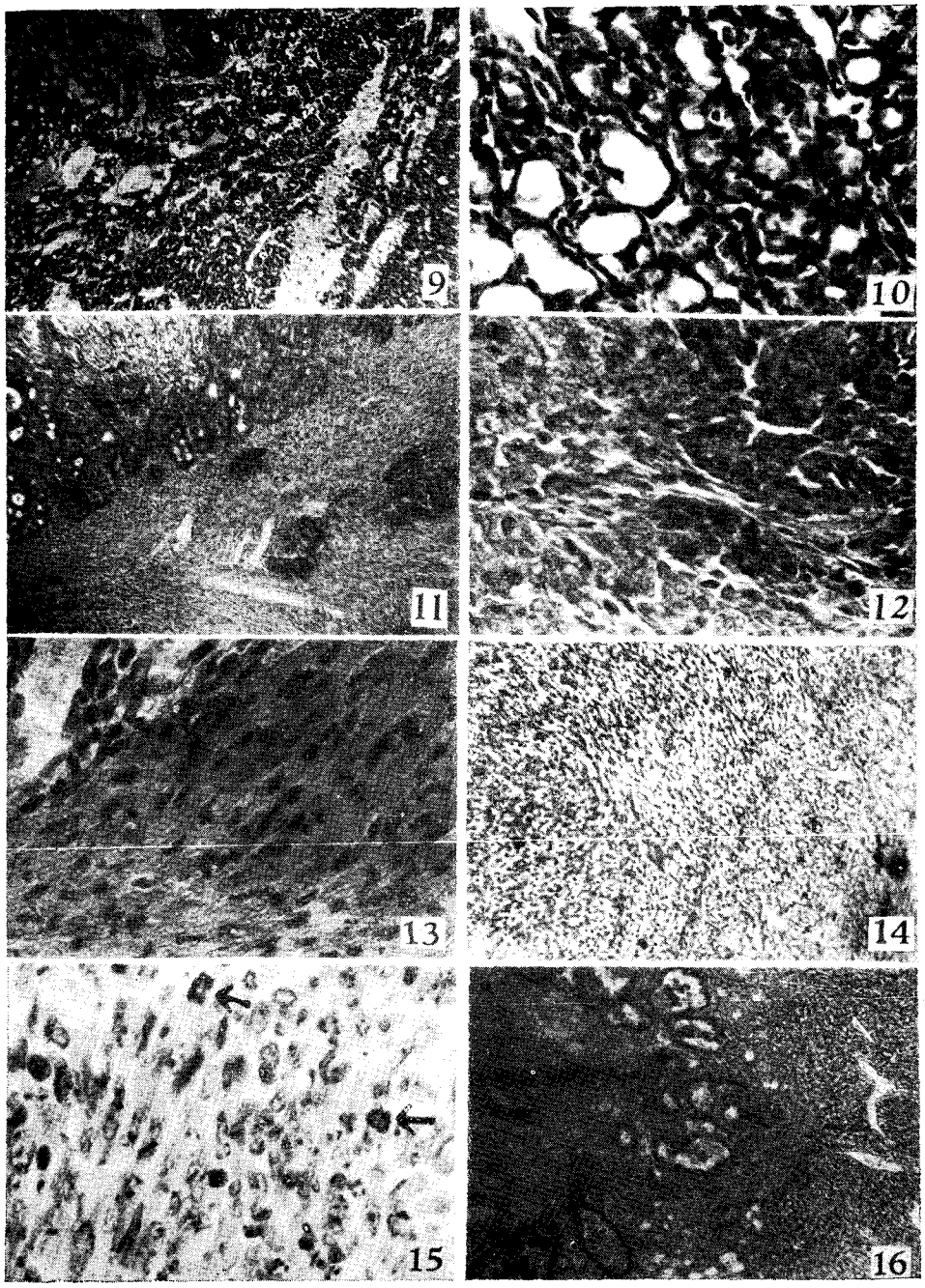
All are photomicrographs taken with "Leitz Mikas". Figs. 1, 3 and 6, ×100. Figs. 2, 4, 5, 7 and 8, ×600. Figures illustrate histological structure of spontaneous tumors in mice. **Figs. 1-2.** Gland cell carcinoma from No. 3. **Figs. 3-4.** Gland cell carcinoma from No. 5. **Fig. 5.** Gland cell carcinoma from No. 6. **Figs. 6-7.** Gland cell carcinoma from No. 8. **Fig. 8.** Gland cell carcinoma from No. 9.

Explanation of Plate IV

All are photomicrographs taken with "Leitz Mikas". Figs. 9, 11, 14 and 16, ×100. Figs. 10, 12 and 15, ×600. Figures illustrate histological structure of spontaneous tumors in mice. **Figs. 9-10.** Gland cell carcinoma from No. 10. **Figs. 11-13.** Gland cell carcinoma from No. 11. Fig. 13 shows neoplastic growth of spindle cells occurring in a certain region of this tumor. **Figs. 14-16.** Spindle cell sarcoma from No. 4. Fig. 16 shows neoplastic growth of gland cells occurring in a part of this tumor.



T. H. Yosida: Spontaneous Tumors occurring in Inbred Mice



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