Growth and metastasis of fresh human benign and malignant tumors in the head and neck regions transplanted into scid mice

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Running title: Human benign and malignant tumors in scid mice

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Abbreviations: SCC, squamous cell carcinoma; scid, severe combined immunodeficiency. Gene symbol, scid used in the text indicates scid/scid homozygote unless otherwise described.

Abstract

Surgically resected fresh human tumors (16 malignant and one benign) in the head and neck regions were subcutaneously transplanted into scid mice. All malignant tumors (12 squamous cell carcinomas, 2 papillary adenocarcinomas and one adenoid cystic carcinoma) except one heavily irradiated squamous cell carcinoma could grow in scid mice. However, all of 3 squamous cell carcinomas of the maxillary sinus grew only in 50 % of scid mice, and two of them failed to grow in the second transfer. Remainders were successfully transplantable for further generations except one accidental case. A benign tumor, follicular adenoma of the thyroid gland, was also accepted in scid mice and transplantable for further generations, although its growth was very slow. Distant metastases were found in the lung only when poorly differentiated carcinomas were transplanted into scid mice, but did not occur until 90 days after the transplantation of well and moderately differentiated carcinomas. Histological characteristics of both malignant and benign tumors well remained in all of the xenografts and metastatic lesions. Thus, scid mice seem useful to investigate not only the properties of benign and malignant human tumors but also the metastatic spread of tumors which threatens the life of cancer patients.

Introduction

Athymic nude mice have long been used for investigating the progression and metastasis of human malignant tumors and for testing therapeutic modalities. However, about half of fresh human malignant tumors have never been transplantable in athymic nude mice, nor transplanted tumors have metastasized spontaneously to distant organs without immunological modifications (1-6).

Recently, an autosomal recessive mutation that severely impairs lymphopoiesis has been found in mice, and used as an animal model for human severe combined immunodeficiency (SCID) (7-10) and for the transfer of a functional human immune system (11, 12). In these scid mice which are deficient for immune functions mediated by both T and B lymphocytes, human malignant tumors such as retinoblastoma, osteosarcoma and embryonal carcinoma could grow more effectively or rapidly than in athymic nude mice (13, 14, 15). Especially, xenograft acceptance occured for fresh human classic seminoma which had never grown in nude mice (14, 15, 16). Furthermore, ectopically transplanted human malignant tumors metastasized spontaneously to distant organs, while such metastatic spread has never been observed in athymic nude mice (14, 15).

The present study extended the previous works to varieties of surgically resected fresh human tumors (both malignant and benign) in the head and neck regions, and their growth, metastatic spread and histological feature were examined to know the validity of using scid mice for the study of human neoplasia.

Materials and methods

Mice

Breeding pairs of C.B17-scid/+ and C.B17 mice were kindly provided by Dr. M. J. Bosma, Fox Chase Cancer Center, Philadelphia in 1986. From a cross of C.B17-scid/+ male and female heterozygotes, C.B17-scid/scid homozygotes were screened by the level of serum IgG (<1 μg/ml), and thereafter scid/scid homozygotes have been maintained by brother × sister mating under the germ-free condition in the Department of Radiation Biology, Osaka University. Sera were taken at about 4 to 6 weeks after birth to measure IgG and IgM (17), and scid/scid homozygotes showing serum IgG and IgM less than 1 μg/ml were used for breeding pairs at each generation (15). Most of C.B17-scid/scid mice (91.9% and 90.7%) showed very low level of serum IgG and IgM ($\leqq 10\mu$g/ml), respectively, while those of wild type C.B17-+/+ mice were more than 2,000 and 700 μg/ml, respectively. Two to three-month-old C.B17-scid/scid mice at F_{10-12} generations showing both serum IgG and IgM less than 10μg/ml (87.1% of C.B17-scid/scid mice) were used for the present study, since high incidence of thymic lymphocytic leukemia was known to develop in leaky scid mice at older ages (14, 15, 18). T and B cell functions analysed by Fluororescence Activated Cell Sorter (FACS) and mitogen stimulation of splenic lymphocytes were reported previously (15). All mice were maintained with mouse diet CRF-1 (Charles River, Kanagawa, Japan) and chlorinated water in the complete barrier condition at 23 ± 1 ℃.

IgG and IgM quantitation

An enzyme-linked immunosorbent assay (ELISA) was used to quantitate serum IgG and IgM concentrations (17) for prescreening scid mice. Microtiter wells (ELISA plate, Sumitomo-Bakelite Ind., Tokyo, Japan) were first coated with rabbit anti-mouse IgG (Zymed, San Francisco, U.S.A.) or rabbit anti-mouse IgM (Zymed), and then serially diluted mouse test sera or standard sera (IgG, ICN Biomedicals, Inc., Costa Mesa, California, U.S.A. or IgM, Cappel Organon Teknika Corp., West Chester, U.S.A.) were added. After incubation and washing, peroxidase-conjugated rabbit anti-mouse IgG (Zymed) or rabbit anti-mouse IgM (Zymed) were added and relative amounts of bound enzyme conjugate in each well was ascertained by addition of o-phenylenediamine (Wako Pure Chemical Ind. Ltd., Osaka, Japan). The extent of hydrolysis was measured at 490 nm with a Microplate Reader MPR A4 (Toso Inc., Tokyo, Japan). Serum IgG and IgM concentrations were calculated from the standard curves.

Human tumors

Used human tumors were 17 fresh tumors in the head and neck regions taken by surgical operation. A total of 17 head and neck tumors were composed of 13 squamous cell carcinomas at the varieties of histological malignancy of the maxillary sinus, nasopharynx, hypopharynx, larynx and tongue, 3 adenocarcinomas of the parotid and thyroid glands, and one benign follicular adenoma of the thyroid gland. These tumors were all obtained at the time of ablative surgery from March 1990 to July 1991 at the Department of Otolaryngology, Osaka University Hospital. Four tumors including one recurrent tumor came from the patients who had undergone radiation therapy prior to surgical resection. Details of clinical and histopathological data

were given in Table I .

Heterotransplantation

Fresh tumor tissues were taken with care to exclude both normal marginal and necrotic tissues in the operation room immediately after surgical resection, and then put into 20 ml of tissue culture medium at 4℃ (RPMI 1640, Gibco, Grand Island, New York, U.S.A. or DMEM, Nikken Bio. Med. Lab., Kyoto, Japan). The tumor tissues were cut to about 2 to 3 mm cubic mass in 0.9% NaCl solution containing antibiotics [penicillin G 10,000 units/ml (Gibco) and streptomycin sulfate 50 mg/ml (Wako)] in the mouse laboratory, and two pieces of them were transplanted subcutaneously into the back of C.B17-scid/scid mice following a small skin incision under anesthesia with 0.77% tribromoethanol solution (Aldrich Chem., Co. Ltd., Milwaukee, WI, U.S.A.).

Maximum and minimum diameters were measured twice a week with a slide caliper, and tumor volume was estimated by means of Houchens' formula (19). Mice were observed up to about 90 days after transplantation, and sacrificed by cervical dislocation. Gross pathological lesions were examined macroscopically, especially for metastases to distant organs, and all were submitted to microscopic examinations to confirm the xenograft acceptance and metastatic spread.

Results

All of fresh human malignant tumors in the head and neck regions except one hypopharyngeal carcinoma (HPC-2) which had been heavily irradiated could grow in C.B17-scid/scid mice. As for the primary acceptance, no relationships were found to histological patterns of original tumors. However, it was slightly low in squamous cell carcinomas of the maxillary sinus, and two of them (MSC-1,-3) were lost in the second transfer. One squamous cell carcinoma of the tongue (TC-3) was also lost in the second transfer, because the recipient animal died of accidentally occured thymic lymphocytic leukemia. The remainders were successfully transplantable for further generations (Table‖). A benign tumor, follicular adenoma of the thyroid gland (TGFA-1), could grow in C.B17-scid/scid mice, and has been maintained for further generations.

Distant metastases to the lung were observed only in the scid mice engrafted with poorly differentiated squamous cell carcinomas (NPC-1 and LC-3), while no metastatic spread occured until 90 days after the transplantation of well and moderately differentiated squamous cell carcinomas and adenocarcinomas (Table‖). Although the right upper mediastinal and the right hilar lymph nodes were slightly swollen (approximately 1 mm in diameter) in most of the recipient animals, metastases were not found by the microscopic examinations.

Figure 1 indicates the representative patterns of growth curves of transplanted human tumors. Xenografts of malignant tumors became palpable about two weeks after transplantation and then grew steadily, e.g., two

squamous cell carcinomas, HPC-1 and LC-1, grew to 5 and 3 cm^3 at about 60 and 90 days after transplantation, respectively. Large difference was observed in the growth rate between benign and malignant tumors. Follicular adenoma of the thyroid gland (TGFA-1) grew very slowly in scid mice (Figure 1). The doubling time estimated at the exponentially growing phase was 11.3 $\pm$ 1.1, 16.1$\pm$ 0.9 and 23.7$\pm$ 1.5 days (mean$\pm$ SE) in HPC-1, LC-1 and TGFA-1, respectively.

Histological feature of the xenografts (Figure 2 A-F) and metastatic lesions in the lung (Figure 3) was consistent with that of the original tumors in the patients.

Discussion

In the present study, scid mice accepted 15 of 16 surgically resected fresh malignant tumors (93.8%). One exception was squamous cell carcinoma of the hypopharynx which had been heavily irradiated before transplantation. This acceptance frequency was very high when compared with the average acceptance of human malignancies in athymic nude mice (1-6, 13). The present study showing the high frequency of xenograft acceptance extended the previous studies (13-16), since common types of tumors in humans, i.e., squamous cell carcinoma, papillary adenocarcinoma and adenoid cystic carcinoma in the head and neck regions could grow in scid mice. Moreover, a benign tumor, follicular adenoma of the thyroid gland, could grow in scid mice. It was transplantable up to third generation, although the growth was very slow. We believe this will be the first report showing that human benign tumor was accepted in experimental animals, although further elucidation remains with other benign tumors. Such study is in progress.

Distant metastases were found in the lung only when poorly differentiated human carcinomas were transplanted subcutaneously into scid mice. Our previous finding with specific tumor, classic seminoma (14, 15), was confirmed with a common type of human cancer. However, no metastatic spread has been detected up to 90 days after the subcutaneous transplantation of well and moderately differentiated carcinomas (Table II). Longer observation might be necessary for the metastatic spread of these tumors. Alternatively, such a difference in the metastatic spread may be caused by the tumor cell properties, i.e., oncogene activation (20, 21) modifying the degree of the

differentiation of tumor cells, metastasis suppressive gene (22, 23), etc. in addition to the host immune status (15). During the preparation of this paper, our past findings (14, 15) were also confirmed in part with human malignant melanoma cells which showed metastatic spread in scid mice (24). These advantages to use scid mice for investigating human tumors might depend on the deficiency of B-cell associated functions, directly or indirectly (15), in addition to deficient T-cell function, since NK cells and macrophages opperate normally in scid mice (25, 26). Alternatively, unknown immunodeficiency might be involved in scid mice. Further study remains in future.

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Table I Clinical and pathological feature of fresh human head and neck tumors transplanted into scid mice

Case	Primary site	Age/sex	Stage[a]	Treatment[b]	Histology of original tumor
A.Malignant tumor					
MSC -1	Maxillary sinus	50/M	T3 N0 M0	None	Moderately differentiated SCC
-2		32/M	T4 N0 M0	None	Moderately differentiated SCC
-3		79/M	T3 N0 M0	None	Well differentiated SCC
NPC -1	Nasopharynx	59/M	recurrence	70Gy[c]	Poorly differentiated SCC
HPC -1	Hypopharynx	55/M	T3 N2b M0	39.6Gy[c]	Well differentiated SCC
-2		35/M	T3 N2b M0	40Gy[c]	Well differentiated SCC
LC -1	Larynx	78/M	T3 N0 M0	None	Well differentiated SCC
-2		72/M	T3 N0 M0	None	Moderately differentiated SCC
-3		73/M	T3 N0 M0	None	Poorly differentiated SCC
TC -1	Tongue	44/M	T1 N0 M0	None	Well differentiated SCC
-2		51/M	T3 N2c M0	None	Well differentiated SCC
-3		56/M	T3 N0 M0	40Gy[c]	Well differentiated SCC
-4		54/M	Tis N0 M0	None	Well differentiated SCC
PGC -1	Parotid gland	38/F	T2 N0 M0	None	Adenoid cystic carcinoma
TGC -1	Thyroid gland	55/M	T3 N2b M0	None	Papillary adenocarcinoma
-2		70/M	T4 N0 M0	None	Papillary adenocarcinoma
B.Benign tumor					
TGFA-1	Thyroid gland	29/F	—	None	Follicular adenoma

[a] TNM classification (27).

[b] Treatment prior to surgical resection.

[c] Dose of lineac irradiation. Intervals between the last irradiation and the time of surgical operation were 1550, 18, 19 and 24 days for NPC-1, HPC-1, HPC-2 and TC-3, respectively. 2 Gy was given daily (5 days per week).

Table II Local growth and distant metastasis of human head and neck tumors transplanted into scid mice

Case	Local growth		Distant metastasis
	Incidence(%)[a]	No. of generation	Incidence(%)[b]
A.Malignant tumor			
MSC -1	1/2 (50.0)	1	0/1 (0.0)
-2	2/4 (50.0)	2	0/2 (0.0)
-3	1/2 (50.0)	1	0/1 (0.0)
NPC -1	7/8 (87.5)	4	2/6 (33.3)[c]
HPC -1	19/20 (95.0)	5	0/19 (0.0)
-2	0/2 (0.0)	-	-
LC -1	20/20 (100)	4	0/13 (0.0)
-2	6/7 (85.7)	4	0/6 (0.0)
-3	5/5 (100)	2	1/4 (25.0)[c]
TC -1	3/5 (60.0)	2	0/3 (0.0)
-2	3/5 (60.0)	2	0/3 (0.0)
-3	3/3 (100)	1[d]	0/3 (0.0)
-4	6/6 (100)	2	0/6 (0.0)
PGC -1	8/10 (80.0)	3	0/8 (0.0)
TGC -1	5/6 (83.3)	3	0/5 (0.0)
-2	9/11 (81.8)	4	0/9 (0.0)
B.Benign tumor			
TGFA-1	6/8 (75.0)	3	0/6 (0.0)

[a] Numbers of scid mice with local tumor growth among all tumor-engrafted scid mice. Xenograft acceptance was determined when the growth was confirmed histologically and xenografts grew in the next transfer.

[b] Numbers of mice with distant metastases among mice killed more than 40 days after transplantation.

[c] Distant metastases to the lung.

[d] Xenografts were lost in the next transfer by the accidentally occured murine thymic lymphocytic leukemia in the recipient animal.

Legend to figures

Fig. 1. Growth curves of fresh human tumors in the head and neck regions transplanted into C.B17-scid/scid mice. Vertical bars show mean± SE computed from the t distribution of the mean. ▲, SCC of the hypopharynx (HPC-1); ■, SCC of the larynx (LC-1); ●, follicular adenoma of the thyroid gland (TGFA-1).

Fig. 2. Microscopic view of the surgically resected human tumors and their xenografts in C.B17-scid/scid mice. (A) well differentiated squamous cell carcinoma of the larynx (LC-1) and (B) its xenograft in scid mice. (C) adenoid cystic carcinoma of the parotid gland (PGC-1) and (D) its xenograft. (E) benign follicular adenoma of the thyroid gland (TGFA-1) and (F) its xenograft. Hematoxylin and eosin, × 100.

Fig. 3. Microscopic view of the poorly differentiated squamous cell carcinoma of the nasopharynx (NPC-1) and its metastatic lesion in the lung of C.B17-scid/scid mice. (A) original tumor in the patient. (B) lung metastasis in scid mice. Hematoxylin and eosin, × 200

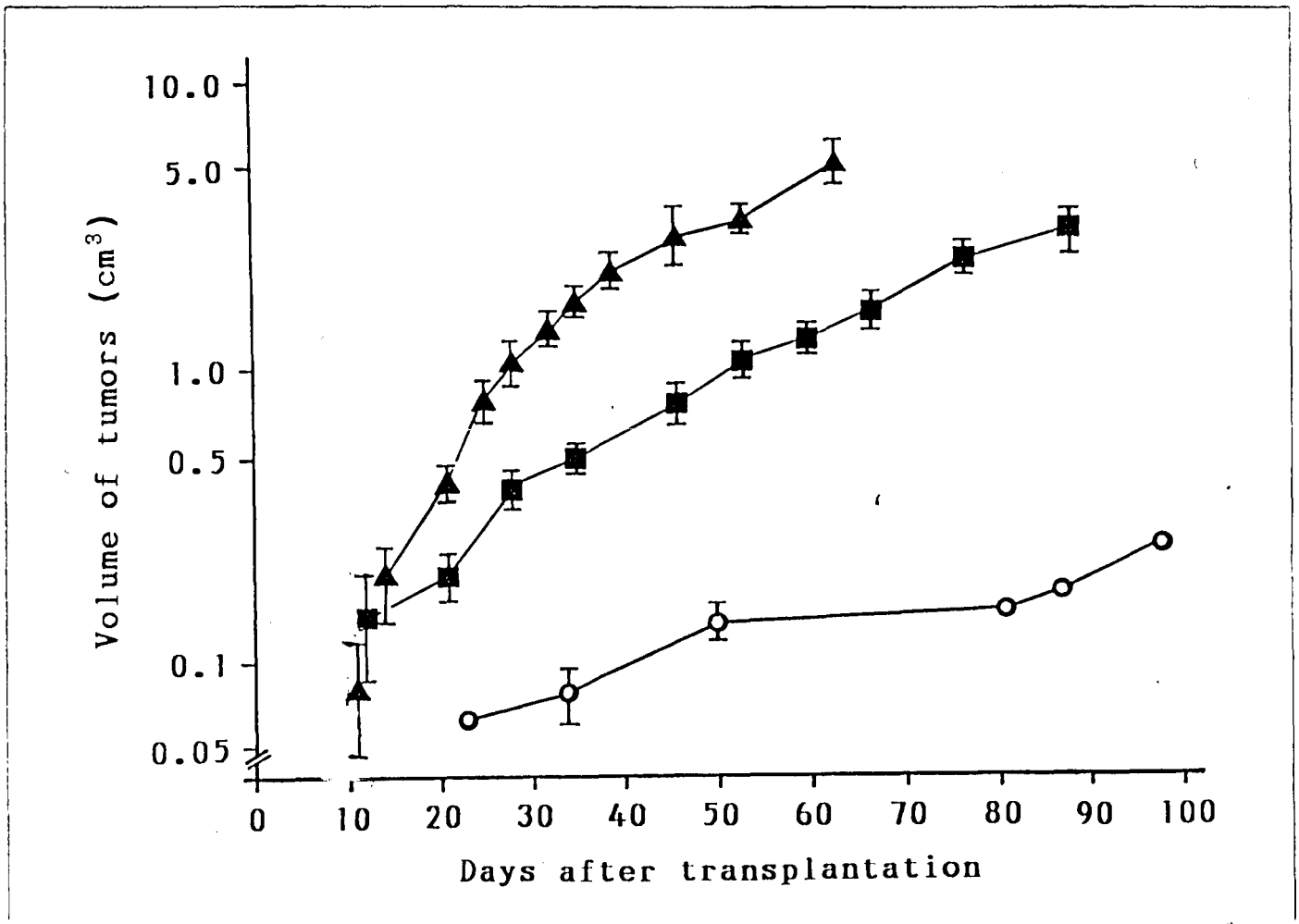
10.0
5.0
1.0
0.5
0.1
0.05
Volume of tumors (cm³)
0
10
20
30
40
50
60
70
80
90
100
Days after transplantation

Fig. 1

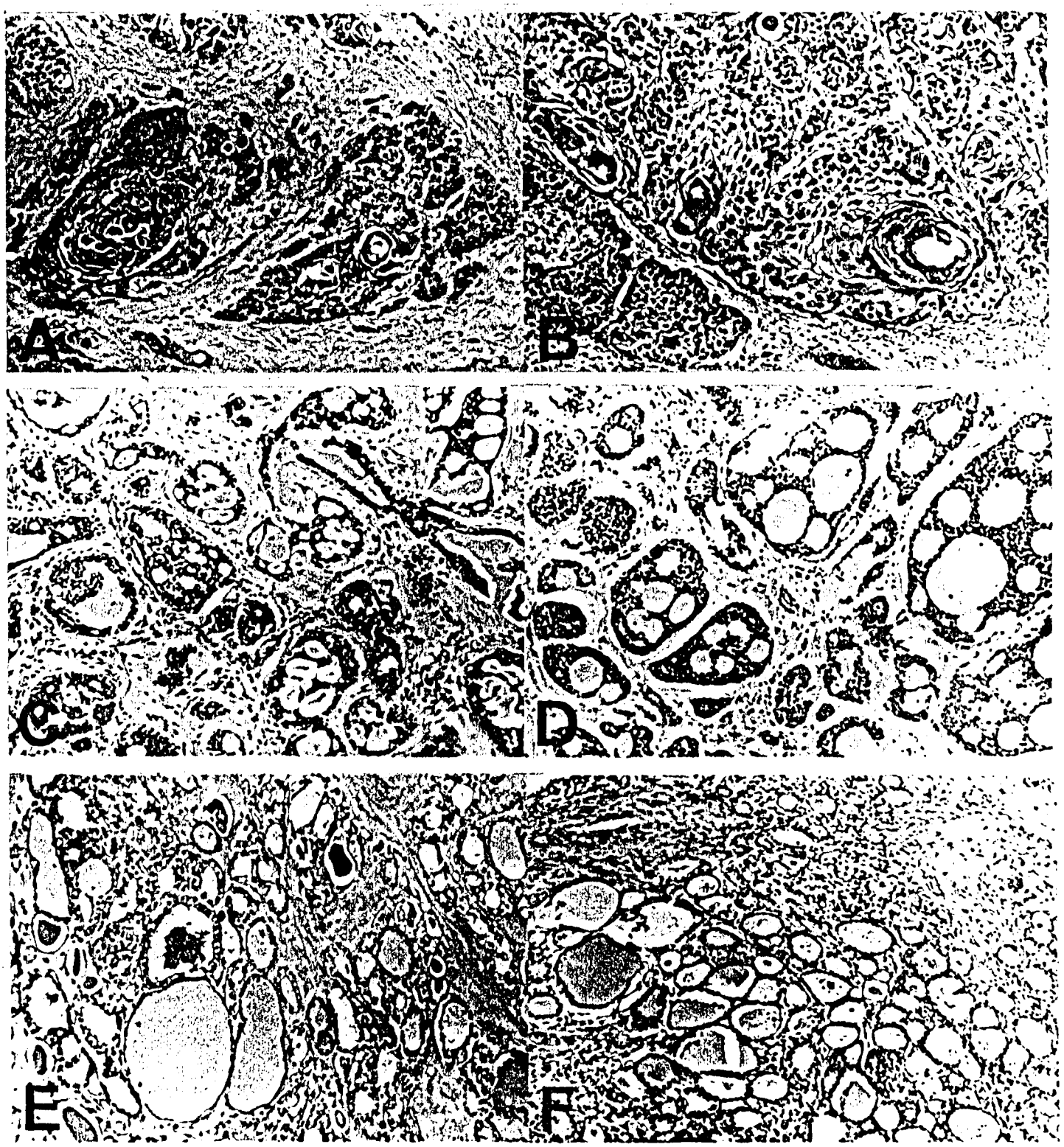

Fig. 2

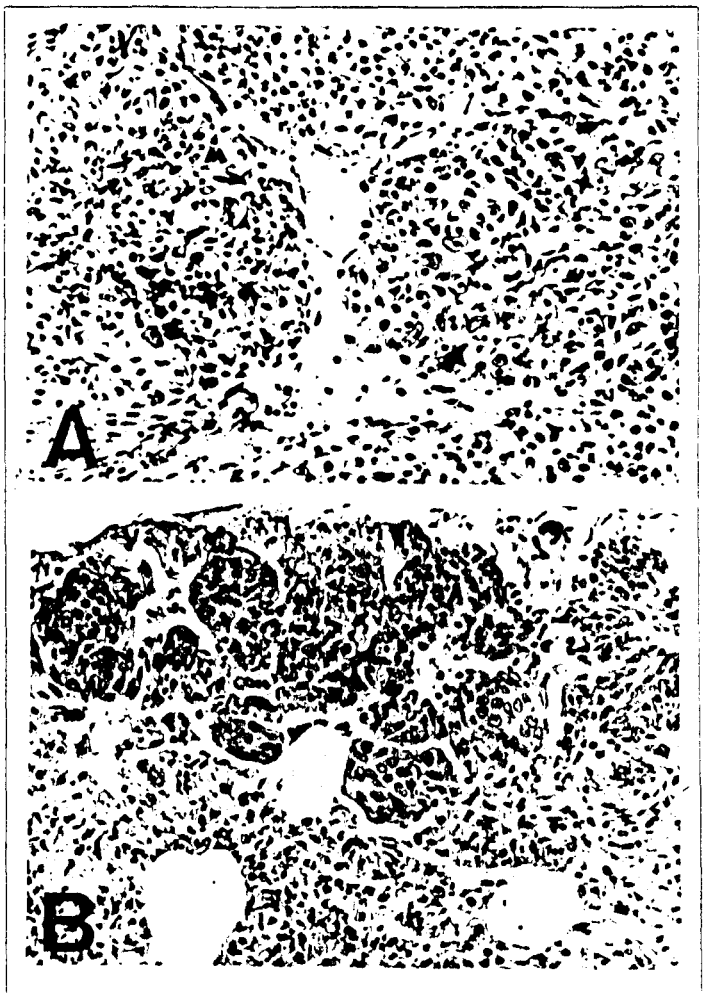

Fig. 3