Continuous Cultivation of Malignant Cells from a Canine Testicular Tumor. (24636)

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This paper describes development in tissue culture of a continuous line of malignant cells from a canine Sertoli cell tumor (SCT) and reports susceptibility of these cells to different viruses.

Materials and methods. The Small Animal Surgery Clinic, School of Veterinary Medicine, Univ. of Pa., received a 13-year-old, obese, male West Highland terrier. The owners had noted a mass in the scrotum about one month previously. Physical examination revealed enlarged mammary glands with hyperpigmented nipples, symmetrical alopecia of inguinal, abdominal and thoracic areas, hyperpigmentation about the anus, scrotum and abdomen. The right testis was firm and greatly enlarged while the left was atrophic. After castration, specimens of testis were retained for study.* Histopathological examination revealed a Sertoli cell adenocarcinoma.† Tumor material was removed aseptically from the surgical specimen and immediately placed in screw cap centrifuge tubes containing 20-30 ml Hanks' Balanced Salt Solution (BSS) and Penicillin, 100 units/ml and Streptomycin 100 μg/ml. After 2 washings in fresh BSS containing antibiotics, approximately 5 mm^3 was removed from center of tumor and placed in 10% formalin. Remaining tissue was cut into 1-2 mm pieces and transferred to sterile 500 ml Erlenmeyer flask containing sterile magnet and 100 ml of 0.5% trypsin. An electrically heated jacket to maintain constant temperature of 37°C was secured around the flask and the entire assembly then placed on magnetic stirrer and agitated 25 minutes. The cellular suspension was separated aseptically from tissue fragments by straining through 2 layers of sterile cheesecloth. Fresh trypsin was then added to flask and this process repeated at 25 minute intervals until practically all tissue was disintegrated. Strained suspensions were stored in ice bath, then centrifuged in the cold at 600 rpm for 30 minutes. The cells were then seeded into conventional screw-cap milk-dilution bottles after cellular suspension had been adjusted to contain one million cells/ml. The count was made in standard haemocytometer counting chamber. Three media were used for growth of these cells—medium 199 plus 10% inactivated horse serum, Basal Medium Eagle (BME) plus 10% inactivated horse serum and Enders' Medium (1). Each medium contained 100 units Penicillin, 100 μg Streptomycin and 50 units Mycostatin/ml. Incubation was at 37°C. Growth media were replaced with fresh media at 3-day intervals. After 3-4 days incubation a fibroblast-like cell appeared and predominated in all cultures. Within 1 to 2 weeks most of these spindle-like cells separated, enlarged greatly and disintegrated. Continued "blind" feeding with fresh growth media for 3 to 4 more weeks resulted in appearance of a few clumps of epithelial-like cells in several bottles. These cells grew fairly rapidly and soon became visible macroscopically. At this time, the cells in one bottle with approximately one-third of its surface covered with isolated clumps of cells, were trypsinized and a new milk-dilution bottle inoculated. This redistribution of cells throughout entire bottle resulted in even seeding. Serial passages carried out by adjusting cellular suspensions of the seeding material to contain 100,000 cells/ml resulted in appearance of full monolayers of cells in 3-4 days. A cytological record of stained cells were kept after each trypsinization step. Coverslips in Leighton tubes were seeded with 1 ml of suspension and allowed to grow for 3-4 days. Coverslips were then fixed in methyl alcohol for 15 minutes and stained with Giemsa. At time of this report the cell line has undergone 55 passages.

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TABLE I. Effect of Maintenance Media and Storage for 2 Months on Survival of SCT Cells *In Vitro*.

Medium No.	Composition	%	Storage temp., °C	Survival*
1	Balanced salt sol.	37.5	4	0
	Bovine amniotic fluid	37.5		
	Horse serum	20	37	0
	Bovine embryo extr.	5		
2	Medium 199	98	"	4+
	Horse serum	2		
3	Medium 199	98	4	3+
	Calf serum	2	37	4+
4	Medium 199	100	"	2+
5	Basal medium, Eagle	98	"	0
	Calf serum	2		
6	Basal medium, Eagle	98	"	0
	Horse serum	2		
7	Balanced salt sol.	95	"	0
	Horse serum	5		
8	Basal medium, Eagle	90	4	3+
	Horse serum	10	R.T.†	1+
9	Medium 199	90	4	3+
	Horse serum	10	R.T.	3+

* Survival was determined by sampling aliquots of cells after 2 mo storage at various temperatures. Appearance of viable cells and degree of growth obtained in tubes or bottles after reincubation for 24 hr and then refeeding with fresh growth media was graded. 0 indicates no growth; 1+ to 3+, intermediate degrees of growth, and 4+, a confluent sheet of cells. All bottles incubated at least 7 days at 37°C.

† Room temperature.

Results. Cells (SCT) were cultivated on glass surface of roller tubes. The citric acid-crystal violet technic was used for determination of viable cells. The media studied were: medium 199 + 10% horse serum, BME + 10% horse serum and Enders' medium. Although growth rate in Blake bottles with medium 199 was fairly similar to growth rate in BME, appearance of cells in each medium was different. In BME the cells tended to grow in discrete patches while in medium 199 the cells were evenly distributed. There was no appreciable growth in Enders' medium.

Table I shows results of these experiments as well as effects of storage. Of media tried only those incorporating medium 199 with small amounts of serum had any beneficial effect in maintaining the cells in storage. Attempts to store the cells at −70°C were unsuccessful.

Animal inoculation. Since an important criterion in the definition of malignancy is establishment of a tumor in a susceptible host (2), varying numbers of SCT cells (250 thousand - 12.5 million) suspended in medium 199 were inoculated into mice, rabbits, guinea pigs, and embryonated eggs. These hosts were refractory to the tumor. SCT cells of 7th and 10th tissue culture passage were inoculated into cheek pouches of Syrian hamsters, conditioned according to technics described by Toolan(3), and Lutz, *et al.*(4), with negative results, until cells of 24th tissue culture passage were used. At this passage level, 9 out of 12 hamsters developed tumors approximately 0.2 cm^3, within 5 days. However, one week after inoculation tumors in all animals except one regressed. Attempts serially to pass this remaining tumor in the hamster as well as in tissue culture failed. These negative results prompted us to investigate the possibility of a tumor response in the homologous host. Accordingly, three, 3-4 month old, male mongrel dogs were inoculated with trypsinized, washed SCT cells of the 10th passage. As controls, male dogs, 3-4 months old were inoculated with diluent. Immediately following inoculation, all dogs were treated with metacortelone acetate (Schering) 75 mg/dog for the first 5 days, and 20 mg/dog for the next 5 days.

The results (Table II) show that tumors were obtained in 2 out of 3 dogs inoculated

TABLE II. Effect of Tissue Culture Propagated SCT Cells on Tumor Formation in Dogs.

Dog No.	Route*	No. SCT cells inj./dog ($\times 10^6$)	Tumor formation and size
1	IP	50	Neg.
	IM	20	Pos. 10 × 20 mm
	IT	2.5	Neg.
	AE	1	"
2	IP	50	Pos. Numerous masses 5 × 10 mm in size
	IM	20	" 40 × 20 × 10 mm
	IT	2.5	Neg.
	AE	1	"
3	IP	50	"
	IM	20	"
	IT	2.5	"
	AE	1	"
4 & 5	IP, IM, IT, AE	None	"

* IP, intraperitoneal; IM, intramuscular; IT, intratesticular; AE, anterior chamber of eye.

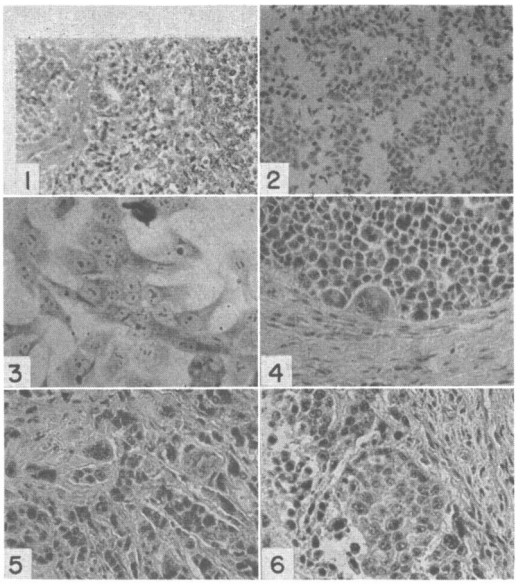

FIG. 1. Histological section of original dog tumor (H & E × 72).

FIG. 2. Forty-eight hour culture of SCT cells of 10th tissue culture passage (Giemsa × 72).

FIG. 3. Same (Giemsa × 288).

FIG. 4. Histological section of intramusc. tumor removed from dog #1 inj. intramusc. with SCT cells of 10th tissue culture passage (H & E × 180).

FIG. 5. Same from dog #2 inj. intraper. with SCT cells of 10th tissue culture passage (H & E × 180).

FIG. 6. Section of intramusc. tumor from dog #2 previously inoculated intramusc. with SCT cells of 10th tissue culture passage (H & E × 180).

with 10th tissue culture passage SCT cells. Histological and pathological examination of H & E- and Giemsa-stained preparations from these tumors revealed nodular neoplastic tissue arranged in cords and nests separated by loose fibrous tissue. Tumor material derived from intramuscular inoculation revealed tumor cells varying greatly in size, shape, nuclear pattern and affinity for hematoxylin. Many multinucleated tumor giant cells and giant nuclei were present. In the less anaplastic areas the cells retained some similarity to Sertoli cells. Examination of control material from each control dog at each site of inoculation revealed no such change of appearance of normal cells.‡

Fig. 1-6 show histopathological and cytological characteristics of this tumor at time of castration, at 10th tissue culture passage and after removal of tumors from 2 dogs inoculated with 10th passage SCT cells.

Tumor cell susceptibility to viruses. A number of viruses have been tested for their capacity to produce cytopathogenic changes in 40th tissue culture passage of this cell line. One tenth ml of 10^{-1}, 10^{-3} and 10^{-5} dilution of each virus was inoculated into SCT cells. Infected cultures were observed daily for 7 days for appearance of maximal cytopathogenic effects. Cultures designated negative, represent 3 successive passages in SCT cells. Table III shows the viral spectrum of this SCT line.

Cytological changes in SCT cells were generally observed 2 to 7 days after inoculation of roller tubes with serial 10-fold dilutions of each test virus. Control cells were invariably

TABLE III. SCT Cells Susceptibility to Viruses.

Virus	Type or strain	Virus source	Cytopathogenic effects
Newcastle	"B"	Chick allantoic fluid	+
Semliki Forest		10% mouse brain	+
Vaccinia		Bovine embryonic skin TC	+
Herpes simplex		Rabbit kidney TC	+
Canine hepatitis		Canine kidney TC	+
Influenza	Jap. 305	Chick allantoic fluid	—
	Formosa	*Idem*	—
	PR-8	"	—
Adenovirus	7	Monkey kidney TC	—
Measles	Edmonston	*Idem*	—
	"	Human amnion TC	—
Echo	6	Monkey kidney TC	—
	9	*Idem*	—
Coxsackie	B	"	—
Rabies		10% mouse brain	—
Canine distemper		Commercial chick embryo vaccine	—
Rous sarcoma		Chick embryo CAM	—

‡ Acknowledgement is gratefully given to G. Keller and Dr. W. Beckfield, of our Inst. for histological examination of these tumors.

polygonal but occasionally round cells were observed. Upon staining, these latter cells were in the process of division. Action of vaccinia virus was characterized by rounding of the cells 48 hours after inoculation, followed on 3rd or 4th day by noticeable swelling and distention of cytoplasmic elements of cells. By the 5th day cells lysed. With herpes simplex, semliki forest and newcastle disease viruses, distinct foci of degeneration first appeared within 64 hours then gradually progressed over entire culture in 148 hours. Infection with canine hepatitis virus was not evident until 5-7 days after inoculation. At this time, cell damage was characterized by appearance of numerous masses of round cells, packets of clumps of cells and eventual destruction of most cells 3-5 days after first signs of infection.

Summary. Growth characteristics of a canine Sertoli cell adenocarcinoma in tissue culture are described. Histopathological and cytological picture of the original tumor, of cells grown in tissue culture and material removed from dogs inoculated with tissue culture cells are shown. According to histopathological criteria, the original tumor, tissue culture cells and material removed from dogs inoculated with 10th passage SCT cells are all malignant. The viral spectrum of this cell line is presented.

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Effect of Continuous Low Dose Gamma Irradiation on Bactericidal Activity of Phagocytic Cells of Mice.* (24637)

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Previous studies(1,2) on bactericidal activity of phagocytic cells of mice indicated that this function was impaired in cells obtained from mice which had been subjected to whole body x-radiation in a single acute exposure within the range of 500-700 r. It was of interest, therefore, to determine whether or not bactericidal activity would be similarly impaired by continuous exposure to relatively low doses of γ irradiation.

Materials and methods. Procedures and reagents for studying bactericidal activity of phagocytic cells from mice (Carworth, CF-1) were essentially those previously described (1). Peritoneal exudates were induced in mice with gelatin. Sixteen hours later, *Pseudomonas aeruginosa* organisms were injected into peritoneal cavities so that phagocytosis could occur *in vivo*. Antibiotics were then injected to kill extracellular organisms and the phagocytes were harvested, pooled, washed, and suspended (10^6 cells/ml) in tissue culture medium with antibiotics. Immediately, and at 2 and 4 hours thereafter, aliquots of the phagocyte suspension were ground and cultured to determine number of viable bacteria/ml. A total of 150 mice were housed in groups of 10 in plastic cages (7½ x 12 x 7 inches) surrounding a nominal 10 curie cobalt 60 source. The cages were placed on curved wooden racks so that their centers were 127

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