GROWTH OF CULTURED HUMAN TUMOR CELLS IN CONDITIONED ANIMALS*

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As early as 1775, Peyrilhe[20] reported an attempt to transfer human cancer to a dog. Since that time, a few investigators have made efforts at heterologous transplantation of human tumors, but without success until 1938, when Green[4] found that human adenoma and adenocarcinoma could be transplanted several times. In 1944, Green[5] also succeeded in transplanting many kinds of human tumor to the anterior chamber of the eye of the guinea pig.

A new approach to the problems of heterologous transplantation was adopted by Toolan[18]. In 1951, she reported 90% survival and proliferation of many human tumors in rats and hamsters treated with X-rays and cortisone. Recently, since the establishment *in vitro* of many cell lines from normal and cancerous sources, several investigators[2][10][11] have administered intraperitoneal or subcutaneous injections of altered tissue-cultured cells to conditioned animals, and have induced tumor formation by this method. Handler *et al.*[6][7] reported that they had succeeded in transplanting human tumors to mice bearing an ACTH-secreting mouse tumor.

In our laboratory, six permanent cell lines from human tumors have been established, and have been cultured successfully for two to three and a half years. In the present investigation, we have used cells from our cultures to produce tumors, perhaps permanetly transplantable ones, in the subcutaneous tissue of conditioned rats and mice. For conditioning of animals, two different systems were employed, and tumor "take" in each system was compared.

MATERIALS AND METHODS

As Table 1 shows, cells from six cell lines have been cultivated as monolayers. Cells were harvested, usually after cultivation for three days, with 0.25% trypsin solution. The cells were centrifuged, washed to free them of trypsin, and then resuspended in medium 199 without serum. Viable cells were counted by staining with nigrosin[9], and their concentration was then adjusted to provide 10^3 to 10^7 cells in 1.0 or 0.5 ml of medium 199. Exact numbers of tumor cells were injected into subcutaneous tissue of the back of conditioned animals. The injected site was palpated for tumor growth every second or third day.

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TABLE 1. Cell Lines Established in Tissue Culutre

Human cell line RPMI #	Origin	Source	Date established
191	Gall bladder carcinoma	Ascites	11-15-58
212	Mesothelioma	Ascites	1- 5-59
41	Osteogenic sarcoma	Ascites	1-15-59
131	Ovarian carcinoma	Ascites	3-11-59
1922	Liposarcoma	Solid tumor	4-21-60
1938	Carcinoma of kidney	Solid tumor	6-14-60

Animals were sacrificed 10 to 30 days after inoculation of tumor cells, and tumor specimens were fixed in formalin solution for histological confirmation.

1. Subcutaneous inoculation of cells into rats treated with X-rays and cortisone:—In each experiment, 5 to 10 weanling Long-Evans rats were conditioned with 250 r of X radiation four days before cell inoculation, with 5 mg of cortisone acetate* the next day, and with a second 5 mg of cortisone acetate one day before cell inoculation. Radiation was produced with a 250-kv. G. E. Maxitron, using 250 kv., 30 ma., 0.25 mm Cu and 1 mm Al filter, air dose 159 r/min.

2. Subcutaneous inoculation of cells into mice bearing an ACTH-secreting tumor:—At-T 20, a solid tumor which secretes ACTH, was originated by J. Furth in 1956, and has been carried in adrenalectomized LAF_1 mice (C 57 L ♀ × A/He ♂). A piece of this tumor was minced, and 0.2 ml of 20% cell suspension ($4–8 \times 10^7$ tumor cells per ml) was injected into the right thigh. After At-T 20 inoculation, peripheral white blood cells were counted daily for 20 days. The average results obtained with 6 mice are shown in Figure 1. Soon after At-T 20 inoculation, the peripheral white blood cell counts fell below 2000/cu.

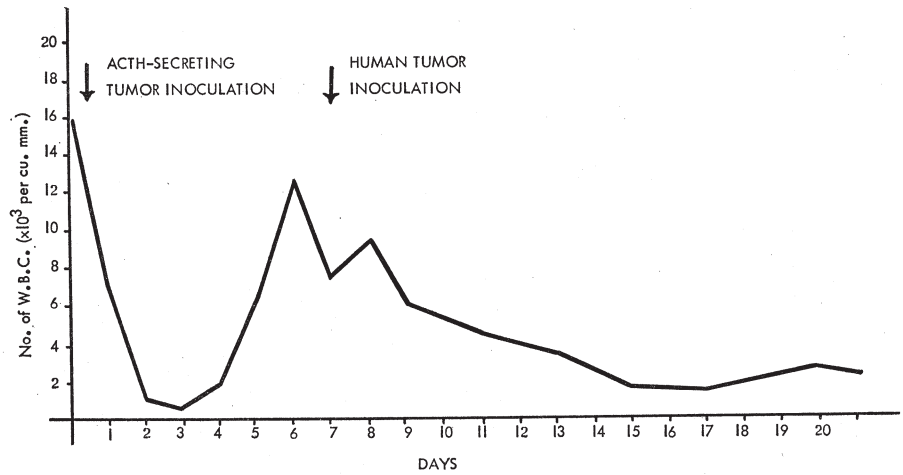

Fig. 1. White blood cell counts following inoculation of ACTH-Secreting tumor cells.

* Cortogen Acetate (Cortisone Acetate, Schering) was used.

mm, but on the sixth day it returned to the nomal range, and then gradually fell again to a point below 3000. Human tumor cells were inoculated on the 7th day. LAF_1 mice bearing At-T 20 received chloromycetin-treated drinking water ad libitnm.

3. Hormone responsivenesss of tumor cells in oophorectomized and cortisone-conditioned mice:—Thirty adult female Swiss mice, divided into three groups, were used for each cell line. On the first day, a sham operation was performed on the mice of group A, and bilateral oophorectomy on those of groups B and C. On the third day, 2.5 mg of cortisone acetate was given to each mouse. On the fourth day, 6×10^6 tumor cells were given subcutaneously. To Group C mice, 50 mcg of estrogen was given daily from the seventh to eleventh day. On the fiffeenth day, all animals were sacrificed, and tumors produced in subcutaneous tissues were weighed.

4. Attempts to provoke acquired tolerance in mice:—Newborn Swiss mice were given intravenous injections of 10^5 cultured tumor cells or 10^5-10^6 human lymphocytes within 24 hours after birth. In another group, the abdomens of middlestage pregnant Swiss mice were opened under light ether anesthesia, and 10^5 tumor cells or 10^5–10^6 human lymphocytes were inoculated into the abdomens of fetuses through the uterus wall and the amnion. The abdominal wall was closed in layers. Human lymphocytes were prepared from thoracic duct lymph or from normal lymph nodes in surgical specimens. The difinitive injection of tumor cells was given 4 to 6 weeks later.

RESULTS

1. Subcutaneous inoculation of cells into coditioned rats:—Cultured tumor cells from the first inoculation produced tumors in subcutaneous tissue. When more than 8×10^6 cells were injected, tumors about 1 cm in diameter were produced in one week to ten days. The tumor was encapsulated, and had a good blood supply (Fig. 2). Table 2 summarizes the results in this group. When more than 10^6 cells were inoculated, the tumor take was 40 to 83% for all cell lines; and when more than 10×10^6 cells were inoculated, the take was 100%. Serial transplantation of a tumor beyond the second generation was unsuccessful

TABLE 2. Tumor Growth in Conditioned Rats*

Human tumor cell lines RPMI #	Number of cells inoculated				Total	%
	1×10^5	1×10^6	5×10^6	1×10^6	10^6–10^7	
212	0/5	2/5	3/4	9/9	14/18	78
41	1/5	4/7	11/13	9/9	24/29	83
131	0/4	0/10	3/5	5/5	8/20	40
1938	—	2/5	4/5	4/4	10/14	72
191	0/5	0/5	6/7	5/5	11/17	65
1922	—	2/4	4/5	5/5	11/14	79

* No. of rats with tumor
No. of rats inoculated

except in one cell line that arose from an osteogenic sarcoma (RPMI #41). This line was transplantable for 3 passages in the same conditioned rats. Some rats with this tumor developed pulmonary metastases.

The tumor tissue eventually starts regression about 10 days after transplantation, and completes the process in 3 to 4 weeks. Histologically, the tumor is encapsulated with very active fibrous tissue, and has malignant characteristics like those of tumors of epithelial origin, but differs very much from the original human tumor (Figs. 3-5). Most sections showed the stroma of very active fibrous tissue, with occasional mitotic figures, and also foreign-body giant cells and calcification.

2. Subcutaneous inoculation of tumor cells into LAF_1 mice bearing At-T 20 in the thigh:—Table 3 summarizes the results in this group. When more than 10^6 tumor cells were injocted, the tumor take was 60 to 94% for all cell lines, and a tumor about 1 cm in diameter was produced in about 2 weeks (Fig. 6). In mice bearing At-T 20 in the thigh, transplanted tumors grew better than in conditioned rats, and survived longer. Mice were usually sacrificed in 3 weeks after tumor transplantation. Necrosis with this tumor was not seen as much in the mice as in rats. When necrosis did develop in the mice, however, they died in 5 to 6 weeks, before the process was completed. The histological pattern of this tumor was much the same as in the same tumor in rats, but showed much less fibrous tissue than in tumors in conditioned rats (Figs. 7 and 8).

TABLE 3. Tumor Growth in LAF_1 Mice Bearing ACTH-Secreting Tumor*

Human tumor cell lines RPMI #	Number of cells inoculated					Total	%
	1×10^3	1×10^4	1×10^5	1×10^6	1×10^7	10^6–10^7	
212	—	0/5	0/5	2/5	4/5	6/10	60
41	0/5	1/9	3/10	8/10	4/5	12/15	80
131	—	1/5	1/5	4/5	13/13	17/18	94
191	—	0/5	2/10	6/8	7/10	13/18	72

* No. of mice with tumor
No. of mice inoculated

3. Hormone responsiveness:—One cell line (RPMI #131) arising from ovarian carcinoma grew better in oophorectomized mice. The average weights of the tumors were 0.24 gm. in Group A, 0.39 gm. in Group B, and 0.05 gm. in Group C. Other cell lines did not undergo modification in the course of growth.

4. Attempts to provoke acquired tolerance:—Acquired tolerance was not obtained in mice injected intravenously with either tumor cells or human lymphocytes. Table 4 and 5 summarize the results in this group.

DISCUSSION

Previous workers[2)11)] have reported that about 10^6 tumor cells are needed to produce intraperitoneal tumors in conditioned rats. In our study, almost

TABLE 4. Tumor Growth in Mice Injected I. V. with Tumor Cells or Lymphocytes within Twenty Four Hours After Birth

Number of mice	Primary injection	Second injection	Time for 2nd inject. (weeks)	Tumor take
10	RPMI # 41	RPMI # 41	4	Negative
9	RPMI #191	RPMI #191	5	Negative
15	Lymphocytes	RPMI # 41	4	Negative

TABLE 5. Tumor Growth in Mice Injected I. P. with Tumor Cells or Lymphocytes at Their Fetus Stage

Litter No.	Number of mice		Primary injection	Second injection	Time for 2nd inject. (weeks after delivery)	Tumor take
	Inject.	Deliv.				
1	8	5	RPMI # 191	RPMI # 191	4	Neg.
2	12	10	RPMI # 191	RPMI # 191	4	Neg.
3	11	11	RPMI #1922	RPMI #1922	4	Neg.
4	10	6	Lymphocytes	RPMI # 41	4	Neg.
5	11	6	Lymphocytes	RPMI # 191	4	Neg.
6	12	7	Lymphocytes	RPMI # 212	4	Neg.
7	10	7	Lymphocytes	RPMI # 131	4	Neg.
8	12	0	RPMI #131	—	—	—
9	10	0	RPMI # 41	—	—	—
10	9	0	RPMI #131	—	—	—

the same number of cells was necessary for tumor production in subcutaneous tissue. From the standpoint of experimental technique, subcutaneous tissue is the optimal site for tumor production, because palpation is easy and mortality is low. It should be borne in mind, however, that "palpable tumors" sometimes prove to be fibrous tissue surrounding necrotic inoculated cells, and that histological confirmation is thus necessary.

Serial transplantation of these tumors in both conditioned rats and conditioned mice was unsuccessful after the second generation, except in the case of the osteogenic sarcoma cell line (RPMI #41), which was transplantable for three passages. When tumor cells are carried in tissue culture for a long period of time, they seem to lose their tumor-producing capacity. Sandford *et al.*[15] reported that when a transplantable mouse tumor was maintained *in vitro*, the tumor-producing capacity, which had increased after the mouse passages, subsided partially or completely after 8 months *in vitro*. This may be one of the reasons why such tumors are not transplantable serially. Manuelidis[10] also failed in his attempts at serial transplantation of tumor produced with cultured tumor cells in conditioned mice, but succeeded in serial transplantation to the brain of the mouse or to the anterior chamber of the eye of the guinea pig.

To obtain permanently transplantable human tumors, even with surgical specimens in conditioned animals, seems to be very difficult. Toolan[19] reported that one epidermoid carcinoma out of 101 human tumors could be transplanted permanently in conditioned animals. Skiff *et al.*[17] also reported that 5 out of 55 human tumors could be transplanted permanently into the cheek pouches of conditioned hamsters. Recently, Schulte[16] *et al.* reported that 8 of 126

surgical specimens could be transplanted for more than 5 generations into the cheek pouches of conditioned hamsters, and that two of these transplanted tumors were regarded as established permanent strains.

In order to reduce the potentiality for antibody formation, splenectomy has been performed before treatment with cortisone and X rays, but has failed to accelerate tumor growth. All of the tumors in conditioned rats start regression about ten days after transplantation. Therefore, if human tumors are to be transplanted into other experimental animals, the second transplantation must be done before the tenth day. In some studied, an additional dose of cortisone was administered between the seventh and tenth days, in an effort to prevent this regression, but without success.

Appreciable histological differences between the original human tumor and the tumors produced in conditioned animals may be explained on the basis of changes resulting from the cultivation of cells *in vitro* for a long period of time, since the tumors resulting from transplantation of surgical specimens are very similar to the original tumors, and the same is also true of those produced from ascites cells. It is well known[3] that normal human tissues may change in tissue culture and become malignant after a long period of time *in vitro*. When our cell lines were inoculated into human volunteer patients, the histological patterns of tumors produced in subcutaneous tissue were very similar to those of tumors produced in conditioned rats.

Tumor "take" in LAF_1 mice bearing At-T 20 seems to be better than in conditioned rats. Handler and Fauci[7] reported that human tumors which are transplantable in cortisone-treated hamsters grew vigorously in these conditioned mice. Human tumors in these mice bearing At-T 20 have survived much longer than in conditioned rats. This may be explained by the fact that the ACTH-secreting tumor produces continuous suppression of immunological responses. Handler and Fauci[7] reported that massive doses of cortisone or ACTH alone cannot suppress immunological response as successfully as At-T 20 does. In this respect, mice bearing At-T 20 must have not only a high level of cortisone but also a high level of other corticosteroids which may play important roles in the suppression of immunological response.

One cell line arising from ovarian carcinoma responded to oophorectomy. Under the circumstances, this cell line is considered to be responsive to gonadotropic hormone.

Several investigators have employed the principle of immunological tolerance in the transplantation of heterologous tumors. Some workers[12~14] have succeedd in transplanting animal tumors. No one has yet succeeded in transplanting human tumors to animals except Agneenko[1] and Iversen and Sorensen[8], who have reported that they obtained partial tolerance of human tumors in animals. In our study, such transplantation could not be proved. This problem is being given further study.

SUMMARY

Six human tumor cell lines have been established in our tissue culture laboratory. Cells from these lines have produced tumors in the subcutaneous

ssue of conditioned animals. Tumor take seemed to be better in mice bear g an ACTH-secreting tumor than in rats conditioned with X-rays and corti- ne. No tumor permanently transplantable in conditioned animals conld be otained. Histologically, the resulting tumors have malignant characteristics milar to those of tumors of epithelial origin. One cell line arising from an arian carcinoma grew better in oophorectomized and cortisone-treated mice. cquired tolerance was not provoked in mice by the injection of tumor cells id lymphocytes.

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LEGENDS FOR FIGURES 2-8

FIG. 2. Tumor (RPMI # 191) grown in subcutaneous tissue of conditioned rat, 10 days after tumor inoculation.

FIG. 3. Low-power magnification (×200) of tumor (RPMI # 191) grown in conditioned rat.

FIG. 4. High-power magnification (×600) of tumor (RPMI # 191) grown in conditioned rat.

FIG. 5. High-power magnification (×600) of tumor (RPMI # 131) grown in conditioned rat.

FIG. 6. Tumor (RPMI # 131) grown in subcutaneous tissue of LAF_1 mouse bearing ACTH-secreting tumor, 14 days after tumor inoculation.

FIG. 7. High-power magnification (×400) of tumor (RPMI # 191) grown in LAF_1 mouse bearing ACTH-secreting tumor.

FIG. 8. High-power magnification (×400) of tumor (RPMI # 131) grown in LAF_1 mouse bearing ACTH-secreting tumor.

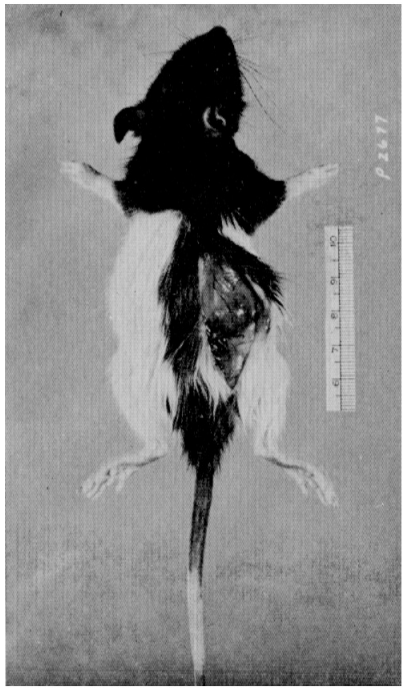

FIG. 2

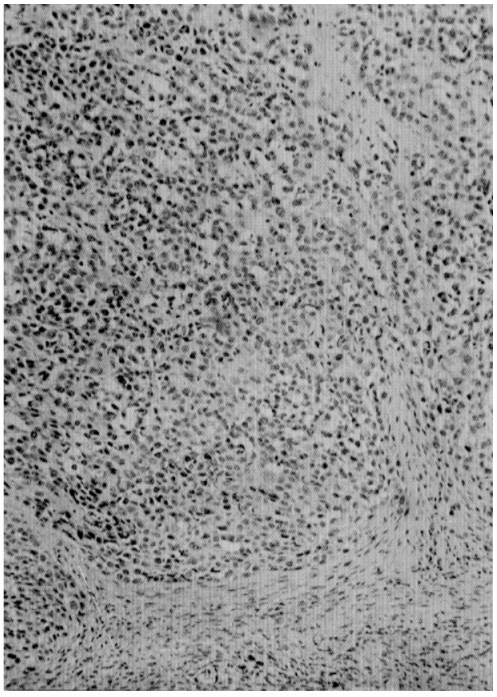

FIG. 3

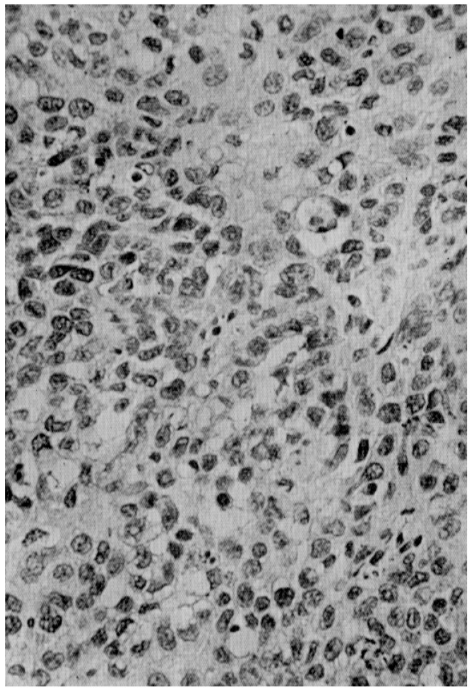

FIG. 4

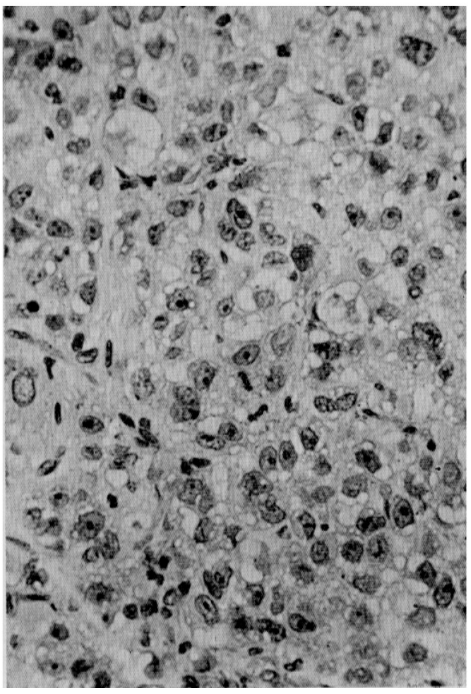

FIG. 5

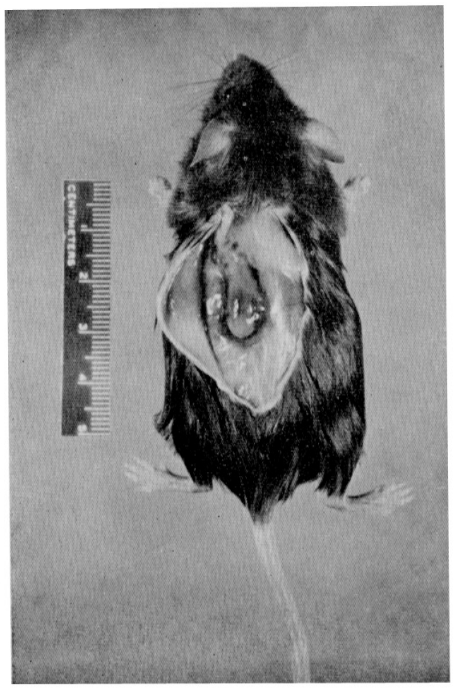

FIG. 6

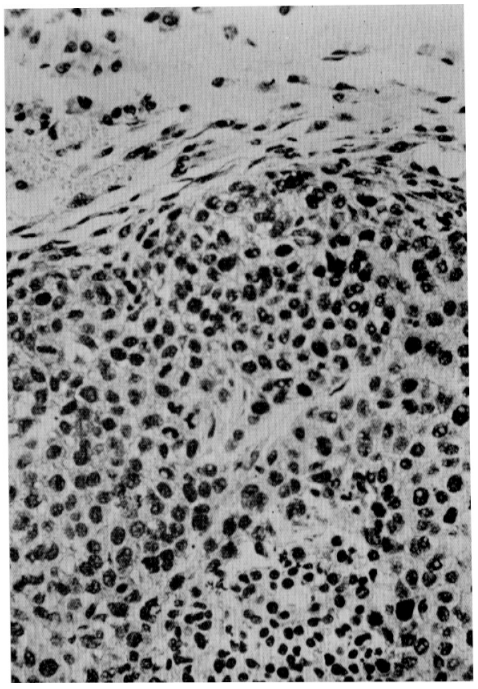

FIG. 7

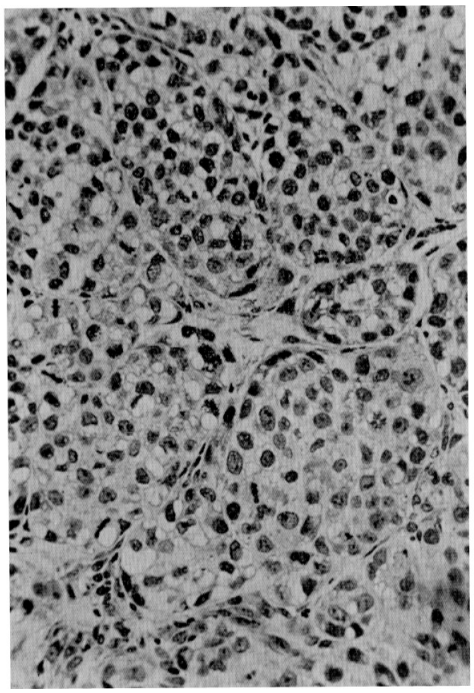

FIG. 8