# Stable Cell Line of Rat Nephroma in Tissue Culture.* (31705)

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This is a report on the establishment of a stable tissue culture cell line of a rat nephroma. The only nephroma line that has been reported previously, to our knowledge, is a human line of Wilm's tumor cultivated by Dobrynin(1) and listed in the American Type Culture Collection (cell repository number CCL 31), but its cultural characteristics and transplantability are not described.

*Origin of the culture.* The original rat tumor was embryonal nephroma (Wilm's tumor). It was maintained by serial homotransplantation in suckling rats, and still retained some of the characteristics of the original tumor(2). The tissue culture cell line was derived from the 13th rat passage of the tumor in December 1960. The tumor was minced in Gey's balanced salt solution (3), centrifuged slowly to sediment the cells and tissue fragments, resuspended in culture medium (see below), and planted in milk dilution bottles. These bottles, lying on their sides, provided a 10 × 4 cm glass surface for growth of the cells. These original cultures were incubated at 37°C and were refed with 10 ml of medium at 7- to 10-day intervals for 3 months before cell growth became dense enough to require subculturing. Thereafter, the cultures were passed by trypsinizing and they soon developed the present pattern of rapid growth.

*Tissue culture characteristics.* The cell line has been maintaind without difficulty in either Eagle's basal medium(4) or Eagle's minimum essential medium(5) supplemented with 0.01 mM glutamine and 20% calf serum or fetal calf serum or newborn "agamma" calf serum. Tissue culture solutions and sera were purchased from commercial suppliers. The cells grew poorly in Puck's medium(6) regardless of the amount or type of serum supplement that was used.

For the past 5 years this cell line has grown rapidly, increasing 5- or 6-fold in a week. Most of the cells were spindle-shaped but some were polygonal (Fig. 1). The cultures were usually trypsinized and passed at weekly intervals, but it was possible to maintain a culture without passage for as long as 4 months by refeeding at approximately 10-day intervals. The cells have been successfully stored in the frozen state in ampules containing 2 to 7 million cells in 1 ml of complete tissue culture medium either without glycerine or with 5%, 10%, or 15% glycerine added. They were frozen in a Canalco programmed freezer at the rate of 1° cm each minute down to —40°C, and were then stored in a dry-ice box. They were thawed at room temperature and used to

TABLE I. Transplantation of Rat Nephroma Tissue Culture Cells into Allogeneic Rats.

| Route of transplantation | # of cells | 5-11-day-old rats | | | 18-34-day-old rats | | |
|---|---|---|---|---|---|---|---|
| | | Injected | Takes* | Regressed | Injected | Takes* | Regressed |
| Subq. | $10^7$ | 24 | 23 | 9 | 14 | 13 | 13 |
| " | $10^6$ | 13 | 9 | 8 | 9 | 8 | 8 |
| " | $10^5$ | 6 | 0 | — | 2 | 1 | 1 |
| I.P. | $10^7$ | 9 | 9 | 0 | 2 | 0 | — |
| " | $10^6$ | 8 | 8 | 0 | — | — | — |
| " | $10^5$ | — | — | — | 2 | 0 | — |

* A "take" following subcutaneous transplantation was defined as the development of a palpable tumor mass at site of injection. These grew to diameters of 1 to 5 cm, but many eventually regressed. Intraperitoneal tumor "takes" were detected by palpation and were confirmed at autopsy and all were lethal. It is possible that smaller i.p. tumors grew and regressed in some rats but were not detected by palpation.

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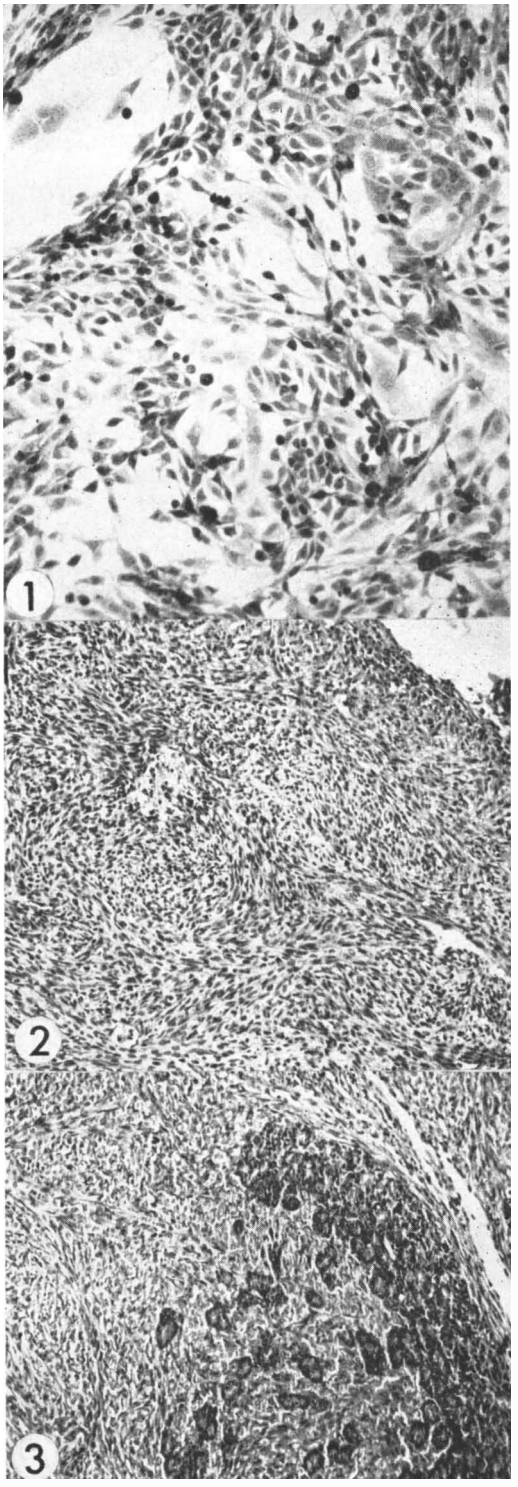


FIG. 1. Eleven-day monolayer tissue culture of the rat nephroma cell line 3½ years (13 passages) after its intiation. Note presence of spindle-shaped and polygonal cells. May-Grunewald-Giemsa stain. 70 ×.

FIG. 2. Histologic section of subcutaneous tumor resulting from transplantation of $10^7$ cells of the rat nephroma line into 11-day-old rat after 16 months in tissue culture. Rat was autopsied 44 days after imp'antation. Hematoxylin and eosin stain. 70 ×.

FIG. Histologic section of intraperitoneal tumor resulting from transplantation of $10^7$ cells of rat nephroma line into 10-day-old rat after 11 months in tissue culture. Rat was autopsied 15 days after implantation. Tumor is invading the pancreas. 70 ×.

establish new cultures.

*Transplantation of the cultured cells.* Tissue-cultured cells were injected into Wistar rats from our randomly bred colony in which the tumor had originated. Results are presented in Table I.

Subcutaneous transplantation of 1 million or more cells into rats of from 5 to 34 days of age produced tumors in 88% of the 60 recipients. Maximum tumor size ranged from 1 to 5 cm diameter. A dose of 100,000 cells produced a tumor in only 1 of 8 recipients. The tumors often regressed in the younger recipients (5 to 11 days old), and always regressed in the older recipients (18 to 34 days old). Regression was often evident as early as the 3rd week after implantation and was usually complete by 6 to 7 weeks if it occurred at all. Regression is not unexpected in homotransplanted tumors, but the frequency of regression of tumors initiated by these tissue-cultured cells was much greater than occurred with tumor established by rat-to-rat passage of a similar dose of cells. Subcutaneous tumors that did not regress caused death of the animals, usually between 7 to 13 weeks after inoculation. No metastases were noted on gross examination; death was apparently the result of local tumor growth and necrosis.

Histologically, the subcutaneous tumors consisted of spindle-shaped sarcoma cells, often arranged in bundles and whorls (Fig. 2). Central ischemic necrosis or scattered foci of necrosis were frequent in nodules 1 cm or greater in diameter. In these characteristics the tumors resemble the spontaneous tumor from which the cell line originated and the tumors produced by rat-to-rat transplantation (illustrated in reference 2). It is not

possible, however, to identify these transplanted tumors as nephromas. Only occasionally are there areas suggestive of abortive tubule formation, and no glomerulus-like formations have been observed.

Intraperitoneal transplantation into the 5- to 11-day-old rats produced lethal tumor growth in all of the animals injected with a million or more cells. Smaller numbers of cells were not tested i.p. There was no evidence of growth on i.p. transplantation into 18- to 34-day-old rats but only 2 rats were tested with more than 1 million cells.

The intraperitoneal tumors grew as nodules and sheets of tumor cells attached to the peritoneal surfaces of diaphragm, omentum, and abdominal viscera. Histological examination revealed some areas of direct invasion through the peritoneum into underlying viscera (Fig. 3) but usually growth was not infiltrative.

*Summary.* An embryonal nephroma, which occurred spontaneously in a rat and had been maintained by serial transplantation, has now been established in tissue culture as a stable cell line and has been maintained in continuous passage for more than 5 years. It is readily transplantable to young rats, subcutaneously or intraperitoneally, by inoculation of a million or more cells but such transplants often regress spontaneously.

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## The "Free" and "Buried' Tryptophan and Tyrosine Residues of Human Plasma $\alpha_1$-Acid Glycoprotein.* (31706)

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The determination of the "free" and "buried" amino acid residues of proteins is known to contribute greatly to our understanding of the structure of this class of biologically important macromolecules. Recently, such studies have been reported for certain enzymes(1,2,3,4) relating activity to some of their "free" amino acid residues. However, apparently no glycoprotein has been investigated by such techniques. In this report we present the results of the determination of the "free" tryptophan and tyrosine residues in $\alpha_1$-acid glycoprotein (orosomucoid)(5,6), a well characterized glycoprotein of normal human plasma.

2-Hydroxy-5-nitrobenzylbromide which has been demonstrated by Koshland *et al*(3) to be a highly specific reagent for "free" tryptophan residues, and native $\alpha_1$-acid glycoprotein isolated from Cohn Fraction VI of pooled normal human plasma(5) were used for the first part of this study. The sialic acid-free glycoprotein was prepared by treatment of the native protein with neuraminidase. The reaction of $\alpha_1$-acid glycoprotein with this reagent was carried out essentially as described by Koshland(3,8). In certain experiments the solvent contained increasing amounts of 2-chloroethanol. The excess of the reagent and solvent was removed by filtration through a Sephadex G-25 column. The effluent containing the protein was dialyzed against water and lyophilized. An aliquot of the resulting protein powder was dissolved in 0.1 N NaOH and its absorbency was determined at 410 m$\mu$. Using the molar extinction coefficient of 18,900 for the Koshland reagent(3), the

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