TABLE I. Catecholamine Content in Culture Media of Pheochromocytoma and of Adrenal Medulla.

Days after expl.	Pheochromocytoma NA, μg/ml I	Pheochromocytoma NA, μg/ml II	Pheochromocytoma A, μg/ml* I	Pheochromocytoma A, μg/ml* II	Adrenal medulla NA, μg/ml I	Adrenal medulla NA, μg/ml II	Adrenal medulla A, μg/ml* I	Adrenal medulla A, μg/ml* II
1	1.88	2.2	1.34	7.75	0	0	.075	.075
2	.50	0	1.70	.75				
4	.125	.25	.55	1.00	0	0	.15	.09
6	0	0	.75	.45				
7	0	0	.15	.30				
9	0	0	.30	.30				
15	0	0	.08	.15	0	0	.08	.15
20	0	0	.15	.15	0	0	0	.30
41	0	0	.15	.15		0		.30
62	0		.037		0		0	

* The method of analysis as used did not reliably separate NA and A in quantities smaller than 0.02 μg/ml.

not quite reliable and all catecholamines tend to appear as A. Failure to find NA in culture fluids should not necessarily be attributed to the method used, since it was shown previously (3) that in adrenal glands of adult humans the largest portion of catecholamine is A.

Summary. Human adrenal medullary tumors and adrenal medulla produced catecholamines in tissue culture for at least 40 days.

1. Symposium on Catecholamines (Section II, 307-374), Williams and Wilkins, Baltimore, 1959.

2. v. Euler, U. S., Lishajko, F., *Acta Physiol. Scand.*, 1959, v45, 122.

3. West, G. B., Shepherd, D. M., Hunter, R. B., *The Lancet*, 1951, v2, 966.

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Continuous Cultivation of Cells Arising from Human Case of Glioblastoma Multiforme (Glioma T.C. 178).* (25365)

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A variety of normal and malignant cells has been successfully cultivated *in vitro* and propagated in continuous subcultivations. There is, however, no report about continuous subcultures of cells arising from a glioma, although neoplasms of the nervous system have been cultivated for limited periods of time (1-6). In this communication we describe a line of cells derived from a human case of glioblastoma multiforme. These cells have been growing in continuous subcultures in this laboratory since Sept. 4, 1957.

Materials and methods. Fresh tumor tissue (5x5 mm) was immediately obtained from the operating room during a right temporal craniotomy for what was later histologically diagnosed as a glioblastoma multiforme of the right frontal lobe. The tumor was minced with uterine scissors and washed several times with balanced salt solution until supernatant fluid was clear. The small quantity of tumor fragments was evenly divided and distributed into three 8 oz. wide-mouthed Blake bottles. Approximately 20 cc of medium (composed of Eagles medium,† glutamine† 1%, Penicillin 100 units/cc, and Streptomycin 100 μg/cc), containing 30% human serum, was added to each bottle. During cultivation of cells, constituents of medium were kept constant in amount, the only variation being source and quantity of serum. The bottles were placed in 37°C incubator. On second day growth was observed around the explants. This continued slowly until 3 weeks later when all bottles showed luxuriant growth; the glass surface was not entirely covered with cells. At that time the original medium and floating necrotic fragments were discarded and 20 cc of new medium containing 20% human serum and 5% new-born human brain cell-free extract was added; Mycostatin (100 units/cc) was also added to eliminate the possibility of fungal growth. When surface of the Blake bottle was covered with cells, the culture was scraped with a rubber policeman, the cells centrifuged at 500 rpm for 5-10 minutes, resuspended in new medium, and planted in new bottles. The

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† Available from Microbiological Assoc., Washington, D.C.

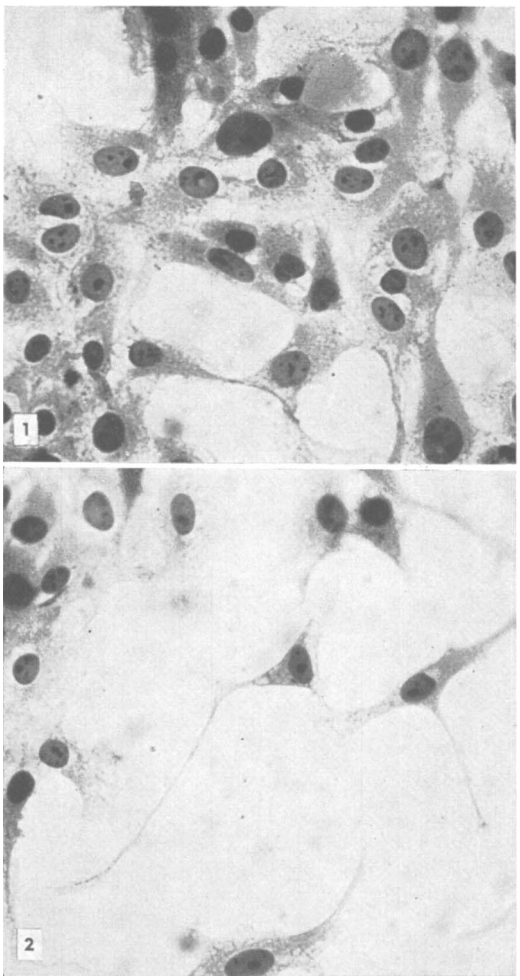

FIG. 1. Round, oval, and elongated nuclei are seen with varying numbers of nucleoli-like chromatin condensations. Hematoxylin and Eosin stain × 690.

FIG. 2. In the center of this picture cells are seen with irregular cytoplasmic processes. Hematoxylin and Eosin stain × 690.

cells were transferred in this manner from bottle to bottle 7 times in 20% human serum for 4 months. For the next 3 months growth was adequate using medium containing 10% human serum. Seven months from date of growth in tissue culture and with excellent growth in 10% human serum, growth was attempted in 10% horse serum. At first the cells grew slowly and much debris was noted in the bottles. Gradually the cells grew in horse serum and have been maintained in that medium to the present. For histological examinations cells were grown on coverslips in Leighton flat tubes. Coverslips were fixed in a variety of fixatives, dehydrated, and covered with a thin layer of paraffin. Using this technic, one may fix large numbers of coverslips and stain them at one's convenience according to the desired histological or histochemical technics. *Histology.* Tissue culture cells revealed pleomorphism; elongated, triangular, and pyriform cells were observed. Some cells were extremely large. Occasional multi-nucleated giant cells were seen. Size of nuclei varied considerably; some were very large; some oval, others round or often elongated. Variable numbers, often up to 7, of nucleoli-like condensations of chromatin were seen. Cytoplasmic details were to some extent obscured in regions of intense growth (Fig. 1). In periphery of culture and in other regions where growth was not so dense, the cells had elongated cytoplasmic processes (Fig. 2) that arose, in many instances, from one part of the cytoplasm and then bifurcated to form 2 unequal branches. Some cells had more than one cytoplasmic process. Often the cytoplasmic process of one cell touched the cytoplasmic process of adjacent cell. In some instances there were cytoplasmic bridge-like structures between neighboring cells. Very often the cytoplasmic processes revealed irregularities and varicosities; part of the process next to cell body was narrow and became enlarged in the periphery. The processes of cells stained with silver when impregnated with silver carbonate.

Results. Growth rate of glioma cells was estimated by nuclei counts and total protein determinations as follows: From healthy cultures in log phase of growth, 64, 3 oz. Brockway bottles were prepared each containing approximately 250,000 cells suspended in 10 cc of medium with 10% horse serum. Beginning with 24th hour, and every 12 hours thereafter to the 108th hour, 4 bottles were taken for nuclei counts and 4 bottles were simultaneously taken for total protein determinations. Average total nuclei count/bottle was determined by Rappaport's modification of the method of Parker *et al.*(7). Average amount of total protein/bottle was determined by the method of Oyama and Eagle(8).

Using exactly the same technics and me-

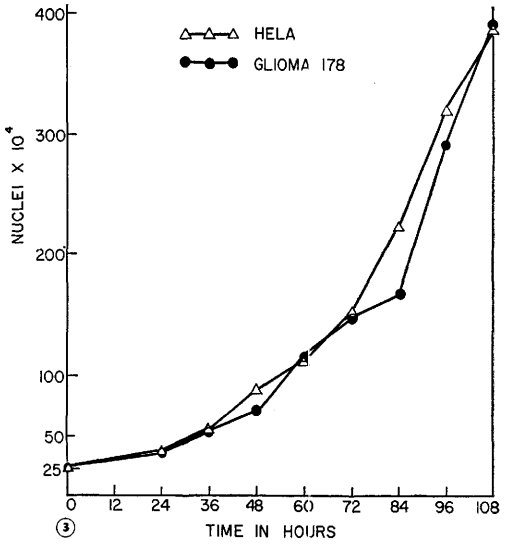

FIG. 3. Avg total nuclei count/bottle ($\times 10^4$) is given for Glioma T.C. 178 cells and HeLa (S-3) cells grown under identical environmental conditions.

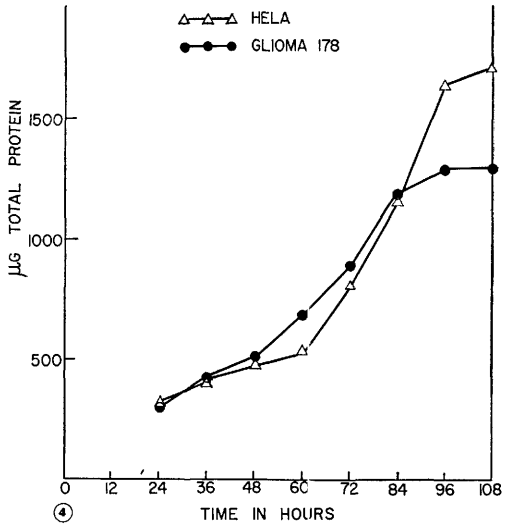

FIG. 4. Avg total protein, µg/bottle, is given for Glioma T.C. 178 cells and HeLa (S-3) cells grown under identical environmental conditions.

dium as that previously described above for the glioma cells, rate of growth was determined for HeLa cells (Strain S-3). The comparative results of nuclei counts of Glioma T.C. 178 and HeLa cells are given in Fig. 3 and total protein determination for both is given in Fig. 4. From these graphs it is evident that rate of growth, in log phase under identical nutritional conditions, is approximately the same for Glioma T.C. 178 cells and HeLa cells; generation time for each is approximately 26 hours.

Preliminary experiments indicate that both West Nile and Poliomyelitis viruses may be propagated in Glioma T.C. 178 cells. Cells of Glioma T.C. 178, injected subcutaneously into the flank of X-irradiated cortisone-treated hamsters according to method of Toolan(9), cause development of small tumors at site of injection. These observations and results of heterologous transplantation of the glioma cells into guinea pig brains will be reported.

Summary. A line of tissue culture cells originally arising from a histologically verified human case of glioblastoma multiforme has been propagated in continuous subcultures for approximately 2 years. Morphological characteristics and preliminary observations concerning susceptibility of these cells to viruses are given. Rate of growth was measured by nuclei count and total protein determination and was compared with rate of growth of HeLa cells under identical conditions.

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1. Buckley, R. C., *Am. J. Path.,* 1929, v5, 467.
2. Cox, L. B., Cranage, M. L., *J. Path. and Bact.,* 1937, v45, 477.
3. Kredel, F. E.. *Arch. Surg.,* 1929, v18, 2008.
4. Pinkus, H., *Am. J. Cancer,* 1937, v29, 25.
5. Pomerat, C. M., *J. Neuropath. and Exp. Neurol.,* 1955, v14, 28.
6. Russel, D. S., Bland, J. O. W., *J. Path. and Bact.,* 1934, v39, 375.
7. Parker, R. C., Healy, G. M., Fisher, D. C., *Canad. J. Biochem. & Physiol.,* 1954, v32, 306.
8. Oyama, V. I., Eagle, H., PROC. SOC. EXP. BIOL. AND MED., 1956, v91, 305.
9. Toolan, H. W., *Cancer Res.,* 1954, v9, 660.

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