Catecholamine Production in Tissue Cultures of Human Adrenal Medullary Tumors and of Adrenal Medulla.* (25364)

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While metabolism of catecholamines of the adrenal medulla has been extensively investigated in various animal species *in vivo* or by utilizing tissue slices or cell fractions *in vitro* (1), no studies of hormone production in tissue cultures of adrenal medullary cells have previously been reported.

Methods. The material for tissue cultures was obtained from 2 cases of pheochromocytoma removed in University of Virginia Hospital last year. The first patient was a 24-year-old white female. Her urinary catecholamine excretion before operation was 651 μg noradrenaline (NA)/24 hr and 364 μg adrenaline (A) 24 hr. A pheochromocytoma of the right adrenal weighing 153 g and measuring 9 x 6 x 5 cm was removed on July 28, 1958. One week after the operation, the patient excreted 28.8 μg NA/24 hr and 20.7 μg A/24 hr. The second patient was a 29-year-old white female with preoperative urinary catecholamine excretion of 670 μg NA/24 hr and 451 μg A/24 hr. A pheochromocytoma of the left adrenal, removed May 14, 1959, weighed 63 g and measured 6 x 6 x 4 cm. The bloody fluid which exuded from the tumor and from the adrenal into the Petri dish in which the specimen was transported to the laboratory contained more than 1675 μg NA/ml and 1950 μg A/ml. One week after the operation, catecholamine excretion was 170 μg NA/24 hr and 28.4 μg A/24 hr. It returned to normal after 1 month (8.6 μg NA/24 hr and 3.6 μg A/24 hr). Explantations of tissues were started within 5 minutes after removal of tumor. Fragments of the tumor and of tumor-free parts of the adrenal medulla were grossly separated from the cortex and trypsinized for 10 min. before explantation into 40% human serum-Hank solution medium containing penicillin and streptomycin. During the first days the culture media became deeply colored by a brown-black pigment which may have been melanin. The explants both of tumors and of adrenals showed outgrowths of elongated large cells, 360-400 μ long, with large nuclei and nucleoli, 3 to 5 days after explantation. The cytoplasm contained a large number of faint yellow granules seen in unstained preparations. The outgrowths were more abundant in the adrenal than in the tumor explants. During 2 months cultivation the cells retained their morphological characteristics, although they became smaller. Two subcultures in series were obtained from the adrenal but none from tumor explants. After 7 weeks most cultures deteriorated and were discarded. The catecholamines were estimated by the method of v. Euler and Lishajko(2). One or 2 ml of culture medium was diluted to 25 ml with distilled water and treated as 25 ml urine in the above method. Catecholamines in the medium of first patient's tumor culture were determined on third day only. The culture medium was darkly pigmented and contained 0.56 μg NA/ml and 0.26 μg A/ml. In the second case of pheochromocytoma the catecholamines were determined for 2 parallel groups of tumor and adrenal explants. The media from 3 tubes were pooled first daily and later over periods of several days. Every tube contained 5-10 pieces (about 3 mm^3 each) of tissue and their outgrowths of varying size.

Results. Representative values of the catecholamine content of the media are given in Table I. The media from adrenal medulla cultures never contained detectable amounts of NA. The media from tumor cultures contained large amounts of NA on the first and smaller amounts on following 3 days. Catecholamines were found in fluids from both tumor and medulla cultures for at least 40 days. Experiments in which mixtures of small amounts (<0.2 μg/ml) of NA and A were estimated showed that by the method used the differentiation between NA and A is

* Supported by Research Grants from Am. Cancer Soc. and Virginia Heart Assn.

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.

Received September 14, 1959. P.S.E.B.M., 1959, v102.

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

* Supported by U.S.P.H.S. Grant.

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