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Incorporation and Release of H^3-Catecholamines by Cultured Fetal Human Sympathetic Nerve Cells and Neuroblastoma Cells.* (32258)

MILTON N. GOLDSTEIN†
St. Jude Research Hospital, Memphis, Tenn.

Children with neuroblastomas have an increased urinary excretion of dopa, dopamine, norepinephrine and the metabolites of these catecholamines(1-3). The excretion of the amines is not constant; there may be a variation in the quantities of the various amines and their metabolites as the disease progresses. The excretion of these amines has not been correlated with the Bright Field histology of the tumor cells(1). La Brosse and coworkers(4) reported that 3-O-methylated metabolites of H^3-norepinephrine were found in media after adding H^3-norepinephrine to cultures of neural crest tumor cells. Their studies indicated that these tumors contained enzymes which could rapidly degrade norepinephrine and other precursors of this amine.

The purpose of the present study was to compare the incorporation and retention of H^3-dopamine and H^3-norepinephrine by neuroblastoma cells and by human fetal sympathetic neuroblasts growing in tissue culture.

Material and methods. Neuroblastoma cells from 8 children whose tumors were composed of immature, small neuroblasts were explanted on the surface of collagen coated cover slips in 30 mm plastic petri dishes. A dense suspension was made from the friable tumors in growth medium. One-tenth cc of the suspension, consisting of small clumps and many isolated tumor cells, was placed on the collagen. One and one-half cc of medium, composed of 30% calf serum and 70% 199 containing 200 units/ml of penicillin G, was added after the cultures had been incubated at 37°C for one hour in a humidified incubator gassed with 5% CO_2 and air.

The sympathetic chains were dissected from 10 human fetuses aborted between 12 and 14 weeks gestation. The chains from a fetus were cut into small fragments after removing the capsule of connective tissue and incubated with intermittent shaking at 37°C in 0.5% crude trypsin in Hanks' solution for 90 minutes. The nerve cells were dissociated by pipetting and the cells were centrifuged at 1,000 RPM for 10 minutes. The super-

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† Present address: Dept. of Anatomy, Washington Univ. School of Med., St. Louis, Mo.

natant trypsin solution was discarded and the cells were washed twice in Hanks' solution, suspended in 2 cc of medium, and 0.2 cc was added to each of 10 collagen coated cover slips in 30 mm plastic petri dishes. The cultures were incubated for 1 hour to allow for the attachment of cells, and 1½ cc of medium was then added.

After 10 and 20 days in culture, cells were pulse labelled for 10 minutes with 5 μc/ml of H^3-dopamine or 1-5 μc/ml of H^3-norepinephrine (New England Nuclear). The cultures were rinsed in 3 changes of Hanks' and fixed in 3% glutaraldehyde in Hanks' for 1 hour. Other cultures were chased after the pulse for 1-24 hours in media free of labelled catecholamines. In some experiments, 0.1 μg/ml of unlabelled dopamine or norepinephrine was added to the chase medium to inhibit further incorporation of labelled catecholamines. After fixation, cover slips were mounted on 3″ × 1″ microslides with adhesive tape and covered with Kodak AR 10 stripping film. Preparations were developed after storage at 10°C for 8 days, stained with 0.02% Toluidin Blue for 3 minutes, rinsed in distilled water and dried at 37°C. Cover slips were removed from the slides and remounted, film side down, in DPX on slides.

Results. The distribution of the grains was the same over cells pulsed with dopamine or norepinephrine and the disappearance of the grains from the cells which incorporated these catecholamines occurred in exactly the same sequence. The addition of either cold dopamine or cold norepinephrine (1-5 μg/ml) to the pulse medium completely inhibited the incorporation of either H^3-catecholamine into normal or malignant sympathetic nerve cells.

Only 5 of 8 neuroblastomas showed intense labelling over cell bodies and axons after pulsing with the H^3-catecholamines (Fig. 1, Table I). The concentration of grains rapidly decreased as cells in these cultures were chased. After 10 hours, only a few grains were seen over cell bodies and axons, and none were observed in any of the cells after 20 hours. Only about 1% of the cells in one of the neuroblastomas incorporated and bound label (Fig. 2), and no graining could be seen in any of the cells in these cultures

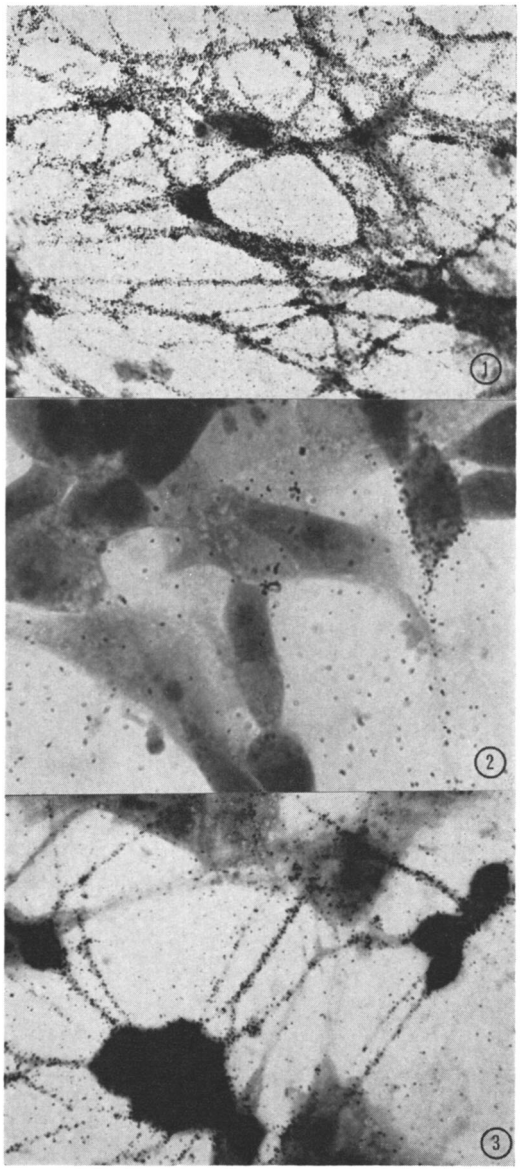

FIG. 1. Autoradiograph of neuroblastoma NJB in culture 20 days. Pulsed 10 min with H^3-norepinephrine. All the cell bodies and axons are heavily labelled. Toluidin Blue. ×400.

FIG. 2. Autoradiograph of neuroblastoma SNB in culture 20 days. Pulsed 10 min with 5 μc/ml of H^3-norepinephrine. Chased 30 min. Only one cell and its axon is labelled. Toluidin Blue. ×550.

FIG. 3. Autoradiograph of dissociated human sympathetic ganglion cells from 12-week aborted fetus. 20 days *in vitro*. The cell bodies and axons of the neuroblasts are still heavily grained after a 10 min pulse with 1 μc/ml of H^3-norepinephrine and an 8 hr chase in nonradioactive medium. Toluidin Blue. ×450.

TABLE I. Incorporation of H^3-Amines into Neuroblastoma Cells and Fetal Human Sympathetic Nerve Cells.

Patient	Age (yr)	Source of tissue	X-ray and chemotherapy	Labelling with H^3-catecholamines
JB	1	lymph nodes	yes	++++
JH	2½	bone marrow	"	++++
TD	4	lymph nodes	"	++++
CE	4½	abdominal mass	no	++++
KK	6	lymph nodes	yes	++++
SNB	1½	abdominal mass	no	+
CM	6	lymph node	yes	0
MS	8	bone marrow	"	0
Fetal symp. gang.	12-14 wk			++++

after a 10-hour chase. It should be emphasized that the pattern of outgrowth and Bright Field histology of these neuroblastoma cells was identical to those which incorporated and bound H^3-catecholamines. Neuroblastomas CM and MS showed no evidence of incorporation. Some of these cultures were incubated with the H^3-catecholamines for as long as one hour, but there were still no grains over any of the tumor cells.

All of the sympathetic nerve cells explanted in culture from 10 human fetuses showed evidence of rapid incorporation and binding of the H^3-catecholamines (Fig. 3). Heavy graining was observed over the cell bodies and axons at the 10th hour and many cells still contained grains 20 hours after pulse labelling.

Discussion. Fischer and Snyder(5) have described the disposition of norepinephrine in normal and denervated superior cervical ganglia of the rat *in vivo*. In their experiments H^3-norepinephrine disappeared more slowly from denervated ganglia. There was considerable radioactivity in the denervated ganglia of the rat after 24 hours. The normal human sympathetic ganglion cells in culture retained catecholamines as indicated by the graining over the cells and like the *in vivo* experiments(5) retained labelled amine 20 hours after the pulse.

Some of the neuroblastomas rapidly incorporated catecholamines, but these compounds disappeared much more rapidly from the malignant cells in culture. The disappearance of the label may be an indication of the more rapid degradation and release of the metabolites of dopamine and norepinephrine. The loss of label may also be due to inability of the tumor cells to store these compounds. Both mechanisms may be operative in malignant neuroblastomas. La Brosse and coworkers(4) and others(3) have shown that enzymes are present in neuroblastoma cells which can degrade these amines. Many functioning tumor cell populations growing *in vivo* or *in vitro* have lost the rigid control mechanisms which regulate synthesis, storage and release of differentiated products. Those neuroblastoma cells which did not incorporate amines may have lost the ability to store these amines or the labelled amines were degraded as rapidly as they were incorporated. This aspect of the problem may be determined by identifying the catecholamines and their metabolites in media of those neuroblastomas in culture which showed no evidence of incorporation.

Summary. Neuroblastoma cells from 8 children and dissociated human sympathetic nerve cells from 10 fetuses were grown in tissue culture. Sympathetic nerve cells from all of the fetuses and 5 of 8 neuroblastomas rapidly incorporated H^3-norepinephrine or its precursor, H^3-dopamine. There was a more rapid turnover of these catecholamines in the tumor cells than in the normal fetal human sympathetic nerve cells. Three neuroblastomas showed little or no incorporation of these amines.

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Enhancement of Interferon Titers by Poly-L-Ornithine.* (32259)

JEREMIAH G. TILLES (Introduced by Maxwell Finland)

Thorndike Memorial Laboratory, Second and Fourth (Harvard) Medical Services, Boston City Hospital, and Department of Medicine, Harvard Medical School, Boston, Mass.

Protamine was shown by Amos(1) to enhance uptake of RNA by cultured chick cells. Subsequently, Ryser and Hancock(2) were able to stimulate uptake of albumin by tumor cells in culture with the addition of histones or basic polyamino acids. These findings suggested a possible means of enhancing the sensitivity of the interferon assay. If this were successful, the method would shed some light on the controversial question of the uptake of interferon by cells(3,4).

Materials and methods. Media. The growth medium consisted of Geys-Tris solution containing 5% lactalbumin hydrolysate, 5% fetal calf serum, 150 μg/ml potassium penicillin G and 250 μg/ml streptomycin sulfate. The same medium without fetal calf serum was used as diluting fluid. The agar overlay consisted of growth medium, 0.9% Bacto Noble agar, and 1:10,000 neutral red. *Poly-L-ornithine* (PLO) was employed as a crystalline powder, M.W. 140,000, obtained from Pilot Chemical Co., Watertown, Mass. *Chick embryo cells* were used as 24-hour, confluent monolayers in 60 mm plastic culture plates (Falcon) for interferon assays and virus titrations, and in 200 ml plastic culture flasks (Falcon) for production of virus and interferon. *Viruses.* Chickungunya virus (CV) from a mouse brain passage, and Sindbis virus grown in chick cells, were stored in sealed capillary tubes at −80°C; their titers on chick embryo cell monolayers were 2×10^7 and 2×10^8 plaque-forming units (PFU) per ml, respectively. *Interferon* was produced by inoculating chick cell monolayers with CV at a multiplicity of 0.1 PFU per cell. After incubation at 37°C for 48 hours, the tissue fluid was harvested and the virus inactivated by dialysis against 2 M KCl at pH 2.0 for 24 hours. The pH was readjusted to 7.4 by dialysis against Geys-Tris solution for 24 hours. Specimens of interferon were pooled and 1.0 ml aliquots were quick-frozen and stored at −80°C. For each experiment, the required number of aliquots were thawed and pooled, and portions were diluted with and without the indicated concentrations of PLO in the diluting fluid. The *standard interferon assay* was done in quadruplicate: growth medium was removed from plates with monolayers of chick embryo cells and replaced with 4 ml aliquots of diluting fluid or with a dilution of interferon. The plates were then incubated for 3 hours at 37°C. After the fluids were again decanted, 2 ml of an appropriate dilution of a Sindbis virus suspension, to contain 50-100 PFU, was delivered to each monolayer. Virus adsorption proceeded at 37°C for one hour, after which unadsorbed virus was removed and 6 ml of agar overlay was applied to each plate. Plaques were counted after incubation for 48 hours at 37°C. The interferon titer was expressed as the reciprocal of the dilution causing 50% reduction in plaques of Sindbis virus when compared to controls exposed to diluent alone.

Results. Although the primary interest in this study was on interferon, any concomitant effect of PLO on the virus challenge also had to be considered. To minimize any direct effect of PLO on the virus, the tissues were subjected to the fluids containing this polyamino acid only during the first hour of the standard 3-hour exposure to interferon. At

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