Roeder, *Biol. Bull.* **118**, 111 (1960); N. S. Milburn and K. D. Roeder, *Gen. Comp. Endocrinol.* **2**, 70 (1962); S. Ozbas and E. S. Hodgson, *Proc. Nat. Acad. Sci. U.S.* **44**, 825 (1958); J. E. Steele, *Nature* **192**, 680 (1961).
3. M. L. Cameron, *Nature* **172**, 349 (1953).
4. K. G. Davey, *Gen. Comp. Endocrinol*, **1**, 24 (1961); *Nature* **192**, 284 (1961); *J. Insect Physiol.* **9**, 375 (1963).
5. L. Barton-Browne, L. F. Dodson, E. S. Hodgson, J. K. Kiraly, *Gen. Comp. Endocrinol.* **1**, 232 (1961).
6. C. L. Ralph, *J. Insect Physiol.* **8**, 431 (1962).
7. E. H. Colhoun, *Experientia* **19**, 9 (1963); M. Gersch, F. Fischer, H. Unger, W. Kabitzer, *Z. Naturforsch.* **166**, 351 (1961).
8. B. E. Brown, *Gen. Comp. Endocrinol.* **5**, 387 (1965).
9. J. W. S. Pringle, *J. Exp. Biol.* **15**, 101 (1938).
10. R. B. Willey, *J. Morphol.* **108**, 219 (1961).
11. Supported by NSF grant GB-4847, NIH grant NB-04989, and a Title IV NDEA fellowship. I thank Prof. Dietrich Bodenstein, Dr. DeForest Mellon, and Dr. Michael Garrick for their comments and advice on these experiments.

6 February 1968

Tyramine-H^3: Deaminated Metabolites in Neuroblastoma Tumors and in Continuous Cell Line of a Neuroblastoma

Abstract. *Neuroblastoma tumors, as well as cultured cells of neuroblastoma, contain high monoamine oxidase activity. The major deaminated metabolite of tyramine-H^3 in the incubation mixtures with the tumors or with the cultured cells is* p-*hydroxyphenylacetaldehyde. Upon addition of reduced nicotinamide-adenine dinucleotide phosphate, the aldehyde was further metabolized by the reductive pathway to* p-*hydroxyphenylethanol, whereas upon addition of nicotinamide-adenine dinucleotide phosphate the aldehyde was only metabolized to a minor extent by the oxidative pathway to* p-*hydroxyphenylacetic acid. Aldehyde dehydrogenase activity is very low in the neuroblastoma tumors and in the cultured neuroblastoma cells. The generation of aldehydes and alcohols by the action of monoamine oxidase suggests that the deaminated metabolites of biogenic amines might exhibit some toxic effects in neuroblastoma patients.*

In view of the common histogenetic origin of the chromaffin cells and of the sympathetic nerve cells it was not unreasonable to assume that the tumors arising from the latter might also be metabolically active. However, only in the last decade has the increased excretion of catecholamines and their metabolites been described in patients with neuroblastomas (*1*). The sympathetic manifestations, such as hypertension, frequently observed in pheochromocytoma patients are rather uncommon in patients with neuroblastoma. These clinical observations, as well as the excretion spectrum of catecholamine metabolites in neuroblastoma patients, suggest that the tumor mainly secretes the breakdown products of the corresponding catecholamine (*2*). Therefore, we undertook the determination and identification of the metabolic breakdown products of biogenic amines in these tumors. We have investigated the deamination of tyramine by monoamine oxidase and have identified the deaminated products which were formed in a continuous cell line of neuroblastoma and in fresh neuroblastoma tumors.

The neuroblastoma cells were in a continuous culture for 2 years. The line was started from a lymph node obtained from a 2-year-old child immediately after its death. The fresh neuroblastoma tissues were obtained immediately after surgery and they were kept at −10°C.

Tissue culture cells of neuroblastomas were suspended in 0.5*M* phosphate buffer (*p*H 7.4) and incubated with tyramine-H^3 at 37°C for 2 hours. Neuroblastoma tissue tumors were homogenized in 0.5*M* phosphate buffer (*p*H 7.4) and incubated under the same experimental conditions as the tumor culture cells. At the end of the incubation period, the reaction mixtures were stopped by addition of 0.1 ml of concentrated HCl. Upon deproteinization the supernatant was adjusted to *p*H 2, and the deaminated products were extracted into ethylacetate. Samples of the ethylacetate fraction were counted in a liquid scintillation spectrometer. The deaminated tyramine-H^3 metabolites were analyzed and identified by the following procedures. Samples of the ethylacetate fraction were subjected to paper chromatography in a mixture of isopropyl alcohol, ammonia, and water (8:1:1). In this solvent system *p*-hydroxyphenylacetic acid was separated from *p*-hydroxyphenylethanol and from *p*-hydroxyphenylacetaldehyde. The radioactive peak which contained *p*-hydroxyphenylethanol and *p*-hydroxyphenylacetaldehyde was eluted with methanol and the radioactive material was acetylated (*3*). Upon acetylation, the radioactive material was submitted to paper chromatography in the "C" solvent system of Bush (*4*). This system separates the acetylated alcohol from the acetylated aldehyde. Other samples of the ethylacetate fraction were chromatographed in toluene, methanol, ethylacetate, and water (1:1:1:1). In this system *p*-hydroxyphenylacetaldehyde separates from *p*-hydroxyphenylethanol and from *p*-hydroxyphenylacetic acid. The R_F values of the deaminated tyramine metabolites in these different solvent systems are presented in Table 1.

Table 1. The R_F values of deaminated tyramine metabolites in different solvent systems for paper chromatography. (A) Isopropyl alcohol, ammonia, and water (8:1:1); (B) toluene; ethylacetate, methanol, and water (1:1:1:1); (C) toluene, ethylacetate, methanol, and water (1:0.1:0..5:0.5) [Buch "C" solvent system (*4*)]. The R_F values in the "C" solvent system are listed for the acetylated metabolites.

Metabolite	R_F in different solvents		
	A	B	C
p-Hydroxyphenylacetic acid	0.36	0.60	
p-Hydroxyphenylethanol	0.90	0.64	0.75
p-Hydroxyphenylacetaldehyde	0.90	0.90	0.95

A further identification of *p*-hydroxyphenylacetaldehyde was achieved by the addition of aldehyde dehydrogenase into some of the incubation mixtures. The enzyme aldehyde dehydrogenase which catalyzes the conversion of the aldehydes into the corresponding acids was isolated and purified from guinea pig kidney as previously described (*5*).

Neuroblastoma tissue, as well as cells of neuroblastoma tissue cultures, contain monoamine oxidase activity (Table 2). The enzyme activity is inhibited by such known inhibitors of monoamine oxidase as pheniprazine or iproniazid, as well as by sulfhydryl blocking agents and by disulfiram.

In incubation mixtures with neuroblastoma tumors as well as with cells of neuroblastoma tissue cultures, the only deaminated metabolite which was formed from tyramine-H^3 was *p*-hydroxyphenylacetaldehyde-H^3 (Table 2). Upon addition of reduced nicotinamide-adenine dinucleotide phosphate (NADP) to the incubation mixtures, two radioactive deaminated metabolites of tyramine-H^3 were formed. The major one was *p*-hydroxyphenylethanol-H^3 and the minor was *p*-hydroxyphenylacetaldehyde-H^3. Upon addition of NADP also two radioactive deaminated metabolites of tyramine-H^3 were

Table 2. The deaminated metabolites of tyramine-H^3 in incubation mixtures with neuroblastoma tumors (tissue) and with continuous cell lines of neuroblastoma (cells). Neuroblastoma tissues (20 mg) or neuroblastoma cells (5 mg of protein) were incubated with 100 nmoles of tyramine-H^3 (uniformly labeled with a specific activity of 5 μc per 0.1 μmole) in a total volume of 1 ml for 2 hours at 37°C. Nicotinamide-adenine dinucleotide phosphate (NADP) (1 μmole), reduced NADP (1 μmole) and aldehyde dehydrogenase (5 mg of protein) were added to the incubation mixtures as indicated in the table. The following abbreviations were used: *p*-HPAA, *p*-hydroxyphenylacetic acid; *p*-HPEA, *p*-hydroxyphenylethanol; *p*-HPAAL, *p*-hydroxyphenylacetaldehyde; N.D., not detectable > 0.1 μmole. The results are averages obtained mixtures and the standard errors of the means.

Incubation mixture	Metabolites formed in the incubation mixtures (nmole)		
	p-HPAA	*p*-HPEA	*p*-HPAAL
Tissues	N.D.	N.D.	45 ± 5
Tissues + NADP	2.5 ± 0.5	N.D.	55 ± 5
Tissues + reduced NADP	N.D.	50 ± 5	15 ± 3
Tissues + NADP + aldehyde dehydrogenase	50 ± 5	N.D.	10 ± 2
Cells	N.D.	N.D.	35 ± 5
Cells + NADP	2.0 ± 0.4	N.D.	40 ± 5
Cells + reduced NADP	N.D.	40 ± 5	10 ± 2
Cells + NADP + aldehyde dehydrogenase	45 ± 5	N.D.	5 ± 0.5
Aldehyde dehydrogenase + NADP	2.5 ± 0.5	N.D.	N.D.

formed. The minor one was identified as *p*-hydroxyphenylacetic acid-H^3, and the major as *p*-hydroxyphenylacetaldehyde-H^3. The addition of aldehyde dehydrogenase to the incubation mixtures resulted in the disappearance of *p*-hydroxyhenylacetaldehyde-H^3 and in the formation of *p*-hydroxyphenylacetic acid-H^3. This finding demonstrates that aldehyde dehydrogenase is not present in a sufficient concentration in the neuroblastoma tumors and in the neuroblastoma cell cultures to convert the aldehydes generated by the monoamine oxidase action into the corresponding acids. In incubation mixtures with rat brains or other tissues the major deaminated metabolite of tyramine-H^3 is *p*-hydroxyphenylacetic acid-H^3, whereas *p*-hydroxyphenylethanol-H^3 and *p*-hydroxyphenylacetaldehyde-H^3 are only minor metabolites (5 to 10 percent of the total deaminated metabolites).

Our results show that the deamination of tyramine-H^3 proceeds by the same metabolic pathway in neuroblastoma tumor tissues as in the continuous cell line of neuroblastoma. The studies on the formation of catabolic products from biogenic amines in the continuous cell line of neuroblastoma allow us to determine the deamination pathway without interference by endogenous amines present in the tumors.

The finding that tyramine in neuroblastoma tumors and in the cell cultures of neuroblastomas is metabolized to the corresponding aldehyde and alcohol demonstrates that the oxidative pathway, catalyzed by aldehyde dehydrogenase which is predominant in most tissues is only a minor one in neuroblastoma tumors. Other biogenic amines (that is, norepinephrine and dopamine) are also predominantly deaminated to the corresponding aldehydes and alcohols in neuroblastoma tumors.

The formation of aldehydes and alcohols from the biogenic amines in the tumor tissues raises the question of whether by the action of monoamine oxidase some toxic metabolites are generated in the tumors. Aldehydes generated by monoamine oxidase are active in the stimulation of glucose-1-C^{14} oxidation and therefore may influence the metabolism of glucose (*6*). It was also reported that in mice β-phenylethanol, a deaminated metabolite of phenylethylamine, causes injuries to the central nervous system (*7*). Thus, the deaminated products of biogenic amines formed in neuroblastoma tumors might be responsible for some toxic effects associated with this syndrome.

M. GOLDSTEIN
B. ANAGNOSTE
Department of Psychiatry and Neurology, New York University Medical Center, New York
M. N. GOLDSTEIN
Department of Anatomy, Washington University, St. Louis, Missouri

References and Notes

1. G. A. Mason, J. Hart-Mercer, E. J. Millar, L. B. Strong, N. Wynne, *Lancet* **1957-II**, 322 (1957).
2. H. Kaser, *Pharmacol. Rev.* **18**, 659 (1967).
3. M. Goldstein, A. J. Friedhoff, C. Simmons, N. N. Prochoroff, *Proc. Soc. Exp. Biol. Med.* **103**, 137 (1960).
4. I. E. Bush, *Biochem. J.* **50**, 370 (1951).
5. H. Weissbach, B. J. Redfield, S. Udenfriend, *J. Biol. Chem.* **229**, 953 (1957).
6. S. H. Barondes, *ibid.* **237**, 204 (1962).
7. P. G. Stecher, M. J. Finkel, O. H. Siegmund, Eds., *The Merck Index of Chemicals and Drugs* (Merck, Rahway, N.J., 1960), p. 792.
8. Supported by grant T-397 from the American Cancer Society, by PHS grant MH-02717, and by NSF grant GB 5941.

12 February 1968

Gallamine (Flaxedil) and Synaptic Transmission in the Spinal Cord

Abstract. *A paralyzing dose of gallamine (Flaxedil) (1 to 2 milligrams per kilogram of body weight) has no effect on synaptic transmission in the cat's spinal cord. In spinal cats ventilated with oxygen, we stimulated a dorsal spinal root and recorded the compound ventral root potential. The reflex potential was not affected by 6.25 milligrams of gallamine per kilogram. Giving 12.5 milligrams of gallamine per kilogram had no significant effect on the monosynaptic spike height, but the polysynaptic response rose briefly to 12 percent above control. Increased magnitude of the polysynaptic response appeared related to a concomitant rise in blood pressure.*

Gallamine triethiodide (Flaxedil), a neuromuscular blocking agent similar to curare, is used extensively in experimental preparations to prevent reflex movement. Use of this drug in studies of the central nervous system presupposes that it has no effect on neural elements. Yet Mountcastle *et al.* (*1*) have warned that neuromuscular blocking drugs may depress central synaptic transmission, and thus should be given only in small doses. Moreover, gallamine enhances and prolongs the afterdischarge in isolated cortex (*2*), and it increases the rate of firing of cuneate neurons (*3*). These reports led us to examine the effect of gallamine on synaptic transmission in the spinal cord.

In 12 adult cats anesthetized with halothane, the trachea, femoral artery, and saphenous vein were cannulated, and the carotid arteries were ligated. The spinal cord was transsected at the atlanto-occipital junction. The lungs were then mechanically ventilated with oxygen to maintain the arterial CO_2 tension between 27 and 35 mm-Hg. Balanced electrolyte solution with 5 percent glucose was given intravenously to support circulation; body temperature was maintained between 37° and 38°C. The lumbosacral portion of the spinal cord was exposed, and the L_6 or L_7 ipsilateral dorsal and ventral roots were cut distally. The roots were each placed on paired platinum electrodes and submerged in a pool of mineral oil kept at 37°C. The dorsal root was stimulated with supramaximal rectangular pulses 0.2 msec long. The compound reflex action potential was led from the ven-