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.

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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-

tral root, amplified, and photographed from an oscilloscope screen.

After control records were made, gallamine was given intravenously in 20 seconds. Because a dose of 6.25 mg per kilogram of body weight had no appreciable effect on the reflex response in the first two cats, we gave 12.5 mg/kg to the next ten cats. The ventral root reflex potential was recorded every half minute for the first 5 minutes, and every minute thereafter for a total of 60 minutes. We measured the amplitude of the monosynaptic spike and computed the area under the polysynaptic component of the reflex by rectangular integration. Results were expressed as percentage changes from control values and tested for significance by means of the paired *t*-test.

Results obtained on ten cats given 12.5 mg of gallamine per kilogram of body weight are shown in Fig. 1, where the mean amplitude of the monosynaptic spike, mean area of the polysynaptic response, and mean blood pressure are plotted against time. Neither the initial fall of the monosynaptic spike height nor its subsequent rise were significantly different from the control value ($P > .2$). The polysynaptic response fell first, then rose above control value, and returned in 5 to 10 minutes to its baseline. The maximum mean value of the polysynaptic response (112 percent) was reached 2.5 to 5 minutes after the injection of gallamine; this peak differed significantly ($P < .05$) from control (the other observations were not significantly different from control). Mean arterial blood pressure rose to a peak between 1 and 2 minutes after the injection of gallamine, then rapidly returned to its base line. When 6.25 mg of gallamine were given per kilogram of body weight, neither the reflex responses nor the arterial pressure changed significantly from their control values.

In our experience, the average dose of gallamine which effectively blocks neuromuscular transmission in the cat is 1 to 2 mg per kilogram of body weight. We used 12.5 mg of gallamine per kilogram here, because Halpern and Black (*2*) found that one-half this dose (6.25 mg/kg) increased the duration of the cortical afterdischarge in isolated cerebral cortex. Our observations show that the monosynaptic spike height is not altered significantly by gallamine. The increase in magnitude of the polysynaptic response—associated with a concomitant rise in mean blood pressure (Fig. 1)—was brief and observed only when a large dose of gallamine was given.

Halpern and Black (*2*) also noted a delay of 10 to 20 minutes in the onset of the central action of gallamine, which they attributed to slow passage of the drug across the blood-brain barrier. In our preparation, the ventral reflex potentials had recovered long before that time. Galindo *et al.* (*3*) showed that iontophoretic application of gallamine enhances the repetitive firing of cuneate neurons. Whether spinal neurons differ from cortical and cuneate neurons in their response to gallamine, or whether gallamine does not reach spinal neurons in sufficient concentration cannot be determined from our experiment; neither can we eliminate the possibility that repetitive firing of spinal interneurons aided in maintaining the area under the late component of the ventral root potential. Because temperature, respiration, and circulation were well controlled, it seems unlikely that these factors affected the results (with the exception of the brief rise in blood pressure and associated increase of the polysynaptic response—attributable likely to the tachycardia produced by gallamine). Using a different technique, Davis and co-workers (*4*) also showed that gallamine has no important effect on evoked responses in the central nervous system; though other neuromuscular blocking agents, such as decamethonium (Syncurine) and *d*-tubocurarine, have a considerable depressant effect on impulse transmission in the spinal cord (see *4*, *5*).

We conclude that gallamine, given in usual paralyzing doses, has essentially no effect on impulse transmission in the cat's spinal cord.

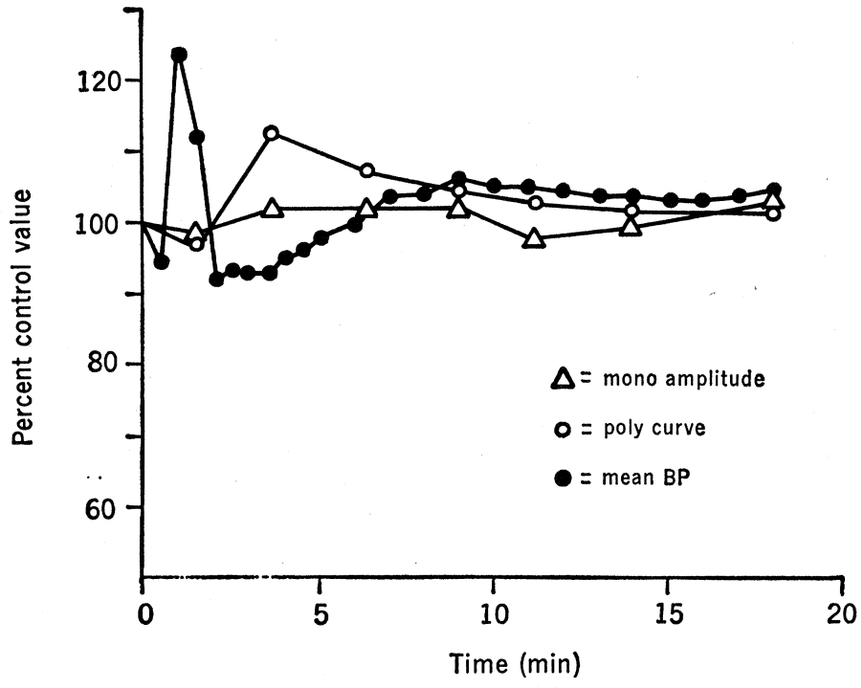

Fig. 1. Mean amplitude of the monosynaptic spike (mono), mean area under the polysynaptic response (poly), and mean arterial pressure (BP) as functions of time. Data were obtained on ten spinal cats given 12.5 mg of gallamine per kilogram of body weight. Peaks of the polysynaptic response and mean arterial pressure differ significantly from their control values.

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