ation of the chalices of Held in the posterior part of the right (contralateral) nucleus of the trapezoid body.

In the control animal, MH12, and in rats P16, P23, and P27, the lesions of the cochlea, or lesions which failed to damage the cochlear nuclei, were without effect upon the chalices of Held in the nuclei of the trapezoid body. The chalice count of rat P23 is shown in Fig. 3, as an example.

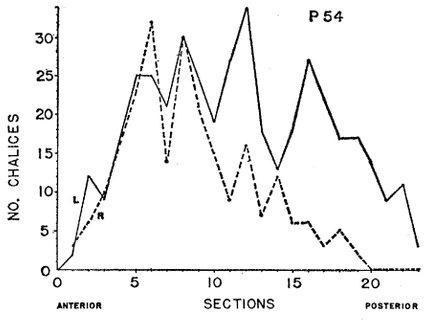

Fig. 2. Rat P54. Count of synaptic endings (chalices of Held) in the nuclei of the trapezoid body (transverse section). The posterior ventral cochlear nucleus was completely destroyed on the left side; the anterior ventral cochlear nucleus was intact on the same side (*L*, left; *R*, right).

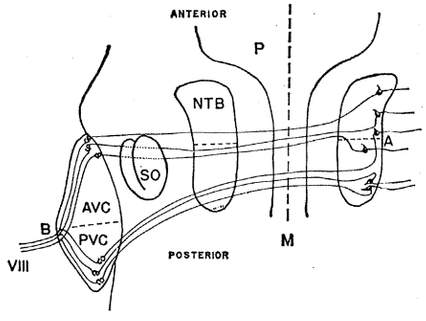

Fig. 3. Rat P23. Count of synaptic endings (chalices of Held) in the nuclei of the trapezoid body (transverse section). This animal sustained no damage to auditory structures and constitutes a control for the data Figs. 1 and 2. (*L*, left; *R*, right).

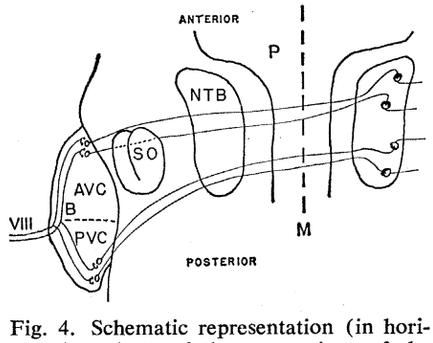

Fig. 4. Schematic representation (in horizontal section) of the connections of the nucleus of the trapezoid body. *A*, line dividing nucleus into anterior and posterior parts; *AVC*, anterior ventral cochlear nucleus; *B*, bifurcation of fibers of acoustic nerve; *M*, midline; *NTB*, nucleus of the trapezoid body; *P*, pyramidal tract; *PVC*, posterior ventral cochlear nucleus; *SO*, superior olive; *VIII*, acoustic nerve.

These results show that the nucleus of the trapezoid body receives afferent fibers from two sources. The anterior half of the nucleus receives fibers from the contralateral anterior ventral cochlear nucleus. These are large fibers which travel in the anterior half of the trapezoid body. The posterior half of the nucleus receives fibers from the posterior ventral cochlear nucleus. These are also large fibers which travel in the posterior half of the trapezoid body. Our data also suggest that the two projection fields overlap to some extent in the middle region of the nucleus. The anatomical arrangement is shown in Fig. 4.

The discovery of dual innervation led us to reexamine the nucleus of the trapezoid body to see if it has the appearance of two nuclei. The nucleus appears quite homogeneous, however, when examined in transverse, sagittal, and horizontal sections.

The implications of our data for understanding the projection of the cochlea upon the nucleus of the trapezoid body can be arrived at by considering the connections of the auditory system. The fibers of the acoustic branch of the stato-acoustic nerve bifurcate upon entry into the medulla into an ascending and a descending branch. The ascending branch terminates in the anterior ventral cochlear nucleus and the descending branch terminates in the posterior ventral cochlear nucleus (*3*). Thus, the cochlea projects upon both the anterior and posterior ventral cochlear nuclei. We have shown that the anterior and posterior ventral cochlear nuclei both project to the nucleus of the trapezoid body, hence the cochlea is represented twice in this nucleus. One representation is in the anterior half of the nucleus and the second is in the posterior half (Fig. 4).

Electrophysiological studies of the nucleus of the trapezoid body have not indicated any clear topographical representation of frequency in the nucleus (*4*). Our data clearly indicate that a single representation of frequency is not to be expected.

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5. Supported in part by the National Science Foundation (NSF GB 929) and in part by the Graduate School of Boston University.

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Identity of Tarichatoxin and Tetrodotoxin

Abstract. *Tarichatoxin ($C_{11}H_{17}N_3O_8$), a potent neurotoxin recently isolated in pure form from the eggs of the California newt,* Taricha torosa, *has been found to be identical to tetrodotoxin from the ovaries of* Sphoeroides rubripes, *the Japanese Fugu or puffer fish. As yet this substance has been detected only in a single family of fish,* Tetraodontidae, *and in a single family of amphibia,* Salamandridae.

A remarkable similarity has been noted already between the chemical (*1*) and physiological (*2*) properties of tarichatoxin isolated from the eggs of the California newt *Taricha torosa* and tetrodotoxin (*3*), which was first isolated in crystalline form in 1950 by Yokoo and Morosawa (*4*) from the ovaries of a species of Japanese puffer fish or globe fish, *Sphoeroides rubripes*. We have now carried out a direct comparison of these two substances in their crystalline form and find them to be indistinguishable. Furthermore, we find no difference in the properties of two crystalline acetates which have been prepared both from tarichatoxin (*5*) and tetrodotoxin.

In addition to the close similarity in pharmacological properties already noted (*2*), we find that a massive dose (crude toxin equivalent to as much as 1000 μg of pure material per kilogram of body weight) of either tarichatoxin or tetrodotoxin injected intraperitoneally into *Taricha* fails to paralyze or kill these animals. On the other hand, mice, frogs (*Rana pipiens*), gold fish, and tiger salamanders (*Ambystoma*) are killed by far smaller doses of the two toxins. The LD_{50} in mice is about 10 μg/kg for both toxins with intraperitoneal administration. Tarichatoxin blocked the action potential of desheathed frog nerves in a few minutes when applied in concentrations of 1 to 10 μg/lit. When sci-

atic nerves from *Taricha granulosa* were desheathed in the same way, concentrations of either tarichatoxin or tetrodotoxin as high as 30,000 μg/lit. produced only partial block after 20 to 30 minutes.

This unique insensitivity of the newt (*Taricha*) to toxin from the puffer fish (*Sphoeroides*) shows that in the newt tarichatoxin and tetrodotoxin cannot be distinguished and reinforces the conclusion that the two toxins are identical.

The chemical and physical evidence for identity rests on the nuclear magnetic resonance spectra, infrared spectra, ultraviolet spectra, mass spectra, and optical rotation of the two toxins and their acetate derivatives, as well as on their chromatographic behavior on thin-layer chromatography with several solvent systems.

Tetrodotoxin has been reported to occur only in fish of the family Tetraodontidae (and possibly in some closely related species), while we have thus far found tarichatoxin only in Salamandridae and not in salamanders of other families. This occurrence of identical toxins only in completely unrelated families of two classes of animals is a most remarkable biogenetic fact.

Tarichatoxin, isolated as previously described (*1*) from the eggs of *Taricha torosa*, was brought to a high state of purity as indicated by the appearance of a single spot, when tested by thin-layer chromatography (Merck silica gel G, 96 percent ethanol and 4 percent acetic acid) and as indicated by a subcutaneous toxicity in mice of approximately 7000 mouse units per milligram (*1*, *5*, *6*). Final purification was accomplished by bubbling carbon dioxide through a water suspension of the toxin until solution was essentially complete. This solution (approximately 0.1 percent in toxin) was centrifuged and the supernatant was allowed to stand in a centrifuge tube at room temperature in a closed vessel over aqueous 0.01*N* ammonium hydroxide. The toxin crystallized near the surface of the solution. The nuclear magnetic resonance spectra of a sample of tarichatoxin (28 mg dissolved in 0.30 ml of deuterium oxide and acidified with 0.02 ml of perdeuteroacetic acid) and of a sample of tetrodotoxin furnished by K. Tsuda and purified in the same way were identical at both 60 and 100 mcy/sec. The rotation $[\alpha]_D^{25}$, -7.8 ± 1.0 ($c = 3.33$, D_2O, D_3CCOOD) and $[\alpha]_D^{25}$, -5.0 ± 1.0 ($c = 7.5$, D_2O, D_3CCOOD) on two different determinations was close to that reported (*7*) for tetrodotoxin $[\alpha]_D^{25}$, $-8.64°$ ($c = 8.55$, dilute acetic acid), and the infrared spectra, taken in a KBr pellet, were as previously reported (*1*, *8*, *9*) and were superposable (Fig. 1). Both toxins showed only end absorption in the ultraviolet region down to 200 mμ when the solutions were freshly prepared (0.01*N* acetic acid) and the spectra were taken immediately. Both toxins began to darken at approximately 220°C and charred without melting at higher temperatures; they were not volatile enough to give meaningful mass-spectral data even with direct vaporization into the ion beam (*10*). Samples of the two toxins had the same behavior on thin-layer chromatography on silica gel with four different solvent systems, 4 percent acetic acid in ethanol; butanol, acetic acid, water (50:3:10); 70 percent collidine in water; phenol, ammonium hydroxide, and water (13:1.8:22).

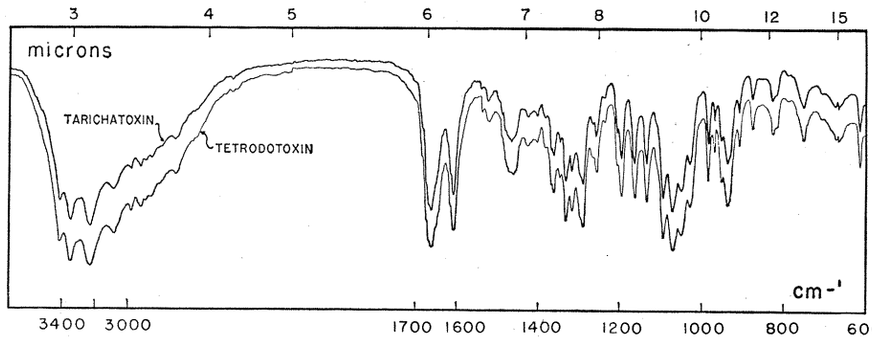

Fig. 1. Infrared spectra of tetrodotoxin and tarichatoxin.

Either tarichatoxin or tetrodotoxin (*11*), upon treatment with acetic anhydride and pyridine at 80°C for 20 hours, gave a mixture of acetates which was purified by chromatography on a silicic acid column with chloroform progressively enriched in methanol. The first acetate to be eluted—mp 188.5°–191.5°C; $[\alpha]_D^{30}$, $+15.8°$ ($c = 0.39$ $CHCl_3$); $\lambda_{max}^{H_2O}$ 236 mμ ($\epsilon = 9100$), 210 mμ ($\epsilon = 8750$)—was a heptaacetate as shown by its nuclear magnetic resonance spectrum, mass spectrum (parent ion peak $m/e = 595$), and analysis. The second acetate to be eluted—mp 205.5°–207°C; $\lambda_{max}^{H_2O}$ 235 mμ ($\epsilon =$ 14,000), was a pentaacetate as shown by its nuclear magnetic resonance spectrum and mass spectrum (parent ion peak $m/e = 511$). Neither the heptaacetate nor the pentaacetate was completely stable to crystallization. A third acetate precipitated from the mother liquors upon standing. We found this acetate only after K. Tsuda in private correspondence pointed out to us its method of generation. This acetate is insoluble in methanol, does not melt but begins to darken at 215°C, and does not absorb in the ultraviolet region above 220 mμ; from its mass spectrum (parent ion peak m/e 368) and NMR spectrum it is clearly a *diacetate*. From our examination of these data it seems conclusive that tarichatoxin and tetrodotoxin yield identical acetate derivatives, and these toxins are indeed the same chemical substance.

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3. We thank Professor K. Tsuda, Institute for Applied Microbiology, University of Tokyo, for a generous sample of crystalline tetrodotoxin isolated from the ovaries of *Sphoeroides rubripes* used for these comparisons.
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10. We are grateful to Dr. H. Budzikiewicz and Mamoru Ohashi, Stanford University, for these mass spectra determinations.
11. None of these acetates correspond in physical properties to those already described for tetrodotoxin by K. Tsuda, M. Kawamura, and R. Hoyatsu [*Chem. Pharm. Bull. Tokyo* **8**, 257 (1960)]. According to the private communication of Prof. Tsuda, he and his colleagues have confirmed that tetrodotoxin gave a polyacetate, which could be converted into a pure diacetate and that, therefore, each of three acetates reported is a mixture.
12. This acetylation and purification procedure is described in detail by Brown (*5*).
13. Supported by U.S. Public Health Service research grant NB 03381.

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