

served during purification (Fig. 1)? Proteolytic activity has been associated with muscle fractions other than myosin; thus, we may be dealing in the initial purifications with a separation of several proteases of differing specific activity, that of myosin being the weaker. Preliminary evidence for this separation of activities has already been gathered.

Dilution of the enzyme increased the specific activity at all stages of purification, an increase which suggests (Fig. 1) the persistent presence of a precursor, inhibitor, or an enzyme-enzyme masking interaction that reduces the activity of the preparation.

We have examined the effects of diverse small molecules on the activity of the third reprecipitation fraction. Thus, $10^{-3}M$ cysteine, magnesium ion, and ferrous ammonium ions all potentiate its activity. The positive effect of cysteine allows us to distinguish the activity we observe from the muscle cathepsin extractable with KCl which Snoke and Neurath examined (6). Cupric ions do not inhibit our enzyme at concentrations reported to inhibit the catheptic activity associated with actin and described by Drabikowski (5).

The most notable effect is the potentiation obtained from *l*-adrenaline at physiological and lower than physiological dose levels (Fig. 2). Although equal to *l*-adrenaline in its potentiating effect at concentrations above $1 \mu g/ml$, *d*-adrenaline is inactive in the lower dose range. This relative insensitivity of the *d*-form parallels the reduced effect that the *d*-form of adenaline exerts on physiological systems in vivo (11). The muscle catheptic enzyme under investigation, inasmuch as it is as sensitive to adrenaline as the natural receptor system, may itself be an adrenaline receptor in vivo, a phenomenon possibly related to the powerful effect exerted by adrenaline on the mechanisms of muscle contraction.

The unusual multiple activity peaks observed for the dose-response curve of adrenaline deserve comment. They are reproducible and depend for their specific relation to dose on the length of time employed for the prior triple reprecipitation (12).

PAUL GORDON

*Institute for Medical Research,
Chicago Medical School,
Chicago, Illinois*

RADOVAN ZAK

*Northwestern University Medical
School, Chicago*

References and Notes

1. P. Gordon, *Fed. Proc.* **21**, 345 (1962).
2. A. L. Tappel, H. Zalkin, K. A. Caldwell, I. D. Desai, S. Shibko, *Arch. Biochem. Biophys.* **96**, 340 (1962).
3. R. Zak, thesis, Czechoslovak Academy of Sciences, Prague (1960).
4. R. M. Nardone, *Proc. Soc. Exptl. Biol. Med.* **80**, 756 (1952).
5. W. Drabikowski, *Acta Biochim. Polon.* **8**, 3 (1961).
6. J. E. Snoke and H. Neurath, *J. Biol. Chem.* **187**, 127 (1950).
7. D. J. Ingle, V. Flores, G. Torralba, *Proc. Soc. Exptl. Biol. Med.* **90**, 217 (1955).
8. R. L. Noble and E. Papageorge, *Endocrinology* **57**, 492 (1955).
9. W. F. H. M. Mommaerts, in *Methods in Medical Research*, J. V. Warren, Ed. (Year Book Publishers, Chicago, 1958), vol. 7; W. F. H. M. Mommaerts and R. G. Parrish, *J. Biol. Chem.* **188**, 545 (1951).
10. T. P. Waalkes and S. Udenfriend, *J. Lab. Clin. Med.* **50**, 733 (1957).
11. H. E. Dubin, H. B. Corbitt, L. Freedman, *J. Pharmacol.* **26**, 233 (1925-26).
12. Supported by U.S. Public Health Service grant AM-05588-02S1.

11 February 1963

Tarichatoxin: Isolation and Purification

Abstract. *The potent neurotoxin occurring in the embryos of the California newt, Taricha torosa, has been obtained in crystalline form. With a lethal subcutaneous dose of approximately 0.14 micrograms for a 20-gram mouse, it is, along with saxitoxin and tetrodotoxin, one of the most toxic nonprotein substances known.*

In connection with transplantation experiments, Twitty and Johnson (1) in 1934 discovered that the eggs and embryos of the California newt *Taricha torosa* (formerly *Triturus torosus*) carried a toxic substance which, upon injection into other species of newts and salamanders, caused paralysis. This substance caused death when injected into other amphibians and mammals. The purification of this neurotoxin was undertaken in 1940 by Horsburgh, Tatum, and Hall (2), and in 1942 by van Wagendonk, Fuhrman, Tatum, and Field (3) who purified it so that it had a toxicity of approximately 70 mouse units (2) per milligram—that is, $1/70$ mg would kill a 20-gram mouse in 10 minutes when injected subcutaneously. Chemical work established that this preparation was dialyzable, soluble in water, soluble in methanol and ethanol, insoluble in other organic solvents, and unstable in strong acid and base. Its mode of action has been studied by Fuhrman *et al.* (4).

We have renewed the chemical investigation of this toxin, which we call tarichatoxin, and have isolated a crys-

talline material with an activity of approximately 7000 mouse units per milligram. By comparison, the previous preparation (2) was only about 1 percent toxin. Thus, tarichatoxin ranks with saxitoxin (5) from shellfish and tetrodotoxin (6) from the Japanese Fugu as one of the most toxic non-protein substances known.

Purification resulted from the following: *Taricha torosa* eggs, 95 liters, collected (7) during the spawning season in the vicinity of Stanford University, were ground with 47 liters of water, and 1.2 kg of sodium chloride in 1-gallon batches in a Waring blender. This homogenate was treated with 40 percent of its volume of acetone; the coagulated gelatinous solid which floated to the top was filtered through a mat of glass wool and washed with methanol. The extracted solids, which contained only a few percent of the original toxic activity, were discarded. The filtrate and washings were concentrated to 2 to 3 liters and then dialyzed against three successive 10-liter portions of distilled water. The dialysates were evaporated and extracted with methanol to give 113 g of material with an activity of 10 to 20 mouse units per milligram. This crude toxin was stirred with 200 ml of methanol; 18 g of almost inactive material, which did not dissolve, was removed by centrifugation. The soluble portion was deposited by evaporation on an equal weight of silicic acid and chromatographed in three batches on acid-washed silicic acid (Mallinckrodt) which had been exhaustively washed with distilled water and dried at $120^{\circ}C$ for 24 hours. The material was chromatographed with chloroform successively enriched with methanol. The most active fractions, after evaporation, gave material of activity about 1000 mouse units per milligram. During preparative electrophoresis the toxin moved toward the anode with 0.5 percent acetic acid as solvent and electrolyte. After solvent evaporation, this gave 537 mg of material with activity of approximately 3000 mouse units per milligram.

Purification was also followed by thin-layer chromatography on Merck silica gel G with 4 percent acetic acid in absolute ethanol as solvent. The spots could be developed with ceric sulfate reagent or by spraying with 10 percent potassium hydroxide in methanol followed by heating at $120^{\circ}C$ for 15 minutes; this procedure gave yellow

fluorescent spots. The material from electrophoresis gave two spots with R_F values of 0.15 and 0.30; only the faster moving one contained the toxin. Many attempts at further purification were futile until the following procedure was tried. Since the toxin from electrophoresis did not dissolve in water or alcohol, a 90-mg sample was dissolved in 1.2 ml of 1.6 percent aqueous acetic acid, and 3.5 ml of ethanol was added. A small amount of slightly active insoluble material was removed by centrifugation, and the toxin was induced to crystallize by the addition of 2.6 ml of ether. This process, repeated twice, yielded 13 mg of microcrystalline toxin which gave only one spot on thin-layer chromatography; when assayed it showed activity of approximately 7000 mouse units per milligram.

This material began to darken at 225°C but did not melt. The nuclear magnetic resonance spectrum was taken on a 10-mg sample dissolved in a mixture of 0.15 ml of deuterium oxide, 0.01 ml of perdeuteroacetic acid, and a trace of tetramethylsilane. The spectrum showed a broad singlet at 2.72 ppm (relative area 1/2), a doublet centered at 2.98 ppm ($J = 9$ cy/sec, relative area = 1), strong unresolved absorption centered at 4.65 and 4.90 ppm (relative area between 6 and 7) which was not completely resolved from the strong OH- or NH-peak or both at 5.39 ppm, and a doublet centered at 6.15 ppm ($J = 9$ cy/sec, relative area = 1).

This same solution was quantitatively transferred and made up to 0.3 ml with deuterium oxide, $[\alpha]_D^{25} -8.1 \pm 0.6$ ($\alpha = 0.13 \pm 0.01$, $c = 3.3$, D_2O , $l = 0.5$). This solution was quantitatively transferred and made up to 2.0 ml with deuterium oxide; a weak shoulder showed at $\lambda_{280} E^{1\%}$, $C^m = 0.2$ with end absorption cutting off at 215 $m\mu$. The infrared spectrum (KBr) showed characteristic absorption at 3410, 3350, 3230, 1665, 1605 cm^{-1} , and rich discrete absorption in the 900 to 1400 cm^{-1} region at 1330, 1315, 1288, 1193, 1162, 1132, 1091, 1070, 1050, 1028, 980, and 935 cm^{-1} . Analysis on the sample recrystallized a fourth time after these determinations gave the following results. Found, C, 39.73; 39.94; H, 5.76, 5.70; N, 13.0. The ninhydrin test, tests for carbohydrates, and the Elson-Morgan test for amino sugars were negative both on the toxin and on an acid hydrolysate of the toxin, but

the toxin gave positive periodate and permanganate tests.

Tarichatoxin and saxitoxin (5) are chemically quite distinct, but the similarity both chemically and pharmacologically between tarichatoxin and tetrodotoxin (6) has not gone unnoticed (7).

MELANCTHON S. BROWN
HARRY S. MOSHER

Department of Chemistry, Stanford
University, Palo Alto, California

References and Notes

1. V. C. Twitty and H. H. Johnson, *Science* **80**, 78 (1934).
2. D. B. Horsburgh, E. L. Tatum, V. E. Hall, *J. Pharm. Exptl. Therap.* **68**, 284 (1940).
3. W. J. van Wageningen, F. A. Fuhrman, E. L. Tatum, J. Field II, *Biol. Bull.* **83**, 137 (1942); F. A. Fuhrman and J. Field II, *Proc. Soc. Exptl. Biol. Med.* **48**, 423 (1941); R. S. Turner and F. A. Fuhrman, *Am. J. Physiol.* **105**, 325 (1947).
4. C. Y. Koa and F. A. Fuhrman, *J. Pharm. Exptl. Therap.*, in press.
5. K. Tsuda and M. Kawamura, *Chem. Pharm. Bull. Tokyo* **2**, 112 (1953), and other papers.
6. E. J. Schantz et al., *J. Am. Chem. Soc.* **79**, 5230 (1957).
7. We thank Mrs. S. Mooreshead for permitting the collection of eggs on her estate. Support by the U.S. Public Health Service. Taken in part from the Ph.D. thesis of M.S.B., Stanford University, 1962.

4 March 1963

Pattern Vision in Newborn Infants

Abstract. *Human infants under 5 days of age consistently looked more at black-and-white patterns than at plain colored surfaces, which indicates the innate ability to perceive form.*

It is usually stated or implied that the infant has little or no pattern vision during the early weeks or even months, because of the need for visual learning or because of the immature state of the eye and brain, or for both reasons (1). This viewpoint has been challenged by the direct evidence of differential attention given to visual stimuli varying in form or pattern (2). This evidence has shown that during the early months of life, infants: (i) have fairly acute pat-

tern vision (resolving 1/8-inch stripes at a 10-inch distance); (ii) show greater visual interest in patterns than in plain colors; (iii) differentiate among patterns of similar complexity; and (iv) show visual interest in a pattern similar to that of a human face.

The purpose of the present study was to determine whether it was possible to obtain similar data on newborn infants and thus further exclude visual learning or postnatal maturation as requirements for pattern vision. It is a repetition of a study of older infants which compared the visual responsiveness to patterned and to plainly colored surfaces (3). The results of the earlier study were essentially duplicated, giving further support for the above conclusions.

The subjects were 18 infants ranging from 10 hours to 5 days old. They were selected from a much larger number on the basis of their eyes remaining open long enough to be exposed to a series of six targets at least twice. The length of gaze at each target was observed through a tiny hole in the ceiling of the chamber (Fig. 1) and recorded on a timer. The fixation time started as soon as one or both eyes of the infant were directed towards the target, using as criterion the superposition over the pupil of a tiny corneal reflection of the target; it ended when the eyes turned away or closed (4). The six targets were presented in random order for each infant, with the sequence repeated up to eight times when possible. Only completed sequences were included in calculating the percentage of total fixation time for each target.

The targets were circular, 6 inches in diameter, and had nonglossy surfaces. Three contained black-and-white patterns—a schematic face, concentric circles, and a section of newspaper containing print 1/6 to 1/4 inch high. The other three were unpatterned—white, fluorescent yellow, and dark red. The relative luminous reflectance was, in decreasing order: yellow, white, newspaper, face and circles, red. Squares

Table 1. Relative duration of initial gaze of infants at six stimulus objects in successive and repeated presentations.

Age group	N	Mean percentage of fixation time						P*
		Face	Circles	News	White	Yellow	Red	
Under 48 hours	8	29.5	23.5	13.1	12.3	11.5	10.1	.005
2 to 5 days	10	29.5	24.3	17.5	9.9	12.1	6.7	.001
2 to 6 months†	25	34.3	18.4	19.9	8.9	8.2	10.1	.001

* Significance level based on Friedman analysis of variance by ranks.

† From an earlier study (2).