

FIG. 1. Rotating sector and irradiation cells in front of Co<sup>so</sup> bomb.

since they are chain reactions exhibiting a nonlinear dependence of rate of reaction on dose rate (3, 4). The dose rate under constant irradiation was 1200 r/hour, and the dark to light ratio was 2:1. Pairs of samples were irradiated in the beam of gamma rays with the sector rotating at various speeds. The amount



FIG. 2. Variation of acid production with length of irradiation period (all results referred to samples given the same dose continuously at the rate of 1260 r/hr) O, chloral hydrate solutions; •, chloroform saturated with water.

of acid found was compared with that found in pairs of samples irradiated in the beam for one-third the time with the sector stationary. The results are recorded graphically in Fig. 2 as

(acid formed when sector stationary)-(acid formed when sector rotating) % diff. = ×100. (acid formed when sector stationary)

The figure indicates that the average free radical chain lifetime in the chloral hydrate solution under these conditions was approximately 0.1 sec and that in the chloroform system was approximately 1 sec. These lifetimes are only approximate, since the reaction mechanisms are not known.

#### References

- NOYES, W. A., and LEIGHTON, P. A. The Photochemistry of Gases. New York: Reinhold, 1937.
   HART, E. J., and MATHESON, M. S. Discussions Faraday Soc., No. 12, 169 (1952).
- 3. ANDREWS, H. L., and SHORE, P. A. J. Chem. Phys. 18,
- 1165 (1950). 4. TAPLIN, G. V., DOUGLAS, C. H., and SANCHEZ, B. Nucleon-
- ics 9, (2), 73 (1951).

Manuscript received November 16, 1953.

## Results of Dialyzing Some Fish Poisons<sup>1</sup>

### Bruce W. Halstead and R. J. Ralls<sup>2</sup>

Department of Ichthyology and Herpetology, School of Tropical and Preventive Medicine College of Medical Evangelists, Loma Linda, California

Clinical reports by Khlentzos (1), Lee and Pang (2), Larsen (3), Watanabe (4), and others indicate that ichthyosarcotoxins from many fish species are powerful neurotoxins. Japanese investigators have studied quite extensively the chemical properties of puffer toxin, which have been reviewed by Yudkin (5). However, the chemical and pharmacological properties of fish toxins exclusive of puffer poison have not been studied to any extent. General reviews of the overall problem of poisonous fishes and ichthyosarcotoxism have been written by Phisalix (6), Pawlowsky (7), and Halstead (8, 9).

The present study was conducted preliminary to more extensive work on the chemistry and pharmacology of Gymnothorax (moray eel) and Lutjanus (snapper) poison. Specimen material consisted of fresh frozen Japanese puffer, Fugu rubripes chinensis (Abe), from Tokyo, Japan; cooked moray eel, Gymnothorax sp. indet., from Kwajalein, Marshall Islands: fresh frozen red snapper, Lutjanus vaigiensis (Quoy & Gaimard), from Palmyra Island; and fresh frozen Caranx melampygus Cuvier, from Palmyra Island. Fugu extract No. 1 (Table 1) was prepared from

<sup>&</sup>lt;sup>1</sup> Supported by a research grant from the Division of Research Grants and Fellowships, National Institutes of Health, Public Health Service, and a contract from the Office of Naval Research, Department of the Navy, Contract No. NONR-205(00).

The authors are indebted to John Field, Department of Physiology, School of Medicine, University of California at Los Angeles, for his helpful suggestions and criticisms.

TABLE 1. Dialyzation results.\*

Fish species	Sample	Original extract		Dialysate		Residue	
		Deaths no. inj.	Av. death time	Deaths no. inj.	Av. death time	Deaths no. inj.	Av. death time
Fugu rubripes chinensis Fugu rubripes chinensis Gymnothorax sp. indet. Gymnothorax sp. indet. Lutjanus vaigiensis	(1) (2) (1) (2) (1)	$     4/4 \\     4/4 \\     4/4 \\     4/4 \\     21/43 $	24 min 3 min 16 min 24 hr 24 hr	8/14 30/32 1/4 0/8 0/54	41 min 4.7 min 16 hr +++ +	0/619/340/44/89/56	+ 15 hr + 24 hr 28 hr
Lutjanus vaigiensis Caranx melampygus	(2)	1/4 4/8	24 hr 30 min	0/12 0/28	++ ++	$0/12 \\ 0/28$	+ +

\* Sublethal response recorded as: + occasional symptoms; ++ mild to acute symptoms in most mice; +++ entire group extremely distressed.

liver, and extract No. 2 from viscera (pooled liver, intestines, and gonads). Gymnothorax extracts Nos. 1 and 2 were from separate batches of muscle. Lutjanus extracts Nos. 1 and 2 were prepared from viscera. No. 1 was stored in the frozen state until tested; No. 2 was tested immediately on preparation. The Caranx extract was a sample of liver and gonads. Two milliliters of distilled water were used for each gram of tissue. The material was then homogenized in a Waring Blendor and centrifuged at 2000 rpm for 25 min. Aliquots of these extracts were dialyzed against distilled water that had been sterilized by autoclaving. Dialysis was conducted in the cold  $(38-42^{\circ} \text{ F})$  for a minimum of 24 hr. The dialyzate was concentrated by distillation under low pressure and the volume adjusted to be equal to that of the original aliquot. The original, the concentrated dialyzate, and the residue were all injected intraperitoneally in 1.0-ml volumes into three separate groups of weanling mice of the California Caviary Strain No. 1, weight 15-23 g. Symptoms were observed for a minimum of 36 hr.

The data show that the dialyzate under the conditions of these experiments has a measurable toxicity. When it is considered that the dose-response curve for these toxins shows a large increment in response for a small increment in dosage, with a very small dose range between  $ED_1$  and  $ED_{100}$  (10), it is obvious that a considerable fraction of the toxin has penetrated the membrane,<sup>3</sup> and it is therefore a fair inference that the toxin is a small molecular species.

There is an apparent difference in percentage distribution of the toxin between dialyzate and residue in the four species tested. This could be explained as either a function of concentration gradients, or actual differences in molecular species. Our present data do not resolve this question, but indicate that the toxin from each of the four species is a small water-soluble molecule.

#### References

- KHLENTZOS, C. T. Am. J. Trop. Med. 30, 785 (1950).
   LEEE, R. K. C., and PANG, H. Q. Ibid. 25, 281 (1945).
   LARSEN, N. P. Queen's Hosp. Bull. 2, 1 (1925).
   WATANABE, M. Spec. Sci. Rept.: Fish. No. 25, Fish and Wildlife Service, 209 (1950).
- 5. YUDKIN, W. H. Bull. Bingham Oceanogr. Coll. 9, 1 (1944).

PHISALIX, M., Animaux Venimeux et Venins, Vol. I, p. 487. Paris: Masson, 1922.
 PAWLOWSKY, E. N. Gifttiere und ihre Giftigkeit, p. 406. Jena: Verlag von Gustav Fischer, 1927.

Manuscript received November 9, 1953.

# Movement of Yolk Cells in the Silkworm (Bombyx mori L.)

## Takeo Takami

#### Physiology Department, Sericultural Experiment Station, Tokyo, Japan

In the silkworm, Bombyx mori L., the egg, as in many other insects, contains a large quantity of yolk which is nonliving and is used for nutrition of the embryo. Covered with this huge amount of yolk, the activity of the yolk cells themselves, which contain the volk and provide for the embryo, is often overlooked.

The behavior of the living yolk cells in hanging drops has been studied and it has been found that the yolk cells show active movement in vitro. This is a fact that has not yet been reported about Bombux mori or presumably the other insects, and seems worth describing.

A drop of 0.9% NaCl solution was spread on the underside of a cover slip. The yolk was added to it, and the cover-slip was sealed to a depression slide with petroleum jelly.

In the silkworm egg, fertilization takes place within 2 hr after deposition. At about the 24th hr the yolk segmentation begins and the yolk mass is divided by thin membranes into many large cells that contain several groups of nuclei surrounded by dense masses of fatty globules and a large quantity of protein globules. These large cells are subdivided into much smaller yolk cells, or so-called "yolk segments," which are mononuclear, as usually seen.

The moving yolk cells observed in vitro show a peculiar shape. They have a concave portion and a satellitic projection at that portion (Fig. 3), and move toward the direction of the headlike extension. The maximum moving velocity in these experiments was 13.18 µ/m.

Many cells cease movement within about 1 hr or so

January 29, 1954

<sup>&</sup>lt;sup>8</sup> Cellophane dialyzing tubing, Braun Corp., No. 25 225, %-in. width.