(latent?) pseudotuberculosis was present in mice obtained from another institution that supplies animals to many laboratories throughout the country. "Activation" of this infection by radiation and other stressing agents (*3*) may invalidate experimental results. It is, therefore, advisable to reemphasize the importance of post-mortem examination of animals that die without any apparent cause, as well as of systematic examination of all dead experimental animals. In our experience, latent pseudotuberculosis in a resident animal colony presents a very serious problem. Eradication of infection in such a herd is a difficult task with uncertain results.

I. L. SHECHMEISTER
*Department of Bacteriology, Washington University School of Dentistry, St. Louis, Missouri*

References

1. Kutscher, *Z. Hyg. Infektionskrankh.* 18, 327 (1894); R. Bongert, *ibid.* 37, 449 (1901).
2. I. L. Shechmeister and F. L. Adler, *J. Infectious Diseases* 92, 228 (1953).
3. W. Antopol, *Proc. Soc. Exptl. Biol. Med.* 73, 262 (1950); C. LeMaistre and R. Tompsett, *J. Exptl. Med.* 95, 393 (1952).

28 December 1955

## Artemia salina as a Test Organism for Bioassay

Bioassay methods for the detection of insecticide residues have a strong appeal because of the broad range of compounds to which they can be applied and because of their high sensitivity. Many bioassay methods have been devised using numerous test organisms (*1–5*). These procedures, with the exception of one or two (*6*), are based on the same fundamental technique.

For most of these bioassay methods it is necessary to maintain a culture of the test organism at all times. Many difficulties are encountered in breeding and rearing these organisms in the laboratory. In an effort to resolve this problem, we instituted a screening of test organisms. One organism, *Artemia salina,* a crustacean, commonly known as the brine shrimp, was superior to all others for this purpose. It not only demonstrated high sensitivity against a broad range of compounds, but it has one other outstanding characteristic—the eggs will remain viable for several years when they are stored in a dry condition. The necessity for maintaining cultures of the organism is eliminated; hatching can be obtained in less than 24 hours. *Artemia salina* has been the subject of physiological studies by zoologists for many years (*7*). More popularly, this organism has found wide use as food for tropical fish, and the dry eggs can be obtained readily at most tropical fish stores under the name "brine shrimp eggs."

Table 1. Composition of the salt solution used for hatching and rearing *Artemia* (in 1000 ml of distilled water and adjusted to a *p*H of 10.0 with sodium hydroxide).

| Compound | Wt. (g) |
|---|---|
| Sodium chloride | 30 |
| Calcium sulfate | 2 |
| Magnesium sulfate | 3 |
| Magnesium chloride | 8.5 |
| Potassium chloride | 0.8 |
| Magnesium bromide | 0.1 |

The eggs used in these experiments were hatched in a shallow glass tray approximately 16 in. long by 12 in. wide. A wooden divider extending across the tray and a short distance down into the solution was placed about 5 in. from one end to prevent the eggs that floated on the solution on the narrow side from drifting to the other side. The newly hatched nauplii swam under the divider and were drawn to the end of the tray by positive phototaxis. Here they were readily collected, free of eggs, by siphoning. The composition of the salt solution used for hatching and rearing *Artemia* is shown in Table 1.

Ordinary yeast made an excellent and convenient food. The yeast was suspended in water, and a few drops were floated on the salt solution. It was added in small quantities every second day. The optimum temperature for *Artemia* was found to be about 30°C. These organisms were reared in concentrations of salt ranging from 4 percent to near saturation. They required at least 3 weeks to reach the adult stage.

When *Artemia salina* was used as a test organism, a sensitivity to certain insecticides of 0.01 parts per million was demonstrated. Acetone solutions of insecticides were suspended in various concentrations in water or in salt solution of the same composition as the hatching or rearing salt solution. Toxicity was measured by placing the brine shrimp directly in the suspension of the test chemical. All age groups of *Artemia* from 24-hour-old nauplii to adults were tested and showed sensitivity to the presence of insecticides in microamounts.

A number of tests were made using adult brine shrimp as test organisms by placing them in measured amounts of water suspensions of insecticides in medicine droppers having an inside diameter of 5 mm.

Table 2. Time required to obtain fall of adult *Artemia* in varying concentrations of insecticides.

| Insecticide | Concn. | | |
|---|---|---|---|
| | 1 ppm | 0.1 ppm (min) | 0.01 ppm |
| Chlordane | 60–120 min | 120–135 | 120–180 min |
| Methoxychlor | 45– 60 min | 45– 60 | 45– 60 min |
| Lindane | 45– 60 min | 60–120 | 60–120 min |
| Toxaphene | 45– 60 min | 90–120 | 18 hr |
| DDT | 60 min | 60 | 60–120 min |
| Acetone control (1:100) | 24– 48 hr | | |
| $H_2O$ control | 26– 50 hr | | |

The shrimp move freely in the columns of liquid and usually take a position at the top air-liquid interface. When the toxic material affects a shrimp, its swimming movements are rapidly curtailed, so that it is unable to remain at the top of the column and sinks to the bottom. Use of this fall as a criterion for the test yielded more rapid results than a mortality test. With the insecticides tested and concentrations used, readings were usually obtained in 45 minutes to 2 hours. Survival of the controls was in excess of 8 hours. Results obtained with lindane, methoxychlor, chlordane, DDT, and toxaphene are shown in Table 2.

*Artemia salina* exhibits a pronounced phototaxis, which, in view of the work of certain investigators (*6*), might also be used as a physiological criterion for a bioassay test. Initial tests using this characteristic have been promising.

A. S. MICHAEL
C. G. THOMPSON
M. ABRAMOVITZ
*Entomology Research Branch, U.S. Department of Agriculture, Beltsville, Maryland*

References

1. A. Viehoever, *J. Am. Pharm. Assoc. Sci. Ed.* 25, 112 (1936).
2. W. M. Hoskins, J. M. Witt, W. R. Erwin, *Anal. Chem.* 25, 555 (1952).
3. K. Nolan and F. Wilcoxon, *Agr. Chemicals* 5, 53 (1950).
4. A. Hartzell and E. E. Storrs, *Contribs. Boyce Thompson Inst.* 16, 47 (1950).
5. R. C. Bushland, *J. Econ. Entomol.* 44, 421 (1951).
6. H. P. Burchfield, J. C. Hilchey, E. E. Storrs, *Contribs. Boyce Thompson Inst.* 17, 57 (1952).
7. D. J. Kuenen, *Arch. néerl. zool.* 3, 24 (1939).

13 September 1955

### Correction

One of the series of "Yale natural radiocarbon measurements, II" [*Science* 122, 954 (1955)], by Richard S. Preston, Elaine Person, and E. S. Deevey, was incorrectly reported through a technical error. The date for sample Y-293A (South Haven, Mich.: peat) was 10,790 ± 200 yr, not 9500 ± 250 yr as reported on page 958.

E. S. DEEVEY
*Geochronometric Laboratory, Yale University*

16 February 1956