opment of the astronomical *G. breve* populations involved in Florida red-tide outbreaks.

If Florida west coast rivers do not provide direct energy sources for multiplication of *G. breve,* the organism's vitamin, trace-metal, and chelator requirements assume added ecological significance as factors potentially limiting population growth. Similar indirect evidence may be seen in extensive field data (*11*), which suggest that phosphate and nitrate are not usually growth restricting to this form in nature.

Vitamins, trace metals, and chelators may be introduced by river waters into coastal environments. Provasoli (*12*) has pointed out broad ecological implications: "It is well known that the waters near estuaries, inlets, and inshore are fertile areas; this fertility should now be considered not only in terms of phosphates and nitrogenous compounds but also of vitamins, trace metals, and chelating agents." The natural fluctuations in levels of such substances may also be a fruitful area for future study of *G. breve* and red-tide ecology.

DAVID V. ALDRICH

Bureau of Commercial Fisheries, Biological Laboratory, Galveston, Texas

References and Notes

1. G. Gunter, F. G. Walton Smith, R. H. Williams, *Science* **105**, 256 (1947).
2. L. B. Slobodkin, *J. Marine Res. Sears Found. Marine Res.* **12**, 148 (1953).
3. J. H. Davis, *Florida Geol. Surv. Geol. Bull. No. 25* (1943).
4. W. B. Wilson and A. Collier, *Science* **121**, 394 (1955).
5. A. Lwoff, *L'Évolution Physiologique* (Hermann, Paris, 1943); M. V. Lebour, *The Dinoflagellates of Northern Seas* (Marine Biol. Assoc. U.K., Plymouth, 1925).
6. D. V. Aldrich and W. B. Wilson, *Biol. Bull.* **119**, 57 (1960). Other than traces of thiamine, cobalamin, and biotin, the medium's organic content consisted of tris (hydroxymethylaminomethane), 18 mg/liter and ethylenediaminetetraacetic acid (disodium salt), 3 mg/liter. The latter two compounds, a *p*H buffer and a metal chelator, can be replaced with $NaHCO_3$ and Na_2S, respectively, without limiting rate of multiplication. Hence these organics may not be considered energy sources for *G. breve.* Growth results are less erratic with the organic agents, which provide closer regulation of *p*H and precipitation in the medium. The technical assistance of Alice Kitchel is gratefully acknowledged.
7. J. H. Finucane and A. Dragovich, *U. S. Fish and Wildlife Serv. Spec. Sci. Rept., Fisheries No. 289* (1959).
8. W. B. Wilson, in preparation.
9. H. A. Barker, *Arch. Mikrobiol.* **6**, 1957 (1935); S. H. Hutner and L. Provasoli, in *Biochemistry and Physiology of Protozoa,* S. H. Hutner and A. Lwoff, Eds. (Academic Press, New York, 1955), vol. 2, p. 17.
10. J. C. Lewin, *Science* **112**, 652 (1950).
11. S. J. Bein, *Bull. Marine Sci. Gulf and Caribbean* **7**, 316 (1957); A. Dragovich, J. H. Finucane, B. Z. May, *U.S. Fish and Wildlife Serv. Spec. Sci. Rept., Fisheries No. 369* (1961).
12. L. Provasoli, in *Perspectives in Marine Biology,* A. A. Buzzati-Traverso, Ed. (Univ. of California, Berkeley, 1958), p. 385.

4 May 1962

Convenient Assay of Xanthine Dehydrogenase in Single Drosophila melanogaster

Abstract. Each fruit fly is homogenized in buffer and treated with charcoal. The enzyme is subsequently assayed by following the conversion of 2-amino-4-hydroxypteridine to isoxanthopterin in a sensitive fluorometer.

Many methods have been devised for the assay of xanthine dehydrogenase (*1*). The most convenient way is to determine the increase in fluorescence which occurs during the oxidation of 2-amino-4-hydroxypteridine to 2-amino-4,7-dihydroxypteridine (isoxanthopterin), a reaction catalyzed by this enzyme (*2*). Interest in xanthine dehydrogenase of *Drosophila melanogaster* stems from the fact that mutants at two loci (maroon-like eye color and rosy eye color) lack this enzyme (*3*). As a consequence, these abnormal flies accumulate the enzyme substrates (hypoxanthine and 2-amino-4-hydroxypteridine) and show no trace of the products (uric acid and isoxanthopterin) formed from these compounds (*4*). Enzyme activity has not been detected in partially purified extracts of the mutants (*5*). The following assay in single flies, weighing 0.7 to 0.8 mg, was developed to avoid the necessity for separating large numbers of the various genotypes of flies derived from crosses (*6*). This procedure is not a microassay; convenient macro volumes are used.

All procedures except the assay are carried out at temperatures of less than 5°C. A single fly is homogenized in 4 ml of 0.1*M* Tris buffer, *p*H 7.5, containing 10 mg of crystalline bovine albumin (Armour) per milliliter. Approximately 10 mg of Norite-A is added, and the mixture is allowed to stand for 60 minutes with occasional stirring. The solution is then centrifuged for 15 minutes at 30,000*g*, and then the supernatant is filtered through sintered glass to remove the last remnants of charcoal. One milliliter is placed in a fluorometer cuvette, which is then kept in a dry bath (Thermoline) until it reaches a constant temperature of 30°C (3 to 5 min), after which 0.02 ml of 0.001*M* methylene blue and 0.01 ml of 0.001*M* 2-amino-4-hydroxypteridine (*7*) are added. The mixture is stirred, and readings are taken at 1-minute intervals for 10 minutes in a fluorometer (Photovolt No. 540) equipped with a 340-mμ primary filter and a 405-mμ secondary filter. The fluorometer is set at maximum sensitivity; thus a solution of $1.4 \times 10^{-6}M$ quinine in 0.1*M* sulfuric acid has a reading of 100. The high blank of the reaction mixture is adjusted to zero with the "zero control" on the fluorometer. During the assay, the cuvette is always returned to the dry bath between readings. The entire procedure can also be used on eggs, larvae, and pupae.

The use of albumin in the homogenizing fluid and the use of Norite-A during the processing of the extracts are important. If albumin is omitted, then the activity of xanthine dehydrogenase in the extracts is lost during filtering. The use of albumin prevents this and has, so far as we can tell, little effect on the rate of the reaction. Norite-A is used to remove endogenous "inhibitors" (such as purines and pteridines) from the extract. By means of experiments involving the addition of various amounts of Norite-A to extracts containing known amounts of *Drosophila* xanthine dehydrogenase, it has been clearly demonstrated that no loss of enzyme activity occurs during the processing of these extracts for assay.

Figure 1 shows that the reaction rate is linear for at least 20 minutes. Other data indicate that the enzyme activity is proportional to the number of flies used in the assay. Thus, in one experiment, one fly had 2.1 units of xanthine dehydrogenase, two flies yielded 4.4

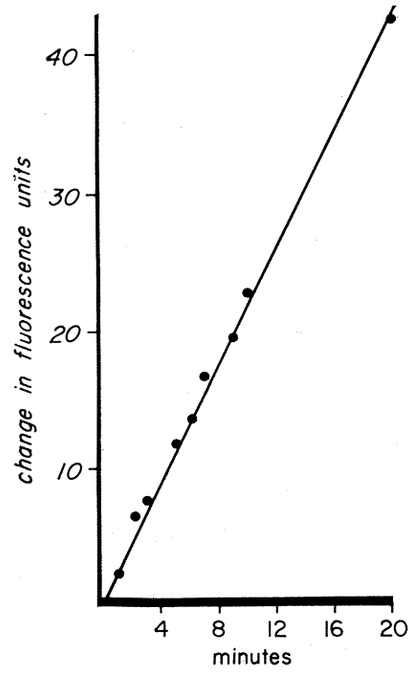

Fig. 1. Linear change in fluorescence units during assay of xanthine dehydrogenase in a single fruit fly.

units, four flies yielded 7.2 units, and eight flies yielded 13.7 units when homogenized together. These results indicate that the assay of xanthine dehydrogenase in a single fly as described herein is a valid one. The assay is now being applied to a variety of problems (*6*). With minor modifications—the use of smaller volumes and more sensitive fluorometers—one might hope to assay individual organs of these flies. This method should also prove applicable to the assay of xanthine dehydrogenase in small amounts of tissue from various sources (*8*).

EDWARD GLASSMAN*
Department of Biochemistry, School of Medicine, University of North Carolina, Chapel Hill

References and Notes

1. B. L. Horecker and L. A. Heppel, in *Methods in Enzymology*, S. P. Colowick and N. O. Kaplan, Eds. (Academic Press, New York, 1955), vol. 2, p. 482; O. H. Lowry, *ibid.* (1957), vol. 4, p. 380.
2. This method was first used by H. B. Burch, O. H. Lowry, A. M. Padilla, and A. M. Combs [*J. Biol. Chem.* **223**, 29 (1956)]; later it was independently applied to *Drosophila* by E. Glassman and H. K. Mitchell [*Genetics*, **44**, 153 (1959)].
3. H. S. Forrest, E. Glassman, H. K. Mitchell, *Science* **124**, 725 (1956).
4. E. Hadorn and I. Schwink, *Nature* **177**, 940 (1956); H. K. Mitchell, E. Glassman, E. Hadorn, *Science* **129**, 268 (1958).
5. E. Glassman and H. K. Mitchell, *Genetics* **44**, 153 (1959).
6. E. Glassman, J. D. Karam, E. C. Keller, Jr., in preparation.
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Strontium-90 Fallout from the 1961 Soviet Nuclear Detonations

Abstract. A steady increase in the strontium-90 concentration in rain was observed at Fayetteville, Arkansas, after the 1961 Soviet nuclear test series. Experimental data indicate that the nuclear weapons tested in 1961 were "cleaner" on the average by a factor of five than those exploded in 1957 and 1958.

After the 1961 Soviet atmospheric test series, it was feared that the Sr^{90} fallout would far exceed the levels previously observed. Judging from the reported explosive powers of some of the super-bombs tested in the fall of 1961, it was thought that the fallout during the spring peak of 1962 would be several times that observed, for example, in the 1959 spring peak period.

It soon became obvious, however, that the yield of fission debris relative to the explosive power of the Soviet bombs was unexpectedly small—the bombs were "clean." We have attempted to compare the ratio of Sr^{90} fallout versus the power (expressed in terms of megaton equivalent of TNT) for the 1961 Soviet tests with the values for previous test series.

We have measured the Sr^{90} concentrations in individual samples of rain and snow collected at Fayetteville and have calculated the monthly average in rain ($\overline{C}$) from the equation:

$$\overline{C} = \Sigma F / \Sigma R$$

where ΣF is the total amount of Sr^{90} (in 10^{-12} curies per square meter) transported by rain and snow during the month period and ΣR is the total rainfall (in millimeters) during the same period.

The experimental results are shown in Figure 1. Prior to the Soviet test series, the concentration in rain was gradually decreasing after the 1961 spring peak, but the trend reversed to a steady increase soon after the tests began in September 1961. The levels during the following several months through March 1962 were intermediate between the levels observed during the corresponding months of 1957–58 and 1958–59. However, a trend indicated that the 1962 peak value might reach or exceed the levels of March and April 1959.

According to the statement issued by the U.S. Atomic Energy Commission on 9 December 1961, the total explosive power for the approximately 50 Soviet atmospheric tests conducted during the months of September and October 1961 is estimated to be equivalent to that of 120 megatons of TNT. Machta and List (*1*) reported that stratospheric injections of Sr^{90}, as estimated by Libby (*2*), were as follows: 1957 spring 3.0, fall 6.0; 1958 winter 3.3, spring 4.0, fall 20.0 (megaton equivalents).

Most of the Sr^{90} injected into the stratosphere appears to be removed during the spring peak of the following year, as first pointed out by Martell (*3*). Hence the level of concentration in rain during the spring peak period of fallout may be considered as roughly proportional to the total amount of Sr^{90} injected into the stratosphere by the major test series during the previous year. Under the above simplifying assumption, the concentration in rain during the spring peak period divided by the total explosive power of the weapons tested during the previous year may be used as a measure of the ratio Sr^{90} production versus the explosive power of the bombs.

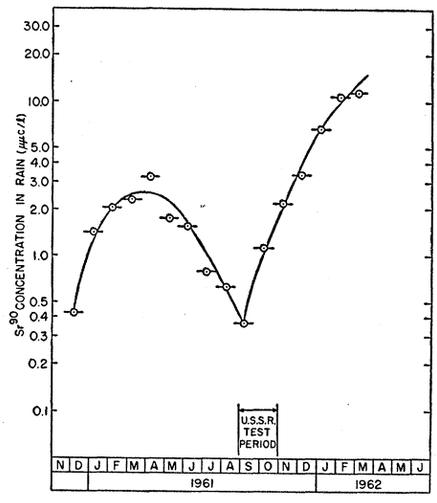

Fig. 1. Variation of the Sr^{90} concentration in rain and snow at Fayetteville, Arkansas.

Figure 2 was obtained by simply dividing the observed monthly values of $\overline{C}$ by the total explosive power of the bombs tested during each year: 9.0 megaton equivalents of TNT in 1957, 27.3 in 1958, and 120 in 1961.

The results indicate that the 1961

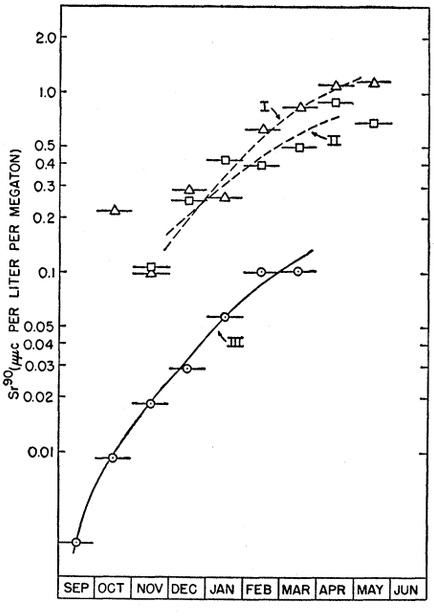

Fig. 2. Comparison of the Sr^{90} concentrations in rain relative to the total explosive power of the bombs exploded in the previous year, expressed in micromicrocuries of Sr^{90} per liter per megaton. Curves I, 1957 to 1958; II, 1958 to 1959; III, 1961 to 1962.