

active form of the pigment, P_{FR} , to revert to the red-absorbing form, P_R , in darkness (1, 7). The assumption is that in short-day plants P_{FR} must remain below a certain critical level for a given time for flowering to occur; light acts only to maintain or restore a high level of P_{FR} . On this hypothesis, once photosynthetic interactions are eliminated, there is no reason why flowering should occur readily under the schedules 11(13) or $\frac{1}{4}(2\frac{1}{2})\frac{1}{4}(2\frac{1}{2})\frac{1}{4}(2\frac{1}{2})\frac{1}{4}(2\frac{1}{4})\frac{1}{4}(13)$ but not under $1(9\frac{3}{4})\frac{1}{4}(13)$. An explanation of this phenomenon in terms of phytochrome may nevertheless be at hand.

The Beltsville group has reported (2, 7, 8) that in some intact etiolated tissues the presence of P_{FR} , brought about by red light, causes an apparent decrease in the total level of phytochrome, at least as assayed by in vivo reversibility. While the actual fate of the phytochrome is completely unknown, these observations can be taken at face value as representing a decrease of phytochrome level after treatment with light; on this admittedly speculative basis, the present results with *Lemna* become explicable. Those plants on schedules in which the "light period" consists largely of uninterrupted darkness would be expected to have more phytochrome than plants on schedules with the usual light periods, since, during the latter, the phytochrome level should decrease. Thus the plants on the first type of schedule would start each night with a higher total phytochrome level, all of it in the P_{FR} state because of the final light exposure. It would then require a longer night to allow the P_{FR} level to fall below the critical level, by reversion to P_R ; hence there is a shift in critical night length (Fig. 1).

Whether or not this is a valid hypothesis will only be determined by direct in vivo assays and extended kinetic studies of the phenomenon itself. A general hypothesis of photoperiodic timing based on the interaction of phytochrome state (P_R or P_{FR}) and total phytochrome level, the type proposed here, has obvious advantages in analyzing many complex light-dark interactions now inexplicable and possibly also certain anomalies in the red, far-red responses of plants such as *Pharbitis* seedlings (9) or *Lemna perpusilla* itself (4). However, the relevant data are at present too scanty to justify an extended discussion.

There are few reports in the literature comparable with the present observations. Krumwiede (10), using the

short-day plant *Kalanchoe* grown photosynthetically, found a $\frac{1}{4}$ -hour increase in the critical night length when part of the light period was of reduced intensity.

More relevant are the results of Könitz (11) who observed a marked inhibition of flowering in the short-day plant *Chenopodium* when the main (13-hour) light period was interrupted by treatments with far-red light for varying periods. In the controls a light period was interrupted with dark for up to 4 hours; such interruptions were ineffective or else produced only slightly increased flowering. Longer interruptions might have given results similar to those reported here, though always with the difficulties and ambiguities encountered when plants are grown under photosynthetic conditions. Könitz concluded that the photophile phase was highly sensitive to far-red light, but his results are equally consistent with the more concrete hypothesis suggested here.

Confirmation of these observations on many plants may prove difficult unless interactions with photosynthesis are eliminated. For this reason, *Lemna* and

other small plants that can be grown under axenic, nonphotosynthetic conditions should be further exploited for analyses of the mechanism of photoperiodic timing, especially in relation to the phytochrome system (12).

WILLIAM S. HILLMAN

Department of Biology, Brookhaven
National Laboratory,
Upton, New York

References and Notes

1. H. A. Borthwick and S. B. Hendricks, *Science* **132**, 1223 (1960).
2. W. L. Butler, H. C. Lane, H. W. Siegelman, *Plant Physiol.*, in press.
3. W. S. Hillman, *Am. J. Botany* **46**, 466 (1959).
4. W. K. Purves, *Planta* **56**, 684 (1961).
5. W. S. Hillman, *The Physiology of Flowering* (Holt, Rinehart, and Winston, New York, 1962), p. 20.
6. E. Bünning, in *Photoperiodism and Related Phenomena in Plants and Animals*, R. B. Winthorpe, Ed. (AAAS, Washington, D.C., 1959), pp. 507, 531.
7. S. B. Hendricks, *Cold Spring Harbor Symp. Quant. Biol.* **25**, 245 (1960).
8. —, W. L. Butler, H. W. Siegelman, *J. Phys. Chem.* **66**, 2550 (1962).
9. S. Nakayama, H. A. Borthwick, S. B. Hendricks, *Botan. Gaz.* **121**, 238 (1960).
10. D. Krumwiede, *Biol. Zentr.* **79**, 257 (1960).
11. W. Könitz, *Planta* **51**, 1 (1959).
12. Research was carried out at Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission. I thank Rosemarie Dearing for technical assistance.

26 April 1963

Rhodium-102 Fallout: Variations in Deposition and Concentrations in Precipitation

Abstract. *Rhodium-102 was produced as a tracer for U.S. high-altitude detonations in August 1958 and has been detected and monitored in precipitation since October 1960. Between January and September 1961, when atmospheric tests were resumed, the contribution of this high-altitude debris in fallout increased.*

Rhodium-102 was produced as a tracer in the Orange shot of the U.S. "Hardtack" nuclear test series 11 August 1958 (1). This detonation was a high-altitude air burst at about 43 km above Johnston Island (lat 16°N, long 170°W) (1). It was hoped that this nuclide could be used as a unique tracer for debris injected into the higher stratosphere. In a similar manner W^{185} was used to trace lower stratospheric equatorial debris (2). The debris was in all probability placed at an altitude above 100 km (1).

The total amount of the Rh^{102} injected was generally estimated to be about 3 megacuries (Mc) (1, 5). In addition about 0.3 Mc of Rh^{102} from other U.S. tests and some from Soviet sources (1) could have contributed to material deposited from the stratosphere in 1958–59.

Scientists have reported (1, 3–11) on

the Rh^{102} high-altitude tracer experiment and the distribution of the radioactivity in the atmosphere; thus far little information has been presented on the occurrence of Rh^{102} in precipitation. Under the auspices of the U.S. Atomic Energy Commission, Rh^{102} has been monitored in rainwater at Westwood, New Jersey, since July 1960. Data from two other stations (Pittsburgh, Pa., and Richmond, Calif.) are available for a shorter period (12).

Volumes of precipitation (obtained with a large rainfall collector) (13) were processed because of the expected low concentrations of Rh^{102} . The minimum volume of water processed in any month was 75 liters. The procedures have been described (14).

Two known isomers of Rh^{102} were believed to have been produced by the Orange shot, one with a half-life of 210 days and another with

a half-life of approximately 4 years (1). Since these isomers decay mainly by electron capture, the radioassay was done by counting the 20–21 kev x-ray of the ruthenium daughter products of the rhodium activities with a thin crystal γ -spectrometer system. Kalkstein's method (11) was used to calculate in our samples the amount of the activity of the 210-day component corrected for decay.

Figure 1 gives the monthly average concentrations of Sr^{90} and Rh^{102} in precipitation corrected for radioactive decay to 12 August 1958. Precipitation has also been plotted on this graph. The trend of the actual Rh^{102} deposition follows that of the concentration curves (14). Sr^{90} exhibits the same pattern of behavior but with higher concentrations than Rh^{102} for the whole period.

Seasonal variations in concentrations of Rh^{102} in precipitation were also generally similar to those of Sr^{90} (Fig. 1) with spring peaks in 1961 and 1962. The Sr^{90} peak in 1962 was very pronounced as a result of U.S.S.R. testing in 1961, but it was expected that a spring peak would have been evident in any event. The peak concentration of Rh^{102} in 1961 was out of phase with the Sr^{90} peak by 1 month (Fig. 1). The maximum concentration was actually attained in June 1961 while the peak for Sr^{90} occurred in May 1961. The Rh^{102} concentrations in May and June 1961 were similar.

When the ratios of the Rh^{102} and Sr^{90} were calculated, the peak ratio in the spring of 1962 was lower than in previous years (Fig. 2) because of large amounts of Sr^{90} from the Soviet tests of 1961. Without these tests the peak concentrations of Sr^{90} might have attained values about 10 percent of those actually observed. The ratios of Rh^{102} to Sr^{90} would, therefore, have been about an order of magnitude higher than those observed (broken curve in Fig. 2).

The variation in isotope ratios with time (Fig. 2) confirm the fact that the composition of the debris at Westwood changed with time during late 1960, 1961, and 1962. It was apparent after the first 3 months of 1961 when Rh^{102} became measurable in precipitation that the composition of the fallout changed to a source richer in Sr^{90} relative to Rh^{102} . After March 1961, even though the Rh^{102} concentrations continued to fluctuate through June 1962, the relative proportion of Rh^{102} in the debris continued to increase. This is probably

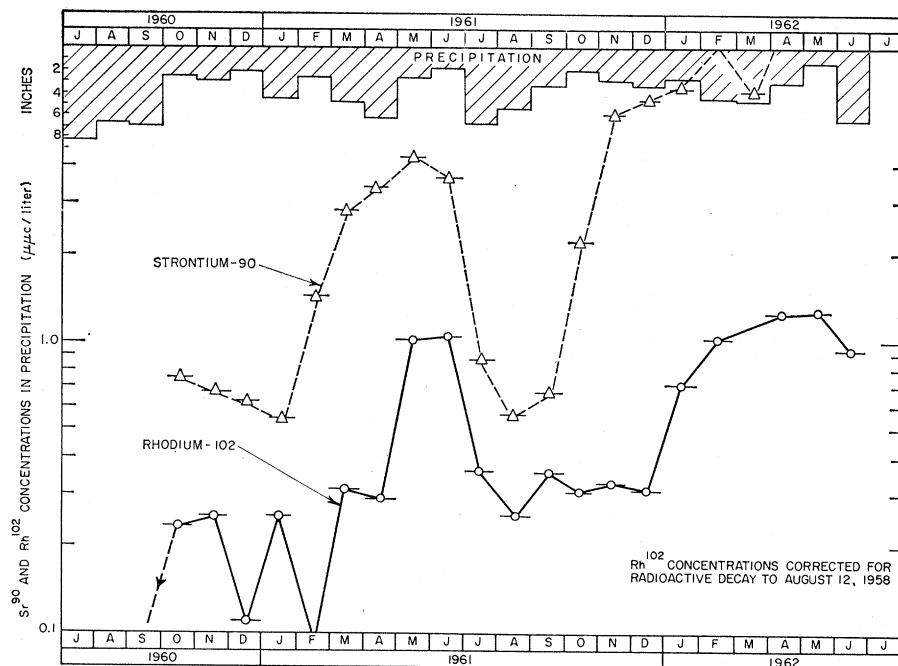


Fig. 1. Concentrations of Rh^{102} and Sr^{90} in precipitation at Westwood from 1960 through 1962.

true for the spring of 1962 if one allows for the contribution of Sr^{90} from the Soviet tests in 1961 (14).

According to Stebbins (5), the initial activity ratio of Rh^{102} to Sr^{90} from mixed Teak and Orange debris, corrected for radioactive decay to 12 August 1958, would be about eight. This arises from the total Rh^{102} yield of 3 Mc and an estimated Sr^{90} yield of about 0.4 Mc. From the activity ratios of these nu-

clides (corrected for decay) in precipitation at Westwood, the highest values observed were close to 0.5 in 1961 and almost unity in 1962. Compared with the theoretical ratio of eight, the observed values appear to be rather low. However, the results are in good agreement with the data reported for activity ratios of these nuclides in stratospheric air filter samples (8).

Knowledge of the quantity of Rh^{102}

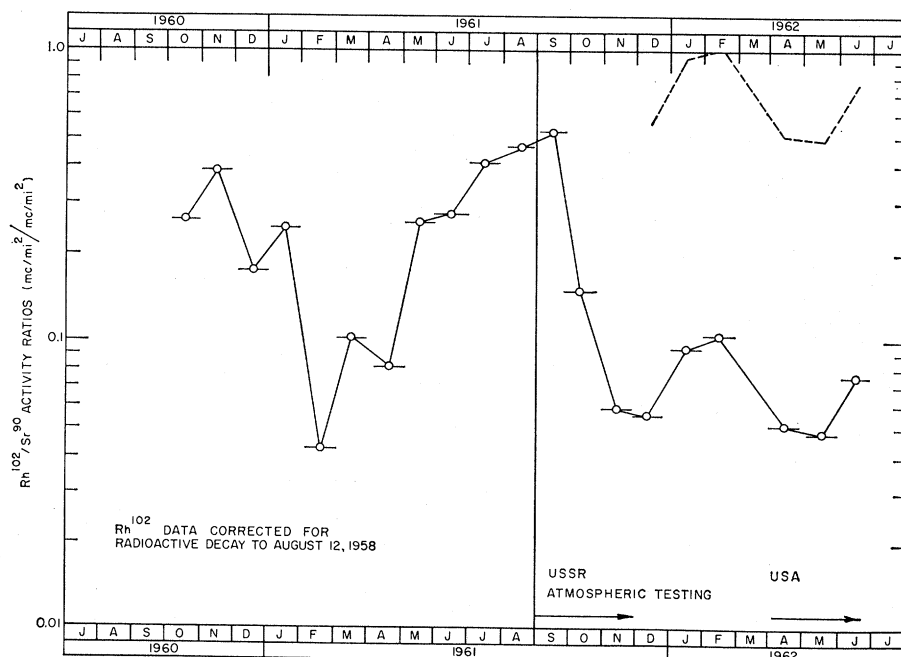


Fig. 2. Activity ratios of Rh^{102} to Sr^{90} in precipitation at Westwood from 1960 through 1962.

deposited since the detonation in 1958 would help us to understand the rate of transfer of material from the upper atmosphere to the surface of the earth, but only a small number of measurements are being made to determine the rate of deposition. However, worldwide deposition of Rh^{102} may be roughly estimated from the Westwood results. Previous fallout data indicate ratios for the Westwood to the total worldwide deposit close to 2 for W^{185} and about 5 for Sr^{90} (15). Both nuclides show greater deposits in the Northern than in the Southern Hemisphere while the Rh^{102} may be expected to be more evenly distributed between the hemispheres than these two radionuclides (1). If a value of unity for the above ratio is assumed in the case of Rh^{102} , a total global deposit by 1 July 1962 of 0.5 Mc Rh^{102} may be calculated.

Uncertainties in this type of calculation are apparent; yet the result is in reasonable agreement with stratospheric inventories of 0.37 and 0.52 Mc of Rh^{102} calculated to be present in the altitude range 40,000 and 70,000 feet (16, 5) in 1960; this material may have been deposited by July 1962.

It may be speculated from our data and the deposition of Rh^{102} in precipitation at Westwood, that the nuclide will remain measurable for a number of years (17).

M. W. M. LEO
A. WALTON

Isotopes, Incorporated,
Westwood, New Jersey

References and Notes

1. M. I. Kalkstein, *Science* **137**, 645 (1962).
2. A. Walton, *Nature* **188**, 220 (1960); H. W. Feely and J. Spar, *ibid.* 1062 (1960).
3. "Mixing and transfer within the stratosphere," *Isotopes, Inc., Quarterly Progress Report, Contract No. DA-29-044-XZ-609* (1960).
4. A. K. Stebbins, III, "Special report on high-altitude sampling program (HASP)," *Defense Atomic Support Agency Publ. No. DASA 532B* (1960), p. 142.
5. —, *ibid.* 539B (1961), p. 94.
6. M. I. Kalkstein, "Results for the Rh^{102} high-altitude tracer experiments," *Sandia Corporation Publ. No. SCR-420* (1961), p. 69.
7. U.S. Weather Bureau, "Global atmospheric radioactivity May, June, and November 1960," *U.S. At. Energy Comm. Report HASL-115* (1961), p. 177.
8. J. P. Friend and H. W. Feely, "Second quarterly report on project star dust," *Defense Atomic Support Agency Publ. No. DASA 1302* (1961), p. 32.
9. U.S. Weather Bureau, "Global atmospheric radioactivity, May-June 1961," *U.S. At. Energy Comm. Report HASL 117* (1961), p. 225.
10. L. Machta, R. J. List, K. Telegadas, *J. Geophys. Res.* **67**, 1389 (1962).
11. M. I. Kalkstein, "Results for the Rh^{102} high-altitude tracer experiment. I. Stratospheric concentrations," *Air Force Cambridge Research Laboratories Publ. No. 62-460 (1)*, (1962).
12. "Fallout program quarterly summary report," *U.S. At. Energy Comm. Health and Safety Lab. Publ. No. HASL-131* (1962).
13. A. Walton and R. E. Fried, "Studies of

nuclear debris in precipitation," *Isotopes, Inc. Seventh Progress Report, Contract No. AT(30-1)-2415*, 15 Aug. 1961.

14. A. Walton and M. W. M. Leo, *ibid.* 15 Nov. 1962.
15. J. P. Friend, H. W. Feely, P. W. Krey, J. Spar, A. Walton, "High-altitude sampling program," *Defense Atomic Support Agency Publ. No. DASA 1300* (1961), vol. 4.
16. R. J. List and K. Telegadas, "The pattern of global atmospheric radioactivity—May 1960," *U.S. At. Energy Comm. Report HASL 111* (1961).
17. Supported by the U.S. At. Energy Comm. contract AT (30-1)-2415. We thank Drs. H. W. Feely, J. P. Friend, and H. L. Volchok for helpful discussions. The laboratory work was performed by the staff of the Analytical Division, Isotopes, Inc., under the management of P. W. Krey. We thank J. Z. Holland, U.S. Atomic Energy Commission, for his encouragement of this work.

10 April 1963

Nitrogen Mustard: Diminution of Toxicity in Axenic Mice

Abstract. *Axenic, or germ-free, mice are more resistant to the delayed lethal effect of nitrogen mustard than normal mice. The resistance is most striking when mustard is administered at pH 2.0. The finding supports the hypothesis that intestinal bacteria play an important role in the systemic toxicity of nitrogen mustard.*

To evaluate the role of intestinal bacteria in the delayed lethal toxicity of nitrogen mustard [methyl-bis (β -chloroethyl) amine-HCl] we have used a strain of axenic mice which has recently become available. We have found these animals to be a useful experimental tool.

Delayed lethal toxicity refers to that form of toxicity resulting in death of animals 4 to 10 days after treatment. It has been found that the pH of a solution of nitrogen mustard at the time of injection plays a critical role in this delayed lethal toxicity (1). Solutions at pH 2.0 have an LD₅₀ (lethal dose for 50 percent of the inoculated group) for mice of approximately 6.5 mg/kg whereas solutions freshly made up and injected at pH 8.0 have an LD₅₀ of 3.25 mg/kg. Nitrogen mustard at pH 2.0 retains full anti-tumor activity and, therefore, the therapeutic index of the compound at pH 2.0 is superior to that found at pH 8.0 (2). Why nitrogen mustard at two pH values should have two different toxicities has not been explained.

The suppression of the normal intestinal flora of rats and mice with oral or parenteral antibiotics has been accompanied by an increased tolerance for whole-body x-irradiation (3, 4).

There is a reduction in the lethal effect of x-rays in Swiss mice when these animals are raised under pathogen-free conditions, with fecal flora containing chiefly lactobacilli (5). Several groups have reported that axenic or gnotobiotic conditions have been accompanied by a reduction in lethal toxicity in mice (6), rats (7), and chicks (8) exposed to whole-body x-irradiation. These reports stimulated an interest in determining whether axenic mice were automatically protected by their bacteria-free condition against the delayed lethal toxicity of nitrogen mustard and whether normal mice could be similarly protected by the administration of antibiotics.

In preliminary experiments Charles River CD mice were placed on ordinary diets and antibiotics were added to the drinking water. Oxytetracycline was mixed and replaced every 12 hours. Neomycin and polymyxin were replaced once daily. A single injection of a solution of nitrogen mustard was given on the seventh day after antibiotics had been started. The volume varied between 0.6 and 1.0 ml. All mice were observed until death or for a minimum of 30 days after injection. With oxytetracycline, high doses of neomycin, and polymyxin, bacterial content of feces decreased somewhat, and the lethal toxicity of nitrogen mustard diminished (Table 1). However, this protection was rarely statistically significant.

The experiment was repeated several times with axenic mice of the same strain. These mice were raised in plastic isolators; air was drawn through bacterial filters, and the mice were fed sterile water and food. Their growth curves are similar to those of normal animals. They are very sensitive to bacterial infection, and this sensitivity served as a check on their "germ-free" status. Mice were weighed and given single intraperitoneal injections of sterile nitrogen mustard solution (0.02 ml/g of body weight). Several dose

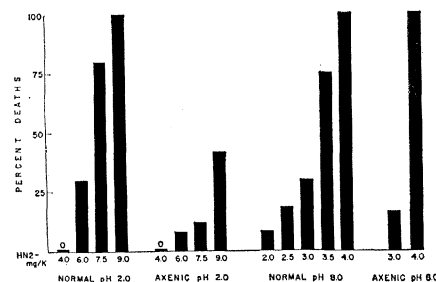


Fig. 1. Lethal toxicity of nitrogen mustard in normal and bacteria-free mice.