the spleen) protected, 16 were still living on the 8th day, but only 5 on the 30th day. (d) Out of 24 rats that received 1130 r with the liver protected and 3 injections of becaptan as in (b) (i.e., *after irradiation*), 14 were living and in excellent condition 30 days after irradiation. If another part of the rat's body (a hind limb, for instance) is protected, cysteamine does not decrease mortality if given after irradiation.

3) Becaptan reduces the weight of the liver in mice, and increases its content by 50% in ascorbic acid (Table 2, Fischer and Bacq, unpublished).

*Other actions.* β-Mercaptoethylamine has a peculiar antimitotic action on tissue cultures of chick embryo. It seems to act mainly on the cytoplasm of cell in interkinesis; the nucleus divides, but the cytoplasm does not; thus binucleated cells are formed (*23*).

Large doses of cysteamine (up to 1 g daily, for 15 days) have been given intravenously in 11 cases of chronic leukemia, with good results in 4 cases. Cysteamine does not change the basal metabolic rate and has no antithyroid action (*21*). It inhibits the tuberculinic reaction in the skin of sensitized humans and increases the time necessary to observe the trypan blue color in the skin of rabbits after local chloroform application, about 500% (Ambrose and de Eds test) (*24*). It also inhibits the allergic passive Arthus reaction in the skin of rabbits.

*Conclusion.* At the present time β-mercaptoethylamine (= cysteamine) thus seems to be the most practical substance to protect mammals against ionizing radiations. It remains active when given after irradiation, if the liver has been protected. It has a remarkable therapeutic action against radiation sickness in cancerous patients locally irradiated. Other observations show that mercaptoethylamine is a molecule of great biological interest, the study of which is being actively pursued in our laboratories.

### References


1. Certain Aspects of the Action of Radiation on Living Cells. *Brit. J. Radiol.*, Suppl. n°1 (1947).
2. Colloques internationaux, Cinquantenaire de la Découverte du Radium, Paris, July 1950.
3. Radiation Chemistry, General discussion, Faraday Society, Leeds, April 1952.
4. Bacq, Z. M., and Herve, A. *Brit. J. Radiol.*, **24**, 617 (1951).
5. Boyland, E., and Gallico, F. *Brit. J. Cancer*, **6**, 160 (1952).
6. Cole, L. J., Bond, V. P., and Fishler, M. C. *Science*, **115**, 644 (1952).
7. Treadwell, A. de G., Gardner, W. V., and Lawrence, J. H. *Endocrinol.*, **32**, 161 (1943).
8. Patt, H. M., *et al. Proc. Soc. Exptl. Biol. Med.*, **73**, 18 (1950).
9. Thompson, T. L., Meffred, R. B., Jr., and Wyss, O. *J. Bacteriol.*, **62**, 39 (1951).
10. Bacq, Z. M., and Herve, A. *Bull. Acad. Méd. Belg.*, VIème série, **XVII**, 13 (1952).
11. Bacq, Z. M., and Herve, A. J. *Suisse Méd.*, **82**, 160 (1952).
12. Hollaender, A., Stapleton, G. E., and Burnett, W. T. Ciba Foundation Conference, Isotopes in Biochemistry, Churchill Ltd., London (1951). Hollaender, A., Barker, W. K., and Anderson, E. H. *Cold Spring Harbor Symposia Quant. Biol.*, **16** (1951).
13. Lecomte, J. *Arch. internat. physiol.*, **60**, 179 (1952).
14. Bacq, Z. M., Mugard, H., and Herve, A. *Acta Radiol. Scand.* (in press).
15. Nizet, A., Bacq, Z. M., and Herve, A. *Arch. intern. physiol.*, **38**, 489 (1952).
16. Desaive, P., Bacq, Z. M., and Herve, A. *Experientia*, **8**, 436 (1952).
17. Cronkite, E. P., Brecher, G., and Chapman, W. H. *Proc. Soc. Exptl. Biol. Med.*, **76**, 396 (1951).
18. Herve, A. *Rev. Méd. Liège*, **7**, 276 (1952).
19. Bacq, Z. M., Fischer, P., and Pirotte, M. *Arch. intern. physiol.*, **60**, 535 (1952).
20. Van Cauwenberge, H., *et al. Ibid.*, **61**, 124 (1953).
21. Bacq, Z. M., Bernard, J., Ramioul, H., and Deltour, G. *Bull. Acad. Méd. Belg.*, VIème série, **XVII**, 460 (1952).
22. Chevallier, A. Radiological Conference, Stockholm (1952).
23. Maisin, J., Mandart, M., Lambert, G., and Maisin, H. *Compt. rend. soc. biol.* (in press).
24. Lecomte, J., and Borenstayn, C. *Ibid.*


Manuscript received December 1, 1952.

## Incorporation of Tritium Oxide Into Growing *Chlorella pyrenoidosa* Cells[1]


**D. Weinberger and J. W. Porter[2]**

***Biology Section, Radiological Science Department, General Electric Company, Richland, Washington***


It is well established that water is the source of hydrogen in green plants and that photosynthesis is the mechanism whereby the hydrogen is incorporated. Presumably, tritium ($H^3$) should follow the same pathway in plants and should be incorporated at the same rate as ordinary hydrogen, if no isotope effect exists. From the work of Reitz and Bonhoeffer (*1*) with deuterium, however, it appears that an isotope effect should be expected with tritium, for these workers found that *Scenedesmus acutis* and *Chlaymdomonas spp.* incorporated deuterium in the nonexchangeable portion of growing algae to the extent of only 30 to 70% of its concentration in the nutrient solution. Deuterium oxide concentrations of 12 to 47% were used.

In the experiments to be described algae were grown under standard conditions for varying periods of time. The cells were harvested, and determinations were made of dry weight, and of hydrogen and tritium content. The results were expressed as mc of tritium per ml of water of combustion of the dry cells. The values were then plotted against the number of weight doublings of the original cells, and comparisons were made with a theoretical curve derived on the assumption that no isotope effect exists. Details of the experimental procedure follow.

The green alga, *Chlorella pyrenoidosa,* was used. The culture, obtained from the American Type Culture Collection, was grown on agar slants enriched with sucrose, and transfers were made at monthly intervals. Algae to serve as inocula in experiments were transferred to a sucrose enrichment culture and then, after growth had taken place, to the completely

[1] This work was performed under Atomic Energy Commission Contract No. W-31-109-Eng-52.

[2] The authors are indebted to the Analytical Unit of the Biology Division for the tritium analyses and to Mrs. M. Newcombe of the Analytical Services Unit, Technical Division, for the hydrogen analyses.

inorganic nutrient solution of Knop (modified). After a growing period of 4 to 7 days under fluorescent light of 300–500 footcandles with aeration by a stream of 10% $CO_2$ in air, the algae were withdrawn for use. They were centrifuged and subsequently resuspended in sterile water for inoculation.

Experimental algae were grown in test tubes as suggested by Myers (*2*). Twenty-five by 150 mm tubes were partially filled with nutrient solution, plugged with cotton, and sterilized by autoclaving. The iron solution was sterilized separately and added to the other salts. Tritium oxide was added to give a concentration of 1 mc/ml in most experiments. (Control experiments indicated that this level of tritium was not inhibitory to algal growth.) Inocula were in the log period of growth, and they were added in amounts of 0.02–0.12 mg dry weight of cells/ml of nutrient. (Optical density was used as a measure of the initial concentration (solids) of algae and as a measure of growth attained during and at the end of an experiment. Other experiments had shown a linear relationship between cell mass and light absorption.) Throughout the growth period of the experiment a mixture of 10% $CO_2$ in air was bubbled into the solutions through sterile 0.5-mm capillary tubing, and the cells were exposed to light of approximately 500 footcandles, supplied by 36-in. fluorescent tubes. The temperature of the solutions was maintained at 25 ± 3° C.

The algae were harvested at intervals within the range of 0.1–8.0 doublings in weight. Light absorption readings were made, and the cells were then centrifuged at approximately 8500 rpm for 2 min. The cells were resuspended in distilled water and then centrifuged. This procedure was repeated twice. (While this procedure will remove some exchangeable tritium from the cells, the amount so lost was shown to be less than 6% of the total tritium present in the cells.) The washed cells were vacuum dried; then they were combusted over copper oxide in a conventional macro furnace. A circle of filter paper was added to the sample to increase the volume of water collected. Combustion water was collected in a U-tube trap cooled with a dry ice-ethanol mixture. Tritium analyses were performed on aliquots of the combustion water. Details of the counting procedure and equipment have been described by others (*3*). Total combustion water was determined on separate samples by the standard microanalytical procedure for hydrogen.

The organically bound tritium content of the algae, expressed as mc/ml of combustion water, is plotted (Fig. 1) against the number of weight doublings of the cells. The number of doublings was calculated from the following equation (adapted from Porter [*4*]):

$$n = 3.3 \log b/B$$

where $n$ = number of doublings in weight, $b$ = weight at the end of an experiment (optical density), and $B$ = weight at the beginning of experiment. A curve showing the theoretical amount of tritium expected in the cells, assuming no isotope effect, is also shown

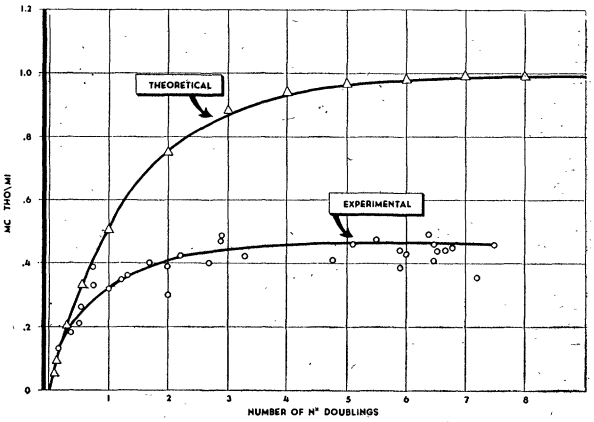


FIG. 1. Uptake of tritium oxide by rapidly growing *Chlorella pyrenoidosa.*

in Fig. 1. The theoretical curve was derived on the assumptions that: (1) all newly formed algal material would incorporate tritium and protium in the same ratio as that present in the nutrient solution; (2) tritium would not replace hydrogen, in significant quantities, in the organic compounds of the inoculum; and (3) hydrogen of the original inoculum would not be lost in significant amounts through metabolism.

Figure 1 shows that after one or two weight doublings of the algae the amount of tritium incorporated is much less than that predicted on the assumption that no isotope effect exists. These results clearly show that tritium is moved more slowly than hydrogen along anabolic pathways of growing algae, and further, they show that the rate for tritium incorporation is between one-third and one-half that for hydrogen. Why the isotope effect is not apparent or as great before one or two weight doublings is not clear at this time. It is quite possible, however, that one or more of the assumptions made in constructing the theoretical curve may not be completely valid during the time the cells of the inoculum first double in weight.

A study has been made of the effect of tritium concentration in the nutrient solution on the relative up-

TABLE 1

TRITIUM CONTENT OF ALGAE GROWING IN MEDIA CONTAINING DIFFERENT LEVELS OF TRITIUM OXIDE

(Results after 5.5 doublings of weight)

| Tritium in medium (mc/ml) | Tritium in Combustion Water | | Per cent of theory |
|---|---|---|---|
| | Theoretical (assuming no isotope effect) (mc/ml) | Experimental (mc/ml) | |
| 0.01 | 0.00975 | 0.00481 | 49 |
| 0.05 | 0.0488 | 0.0231 | 47 |
| 0.10 | 0.0975 | 0.0442 | 45 |
| 0.25 | 0.244 | 0.114 | 47 |
| 1.00 | 0.975 | 0.452 | 47 |
| 4.00 | 3.90 | 1.76 | 45 |

take of tritium and protium by the algae. The results are presented in Table 1. They show that approximately the same isotope effect was obtained at each concentration of tritium.


**References**

1. REITZ, O., and BONHOEFFER, K. F. *Z. physik. Chem.*, **A172**, 369 (1935).
2. MYERS, J. In J. Brunel, G. W. Prescott, and L. H. Tiffany, Eds., *Culturing of Algae—A Symposium.* Yellow Springs, Ohio: Antioch Press (1950).
3. CLARK, R. G., DRIVER, G. E., and EISENACHER, P. L. Ra-det **5**, nos. 3–4, p. 7 (1952).
4. PORTER, J. R. *Bacterial Chemistry and Physiology.* New York: Wiley (1946).



Manuscript received October 16, 1952.


## Extended-Range High-Frequency Radio Communication at Relatively Low Power, by Means of Overlapping Oblique Reflections from Meteor Ionization Trails[1]


O. G. Villard, Jr., A. M. Peterson, L. A. Manning, and V. R. Eshleman

*Radio Propagation Laboratory, Electrical Engineering Department, Stanford University, Stanford, California*


At radio frequencies customarily used for long-range radio communication (6–25 mcs), the following mechanisms are known whereby reasonably consistent signals can be propagated from transmitter to receiver: (1) ground wave, (2) space wave, (3) anomalous tropospheric propagation, (4) ionospheric sky wave, reflected by both regular and "abnormal" layers, and (5) indirect sky-wave transmission, involving backscattering from the earth (*1*). Recently, a new type of transmission explained as ionospheric forward scattering has been demonstrated at a frequency of 50 megacycles, with the aid of transmitting equipment of relatively high power (order of tens of kw), and antennas of high gain (*2*).

It is the purpose of this note to suggest that at frequencies of the order of 15 megacycles and lower, still another mechanism may largely account for a consistent type of propagation which has been found to exist between low-power stations (order of 1 kw) separated by distances of roughly 800 miles. The mechnism proposed is that of overlapping reflections from the large numbers of meteor ionization trails which are present in the E-region of the ionosphere at any given time.

It has been predicted on theoretical grounds (*3*), and demonstrated experimentally to a first approximation (*4*), that the duration of an echo from the most common type of meteor trail is considerably increased when the reflection takes place at oblique, rather than perpendicular incidence. A perpendicular-incidence echo decays in intensity and falls below the threshold of detection not because the causative ionization has disappeared, but rather because contributions to the echo from various portions of the expanding cylindrical disturbance begin to cancel each other out as soon as the separation between any two portions approaches a half wavelength. The onset of this cancellation is considerably delayed when oblique geometry is involved, because the trail must expand further before the pertinent portions become separated by an amount which produces a half-wavelength change in length of path.

The numbers of meteors detectable at vertical incidence at 15 megacycles with continuous-wave powers of the order of one kw, is such that the individual meteor echoes are usually well separated in time.

Over an oblique path of the order of 800 miles, however, the mean duration of received echoes may be expected to be considerably lengthened. For the most favorably situated trails, the increase may be as much as 20 times. When the pertinent statistical and geometrical factors are taken into account, the probability of at least one echo of detectable strength being present at any given time can be shown to be very high.

In order to verify this expectation experimentally, it is necessary so to choose the wavelength, time of day, and season of the year that transmission possibilities (4) and (5) above are ruled out. Elimination of possibility (5) is important, since sporadic-E clouds of ionization located to one side of the transmission path of interest (and so not readily detectable as such) can nevertheless make a perceptible contribution to the total signal observed over the desired path by indirect backscattering from the ground. Over a path of 800 miles, at the frequencies of interest, mechanisms (1), (2), and (3) may not be expected to be of significance.

Experiments have been conducted over paths of approximately this length, at a frequency close to 14 megacycles, with possibilities (4) and (5) ruled out with the aid of a scatter-sounding apparatus which has been described elsewhere (*1, 5*). Background transmission, having characteristics consistent with the meteoric echo explanation, has been found on all occasions on which the experiment could be conducted without ambiguity. Transmitted powers of the order of 500 w were used, together with antenna gains of 6–8 db. With standard communications equipment, at locations reasonably free of noise and reasonably favorable to low-angle radiation, a signal well above receiver noise level may be expected at the distant point.

This signal is subject to strong fading. It will be found to dip occasionally into the noise level, yet often rise very far above. It is consistent enough, however, for hand-keyed telegraphy provided that suitable automatic volume control or limiting circuits are used.

Appreciable increases in transmission distance and


[1] This work was in part supported by the Navy Department (Office of Naval Research), the U. S. Army (Signal Corps), and the U. S. Air Force under ONR Contract N6-ONR-251 Consolidated Task No. 7.