mitters were operating continuously; for the remainder the intervals between data readout were so long that the times of puncture, with the corresponding altitudes, could not be determined. For four of these eight punctures, which occurred while the spacecraft was occulted by Moon, the altitudes can be defined only within limits—the highest and lowest altitudes during the occultation periods. The altitudes at which the eight punctures occurred may then be 305 ± 120, 510 ± 200, 810 ± 90, 1125 ± 675, 1650, 1685, 5100, and 6040 ± 100 km. Study of these data, with due allowance for variation of residence time with altitude (the spacecraft spends less time near perilune than near apolune), indicated no statistically significant variation of hazard with altitude.

The difference between penetration rates near Moon and near Earth should probably be accepted as only tentative since (i) the number of penetrations is (statistically) fairly small, and (ii) the meteoroid flux in the neighborhood of Earth's orbit may vary from one measurement period to another. But the data do indicate with good confidence that the penetration hazard for 0.025-mm beryllium copper near Moon is no greater than near Earth. The data show no evidence of increase in the hazard that might result from secondary flux ("backsplash" from impacts of primary meteoroids on the lunar surface) in the altitude range between 30 and 6200 km. There was no apparent dependence on altitude, and the dependence on direction appeared to agree with that shown by Earth-based radar observations.

C. A. GURTLER
GARY W. GREW
Langley Research Center, National Aeronautics and Space Administration, Hampton, Virginia 23365

References

1. T. N. Nazarova, A. K. Rybakov, C. D. Komissarov, "Investigation of solid interplanetary matter in the vicinity of the moon," before 10th COSPAR meeting, London, July 1967.
2. E. C. Hastings, Jr., Ed., *NASA TM X-949* (March 1964).
3. R. L. O'Neal, Ed., *NASA TM X-1123* (August 1965).
4. F. L. Whipple, *J. Geophys. Res.* **68**(17), 4929 (1963).
5. G. P. Konstantinov, M. M. Bredov, Y. P. Mazets, "Experimental data against the hypothesis of a dust cloud around the earth," in *Rep. Acad. Sci. USSR* **174**(3) (1967); C. Nilsson, *Science* **153**, 1242 (1966).
6. G. S. Hawkins, *Monthly Notices Roy. Astron. Soc.* **116**(1), 92 (1956).
7. D. E. Gault, E. M. Shoemaker, H. J. Moore, *NASA TN D-1767* (1963).

24 June 1968

Tritium Enrichment by Gas-Solid Chromatography: Technique for Low-Level Analysis

Abstract. *A palladium chromatograph was developed that can detect less than 10 atoms of tritium per 10^{18} atoms of hydrogen. Columns operated at ambient temperature have 70-liter hydrogen capacity (50-milliliter water sample) and give 60 percent recovery in the first 500 cubic centimeters of gas evolved.*

Hydrogen isotopes can be separated in a chromatographic column filled with palladium black (*1*). I have developed a tritium-enriching chromatograph, based on this method of isotopic separation, that can detect 2 atoms of tritium (T) per 10^{18} atoms of hydrogen. This analytical system is simpler and faster than the electrolytic (*2*) or thermal-diffusion (*3*) techniques normally used. The technique is specific for tritium and unaffected by trace amounts of other radioactive isotopes.

The entire procedure, beginning with conversion of the water sample to hydrogen and ending with the 500-cm^3 enriched fraction in the proportional chamber, requires about 3.5 hours. Counting time varies with the level of sensitivity desired and with the background of the system; for my system (background of 6.4 count/min in a 2.6-liter chamber), a 30-minute counting period suffices for detection of original sample T:H concentrations greater than $1:10^{17}$ with an error (1σ) of 41 percent. Counting times up to 1000 minutes were used for radioassay of tritium concentrations below $1:10^{17}$.

Replicate analyses at T:H ratios from $4:10^{14}$ to $5:10^{17}$ (the lower concentration was restricted by availability of tritium-free water to make dilutions, and not by column characteristics) resulted in an overall recovery of 62 ± 6.3 percent at the 95 percent confidence level. There was no loss in recovery at the lower concentration.

If one assumes that recovery does not change for concentrations lower by at least one order of magnitude than those tested, Atlantic Ocean water had a T:H ratio of $2:10^{17}$, and Savannah River water, stored in glass, contained 1.3 atoms of tritium per 10^{17} atoms of hydrogen. Both results compare favorably with published values (*4*). The lowest tritium concentration was found in deep-well water freshly sampled and quickly processed before significant exchange with atmospheric water could occur; the estimated initial T:H ratio of $2:10^{18}$ increased to $2.0:10^{17}$ after storage for 6 months at room temperature in a capped polyethylene bottle—because of infusion and exchange of tritium from moisture in ambient air (T:H, $3:10^{15}$). The lowest atomic T:H concentration previously tested for enrichment characteristics, by a chromatographic system, was $1:10^{14}$ (*5*); a molecular-sieve column was used at liquid-nitrogen temperature. The capacity of this column was later increased from 300 ml of hydrogen to 10 liters (*6*), but behavior at low concentrations was not reported.

The enrichment scheme is as follows: The water sample is reacted with magnesium chips at 650°C. The hydrogen generated sorbs directly onto an evacuated palladium column. When a sufficient volume of water has reacted, the furnace is isolated and cooled. No buffer gas or carrier gas is used; movement of hydrogen through the column is maintained by the initial pressure

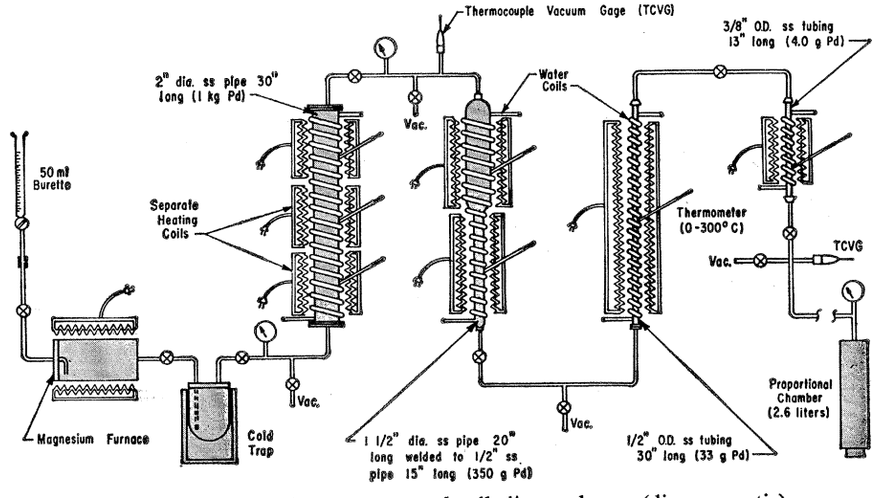

Fig. 1. Experimental arrangement of palladium columns (diagrammatic).

differential (vacuum at exit end and hydrogen partial pressure at feed end), and the additional pressure created by zonal heating of the feed end of the column, causing palladium to release sorbed hydrogen.

Tritium exhibits the highest partial pressure over palladium and diffuses through the column faster than does either deuterium or protium. The first gas evolved, the tritium-rich fraction, is detected by a pressure increase in an evacuated proportional chamber attached to the exit end of the column. The proportional chamber is removed from the system, filled to atmospheric pressure with methane counting gas, and counted for 30 minutes under low-background conditions.

The column is regenerated by increase in the temperature of the entire system to 215°C and by evacuation of the residual hydrogen to less than 0.3 mm-Hg.

The major components of the system (Fig. 1) were designed for processing 40-ml samples. Data reported apply to this feed volume and a final collection volume of 500 cm^3 of gas in the proportional counter (2.6-liter volume).

Certain practical points should be noted regarding operation of the column. Recovery and enrichment are low if any trace of oxygen remains in the system. All leaks must be eliminated, and the columns are stored under nitrogen or other inert gas when not in use. Chemical vapors (for example, halogens) that react with palladium or catalytically combine with hydrogen must be avoided. Two and a half hours are required for the tritium to evolve from the column, but other gases inert to the column pass through in a few minutes. Gases dissolved in water or those released from magnesium upon heating may be pumped from the discharge end of the column without effect on the final result. Approximately 3 percent of the tritium remains in the hydrogen generator and may contaminate subsequent samples if they are significantly lower in tritium, unless the generator is baked at 80°C for 2 hours before reuse.

Recovery proved to be relatively independent of operating conditions. Changes in column temperature (between 20° and 40°C), in rate of feed of the sample to the hydrogen generator (0.2 to 1.0 ml/min), and in elution rate (2 to 2.5 hours) did not affect recovery. However, recovery depended on sample volume (56 percent recovery for a 50-ml sample; 80 percent for 10 ml).

Tritium can be measured by this technique at concentrations corresponding to natural levels. This relatively inexpensive, rapid analytical system facilitates the use of tritium as a tracer in hydrology, oceanography, meteorology, and biology. The principles involved may have application where tritium is to be separated from another gas or for tritium labeling of organic or inorganic compounds.

JOHN E. HOY
Savannah River Laboratory, E. I. du Pont de Nemours and Company, Aiken, South Carolina 29801

References and Notes

1. E. Glueckauf and G. P. Kitt, in *Proceedings of International Symposium on Isotope Separation*, J. Kistemaker, J. Bigeleisen, A. D. C. Nier, Eds. (North-Holland, Amsterdam, 1957), p. 210; J. Chadwick, *Atomic Energy Res. Estab. 1/M-47* (1958).
2. S. Kaufman and W. F. Libby, *Phys. Rev.* **93**, 1337 (1954) .
3. B. T. Verhagen and J. P. F. Sellschop, *Intern. Conf. Peaceful Uses Atomic Energy 3rd* (1965), vol. 12, p. 398.
4. J. P. Riley and G. Skirro, *Chemical Oceanography* (Academic Press, New York, 1965), vol. 2, p. 454.
5. M. B. A. Crespi and H. Perschke, *Intern. J. Appl. Radiation Isotopes* **15**, 569 (1964).
6. H. Perschke, *Nature* **209**, 1021 (1966).
7. Work done under AEC contract AT(07-2)-1.

10 May 1968

Interferon Inducers in vitro: Difference in Sensitivity to Inhibitors of RNA and Protein Synthesis

Abstract. *Interferon can be induced by diverse agents in a variety of mammalian cell cultures through apparently two mechanisms. One results in an early (2 to 10 hours) appearance of interferon and is relatively resistant to inhibition by actinomycin, puromycin, or fluorophenylalanine. A second mechanism results in a late (18 to 24 hours) appearance of interferon and is more sensitive to inhibition by these inhibitors. The molecular basis for each mechanism is unclear. Since each interferon inducer may have multiple effects on the cell, the differences observed may not necessarily reflect a fundamental difference in the mechanism of interferon stimulation.*

Interferon induction may occur through two mechanisms (*1, 2*). One involves the synthesis *de novo* of interferon, and the other involves release of already formed interferon. This suggestion was based on the differing effects of various drugs and procedures on interferon formation by bacterial endotoxins and RNA viruses in vivo. These include: (i) different sensitivities to inhibition of RNA and protein synthesis; (ii) different kinetics of interferon appearance; (iii) different effects on interferon titers by splenectomy, adrenalectomy, change of body temperature, prior immunization with BCG vaccine (Bacille Calmette-Guérin), and cortisone administration. It was also demonstrated (*3, 4*) that interferon induction in vitro by endotoxin and virus (Newcastle disease virus) had different rates of appearance and different sensitivities to actinomycin D. Furthermore, there were different optimum temperatures for interferon induction in macrophage preparations between a fungal polysaccharide and an RNA virus (*5*).

These studies have drawbacks owing to heterogenous cell populations, secondary effects of the various drugs and agents in vivo, use of a relatively weak interferon inducer to demonstrate a mechanism of interferon induction, and little control over the metabolic changes that each inducer produced in the cells.

In our studies, in vitro we used a variety of naturally occurring and synthetic inducers of interferon in different cell lines; according to type the cell lines showed differences in reactivity to inducers. To measure their activity, inducers were incubated at 37°C with monolayers of different cell types in Eagle's minimal essential medium (MEM). After various intervals the antiviral activity of the tissue culture fluids was assayed by plaque inhibition, and interferon was characterized (*6*). Not all inducers were active in stimulating interferon in each cell type (Table 1). Only the viral inducers, namely, Newcastle disease virus (NDV) and statolon (a fungal virus preparation) induced interferon in all cell types. The synthetic double-stranded RNA consisting of polyribocytidylic acid and polyriboinosinic acid (rC/rI), described as an interferon inducer by Field *et al.* (*7*), and pyran, a polycarboxylate copolymer (*8*), failed to induce interferon in mouse L929 cells, but was effective in human skin fibroblasts and mouse peritoneal macrophages. Endotoxin induced interferon only in the mouse peritoneal macrophages. Like L929 cells, HeLa cells responded only to viral inducers; chick embryo fibroblasts, like human skin fibroblasts, responded in addition to rC/