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Tritium Geophysics as an International Research Project

The presence of artificial tritium on the earth's surface provides unique opportunities for environmental research.

Hans E. Suess

It was fortunate that tritium produced by cosmic rays was discovered before the development of the hydrogen bomb. This discovery in 1949 by Faltings and Harteck (*1*) and independently by Libby *et al.* (*2*) made it possible to derive at least an approximate value for the production rate of natural tritium. Evaluation of the data obtained at that time, and more recent measurements of samples dating from before 1952, indicate a rate of cosmic ray production on the order of 30 atoms per square centimeter of the earth's surface per minute (*3*). A few years later, and especially during 1961 and 1962, quantities of tritium greatly in excess of those naturally present were released artificially into the atmosphere.

The amount of tritium added to the atmosphere by the testing of hydrogen bombs can be estimated, from the observed tritium content of precipitation and of the surface water of the oceans, to be of the order of 10^5 grams. This corresponds to a total of 10^{21} tritium disintegrations per minute for the whole earth, or about 300 disintegrations per minute per square centimeter of the earth's surface, a figure about ten times larger than that for the natural tritium produced by cosmic rays. Since 1964, because of the moratorium on atomic bomb testing, artificial tritium has not been added in noticeable amounts. The global tritium activity decreases at the present time with the half-life of tritium of 12.26 years. At the same time the tritium, which was originally present in the atmosphere, is gradually taken up by the vast water masses of the deep oceans.

As an isotope of hydrogen, tritium is essentially present in the form of water. The small differences in the chemical properties of tritium and ordinary hydrogen (protium) are proportionally greater than those of deuterium and protium. Chemical fractionations caused by these differences can be neglected in most instances insofar as the geochemical behavior of tritium is concerned. At this time all the water present on the surface of the earth, in the form of water vapor in the atmosphere, of cloud droplets, precipitation, river- and groundwater on land, or surface water of the oceans, is tagged with a measurable amount of tritium. This situation affords us with a unique opportunity to study the behavior of water of our environment in connection with many problems of great scientific or economic importance.

This opportunity can be best used within the framework of a worldwide, long-range project difficult to initiate on a local or even national basis. Fortunately, during the development of its scientific program in 1958, the International Atomic Energy Agency (IAEA) had endeavored to include international projects of a type which could be carried out only by an international organization. The projects had to be of a direct benefit to the member states, and particularly to those in the developing areas of the world. In this respect the study of tritium in the natural environment appeared particularly appropriate, and this led to the establishment of the joint project now in operation with the World Meteorological Organization (WMO).

The seed for this project was sown by Begemann and Libby in their paper (*4*) which was presented to the Conference on Radioisotopes in Research organized by UNESCO in Paris, in 1957. Henry Seligman was the first to propose that this work be followed up on a global scale, and the planning was carried out under his direction and with the help of consultant groups coming to the IAEA. The project concerns not only the measurement of tritium, but also of the stable isotopes of hydrogen and oxygen in precipitation from all parts of the world. It commenced operation in 1960 and has continued as a result of harmonious collaboration of meteorological services of many countries and a number of environmental isotope laboratories.

The sampling network was set up in collaboration with WMO, which invited the various national meteorological services to take part in this survey. Thus the machinery for collecting the samples was made available, but provision for the analyses had to be made. At that time the IAEA did not have laboratory facilities for these analyses, so that the initial work depended very much upon international collaboration with existing laboratories capable of making these analyses. The following laboratories have participated, or are still participating, in this program: Chalk River Nuclear Laboratories, Canada; Tata Institute of Fundamental Research, Colaba, Bombay, India; Weizmann Institute of Science, Rehovoth, Israel; Institute of Nuclear Sciences, Lower Hutt, New Zealand; Radioactive Dating Laboratory, Stockholm, Sweden; University of California, La Jolla; and University of California, Los Angeles.

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Insofar as stable isotope analyses were concerned, there were at that time relatively few laboratories equipped to carry out the many analyses required; IAEA contracted them to the Physical Laboratory II, H. C. Oersted Institute, University of Copenhagen, Denmark.

During this time the agency was setting up its own laboratory facilities, and it was logical that these should include a capability for measuring tritium. The establishment of a tritium laboratory by the agency was important not only from the viewpoint of being able to provide service analyses of samples coming from member states, but also by virtue of its international character it was possible to organize comparisons of measurements to ensure that all laboratories were working toward a common standard. The U.S. Atomic Energy Commission supported the early stages of this project under a research contract [AT(30-1)-3162].

As an additional incidental result of having established its own tritium laboratory, the agency now receives requests from member states for advice and assistance in establishing similar laboratories. Thus the existence of an IAEA laboratory provides first-hand experience of the different techniques and types of experimental systems used.

The IAEA laboratory now has facilities for electrolytic enrichment of the tritium, together with four gas-counting systems which use ethane as a counting gas (*5*). The potential analytical capacity of the laboratory is about 150 low-level tritium samples per month. The data are published periodically by the agency. In addition, the laboratory also has a carbon-14 counting system used solely to measure natural carbon-14 in groundwater samples. The IAEA-WMO precipitation sampling network comprises about 100 stations which supply the laboratories with samples representing monthly precipitation averages (*6*).

All in all, the IAEA laboratory for the measurement of environmental tritium contributes in a threefold manner to the obligations of the agency: (i) by measuring the tritium in these samples, it obtains data needed in connection with a variety of hydrological investigations; (ii) it facilitates technical assistance to developing countries; and (iii) it helps in the recruitment of qualified personnel. The scientific results that can be derived from the combined observations are reviewed here.

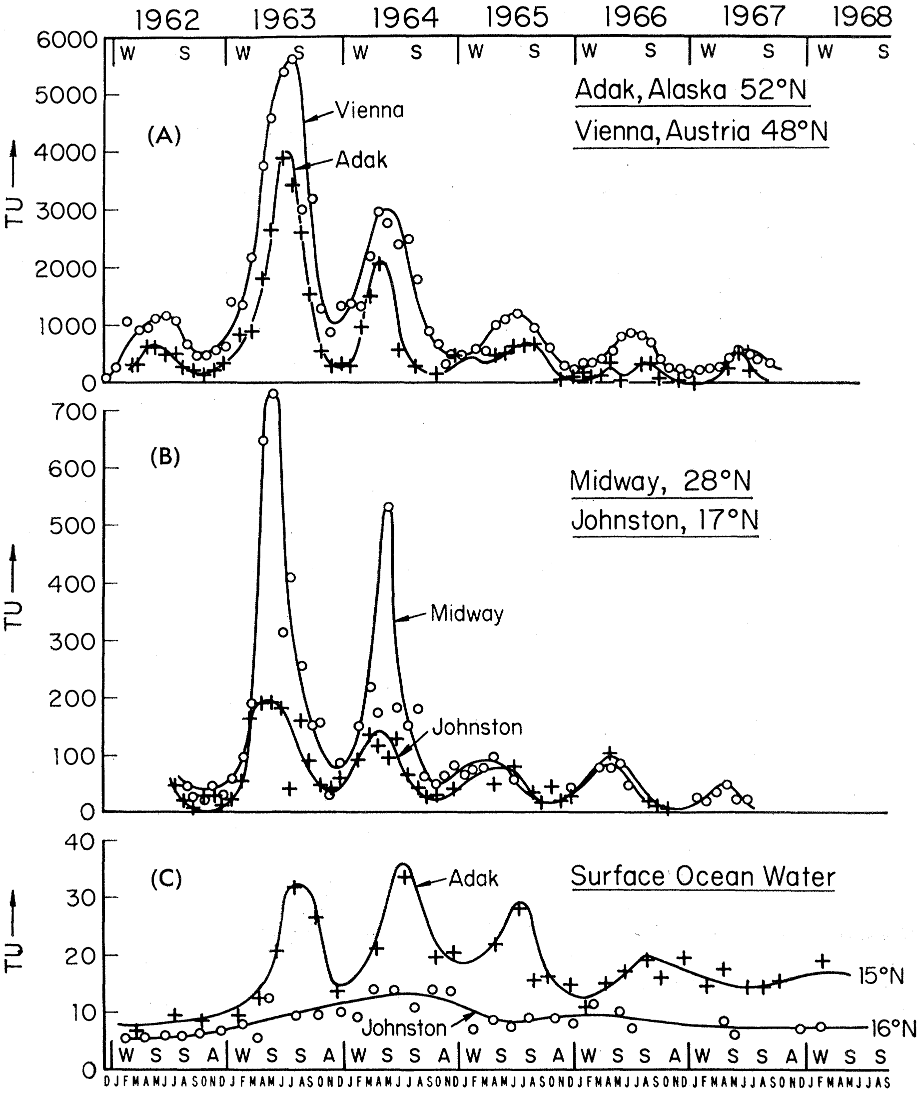

Fig. 1. (A and B) Tritium content of precipitation at Vienna and Stuttgart (average) 48°N, Adak (Alaska) 52°N, Midway Island 28°N, and Johnston Island 17°N; for the years 1962 through 1968. The tritium content shows annual oscillations which are due to the "spring leak" of the tropopause. (C) Tritium content of surface ocean water at Adak and Johnston Island. Tritium values are according to measurements by the IAEA laboratories and the laboratory at La Jolla, California (*15*). The values are given in tritium units (T.U.) = 10^{-18} T/H.

Tritium Meteorology

A most significant meteorological discovery derived from observations of artificial radioactivities in nature was that of the so-called "spring leak." The tritium content in precipitation shows this phenomenon in a most conspicuous way.

The series of high-altitude tests of hydrogen bombs, carried out in 1961 and 1962, introduced large amounts of tritium into the stratosphere at high northern latitudes. The present tritium flux from the stratosphere maintains a near steady-state concentration of tritium in the troposphere, although the tritium in the troposphere is washed out by rains at a relatively rapid rate within a few weeks. The tritium flux from the stratosphere, however, is almost one order of magnitude higher in spring and early summer than in the fall and winter. For this reason, the concentration of undisturbed tritium in precipitation of the Northern Hemisphere shows characteristic annual oscillations (Fig. 1). The amplitudes of these oscillations decrease from the north to the south because most of the tritium was introduced into the stratosphere at high northern latitudes. The tritium concentration gradient with latitude is usually greater over the continents and is much greater in the Northern than in the Southern Hemisphere. Except for high tritium that results from local tropospheric injections, the maximum tritium content in precipitation occurred in the spring and early summer of 1963. Figure 2 shows mean concentrations of the tritium content in precipitation at that time. As an all-time high, concentrations of up to 10,000 tritium units were observed in summer 1963, in rainwater of northwestern Canada. Such concentrations correspond to one disintegration per second per milliliter of rainwater. Since then the amplitude of the annual oscillations has been decreasing with time.

The phenomenon of the "spring leak" has also been observed by numerous measurements of other artificial or natural radionuclides in air, such as strontium-90, cesium-137, and beryllium-7 (*7*). The annual decrease of about a factor of 2 of the amplitude of tritium, as well as of those other radionuclides in rains of the years 1963, 1964, and 1965, indicates an apparent residence time of about 2 years for water vapor and dust particles in the stratosphere (*8*). The change in the tritium amplitude, however, appears to become smaller after 1965, and this indicates that at least a fraction of the total tritium is contained in the stratosphere at a level for which the residence time for mixing into the troposphere is considerably longer than 2 years (*9*). Observations of this type are particularly important in connection with possibilities of stratospheric air pollution by high-flying aircraft and its possible effects on global climate.

Other factors, such as the annual changes in the amount of precipitable water in the troposphere, can cause annual oscillations of the tritium in precipitation. Differences in the tritium content of continental and marine air masses introduce irregular variations, such as those observed by many stations.

For many stations in the Northern Hemisphere, much more regular variations in the tritium content were found after the bomb moratorium than had been observed during the earlier years (Fig. 1). In the Southern Hemisphere, however, the observed variations are quite frequently irregular, which indicates more than one tritium maximum annually. A recent release of tritium in the Southern Hemisphere has undoubtedly changed the situation there. New, prolonged series of measurements will be necessary to recognize possible basic differences in atmospheric mixing patterns of the two hemispheres.

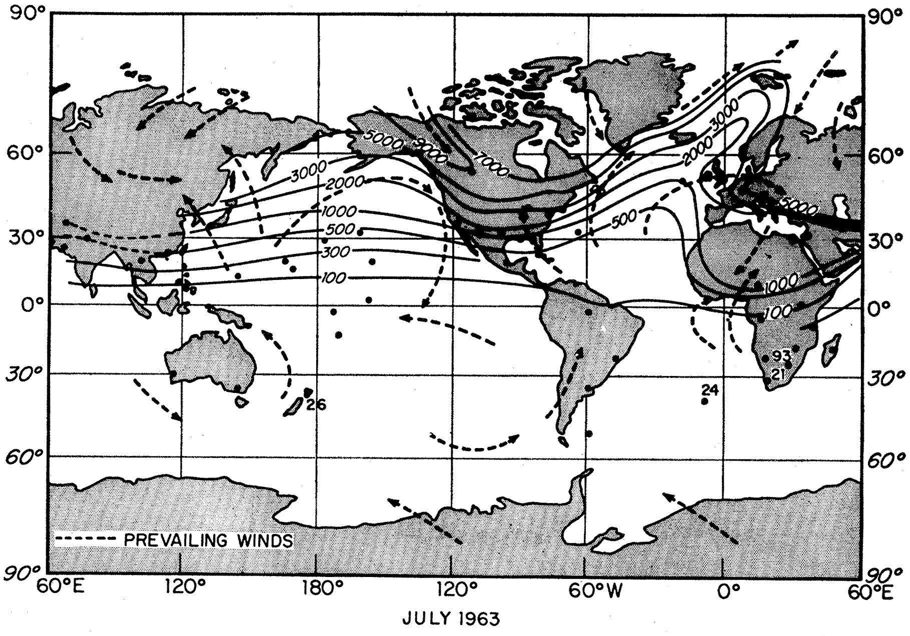

Fig. 2. Tritium content of precipitation at the time of its maximum in July 1963, according to data for samples from the IAEA-WMO network as published in the IAEA tritium lists (*15*).

Variations of the tritium content of precipitation can be used for the study of a variety of localized meteorological phenomena. The steady-state tritium concentration over continental land masses is considerably greater than that of marine air. This is caused by the longer storage time of moisture over land areas, with the result that tritium reaches higher concentrations over land than it does over the oceans, where the relative humidity is higher and where tritium is deposited not only by precipitation are due to varying contributions with the surface water. Irregular fluctuations of the tritium content of precipitation are due to varying contributions of marine and land air masses, and to variations in the length of time an air mass has persisted over a continent. For these reasons the tritium content of rainwater has been found to be related to air-mass trajectories, to radar height of precipitation formation, to rainfall intensity, duration, and other observations characterizing the meteorological situation (*10*). In this connection, the work carried out in Canada [for example, Heer-Amissah (*11*)] is of particular importance. The most promising work of this type is probably that of Östlund on hurricane tritium (*12*). The tritium distributions in the precipitations accompanying hurricane Hilda in 1964 and of hurricane Betsy in 1965 show that the tritium content in the eye of a hurricane is lowered by the admixture of seawater vapor and that the air is well mixed vertically. Quantitative treatment of the data gives valuable information on general features of hurricane dynamics.

The meteorological significance of the extensive data on tritium precipitation obtained by the IAEA sampling network over the past years, in particular since 1964, has not yet been fully evaluated (*13*). Undoubtedly, a general picture of the tritium content of precipitation, as derived from the IAEA-WMO network measurements, will be of great value for investigations of this type.

Tritium Oceanography

Most of the surface of the world's oceans is covered by a thin layer of warm water that floats like a film of oil on the cold main water mass. This so-called "mixed layer" is about 50 to 100 meters deep. Immediately below it is a region where the temperature decreases rapidly with depth, namely, the so-called thermocline. At latitudes above 60°S, and also in the Atlantic at latitudes higher than about 60°N, this warm layer is missing. The thermocline stabilizes the mixed layer against mixing with the deep water, and, except for

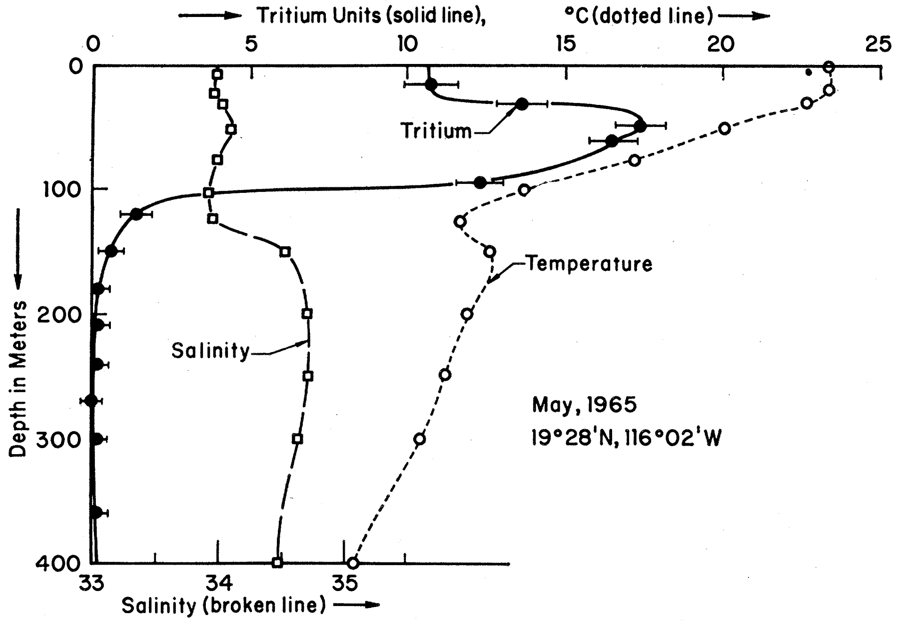

Fig. 3. Unusual tritium profile in the Pacific Ocean (19°28'N and 116°02'W) taken in May 1965, showing a tritium maximum at about 50 meters of depth (*16*).

regions of high southern or northern latitudes, downward mixing and upwelling through the thermocline is slow. The time constants involved in such mixing appear to be of the order of 10 years, but unfortunately they are not well known. The rate of interchange of water through the thermocline, however, is a most important oceanographic quantity in connection with several outstanding problems that still have to be resolved. (i) In many areas, the surface temperatures of the oceans depend on upwelling or downward mixing. It has been found recently that the ocean surface temperatures are decisive factors in the establishment of atmospheric pressure systems and, hence, of weather patterns. (ii) The interchange between surface and deep water is of crucial significance for the distribution of nutrients and for the food chain of marine life. (iii) The carbon dioxide content of the atmosphere is established by equilibration with the bicarbonate dissolved in the oceans. The interchange of the surface water with deep layers has to be considered in the estimations of the rate of uptake of excess carbon dioxide released artificially into the atmosphere by industrial combustion of fossil fuel (*14*). A knowledge of the rate of downward mixing is desirable in connection with all problems of ocean contamination and waste disposal.

The pathway of radioactive fallout from the surface into the depth of the oceans has been studied in many instances. Of all the radioactive isotopes introduced into the oceans, tritium is the most favorable one for the study of ocean-mixing processes because, as an isotope of hydrogen, it follows strictly the behavior of water without complications from adsorption, precipitation, or other interaction with particulate matter.

Measurements of tritium in surface and subsurface waters of the oceans were carried out by three laboratories. Two of them, the Heidelberg (Germany) and the Miami (Florida) laboratories, have investigated water from the Atlantic Ocean; the third one, the La Jolla tritium laboratory (California), has been monitoring, since 1960, tritium in surface water from 12 island stations in the Pacific Ocean and one from the coast of the Caspian Sea (*15*). Measurements of tritium in seawater are more difficult than those in rainwater because the tritium content is, in general, about ten times lower than in precipitation.

As an example of results on tritium in surface ocean water, the tritium content, as measured by the La Jolla laboratory at two stations in the Pacific Ocean, is shown in Fig. 1. The tritium content of seawater offshore Adak (Alaska) reflects in a conspicuous way the changes in the tritium content of local precipitation. Presumably the data reflect the tritium content of only a fraction, perhaps the top 10 to 30 meters, of the mixed layer of the ocean. A mixing time of a few months, for the internal mixing of the mixed layer, can then explain the observed amplitude attenuation and the phase lag of the tritium of the surface ocean water relative to that of the precipitation at Adak. However, this mixing time within the mixed layer can be expected also to show a seasonal dependence. It is longer in summer than in winter, and this greatly enhances the observed seasonal variations in the amount of tritium in the surface water. No seasonal variations are observed in the surface water of the Pacific Ocean at low latitudes. During 1963 the tritium content of the ocean water offshore Johnston Island increased slowly by about 10 tritium units and has subsequently decreased somewhat. It appears now to be practically constant. The actual time of mixing into deeper layers of the oceans cannot yet be deduced from the existing data, and several more years of observations will be necessary before this will be possible. The data obtained so far by various investigators range from a few months to tens of years. The reason for this discrepancy is that the residence time varies greatly with geographic location, and that the concept of a residence time in the case of the mixed layer is not well defined.

One type of difficulty encountered in defining a rigorous residence time can be recognized from Fig. 3, which shows a tritium profile in the mid-Pacific from the surface down to 400 meters (*16*). As generally found in middle and lower latitudes, the tritium introduced since 1962 has not penetrated into depths greater than about 100 meters, an indication that the residence time of surface water in these latitudes is certainly greater than 2 years. Münnich *et al.* (*17*) have found, however, that in the Atlantic Ocean, in latitudes as low as 45°N, tritium can be found at depths greater than 500 meters in concentrations greater than 5 tritium units.

The profile in Fig. 3, however, shows another most remarkable feature—the tritium concentration reaches a maximum at about 50 meters. Such a maximum can be explained only by assuming horizontal movement of a thin tritium-rich layer from the north under a warmer layer with low tritium content. The occurrence of wind-driven movements of thin layers of water over large distances of some 100 kilometers is quite unexpected in view of generally accepted oceanographic concepts, and demonstrates the potential usefulness of tritium as a tracer in oceanography.

Tritium in Hydrology

With a half-life of 12.26 years, environmental tritium is of particular value as a tracer for groundwater of short turnover time and for movement of soil moisture (*3*). Hydrological investigations often pose the question of whether local recharge by precipitation takes place. In view of the relatively high concentrations of tritium in precipitation in recent years, the presence of tritium in groundwater indicates clearly the occurrence of some modern recharge. This fact can be established without a detailed piezometric survey which would be required for the conventional hydrological approach. Similarly, the presence of tritium can indicate whether or not a confined aquifer is leaky. Tritium has also proved very valuable in groundwater flow through fractures and tubular openings, such as limestone and volcanic areas, where flow can be very rapid. In such areas, and in studying the interrelation of surface and groundwater, the maximum benefit from tritium studies is obtained when they are made in conjunction with measurements of the stable isotopes—deuterium and oxygen—of water. Since Urey initiated the study of the geochemistry of these isotopes about 30 years ago, there have been discoveries concerning the factors governing their natural occurrence, such as the characterization of groundwater and whether open water bodies, such as lakes and reservoirs, contribute to groundwater or are the main source of supply to springs.

For practically all hydrological studies, based on measurements of environmental tritium in groundwater, reference must be made to the basic data obtained from the IAEA-WMO precipitation survey. The data provide the fundamental information on the variation, both with respect to geographical location and time, of tritium concentration in precipitation. The data from this global network of stations may be used for estimating with reasonable accuracy the history of tritium concentration in precipitation in areas for which measurements are not available.

Münnich *et al.* (*17*) have estimated the average annual groundwater recharge by measuring the tritium concentration of a soil profile and relating this to the concentration of tritium in precipitation during the months when precipitation is most likely to result in active recharge. Although this method has not been widely applied, it is useful in arriving at a quick estimate of the average annual recharge over a given period of time.

Analysis of the tritium content can provide a useful qualitative guide to the mean transit time and reservoir size. Tritium concentrations comparable to that of recent precipitation are indicative of a system with a relatively short turnover time, whereas low values, which exhibit little variation with time, point to a relatively long mean transit time and considerable reservoir size. These and similar observations are of importance in considering the extent of development of various sources of water, and have been applied in the study of the water resources of the volcanic island Cheju off the coast of South Korea. The work is being supported by an IAEA research contract. The isotope data indicate that infiltration and subsurface flow are rapid. Groundwater discharges at high-altitude springs, but the main recharge is to a well-mixed reservoir of fresh water which discharges at springs near the coast and probably also through submarine springs. Observations of the variation of tritium concentration with time are being made in order to estimate the size of the reservoir.

Deuterium and oxygen-18 have been used to establish the fact that certain lakes are not the principal source of supply to springs. Lake Chala—on the border between Kenya and Tanzania—a volcanic crater lake with no surface inflow or outflow, for example, was found not to be the major source of supply to springs in the area (*18*). A similar study in the Antalya region of Turkey proved that the lakes to the north of the Taurus mountains are not the principal feed to springs on the coastal plain (*18*). In Turkey, the relative turnover times of the various lakes were determined from measurements of the variation of tritium in the lakes with time. In another problem of the same type concerning two lakes, groundwater, and coastal springs in Greece, however, the existence of a common hydraulic system was demonstrated.

The environmental tritium has demonstrated the relative unimportance of local recharge in the Pedro Plains in the southwest of Jamaica (*19*). Analyses of deuterium and oxygen-18 confirmed the hypothesis that groundwater has its origin outside the area. In Niger and Jordan, the existence of local recharge has been shown on the basis of tritium measurements. Information of this type can be obtained easily from a few analyses and is very valuable within the overall context of a hydrological investigation. Estimates of groundwater velocity in a region require more detailed analyses over a longer period, and here the interpretation of the data is more difficult than where recharge is investigated, because of large time variations of the flux of tritium which has been added from the man-made release of tritium. Several different groups are engaged in research on this important question of interpretation of isotope data—work which must be carried out in close collaboration with hydrologists.

Isotope techniques are included in a number of sectors of the program of the International Hydrological Decade. One study is an example of international collaboration between the Hydrological Research Institute in Prague, the University of Pisa, and the International Atomic Energy Agency. The snow hydrology of a small mountain basin in the Krkonoše mountains of Czechoslovakia is being studied with both conventional and isotope methods. Environmental tritium has contributed to the knowledge of the runoff mechanism of melting snow and has provided a method for estimating the relative amounts of snowmelt and base flow in the stream draining the basin (*20*). This study is a good example of what can be done with the use of isotope techniques, which would not be possible with any other approach (*21*).

Summary

The unparalleled presence of artificial tritium on the surface of the earth provides a unique opportunity for the study of a large number of problems in environmental sciences. The environmental tritium can be used as a tracer for water vapor in the atmosphere, precipitation, runoff, and groundwater on land, and for the various water masses of the oceans. To make most use of the opportunities presented by the situation, a worldwide and long-range program is needed. The extent of the program in time and space, as well as its multidisciplinary nature, indicates the necessity for broad-based international and interinstitutional cooperation. Such a program is now in progress at the International Atomic Energy Agency in cooperation with national and university laboratories of several countries.

The program makes use of a sampling network for precipitation, organized jointly with the World Meteorological Organization. In addition to providing data as a basis for future studies, the work in progress is yielding immediate results in the field of hydrology of arid regions and in regard to problems of water supply and water contamination in developing countries.

References and Notes

1. V. Faltings and P. Harteck, *Z. Naturforsch.* **5a**, 438 (1950).
2. A. V. Grosse, W. M. Johnston, R. L. Wolfgang, W. F. Libby, *Science* **113**, 1 (1951).
3. H. Craig and D. Lal, *Tellus* **13**, 85 (1961); F. Begemann, *Chimia* **16**, 1 (1961).
4. F. Begemann and W. F. Libby, *Geochim. Cosmochim. Acta* **12**, 277 (1957); *Proc. Conf. UNESCO Paris* (1957), p. 832.
5. A. E. Bainbridge P. Sandoval, H. E. Suess, *Science* **143**, 552 (1961).
6. For example, see "Tritium and other environmental isotopes in the hydrological cycle," *Int. At. Energy Agency Tech. Rep. Ser. 73* (1967); or *UNESCO Guide Book on Nuclear Techniques in Hydrology* (UNESCO, Paris, in press).
7. C. W. Thomas and N. A. Wogman, "Atmospheric behavior of airborne radionuclides," *U.S. At. Energy Comm. Res. Develop. Rep. BNWL-481, No. 2* (1967), p. 4.
8. P. Fabian, W. F. Libby, C. E. Palmer, *J. Geophys. Res.* **73**, 3611 (1968).
9. B. R. Payne and H. E. Suess, in preparation.
10. R. M. Smith, *Tellus* **20**, 76 (1968).
11. A. Heer-Amissah, thesis, McGill University, Montreal (1964).
12. H. G. Östlund, in "Isotope techniques in the hydrologic cycle," G. E. Stout, Ed., *Geophys. Monogr. Ser.* **11**, 58 (1967).
13. E. Eriksson, *Tellus* **17**, 118 (1965).
14. R. Revelle and H. E. Suess, *ibid.* **9**, 18 (1957).
15. K. O. Dockins, A. E. Bainbridge, J. C. Houtermans, H. E. Suess, in *Proc. Monaco Symp. IAEA Vienna* (1967), p. 29; and IAEA tritium data (preprints).
16. K. O. Dockins, in preparation.
17. O. Münnich *et al.*, *Isotopes in Hydrology*, (IAEA, Vienna, 1967), p. 305.
18. B. R. Payne, in "Isotope techniques in the hydrologic cycle," G. E. Stout, Ed., *Geophys. Monogr. Ser.* **11**, 62 (1967).
19. T. Dincer, T. Florkowski, E. Halevy, B. R. Payne, *Proc. Int. Conf. Water Peace Washington, D.C., 23–31 May 1967.*
20. T. Dincer, B. R. Payne, J. Martinec, G. E. Tongiorgi, T. Florkowski, in preparation.
21. *Guidebook on Nuclear Techniques in Hydrology* (IAEA Doc-10/9, Vienna, 1968).
22. The tritium laboratory in La Jolla, California, is supported by NSF grant GA-1578, I thank B. R. Payne, Hydrology Section IAEA, Vienna, for very material contributions to this paper.

Active Center of DNA Polymerase

The operations are localized and arranged in multiple sites within a single area of the molecule.

Arthur Kornberg

DNA polymerases have now been isolated from a variety of bacterial and animal cells. These enzymes, including those produced specifically in response to virus infection, catalyze the addition of mononucleotide units to the 3′-hydroxyl terminus of a primer DNA chain. Synthesis therefore proceeds in the direction of 5′ to 3′ (Fig. 1) (*1*). There is an absolute requirement for a DNA template, and errors in copying the template are very infrequent. The synthesis of DNA proceeds rapidly, at rates near 1000 nucleotides per minute per molecule of enzyme, with the production of chains several million in molecular weight.

The polymerases are remarkable enzymes. A polymerase takes instructions as it goes along to build a chain according to specifications by a template. Bacterial DNA polymerase will make animal DNA and animal polymerase will make bacterial DNA. The DNA polymerase from *Escherichia coli* has additional catalytic properties. It may degrade DNA progressively from either end (5′ or 3′) of the chain by hydrolysis to produce deoxyribonucleoside monophosphates. Or it may degrade a chain by pyrophosphorolysis with inorganic pyrophosphate to produce deoxyribonucleoside triphosphates.

Until recently we understood little about how this enzyme works because we did not know enough about its physicochemical properties. We knew very little because our supplies of homogeneous enzyme were meager. Now with a simpler method for purification (*2*) and the invaluable use of the large-scale facilities of the New England Enzyme Center, we have had available 600 milligrams of homogeneous DNA polymerase obtained from 200 pounds (90 kilograms) of *E. coli.* The purpose of this article is twofold: (i) to assemble in a brief form the new physicochemical and functional observations concerning the pure enzyme; and (ii) to attempt an interpretation of these data in a model for the active center of the enzyme. This model is of course speculative, but it has helped us reconcile many hitherto confusing details and continues to suggest useful experiments.

Physicochemical Properties

The molecular weight of the homogeneous polymerase, determined by sedimentation equilibrium, is 109,000 (*2*). This large molecular weight and the presence of both polymerase and multiple nuclease activities suggest a subunit structure. However, the molecular weight measured by sedimentation equilibrium under denaturing and reducing conditions (6*M* guanidine hydrochloride and 0.3*M* mercaptoethanol) was found to be the same as that of the native protein. Optical rotatory dispersion and velocity sedimentation studies showed that polymerase loses ordered structure in 6*M* guanidine hydrochloride, and would therefore be expected to be fully dissociated in this solvent.

More than 95 percent of the protein migrated as a single band on polyacrylamide-gel electrophoresis at *p*H 3.5, *p*H 8, and *p*H 11 (with or without 7*M* urea at *p*H 3.5 and *p*H 8). This result is most consistent with a structure composed of either a single polypeptide chain or of two or more identical subunits. However, the possibility of multiple, identical subunits is ruled out by the fact that polymerase contains, per 109,000 molecular weight, a single sulfhydryl group and a single disulfide group. The sulfhydryl group is probably not part of the active site

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