

SELF-DECOMPOSITION OF CARBON-14-LABELED ORGANIC COMPOUNDS

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I. INTRODUCTION

Reviews of the literature of the radiation decomposition of various compounds have dealt generally with the effects of α - and β -particles, γ - and X-rays, neutrons, and other ionizing particles. The behavior of various compounds when subjected to the different forms of *external* radiation has been covered in other reviews (7, 8, 35, 39, 40); chemical changes generally occurring during β -decay processes have also been reviewed (28). Only during the last decade has it been recognized that radiation from isotopes contained *within* a compound could produce profound chemical changes (40).

Some of the classes of pure organic compounds for which there are some radiation decomposition data (39) include amino acids, nitrogen heterocyclic compounds, sterols, aldehydes, sugars, dyes, and metalloorganic compounds. The effects of substituent groups on radiation stability have been studied only slightly, and no data are available to indicate the effect of the following groups on radiation changes: nitro, nitrile, nitroso, sulfonic acid, and hydroxylamine groups and quinones.

This review is limited to the self-degradation of organic compounds containing C^{14} . In general, no attempt is made to consider the behavior of the various compounds when subjected to different forms of external radiation.

Factors causing auto- or self-decomposition and methods of reducing or minimizing this effect are discussed. Equations are given for calculating absorbed dose, per cent decomposition, and *G* values. All data

have been recalculated on a common basis so that they may be compared.

II. CRITERIA FOR CHANGES INDUCED BY RADIATION

Ideally, radiation changes in pure organic compounds can be determined only by extrapolation to zero radiation time (39, 40), since as soon as a compound is irradiated it will be impure. The effect of these impurities on further irradiation can be potentially very important. Before a labeled compound can be used, it is usually necessary to know its purity both chemically and radiochemically. (A radiochemical impurity may be defined (3) as any radioactive species other than the declared isotope in the stated position(s) of the given compound.) This purity will probably have been determined when the compound was prepared. If it is not used at once, it may be subject to significant self-decomposition.

Bayly and Weigel (3) have noted that self-decomposition of a compound labeled with a radioactive isotope can be caused by one or more of the following effects.

1. *The primary (internal) radiation effect* is the decomposition or transformation of a molecule by the disintegration of one of its unstable atomic nuclei. This daughter atom, for example, N^{14} from decayed C^{14} , may or may not be eliminated from the molecule. In either case, if the daughter nucleus is stable (as is the N^{14}), no radiochemical impurity results unless the molecule contains an additional radioactive atom. This effect is governed by the half-life of the radioactive isotope and cannot be controlled. The proportion of the degradation, which may be attributed to it, is insignificant for long-lived isotopes.

(1) Address to where requests for reprints should be sent.

2. *The primary (external) radiation effect* is the decomposition or transformation of a molecule by interaction with a nuclear particle (for example, a β -particle from a nearby decaying C^{14} nucleus). If the molecule is a labeled one, this decomposition or transformation will contribute to the radiochemical impurity.

3. *The secondary radiation effect* is the decomposition or transformation of a molecule of the labeled compound by reaction with reactive species, such as free radicals, produced as a result of primary radiation decomposition. The reactive species may not necessarily be produced from the labeled compound, but from its environment (*e.g.*, residual water in freeze-dried samples).

4. *The chemical effect* is the decomposition or transformation of the radioactive material by chemical reactions not connected with radiation. This can occur with any chemical compound, but there are several reasons why it may be more probable in the case of a labeled one. First, in synthesizing a compound of high specific activity, emphasis is placed on high yield rather than on chemical purity. The amount and even the exact nature of impurities present may not be known, but they may react with the compound. Second, the small amount of radioactive material which is usually synthesized may be chemically decomposed in unexpected ways, not normally observable with larger quantities. For example, it could be due to the atmosphere above a solid or the non-inertness of the container in which it is stored. However, for C^{14} -labeled carbohydrates stored as thin, freeze-dried films in scrupulously cleaned glass vessels, decomposition by chemical reactions is insignificant (30).

III. DETERMINATION OF RADIOACTIVE DECOMPOSITION PRODUCTS

Generally, the amounts of radioactive decomposition products from self-decomposition are too small to be determined by ordinary analytical methods (19). These labeled impurities may constitute a negligible fraction of the total material present, but as they may contain a large fraction of the total radioactivity, their presence may be critical for a specific radiotracer application (3). A comparable amount of inactive impurity may be unimportant

Nonvolatile radioactive impurities may be estimated by isotope dilution analysis, electrophoresis, or column and paper chromatography followed by autoradiography (4, 19, 25, 30, 38). The autoradiograph will have one spot for each labeled species in the chromatographed material; if only one spot is observed, a high degree of radiopurity is indicated. Volatile products may be collected if they have a basic or acidic group and can be made nonvolatile with, respectively, an appropriate acidic or basic solvent. Wherever possible, when discussing the individual compounds later in this review, summaries are given of the experimental

conditions under which they have been stored and analyzed.

IV. CALCULATIONS

In general, the amount of self-decomposition that a labeled compound undergoes depends on the amount of radiation energy which it absorbs, that is, the *absorbed dose*. In this review, the reported values for the *dose*, as well as *per cent decomposition* and *G value*, have been recalculated on a common basis. Before considering the actual data, the methods used in these calculations will be discussed. One should note, though, that the calculations have little real scientific basis, although the results are usually reported without comment. The absorption of energy is not easily measured accurately, and the actual dose received by small samples of material can be only approximately calculated.

A. ABSORBED DOSE

The first papers reporting self-decomposition (19, 38) gave the dose in terms of *roentgens equivalent physical (rep)* = FNE (1.9×10^{-14} g. rep./e.v.)/wt., where F = fraction of the radiation absorbed (arbitrarily assumed = 1), N = total number of events since time of synthesis, and E = average energy of the radiation.

The value of the constant was derived as follows (10c, 17). By definition (26, 31), a *roentgen* (r.) is that quantity of X- or γ -radiation such that the associated corpuscular emission per 0.001293 g. of air (*i.e.*, 1 cm.³ of dry atmospheric air at NTP: 0° and 76 cm.) produces, in air, ions carrying 1 electrostatic unit (e.s.u.) of quantity of electricity of either sign; or 1 r. produces 1.61×10^{12} ion pairs per gram of air. Assuming that the average energy required to produce an ion pair in air, W , is 32.5 e.v., then 1 roentgen would correspond to an energy absorbed in air of

$$\frac{32.5 \text{ e.v.}}{\text{ion pair}} \times \frac{1 \text{ e.s.u.}}{0.001293 \text{ g.}} \times \frac{1 \text{ ion pair}}{4.8 \times 10^{-10} \text{ e.s.u.}} \times \frac{1.60 \times 10^{-12} \text{ erg}}{\text{e.v.}} = 83.8 \text{ ergs/g.}$$

The roentgen equivalent physical (rep) is that amount of radiation incident on tissue which results in the dissipation of 83.8 ergs/g. of tissue (17). Then

$$(1 \text{ g. rep}/83.8 \text{ ergs})(1.60 \times 10^{-12} \text{ erg/e.v.}) = 1.91 \times 10^{-14} \text{ g. rep/e.v.}$$

Examples of values for W other than 32.5 e.v. given in the literature include: 33 e.v. (10a), "about 33 e.v." (32a), probably "between 33 and 35 e.v." (26), 34 e.v. (26), "about 34 e.v." (10b), and 35 e.v. (29). The International Commission on Radiological Units and Measurements (ICRU) currently (1964) (27) recommends 33.7 e.v. for this value, and it was used for the calculations in this review. It should be noted that

this value W varies from gas to gas and, to a small extent, for different kinds of particles; 33.7 e.v. per ion pair is a weighted mean value derived from absolute measurements for electrons in air, and its use is actually recommended for calculations concerning all X- or γ -radiation with quantum energies greater than 20 kev., the energy range over which W is assumed constant. Then, 1 r. = 86.9 ergs/g., and the value of the constant would be 1.84×10^{-14} g. rep/e.v.

Calculation of the absorbed dose in terms of reps is unsatisfactory for several reasons. As noted, the roentgen is defined in terms of the effect of X- or γ -radiation on air. Also, the rep has been defined in terms of corpuscular radiation (32b) or of any type of radiation (31) incident on tissue; besides 83.8 (or 86.9) ergs/g. derived above, values from 93–97 ergs/g. of soft animal tissue have been used (31, 32b). Furthermore, the results considered here are based on the effects of C¹⁴- β 's on organic compounds.

The current procedure is to report the absorbed dose in terms of the *rad* (26, 27, 35a, 36, 37), a unit adopted in 1953 by the ICRU and defined as 100 ergs/g. $\equiv 6.24 \times 10^{13}$ e.v./g. This value is independent of the type of radiation or the material in which the energy is absorbed.

One may convert from rads to reps, and *vice versa*, as follows: $(6.24 \times 10^{13} \text{ e.v./g. rad})(1.91 \times 10^{-14} \text{ g. rep/e.v.}) = 1.19 \text{ rep/rad}$ or 0.84 rad/rep (for 32.5 e.v./ion pair); for the revised value of 33.7 e.v./ion pair, 1.15 rep/rad or 0.87 rad/rep.

The *absorbed dose* resulting from the inherent radiation of a C¹⁴-labeled compound may be calculated as: (specific activity, c./g.)(time)(dis/c. time)(4.5×10^4 e.v./dis)(1.60×10^{-12} erg/e.v.)(1 rad/100 ergs/g.) or

$$\text{rads} = (\text{c./g.})(\text{time})(\text{dis/c. time})(7.2 \times 10^{-10})$$

The average energy for C¹⁴ used in the literature has generally varied between 45 and 51 kev.² In this review, the value is taken as 45 kev. as determined by Jenks and Sweeton (16). It is the only value reported in the latest (1958) revision of the Table of Isotopes (32e, 34).

The units of the conversion factor for the value of the curie must, of course, be chosen to match the units of the storage time, that is, the time since the compound was synthesized. A convenient table follows.

1 curie =	3.70×10^{10} dis/sec.
	3.20×10^{15} dis/day
	2.24×10^{16} dis/week
	9.73×10^{16} dis/month
	1.17×10^{18} dis/year

(2) Forman and Graham (13), however, used (12) 40.2 kev. derived from Catch's statement (7) that 4.7×10^{17} molecules will be destroyed in a year if the radiation in 1 mc. of a compound of $G(-M) = 1$ is completely absorbed in the sample.

It should be noted that in these equations it was arbitrarily assumed that *all* the energy of the emitted β -particles is absorbed by the labeled compound (*i.e.*, $F = 1$). This assumption was made, also, in calculating the absorbed dose for the compounds listed in the tables. (Actually, however, as decomposition proceeds, the amount of radiation expended in the decomposition products increases, and the equations become less exact.)

The equations also assume that the specific activity remains constant for the time of exposure; that is, the half-life of the radioactive isotope is long compared to the storage time. For a short-lived isotope, *e.g.*, P³², the specific activity will vary, even over a brief time period, and t must be replaced by $(1 - e^{-\lambda t})/\lambda$, where λ is the decay constant (3, 36, 37).

B. PER CENT DECOMPOSITION

The amount of decomposition to be expected for a specific compound whose susceptibility to radiation (G value) is known may be calculated from an exponential equation similar to that derived for the *hit* (or *target*) theory of the interaction of radiation with biological substances (see, for example, ref. 5, 15). This equation is similar in form to the equation for the first-order radioactive decay law. The fraction of the initial compound destroyed at time t is $(1 - e^{-kt})$, where k is the proportionality constant. If this fraction is small (less than about 0.10 or 10%, for one-place accuracy), the value of the exponent will be small, and the equation may be reduced to its linear form in accordance with the expression for development of an exponential series (see, for example, ref. 24). However, if the value of the exponent is calculated to be larger than about 0.10, then the linear form of the equation should *not* be used.

The exponential form of the equation may be expressed as (3)

$$\% \text{ decomposition} = (1 - e^{-F\bar{E}GS_0t}) \times 100$$

where F = fraction of its own radiation energy absorbed by the compound; \bar{E} = mean energy of the radiation, e.v.; G = number of molecules irreversibly damaged per 100 e.v. (*i.e.*, the G value); S_0 = initial specific radioactivity of the compound, curies/mole (especially for macromolecules is it important to express this term on a molar, and not on a unit weight, basis); and t = time. As before, for compounds labeled with a relatively short-lived isotope, t should be replaced by $(1 - e^{-\lambda t})/\lambda$.

If the actual G value for a compound is not known, the approximate amount of decomposition to which, theoretically, it may be subject can be calculated by assuming that an ion pair is formed with an average energy loss of 33.7 e.v. (27). This is equivalent to a G value—the same theoretically for all compounds—of 2.97. If it is also assumed (19, 38, 41) that (i) all of the energy of the emitted β -particle is absorbed by the la-

beled compound ($F = 1$) so that the specific ionization is very high, (ii) all decomposition is caused solely by ionization induced by electron impact, and (iii) ionization always results in permanent molecular decomposition, then the *theoretical per cent decomposition* can be calculated.

For a compound labeled with C^{14} , this amount is usually small enough so that the reduced linear form of the equation may be used. Then

$$\begin{aligned} F\overline{EGS}_0t &= (1)(4.5 \times 10^4 \text{ e.v./dis})(2.97 \text{ molecules/} \\ &100 \text{ e.v.})(S_0, \text{ c./mole})(t)(\text{dis/c. time})(6.02 \times \\ &10^{23} \text{ molecules/mole})^{-1} \times 100 = \\ &S_0t(\text{dis/c. time})(2.22 \times 10^{-19}\%) \end{aligned}$$

Thus, a compound of specific activity 1 mc./mmole would in the course of a year destroy itself, theoretically, at the rate of $1 \times 1 \times 1.17 \times 10^{18} \times 2.22 \times 10^{-19} = 0.26\%$. (Wagner and Guinn (41) obtained 0.30% by using 51 kev. for the average C^{14} β -energy and the then acceptable value of 32.5 e.v./ion pair.)

On this basis, too, the average C^{14} β -particle (45 kev.) destroys about 1335 molecules (45,000/33.7) in addition to the molecule originally containing the disintegrating C^{14} atom (41). Recombination of dissociated radicals or ions to the original molecule will decrease this amount; radical or ionic chain reactions will increase it.

The value of F , the *fraction of its own radiation energy absorbed by the compound*, was arbitrarily assumed in these theoretical calculations to equal 1, but it is not necessarily true. The assumption will usually be valid for solids labeled with C^{14} , S^{35} , or H^3 , if the amount of sample is such that its dimensions are large compared with the radiation path length. For energetic β - and for γ -emitters, such as P^{32} or I^{131} , the assumption is not valid because most of the radiation energy will be absorbed outside the sample (*i.e.*, $F < 1$) (36, 37). This is an important point, as this value influences the radiation dose actually received by the compound and the amount of its decomposition due to primary (external) and secondary radiation effects; as noted before, the actual absorbed dose cannot be accurately determined.

Bayly and Weigel (3) suggest that for a pure β -emitter (as is C^{14}) distributed over a relatively large area in a layer of even thickness, l cm., this fraction $F = (pl/2r)[1.5 + \ln(r/pl)]$, where $p =$ density, mg./cm.³, and $r =$ mean range of the particles, mg./cm.². In this it is assumed that r is not less than pl , and the energy of all particles corresponds to their mean energy. A more exact value may be obtained for definite values of p and l using a known β -spectrum, but the error is generally small.

Several empirical range-energy equations have been derived for calculation of r , the *mean range of the particles*. One of the more widely reproduced (10a, 32c) is that derived by Katz and Penfold (18).

$$r = 412E^{1.265 - 0.0954 \ln E}$$

where $E =$ maximum energy of the particles, Mev., and r is in mg./cm.² Although derived for aluminum absorbers with maximum β -energies between 0.01 and 2.5 Mev. (0.1 to 1200 mg./cm.²), the range in other materials is roughly the same. For the maximum energy C^{14} β -particle of 0.156 Mev., the range is calculated to be 28.3 mg./cm.². For the average energy of 0.045 Mev. used in this paper, $r = 3.26$ mg./cm.². These values compare favorably with 29 and 3.2 mg./cm.², respectively, obtained from the well-known Glendenin (14, 32d) curve.

For the freeze-dried sucrose and glucose samples cited in (3), the fraction of the radiant energy absorbed, F , equals 0.20 and 0.13, respectively. (The authors' values of 0.16 and 0.10 were apparently obtained by use of a different value for the average energy.) Tolbert in his recent papers (36, 37) appears to be using a value of 0.93 for this fraction, and he gives a calculated table of *per cent decomposition* for a theoretical organic compound of molecular weight 125 as a function of radiation dose and $G(-M)$ value.

Rads	$G(-M) =$ 5, %	$G(-M) =$ 10, %	$G(-M) =$ 20, %
10^6	0.06	0.12	0.24
10^7	0.59	1.19	2.38
10^8	5.8	11.3	21.4
10^9	45.0	70.2	90.9

The values in the table cannot be derived from the linear form of the decomposition equation. But, if the exponential equation is used, with a value for F of 0.93, one can obtain the values given. Thus, for 10^9 rads and $G(-M) = 20$, decomposition = $100[1 - \exp(0.93 \times 2.59)] = 100[1 - \exp(2.40)] = 90.9\%$; if F were set equal to 1, the result would be 92.5%, and if the linear form of the equation were also used, 259%!

To date, only two groups of authors (3, 30) have reported attempts to estimate values of F for actual experimental samples. In neither instance is anything more than a working approximation claimed for the results.

C. G VALUES

An alternative and more easily comparable method of reporting the amount of decomposition, although it has no theoretical significance, is in terms of the *energy yield* or *G value* (8, 35, 39). This is the *number of molecules changed* (or *permanently altered, i.e., formed or destroyed*) per 100 e.v. of energy absorbed by the system. The G value takes into account the dose and the amount of decomposition and, thus, permits direct comparisons to be made of radiation effects on different compounds or different samples of the same compound. The accuracy of reported G values can vary widely (40), one reason being that the dose actually received by a com-

pound cannot always be determined accurately. Doubt about the value of F is one reason for this.

Another reason is that the calculation assumes the per cent decomposition to be directly proportional to the absorbed dose; that is, the radiation energy is distributed equally over all substances, whether the undecomposed original molecule or a decomposition product. Actually, as the dose increases, the amount of decomposition varies from linearity. As impurities build up, they absorb more and more of the radiation, and the decomposition rate (as far as primary decomposition is concerned) decreases.

The G value may be calculated as: $(6.02 \times 10^{23}$ molecules/mole) (mol. wt., g./mole)⁻¹ (weight % compound destroyed or formed/100) (dose, rads)⁻¹ (1 g. rad/6.24 \times 10¹³ e.v.) (G /molecules/100 e.v.); or

$$G = \frac{\text{wt. \% compd. destroyed or formed}}{\text{mol. wt.} \times \text{rads}} (9.65 \times 10^9)$$

If the value inserted in the numerator is that for *per cent* of the compound *destroyed*, the G value is designated as $G(-M)$.

If the value used refers to *per cent* of a compound among the products *formed* in the decomposition process, the name of the compound or its chemical symbol is used in the parentheses, *e.g.*, $G(\text{H}_2)$, $G(\text{C}_1\text{-C}_4 \text{ hydrocarbons})$. Note that the value for molecular weight in the denominator is that for the initial radioactive compound, not for the product.

However, Forman and Graham (13) calculated their $G(-M)$ values by using (12) the mole % of the product actually present in the sample, rather than the weight % of the original compound destroyed.

V. RESULTS

The tables include all compounds for which sufficient self-decomposition data for computation have been found in the literature. Notes about additional compounds are included in the text. Data have been recalculated on a common basis so that they may be compared. The absorbed dose, in rads, was calculated on the assumption that *all* of the radiation energy was absorbed by the compound. The average C¹⁴ β -energy was taken as 45 kev.

Prior to the appearance in 1953 of the first papers to report on the self-decomposition of C¹⁴-labeled compounds (19, 33, 38, 41), it was believed (19) there would be little such decomposition because of the low average energy of the C¹⁴- β 's. The results, however, showed decomposition to have occurred in many cases to a much greater extent than had been anticipated. Thus, although eight of the fifteen biologically active compounds reported in (38) were observed to have decomposed less than 1%, decomposition among the other seven compounds varied between 1 and 63%.

When originally prepared, the radioactive impurities in these compounds were estimated by paper chromatography and autoradiography to vary between 0 and <1%. After storage as dry solids at room temperature in the dark for the times noted (Table I), the extent of radiation decomposition was estimated by the same techniques. The labeled choline chloride was stored in an evacuated sealed tube; none of the other compounds was stored in sealed tubes so that any volatile decomposition products would not have been detected unless the activity therein was at least 10% of that originally present.

A. CHOLINE METHYL-C¹⁴ CHLORIDE (TABLES I AND II)

This compound is abnormally sensitive to radiation, even when stored in the dark *in vacuo*. Exposure of the compound to different forms of radiation has confirmed this sensitivity (19, 22). The main products are radioactive trimethylamine and inactive acetaldehyde (38, 40), which result from the breaking of the bond between the nitrogen and the carbon atoms of the ethanol group (19). Very small amounts of noncondensable gaseous products have also been obtained (40). The amount of decomposition in ref. 21 was determined by Reinecke salt precipitation. The amount of trimethylamine was determined by paper chromatography followed by autoradiography.

The decomposition was first thought to proceed through a free-radical chain mechanism (20, 35c, 39), but this belief has been abandoned (23). However, the radiation damage can be deferred indefinitely at liquid nitrogen temperatures.

B. CHOLESTEROL (TABLES I AND III)

The unexpectedly large amount of decomposition (~40%) of cholesterol-4-C¹⁴ reported (38) led to a subsequent detailed study (9) to identify the products formed and to evaluate the role of radiation in the decomposition process. Highly purified samples of cholesterol, labeled in the 4-, 24-, or 26-positions, as well as the unlabeled compound, were stored in air and *in vacuo*, in the dark, for various lengths of time. The products were determined by infrared after separation by isotope dilution with fractional elution from chromatographic columns.

The data are summarized in Table III. The results show that when C¹⁴-labeled cholesterol is stored in the presence of oxygen-containing compounds such as air and water, it is very susceptible to radiation and oxidizes on and about the 5,6-double bond. The main products are the epimeric 7-hydroxy, the 7-keto, and the 5 α ,6 β -dihydroxy derivatives. The position of the label made no noticeable difference in the composition of the decomposition products. Both radiation and oxygen are required, as unlabeled cholesterol in air or C¹⁴-labeled cholesterol *in vacuo* is stable. Considering

TABLE I
 SELF-DECOMPOSITION DATA FOR BIOLOGICALLY ACTIVE COMPOUNDS

Compound	Formula weight	Storage time, months	Sp. act., mc./g.	Dose, ^a rads × 10 ⁻⁶	Decomposition, %		G(-M)	Number of nonvolatile radioactive products observed on paper chromatograms	Ref.
					Obsd.	Calcd. ^{a,b}			
Choline methyl-C ₁ ¹⁴ chloride ^c	139.6	9	13.0	8.2	63	0.35	531	0	19, 21, 25, 38-40
Cholesterol-4-C ¹⁴	386.6	18	6.5	8.2	~40	0.97	~122	0	9, 38
Calcium glycolate-2-C ¹⁴	190.2	48	4.0	13.5	22 ^d	0.79	83	1	19, 25, 38, 40
Calcium glycolate-1-C ¹⁴	190.2	48	5.8	19.5	13 ^d	1.14	34	1	19, 25, 38, 40
Norvaline-3-C ¹⁴ ·HCl	153.6	25	17.7	31.0	5	1.47	10	6	19, 25, 38, 40
Norleucine-2-C ¹⁴	131.2	11	17.4	13.4	2	0.54	11	4	19, 25, 38, 40
Valine-4,4'-C ₂ ¹⁴ ·HCl	153.6	15	6.0	6.3	1-2	0.30	10-20	1	19, 25, 38, 40
Stillbamidine diisethionate amidine-C ₂ ¹⁴		24	13.7	23.0	<1				38
8-Azaadenine-4,6-C ₂ ¹⁴		35	4.4	10.8	<1				38
Succinic acid-2-C ¹⁴	118.1	38	3.9	10.4	<1	0.38	<8		38
8-Azaguanine-4-C ¹⁴		35	3.8	9.3	<1				38
Adenine sulfate-4,6-C ₂ ¹⁴	368.3	35	2.9	7.1	<1	0.81	<4		38
Guanine hydrochloride-4-C ¹⁴	187.6	35	2.6	6.4	<1	0.37	<9		38
Glycine-2-C ¹⁴ ·HCl	111.5	14	4.9	4.8	<1	0.17	<18		38
Thyroxine-α-C ¹⁴	776.9	33	1.0	2.3	<1	0.55	<6		38

^a Calculated for total energy absorption ($F = 1$); $\bar{E} = 45$ kev. ^b Calculated using 33.7 e.v./ion pair. ^c Stored *in vacuo*; other compounds stored in air. ^d These percentages include radioactive formate.

the extent of this radiation-induced oxidation and the amount of radiation available from the C¹⁴ present, the reaction is concluded to be of the chain type.

C. CALCIUM GLYCOLATE (TABLE I)

Theoretically, the amount of radiation damage should not depend on the position of the labeled carbon (19), and the G values should be equal. The difference in the G values in (38) may be due to water of crystallization or to the production of volatile radioactive decomposition products that were not detected, as the compounds were stored in air.

The following values (recalculated using 45 kev.) were given in ref. 40, but there were no data about the storage conditions: calcium glycolate-1-C¹⁴, $G(-M) = 184$, $G((COOH)_2) = 68$, $G(HCOOH) = 117$; calcium glycolate-2-C¹⁴, $G(-M) = 196$, $G((COOH)_2) = 62$, $G(HCOOH) = 133$.

D. GLUCOSE-C¹⁴

The decomposition of three preparations of uniformly labeled glucose-C¹⁴ (prepared by photosynthesis using Canna leaves and purified by paper chromatography) was studied (2) under conditions of frozen storage, dry storage, and autoclaving. Glucose-C¹⁴ of high specific activity decomposed extensively during storage in the dry state and during autoclaving except, when during autoclaving, either 0.5 N HCl or sufficient carrier glucose was present. Destruction seemed to be dependent, in part at least, on the presence of an unidentified, nonradioactive, anionic contaminant, at least part of which may be removed by a single pass over a Duolite A-4 anion-exchange column.

E. D-GLUCOSE-C¹⁴ AND SUCROSE-C¹⁴ (TABLE II)

D-Glucose-C¹⁴, uniformly labeled, was prepared by photosynthesis and purified by chromatography and crystallization, after which no radioactive impurities could be detected (4). A freeze-dried sample (481 μ c. in *ca.* 6 mg.) was stored in a vacuum-sealed tube in the dark for 26 months.

Two-dimensional paper chromatography-paper electrophoresis, with subsequent autoradiography, revealed the presence of 37 components. Nine fractions could be identified, including D-glucose; D-arabinose; D-erythrose; D-glycerose; D-glucuronic acid; D-arabonolactone; aldohexonic acids and their keto derivatives, lactones of these; and aldopentonic acids and their keto derivatives. Carrier-dilution techniques were also used to identify some of the self-decomposition products. Others were not determined because of the small amount of the original sample.

Freeze-dried glucose samples are known to contain an appreciable quantity of nonbonded water (4), with which β -particles from a C¹⁴-labeled compound could interact to produce hydroxyl radicals. These, on reaction with more of the labeled compound, would enhance the degree of decomposition. The validity of the theory was tested by comparison with the oxidation of D-glucose using Fenton's reagent, which is known to generate hydroxyl radicals in solution according to the equation: $H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + OH\cdot$. To determine some of the products from chemical oxidation, freshly purified D-glucose-C¹⁴ was treated with Fenton's reagent, and the products were analyzed by carrier-dilution techniques. The results are given in Table IV.

TABLE II
 SELF-DECOMPOSITION DATA FOR VARIOUS COMPOUNDS

Compound	Formula weight	Storage time, months	Sp. act., mc./g.	Dose, ^a rads × 10 ⁻⁶	Decomposition, %		G(-M)	Ref.
					Obsd.	Calcd. ^{a, b}		
Acetylcholine chloride	181.7		117	8.16	0.86	0.46	5.5 ^f	21, 25, 40
Choline methyl-C ₁ ¹⁴ chloride	139.6	9	13.0	8.2	63 ^c	0.35	531	19, 21, 25, 38-40
	139.6	46	0.6	1.9	54.1 ^c	0.08	1930	19, 21, 25, 38-40
	139.6	1.51	10.7	1.1	{ 27.2 ^c 0 ^d }	0.05	{ 1660 ^c 0 ^d }	19, 21, 25, 38-40
D-Glucose-C ¹⁴	180.2	26	80.2	146	20.05	7.80	7.4	3, 4, 30
	180.2	7.82	233	127	0.7-18.9	6.85	0.3-7.9	3, 4, 30
Sucrose-C ¹⁴	342.3	20.24	431	611	1.8-16.4	47.5	0.1-0.8	3, 4, 30
D-Fructose-C ¹⁴	180.2	33	44.6	103	9.33	5.57	4.8	30
D-Mannose-C ¹⁴	180.2	29	111	226	18.54	11.8	4.4	30
D-Ribose-C ¹⁴	150.1	15	38.5	40.4	8.0	1.85	12.7	30
Methanol-1-C ¹⁴	32.0	20.71	281	408	19	4.74	14.0	19, 25, 33, 35b, 39, 40
Methyl-C ¹⁴ iodide	142.0	42	0.845	2.5	0.135	0.11	3.7	19, 39-41
Thioctic acid (in <i>n</i> -hexane-1-C ¹⁴)	206.3	1.41	90 ^e	8.9	6.6	0.57	34.6	1, 35e, 39

^a Calculated for total energy absorption ($F = 1$); $\bar{E} = 45$ kev. ^b Calculated using 33.7 e.v./ion pair. ^c Stored at room temperature. ^d Stored at liquid nitrogen temperature. ^e mc./g. of thioctic acid. ^f $G(\text{Me}_3\text{N}) = 4.4$.

 TABLE III
 SELF-DECOMPOSITION DATA FOR CHOLESTEROL

Compound	Storage	Storage time, months	Sp. act., mc./g.	Dose, ^a rads × 10 ⁻⁶	Decomposition, %		G(-M)	Ref.
					Obsd.	Calcd. ^{a, b}		
Cholesterol (unlabeled)	a. <i>In vacuo</i> , in dark	12	0	0	0	0	...	9
	b. SCV ^c in dark	12	0	0	0	0	...	9
	c. Open to lab. atm., in dark	18	0	0	0	0	...	9
Cholesterol-26-C ¹⁴	SCV ^c	24	1.53	2.6	33-43	0.31	321-418	9, 25, 35d
Cholesterol-24-C ¹⁴	a. Freshly prepared	0	0.18	0	0	0	...	9
	b. <i>In vacuo</i> , in dark	15	0.18	0.19	7	0.023	919	9, 25
	c. 1 month SCV ^c + 14 months under reduced pressure, in dark		0.18	0.01	13	0.0015		
	d. SCV ^c	15	0.18	0.19	17	0.023	2230	9
Cholesterol-4-C ¹⁴	a. SCV ^c	3	3.83	0.80	8	0.096	250	9
	b. <i>In vacuo</i> , in dark	2.3	3.83	0.62	0	0.074	0	9, 25
Cholesterol-4-C ¹⁴	SCV ^c	18	6.5	8.2	~40	0.98	~122	9, 25, 38

^a Calculated for total energy absorption ($F = 1$); $\bar{E} = 45$ kev. ^b Calculated using 33.7 e.v./ion pair. ^c Sample stored in screw cap vial, in the presence of air.

 TABLE IV
 PRODUCTS OF SELF-DECOMPOSITION OF D-GLUCOSE-C¹⁴ AND OF OXIDATION OF D-GLUCOSE-C¹⁴ BY FENTON'S REAGENT

	Yield on self-decompn. for 26 months, %		Yield on oxidation by Fenton's reagent, %
	P.c.-p.e. ^a	Carrier-diln.	
D-Glucose	86.4	79.95	40.12
D-Arabinose	0.44	0.43	0.49
D-Erythrose	0.59		
D-Glycerose	0.12		
D-Gluconic acid		0.62	11.68
2-Keto-D-gluconic acid		0.38	2.32
D-Glucurone		0.10 max.	
D-Arabonic acid		0.07	1.11
Oxalic acid		0.0005 max.	1.02
D-Glucosone	Absent	Absent	12.6 ^b
Formaldehyde			0.02
Carbon dioxide			0.49

^a Two-dimensional paper chromatography-paper electrophoresis. ^b Total osones calculated as D-glucosone.

The similarity between the products of the chemical and radiation-induced reactions suggests that they arise by similar routes, presumably involving participation of hydroxyl radicals. Abstraction of hydrogen by these radicals would yield polymers, keto groups, and carboxyl groups, thus producing gluconic acid and keto-gluconic acids. In conjunction with C-C bond fission, lower aldoses and their acids and keto derivatives would result.

An immobile nonradioactive component was also present on the paper chromatogram of the self-decomposition products; this, the authors suggest, was a non-glycosidic acidic polymer (4).

Bayly and Weigel (3) studied the self-decomposition of D-glucose-C¹⁴ and sucrose-C¹⁴. Both compounds, of high specific activity and uniformly labeled, were prepared by photosynthesis, purified by ion exchange and paper chromatography, and then crystallized. The radiochemical purities were 99.9%. Samples, dis-

pensed from aqueous solutions, were stored in freeze-dried form or uniformly distributed on Whatman No. 3 paper in specially cleaned Pyrex tubes, *in vacuo* or in air, at room temperature and at -80° . Radiochemical impurities were determined by scanning of paper chromatograms.

Glucose and fructose were the principal impurities obtained from sucrose. The amounts of decomposition observed for some of the samples of both sugars were apparently due to secondary radiation effects, probably caused by the presence of moisture. However, self-decomposition of vacuum-stored, freeze-dried sucrose, which can be obtained anhydrous, appears to be due largely to primary radiation effects.

F. D-FRUCTOSE-C¹⁴ (TABLE II)

The compound, uniformly labeled, was prepared by photosynthesis in the presence of C¹⁴O₂ (30). Freeze-dried samples (50 μ c./1.12 mg.), initially free from impurities, were stored 33 months *in vacuo* at room temperature. Two-dimensional paper chromatography revealed the presence of at least 16 components and an immobile, probably polymeric, constituent on the starting line. Isotope dilution analysis identified the following products: D-fructose (90.67%), glucosone (0.29%), two-carbon aldehydic fragments (0.12%), three-carbon aldehydic fragments (0.33%), glycollaldehyde (0.10%), glycollic acid (0.15%), and oxalic acid (0.04%).

Total self-decomposition was determined to be 7.3% by two-dimensional paper chromatography and 9.33% by isotope dilution.

G. D-RIBOSE-C¹⁴ (TABLE II)

The compound, uniformly labeled, was obtained by a several-step process from the D-fructose-C¹⁴, above (30). Freeze-dried samples (50 μ c./1.3 mg.), initially free from impurities, were stored 15 months *in vacuo* at room temperature. Two-dimensional paper chromatography revealed 15 components: 11 neutral, containing 97.61% of the total radioactivity including D-ribose, 93.40%, and four acidic, 2.17%; and an immobile, probably polymeric, material (0.22%).

Isotope dilution analysis identified the following major products: D-ribose (92.03%), D-ribonic acid (0.75%), D-erythrose (0.49%), D-erythronic acid (0.29%), two-carbon aldehydic fragments (0.10% av.), three-carbon aldehydic fragments (0.15%), glycollic acid (0.29% av.), and oxalic acid (0.09%).

Total self-decomposition of the D-ribose-C¹⁴ was indicated to be 6.6% by two-dimensional paper chromatography and 8.0% by isotope dilution.

H. D-MANNOSE-C¹⁴ (TABLE II)

The compound, uniformly labeled, was prepared by photosynthesis with sodium hydrogen carbonate-C¹⁴ as the sole source of carbon (30). The final product

was isolated by paper chromatography. Freeze-dried samples (100 μ c./0.9 mg.), initially free from impurities, were stored 29 months *in vacuo* at room temperature.

Two-dimensional paper chromatography revealed 22 components: 10 acidic, containing 4.62% of the total radioactivity, and 12 neutral, 95.01%, including D-mannose, 85.65%; and an immobile, probably polymeric, material (0.37%).

Isotope dilution analysis identified the following major products: D-mannose (81.46%), D-arabinose (0.50%), D-lyxose (0.34%), D-erythrose (0.18%), D-erythronic acid (0.12%), D-arabonic acid (0.26%), two-carbon aldehydic fragments (0.29%), three-carbon aldehydic fragments (0.34%), glycollic acid (0.55%), and oxalic acid (0.06%). Other decomposition products included 2-oxogluconic acid, four-carbon aldehydic fragments, and two lactones of mannonic acid.

Total self-decomposition was indicated to be 14.35% by two-dimensional paper chromatography and 18.54% by isotope dilution.

The rate of degradation was determined, in a separate set of experiments, to be about 1% per year for samples frozen and stored at -5° and about 7% for samples freeze-dried and sealed in evacuated tubes.

The nature of the products indicates certain similarities between self-decomposition of D-mannose-C¹⁴ and the degradation resulting from γ -irradiation of mannose solutions *in vacuo*. However, there are a large number of additional self-decomposition products present in amounts less than 1% which are not present in the irradiated solution. The self-decomposition products for D-fructose-C¹⁴ and D-ribose-C¹⁴ may also be identified in some instances with the products formed during irradiation of aqueous solutions.

Decomposition of these C¹⁴-labeled sugars (30) may result either from direct degradation due to bombardment by their own β -rays (that is, the primary (external) radiation effect) or from possible secondary radiation effects due to the action of hydrogen atoms and hydroxyl radicals formed by interaction of the β -rays with nonbonded water retained by the freeze-dried samples (as, for example, in the case of the glucose samples (4) discussed previously).

The natures of the products do not permit a clear choice to be made between the two possible degradation mechanisms. Proximate calculations based on the fraction of the radiation energy absorbed by the compounds, that is, the value of F , as derived in (3), and comparisons with the results obtained in (3) for similar freeze-dried samples indicate that the major portion of the decomposition is due to reactive species formed by radiolysis of nonbonded water retained by the samples.

Self-absorption of the emitted β -rays and failure to isolate the initial C¹⁴-carbohydrate discretely from the decomposition products may account for the fact that paper chromatogram values for the amount of decom-

TABLE V
 GAS CHROMATOGRAPHIC ANALYSIS OF THIODAN^a SAMPLES (13)

Sample no.	Storage time, months	Sp. act., mc./g.	Dose, ^b rads × 10 ⁻⁴	Decomposition, %		G(-M)
				Obsd. ^d	Calcd. ^{b,c}	
1, 3, 4, 5	7.36-59.1	Stable	...	1-7
2A ^e	28.3	Stable	...	19 ^e
2B ^e	46.7	Stable	...	2
6A ^f	29.7	5.91	12.3	51	1.54	98.2
6A ^f	48.8	5.91	20.2	77	2.54	90.2
6B ^{f,g}	45.8	5.99	19.2	100 ^h	2.41	123
7 ^f	29.7	1.9	3.96	18	0.50	108
8A ^f	27.4	0.95	1.83	26	0.23	338
8A ^f	45.3	0.95	3.02	48	0.38	377
8B ^{f,i}	0.23	0.95	0.015	12	0.002	18,600
8B ^{f,i}	18.2	0.95	1.21	16	0.15	313

^a C₉H₉Cl₄O₂S; formula weight = 407.0. ^b Calculated for total energy absorption ($F = 1$); $\bar{E} = 45$ kev. ^c Calculated using 33.7 e.v./ion pair. ^d Total Thiodan ether + Thiodan alcohol found. ^e Samples 2A and 2B were portions of the same commercial preparation. 2A was kept in a tightly sealed drum; products included Thiodan alcohol, 17%. ^f Samples 6A, 7, and 8A were solid lumps of 2.3, 14, and 31 g., respectively. Samples 6B and 8B were loose crystals, 0.6 and 0.13 g., respectively. ^g 6B is a sample of 6A recrystallized from hexane shortly after preparation. ^h 60% Thiodan alcohol + 40% Thiodan ether. ⁱ 8B is a column chromatographed sample of 8A. The age of 8B is taken from the time of the chromatogram.

position were consistently lower than the more reliable values obtained by isotope dilution techniques.

The calculations in Table II were made on the basis of total absorption of the C¹⁴-radiation. If values of F adapted from those calculated in ref. 3 and 30 were used, the values for the dose, $G(-M)$, and the theoretical per cent decomposition would, of course, be quite different.

I. DEXTRAN-C¹⁴ SULFATE

The compound (*ca.* 3 mc./mmole; 22.4 mc./g. atom of C) containing about 20 glucose units per molecule was prepared by sulfation of an appropriate C¹⁴-dextran hydrolysate (3). After storage for 3 weeks in the freeze-dried form, it was charred and became a total loss; the inactive compound did not decompose during storage for several months. The decomposition was presumably due to secondary radiation effects resulting from the prior liberation of sulfuric acid, whose concentration was increased, rather than decreased in the process, thus destroying the rest of the material.

J. METHANOL-1-C¹⁴ (TABLE II)

The labeled compound was prepared (33) with a specific activity of 0.250-0.312 c./g. (8-10 c./mole). Samples averaging 101.8 mc. were sealed *in vacuo* in each of six ampoules and stored. When opened, the ampoules contained several atmospheres of gas which was noncondensable at liquid nitrogen temperatures; an average of 0.90 mmole of gas was produced in each ampoule: $G(\text{gas produced}) = 5.86$. Small amounts of a less volatile radioactive product were also obtained. This, on analysis using isotope dilution techniques, was found to consist of ethylene glycol, glycerol, and erythritol in the following proportions, 1360:14.9:1.00. The gas in one of the ampoules, analyzed by infrared and mass spectrometry, contained 98% H₂ and 2% CH₄: $G(\text{H}_2) = 5.7$, $G(\text{CH}_4) = 0.1$.

K. METHYL-C¹⁴ IODIDE (TABLE II)

Samples sealed *in vacuo* and stored 42 months in the dark at room temperature became wine red in color due to liberated iodine (19, 41). Samples of methyl-C¹⁴ iodide, treated similarly, remained colorless. Spectrophotometric techniques were used to determine the amount of decomposition (0.135%). This was reasonably close to the theoretical amount (0.11%) calculated on the basis of no chain reactions.

L. THIOCTIC ACID (TABLE II)

The unlabeled compound was subjected to C¹⁴ β -radiation by dissolving 2.4 mg. in *n*-hexane-1-C¹⁴ (2.4 ml., 90 $\mu\text{c./ml.}$) in a tightly stoppered Beckman spectrophotometer cell (air volume above solution = 0.6 ml.). Absorption at 333 μm was determined every few days for 43 days; the optical density decreased linearly with time. (An inactive sample treated similarly showed no change.) On evaporation of the radioactive hexane, only traces of activity remained on the thioctic acid residue (1). This would indicate that the process of decomposition is one of energy transfer, rather than of reaction of an activated hexane with the thioctic acid molecule.

M. THIODAN-5a,9a-C₂¹⁴ (TABLE V)

Infrared spectra of the C¹⁴-labeled insecticide Thiodan taken shortly after preparation showed no Thiodan ether (13). Spectra taken after sample storage for about 2.5 years in vials in a desiccator in the dark did show the presence of the ether. Gas-liquid chromatography was used to separate the products from a number of nonradioactive technical samples and radioactive samples of Thiodan. The former contained hardly any Thiodan ether, even after prolonged storage. The amount of Thiodan ether found in the radioactive

TABLE VI
 DECOMPOSITION OF FREEZE-DRIED THYMIDINE-2-C¹⁴ (11)

Storage time, months	Sp. act.		Weight of thymidine, mg.	Activity in ampoule, mc.	Storage temp., °C.	Decomposition, %		Dose, ^b rads × 10 ⁻⁴	G(-M)
	mc./mmole	mc./g.				Obsd.	Calcd. ^{b,c}		
33	18.3	75.5	2.4	0.1	-40	2	12.2	175	0.46
33	18.3	75.5	0.24	0.01	-40	2	12.2	175	0.46
25	16.0	66.1	0.15	0.01	0	0	8.28	116	0

^a C₁₀H₁₄N₂O₅; formula weight = 242.2. ^b Calculated for total energy absorption ($F = 1$); $\bar{E} = 45$ kev. ^c Calculated using 33.7 e.v./ion pair.

samples increased with time. When access to moisture was permitted, both types of sample decomposed to Thiodan alcohol. Infrared analysis indicated that the ratio of higher melting to lower melting Thiodan isomers in the radioactive samples had increased from the ratio present shortly after preparation.

The authors suggest that the radiolysis probably occurs by means of free radicals which attack the sulfur atoms and cause rupture of a C-O bond. The resultant radical can form Thiodan ether with expulsion of another radical which decomposes to SO₂ and the original radical. A similar mechanism could result if the radical attacked an oxygen atom.

N. THYMIDINE-2-C¹⁴ (TABLE VI)

The compound was prepared by enzymatic coupling of the correspondingly labeled pyrimidine base, thymine-2-C¹⁴, with 2-deoxy-D-ribose (11). The self-decomposition rate was less than 1% per year and was the same whether the compound was stored on paper, in aqueous solution, or as a freeze-dried solid at +2 or -40°. No thymine-2-C¹⁴ could be detected in these samples. The small amount (1-2%/year) formed on keeping the aqueous solution of the thymidine-2-C¹⁴ at room temperature was probably produced by slow hydrolysis of the thymidine rather than by radiolytic fission of the N-glycoside bond.

For comparison, a sample was kept in tap water in the laboratory exposed to air and occasional sunlight. After 5 months, analysis showed only 8% decomposition; 5% thymine-2-C¹⁴ was found. This remarkable stability contrasts markedly with that of the tritiated compound of comparable specific activity which decomposed to a very much greater extent: 30-50% compared with 0-2% for the C¹⁴-labeled compound stored in the freeze-dried state.

Thymidine is relatively stable chemically, and the C¹⁴-labeled compound can be sterilized by heating in neutral or alkaline solution up to 120° for at least 1 hr. without decomposition.

VI. REDUCTION OF THE AMOUNT OF SELF-DECOMPOSITION

Self-decomposition due to primary (internal) radiation cannot, of course, be controlled. However, that due to other types of decomposition can be reduced or minimized (3, 7, 19, 25, 30, 36, 37, 41). The following

are a number of procedures which have been recommended.

(1) Disperse as a thin layer or film over a large area. As the fraction of its own radiation absorbed by the compound depends on the thickness and density of the sample, dispersion will permit the nuclear particles to escape easily from the body of the material. The compound can be absorbed in a very thin, or even a monomolecular, layer on a solid matrix which in turn will absorb most of the radiation. Suggested for this purpose are paper (as in a paper chromatogram), ion-exchange resins, and clays or Zeolites.

(2) Dilute to interpose inactive molecules into the path of the radiation. (a) Add the inactive form of the radioactive compound (*i.e.*, self-dilution with carrier). This avoids the necessity for subsequent separation, but it reduces the specific activity. However, by use of low-level counters, it is often possible to keep the specific activity below the level where self-decomposition is appreciable.

(b) Add other substances (solid dilution). These will absorb radiation which might produce radioactive impurities. In certain instances, they may terminate chain reactions which would otherwise result in substantial decomposition after the radiolysis of a few radioactive molecules. Labeled solids may be stored as intimate mixtures with a large amount of an inert, finely divided substance such as powdered glass. However, the labeled compound must be very finely powdered so that the nuclear particle can escape. Thus, the maximum range of C¹⁴-β's (0.156 Mev.) is about 28 mg./cm.², and the range of the average particle (0.045 Mev.) is about 3.3 mg./cm.². In a compound such as β-HMX, with a density of 1.9 g./cm.³, the average C¹⁴ β-particle has a range of about 17 μ, and the average particle size would have to be less than this for the method to be effective.

If the foreign substance is unwisely chosen, it may react with the radiation and produce reactive species which will interact with the labeled compound, doing more harm than good. In this connection, it is also important to consider the inertness of the storage containers. The alkalinity of normally washed Pyrex glass is detrimental to the stability of C¹⁴-labeled carbohydrate sirups (3).

Also, the radioactive compound can be dissolved in a protecting medium (solution dilution) so that to a large

degree radiolysis of the solvent instead of the labeled compound will occur (6). Benzene or some other aromatic compound can be used, as can an unreactive ester, aldehyde, acetone, or other double bond containing molecule. In general, water is unsatisfactory because radiation produces long-lived reactive intermediates such as H₂O₂ or HO₂, which will readily attack the dissolved compound.

(3) Convert the radiation sensitive compound to a derivative more stable than itself. Iodides, benzoates, phenolates, and acetates have been suggested.

(4) Low-temperature storage will decrease self-decomposition, as $G(-M)$ values usually decrease with temperature. The mobility of secondary hydrogen and hydroxyl radicals may be reduced in frozen solution (30).

(5) Storage *in vacuo*, as oxygen almost always increases $G(-M)$ values.

(6) Purify the compound as much as possible. This is especially useful for controlling chemical effects.

One may note, in conclusion, that users of labeled organic compounds should check the purity of the compounds before using them, especially if they are of high specific activity, and/or they have been stored for some time. If C¹⁴-carbohydrates of high specific activity must be stored for long periods, then storage in dilute frozen solution, followed by recovery of the sample by freeze-drying, is eminently suitable (30).

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