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REVIEW

Storage and Stability of Compounds Labelled with Radioisotopes — Part II

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SUMMARY

A further review is presented which summarises and comments on recent progress concerning decomposition and control of the self-radiolysis of compounds labelled with radioisotopes. Organic compounds labelled with the radioisotopes carbon-14, hydrogen-3 (tritium), sulphur-35, and phosphorus-32 are dealt with principally, together with some problems concerning their analysis and the effect on their use of the presence of impurities. Tables for the self-radiolysis rates of a number of groups of compounds are included.

INTRODUCTION.

Observations and improvements concerning the storage and the stability of compounds labelled with radioisotopes are continually being assessed. There are currently about 1,500 radiochemicals labelled with the isotopes carbon-14 and tritium being used in research. It is imperative that information derived from the use of these compounds should be valid and not misleading due to the use of impure radiochemicals. Both users and suppliers of radiochemicals need to be aware of the problems involved in order to minimise not only the rate of self-radiolysis, but the chance of drawing the wrong conclusions from experimental data.

Early last year we reviewed [1] and summarised existing knowledge concerning the decomposition of labelled compounds by self-irradiation. Because of the great interest and importance of this subject we have written this publication to keep the research worker informed of recent advances, pitfalls and developments, particularly those concerning methods for controlling decomposition by self-radiolysis. The opportunity has been taken to present further data on the self-decomposition of particular compounds, which, though they are often empirical observations, may give some guidance to research workers as to the rates of decomposition to expect.

Readers are referred to our previous review [1] for a general introduction to the problem, for methods of calculating G(-M) values, and for other basic information. Two points which were made there might bear repetition. The first is that much of the data we reported there, and of that which we give in this paper, is taken from analyses of single batches. This means that in addition to the usual risk of errors and inaccuracies in analysis we are liable to a batch to batch variation which can be considerable, especially when the bulk of the self-decomposition is secondary in nature, as such decomposition is notoriously dependent [2] on even small amounts of impurities, which, if they are inactive, are very difficult to detect. It will be noted that in many of the tables we have calculated a G(-M) value. It might seem this is almost a misuse of this parameter, as apart from the potential inaccuracies already referred to, there are other more fundamental errors. For simplicity in this paper we have assumed complete absorption by the system of the radiation energy and the same assumption was made in many cases in our previous review [1]. However, for freeze-dried solids this assumption is often very erroneous, and indeed use can be, and is, made of this discrepancy in practice. Also one implies in calculating a G(-M) value that decomposition is entirely due to primary or secondary radiation effects. But we know this is certainly not the case, and this source of error is most clearly seen in comparing two batches of a compound with very different specific activities. Particularly if the compound is relatively unstable in its inactive form it will often be found that the "G(-M) value" for the higher specific activity batch will be much lower than that for the lower specific activity batch. It should also be remembered that in storing a high specific activity compound at a similar radioactive concentration as a low specific activity one, the two solutions will have very different chemical concentrations and this will also affect the G(-M) values. In spite of these several severe limitations we have continued to calculate these G(-M) values as giving some means of comparing compounds and different storage conditions, but we trust readers will bear these limitations in mind when using the tables.

The second point which we would stress is the importance of the participation of the labelled compound user in ensuring the purity of the compounds he uses is adequate for his purpose. Whenever possible the user should consider in advance what his purity requirements are. What particular impurities will be likely to be troublesome, and what are the maximum permissible levels of these compounds? What is the minimum acceptable purity for the labelled compound? In cases where he has special requirements he will then need to ensure these are met, usually by conducting his own analyses. Indeed we feel some form of simple control analysis has a lot to commend it, if only because it provides a safeguard against unexpectedly large amounts of decomposition.

The present review is divided into the following sections :

(1) Self-radiolysis of compounds in aqueous solution.

(2) Self-radiolysis of compounds in non-aqueous solvents.
(3) Analytical problems in the detection of impurities.
(4) Effect of impurities on the tracer use of radiochemicals.
(5) Additional tables of decomposition rates.

There is still a very large gap in our knowledge concerning the identity of the products produced by self-radiolysis of labelled compounds in the various physical states of storage. The study of each labelled compound is a major research undertaking and it is regrettable, but understandable, why this information is accumulated rather slowly.

1. Self-Radiolysis of Compounds in Aqueous Solution.

Many radiochemicals used in biochemical research are required in a suitable form for immediate use *viz.* in aqueous solution, and consequently much of the recent studies are concerned with the stability of radiochemicals stored in aqueous solution.

The action of ionising radiation on water is well known [3, 4] to produce hydrogen, hydrogen radicals, hydroxyl radicals, peroxides and hydrated electrons, as "reactive species". It is these "reactive species" which cause self-radiolysis of the radioactive compound, through secondary radiation effects [1].

Because of the necessity to supply compounds in aqueous solution, current investigations are primarily concerned with methods for reducing the damage caused by these "reactive species". Basically such methods reduce to lowering the temperature of the solution, or to the use of "scavengers" which preferentially react with the "reactive species".

(*a*) *Temperature Effects.* — Many research workers are uncertain as to whether aqueous solutions of tritiated or carbon-14 compounds should be kept frozen. In general solutions of carbon-14 compounds are best kept frozen at temperatures of —20° C or below; all the evidence we have suggests that the lower the temperature of storage the lower the decomposition will be, although the gain from going much below —20° C is often small. In contrast, solutions of tritiated compounds should be kept just above the freezing point of the solution, unless facilities are available for storage at —196° C. Storage of tritiated compounds in frozen solutions at temperatures above —100° C (or thereabouts) can often actually cause an acceleration in the rate of self-radiolysis. This unexpected effect was first observed with solutions of tritiated thymidine [5]; at least a partial explanation of it is the heterogeneity of the frozen solution demonstrated by Apelgot and Frilley [6]. These authors also showed that if 10 % glycerol is present in the solution, the heterogeneity of the solute molecules on freezing is reduced considerably.

A difficulty in understanding what is happening when we store labelled compounds in frozen solution is that there are at least two mechanisms whereby the labelled molecules can be decomposed. Either this can occur by solute-"reactive species" interaction at the low temperatures [7], or it can

be due to some build up of certain of these "reactive species" with time and then the reaction of these with the labelled solute when the solution is thawed prior to its use or analysis.

Any complete explanation of the behaviour of labelled compounds in frozen solution must account for :

(i) the increase in decomposition frequently found on freezing solutions of tritium labelled compounds;

(ii) the increased stability of such frozen solutions at -196° C;

(iii) the contrast between the behaviour of carbon-14 and tritium labelled compounds.

Perhaps a clue to the second point is to be found in the behaviour of the radicals produced in water on irradiation at about -160° C. Henriksen [8] has shown that around this temperature the diffusion of the electron spin resonance centres, which are present at lower temperatures, becomes quite significant, and secondary reactions take place quite readily.

If, as is often the case, molecular clustering of the solute occurs on freezing, then it is quite probable that for tritiated compounds such clusters will soon be in juxtaposition to relatively high concentrations of "reactive species" because of the low range of the beta particle emitted by this radionuclide. Such a concentration will result in increased decomposition, whether that decomposition occurs in the frozen state or on thawing. It seems feasible that the "reactive species" will be more dispersed in the case of carbon-14 compounds, though this is probably a gross over simplification of the mechanism.

In Table 1 are shown some results obtained by irradiating thymidine-2-C14 in tritiated water at $+2^{\circ}$ C and in the frozen state at -40° C. At the lower temperature solute-radical interaction is substantially reduced, only 10 % of the thymidine-C14 being decomposed compared with 100 % at $+2^{\circ}$ C. With tritiated thymidine at the same chemical concentration, one is probably observing the effect of molecular clustering coupled with the localisation of "reactive species" on the rate of self-radiolysis.

From a practical standpoint it is clear that more attention needs to be paid to the rate of freezing of solutions of labelled compounds — especially tritiated ones — because of the effect on the heterogeneity of such solutions. Another subject meriting more study is the storage of labelled compounds at -196° C.

Attempts to rapidly freeze tritium compounds by immersion of their aqueous solutions in liquid nitrogen followed by storage at -40° C were not really successful; in many cases decomposition was still faster than at $+2^{\circ}$ C [9, 10].

Actual storage of labelled compounds at -196° C has been shown [9–13] to reduce self-radiolysis considerably in a number of cases. Some further results obtained by storing compounds at -196° C are given in Table 2; comparison

TABLE 1. Decomposition of thymidine-2-^{14}C with beta radiation from tritiated water. Thymidine-2-^{14}C at 35.9 mc/mM.

Wt. of Thymidine-^{14}C μg	Volume of Solution ml	Tritium Activity mc	Temperature of Storage °C	Time of Storage Months	Dose Mega-Rads	Decomposition %
67.5	1	Nil (control)	+ 2	16	0.012	N.D.[a]
67.5	1	10	+ 2	16	1.34	100
67.5	1	1	+ 2	16	0.14	23
67.5	1	10	—40	4	0.35	3
67.5 Thymidine-T	1	10	—40	16	1.34	10
67.5 Thymidine-T	1	1	+ 2	16	0.13	18
67.5	1	1	—40	10	0.08	20

[a] In this and all tables in this paper N.D. = no impurities detected.

of these results with those given elsewhere (e.g. see Table 10 and our earlier review [1]) will emphasise that this method of storage is not a universal panacea.

(*b*) *Effect of Scavengers.* — It is often inconvenient to store aqueous solutions of labelled compounds at liquid nitrogen temperature (—196° C) particularly when relatively large volumes are involved; there is always the danger of the ampoule cracking with the possible loss of an expensive radiochemical. The difficulty of minimising self-radiolysis of compounds in solution has been largely overcome by using "reactive species" scavengers. These have included sodium formate, benzyl alcohol, and ethanol [1, 11, 13], and cysteamine [9, 10]; in fact many other compounds have been tried, and almost any substance which effectively reacts with radicals — hydroxyl radicals in particular — would be a possibility.

Ethanol is perhaps one of the best substances to use as a scavenger as it is easily removed. It has been found [1] especially useful for minimising the decomposition of amino-acids uniformly labelled with carbon-14 at high specific activities. Some recent results obtained under these storage conditions are included in Table 10, and the effect of this stabiliser on the storage of tritium compounds is further illustrated in Table 9.

It should not be assumed that the self-radiolysis of a labelled compound will always be reduced by the addition of ethanol to its solution, although we would recommend it as frequently being "good practice", and to date know of no authenticated cases where it has aggravated the problem. One case that did not respond favourably to this addition is that of L-methionine-

TABLE 2. Self-radiolysis of labelled compounds at —196° C.

Compound	Specific activity mc/mM	Storage conditions	Rad. conc. mc/ml	Storage time months	Decomp. %	G(-M)
S-Adenosylmethionine-(*methyl*-T)	1580	Aqueous solution pH 4	1.0	12	10	0.6
DL-Adrenaline-(*ring*-T(G)) bitartrate	200	Freeze dried solid under vacuum	—	10	10	5.9
DL-*nor*Adrenaline-7-T hydrochloride	1240	Aqueous solution	0.9	4	10	2.2
Cortisol-4-^{14}C	30.4	Ethanolic solution	0.006	10	3	1.2
Cortisol-4-^{14}C	30.4	Methanolic solution	0.006	10	2	0.8
Folic acid-3′,5′-T	25100	Freeze dried solid	—	9	15	0.08
L-Leucine-4,5-T	7600	Aqueous solution	4.8	16	25	0.3
L-Methionine(*methyl*-T)	see Table 4					
Methotrexate-3′,5′-T	6150	Freeze dried solid	—	4	7	0.3
L-Phenylalanine-^{14}C(U)	282	Aqueous solution	0.18	22	2.3	0.05
L-Phenylalanine-^{14}C(U)	282	Aqueous solution containing 30 % ethanol	0.18	22	1.4	0.03
L-Phenylalanine-^{14}C(U)	282	Ethanolic solution containing 5 % water	0.18	22	4.4	0.09
Thymidine-6-T(n)	14800	Aqueous solution	5.6	23	22	0.08
DL-α-Tocopherol-(5-*methyl*-T)	872	Ethanolic solution	1.0	7	6	1.1
DL-Tryptophan-T(G)	3020	Aqueous solution	1.0	9	2	0.08
Vitamin A (*carbinol*-^{14}C)	2.8	Benzene solution containing anti-oxidant[a]	0.2	19	10	24

[a] Antioxidant was 0.05 % butylated hydroxy anisole + 0.05 % butylated hydroxy toluene.

(*methyl*-C14). As can be seen from Table 3, ethanol affords little benefit in minimising the decomposition of methionine-C14 stored in aqueous solution, in agreement with results previously reported [1] for the storage of the corresponding compound labelled with tritium.

In practice L-methionine (*methyl*-C14) is best stored in the freeze dried state (see Table 10), but just why ethanol and other scavengers fail to afford much protection to methionine-C14 or -T is not understood. It was thought that hydrated electrons may be the principal cause of self-radiolysis of the methionine [14]. To test this hypothesis solutions of tritiated methionine-(*methyl*-T) were saturated with nitrous oxide and stored for several weeks. Nitrous oxide is known to convert hydrated electrons into hydroxyl ions and

TABLE 3. Self-radiolysis of methionine (*methyl*-^{14}C).
All samples had a specific activity of 25 mc/mM and a radioactive concentration of 50 μc/ml.

Storage conditions	Temperature °C	G(-M) [a]
In deoxygenated water	20	14.9
	—20	10.3
In deoxygenated water containing 2 % ethanol	20	14.0
	—20	8.8

[a] Calculated from the decomposition rates observed in samples stored for 6 months and for 12 months.

hydroxyl radicals. The results are summarised in Table 4. Nitrous oxide accelerates the decomposition of the labelled methionine in the non-frozen solutions, but the addition of sodium formate as scavenger reduces the rate of decomposition to the same as the controls containing formate alone. This suggests that hydroxyl radicals are causing most of the damage. It is interesting to note the considerable reduction in the rate of self-radiolysis by storing any of the solutions at —196° C, and it is possible there may be some significance in the relatively low value for the solution saturated with nitrous oxide and stored at —40° C.

TABLE 4. Effect of temperature, nitrous oxide, and sodium formate on the self-radiolysis of L-methionine (*methyl*-T).

Specific activity	8.6 curies/mM
Radioactive concentration	5.5 mc/ml
Storage time	7 weeks [a]

Addition to aqueous solution	Decomposition (%) observed at :			
	20° C	2° C	—40° C	—196° C
None	31	26	42	6
1 % Sodium formate	23	20	29	4
Saturated with N_2O	43	37	27	7
Saturated with N_2O + 1 % sodium formate	23	20	24	6

[a] Values given at 20° C and 2° C are extrapolations of measurements made after 6 weeks' storage.

Although the presence of 1 % ascorbic acid minimises the decomposition of tritiated *nor*adrenaline [15] (29 curies/mM) in aqueous solution, it is observed to accelerate the decomposition of methionine (*methyl*-T) in solution, as seen from Table 5.

TABLE 5. Effect of ascorbic acid.

Compound	Specific activity mc/mM	Storage Conditions [a]	Activity concn. mc/ml	Storage time months	Decomp. %
L-Methionine-(*methyl*-T)	4330	Water	1	4	35
L-Methionine-(*methyl*-T)	4330	Water + 1 % ascorbic acid	1	4	90
DL-*nor*Adrenaline-7-T hydrochloride	3680	Water	1	3	15
DL-*nor*Adrenaline-7-T hydrochloride	3680	Water + 1 % ascorbic acid	2	3	6

[a] All solutions stored at 2° C.

We are unaware of any reports suggesting that the addition of compounds such as ethanol to solutions of labelled compounds interferes in their tracer uses. Often, of course, the solution supplied is well diluted before use and the scavenger concentration is then very low indeed. However, it should be borne in mind that some effects are possible and any necessary control experiments should be conducted to find this out. It should also be noted that in order to minimise decomposition of the labelled compound in the diluted solution on storage, it may be necessary to raise the scavenger concentration to that of the original solution.

There have been cases reported [11] as well as others which we have observed, of tritiated L-amino-acids undergoing racemisation on storage. The mechanism of this is far from clear and even the facts themselves are uncertain, as considerable variation has been observed between batches which should be similar. However, it can be stated that no racemisation has been observed in solutions of tritiated amino-acids stored in the presence of small amounts of ethanol. No racemisation has to our knowledge ever been observed to occur as a result of self-radiolysis of amino-acids labelled with carbon-14. This includes a case in which some L-phenylalanine-C14(U) was examined after it had been stored in a purely aqueous solution and had produced 92 % of radiochemical impurity; even so no racemisation was detected by reverse isotope dilution analysis [16].

2. Self-radiolysis of labelled compounds in non-aqueous solvents.

The mechanism of the decomposition of labelled organic compounds by self-radiolysis on storage in non-aqueous solvents is not fully understood. The transfer and absorption of the radiation energy is undoubtedly quite different and produces different forms of "reactive species" from those produced in aqueous solution.

Commonly used solvents include benzene, ethanol or methanol, and it is important that they should be pure. Frankel and Nalbandov [17] have investigated the deleterious effects on the stability of steroids, by using solvents of varying purity, in particular when evaporating very dilute solutions of the labelled steroids; this problem has also been commented on by others [18].

Classes of compounds which are often stored in non-aqueous solvents include steroids, long-chain aliphatic fatty acids and hydrocarbons. Some recent observations on tritiated compounds are given in Tables 6 and 7; results for carbon-14 compounds stored in this way are included in Table 10.

In addition to the results given in Table 6 tritiated anthracene (54 c/mM), 9,10-dimethyl-1,2-benzanthracene (19 c/mM), 3,4-benzpyrene (24 c/mM) and

Table 6. Self-radiolysis of polycyclic aromatic hydrocarbons labelled with tritium.

Compound	Specific activity mc/mM	Storage conditions	Activity mc/ml	Storage Temp. °C	Storage Time months	Decomp. %	G(-M)
3,4-Benzpyrene-T(G)	451	Benzene	2	20	35	1	0.1
3,4-Benzpyrene-T(G)	560	Benzene	10	20	8	4	1.0
3,4-Benzpyrene-T(G)	1330	Benzene	5.5	20	9	5	0.5
3,4-Benzpyrene-T(G)	1760	Benzene	6.7	—40	13	4	0.2
3,4-Benzpyrene-T(G)	3730	Benzene	1	20	8	5	0.2
3,4-Benzpyrene-T(G)	451	Solid	—	—40	36	3	0.2
1,2,3,4-Dibenzanthracene-T(G)	151	Benzene	2.3	20	18	2	0.9
1,2,5,6-Dibenzanthracene-T(G)	834	Benzene	5	20	8	2	0.3
9,10-Dimethyl-1, 2-benzanthracene-T(G)	3,250	Benzene	15	20	14	37	1.2
9,10-Dimethyl-1, 2-benzanthracene-T(G)	361	Solid	—	—80	7	10	4.7
20-Methylcholanthrene-T(G)	346	Benzene	2	20	12	5	1.4
20-Methylcholanthrene-T(G)	400	Benzene	1	20	7	7	2.9
20-Methylcholanthrene-T(G)	471	Benzene[a]	1.1	20	7	18	6.8
20-Methylcholanthrene-T(G)	3860	Benzene	1	20	7	7	0.3
20-Methylcholanthrene-T(G)	3980	Benzene[a]	1.1	20	7	45	2.4

[a] Solution unprotected from light — all other solutions kept in dark.

20-methyl-cholanthrene (44 c/mM), stored for 6 months in benzene solution in the dark at 0-3° C are reported [19] to undergo self-radiolysis at the rate of 12-20 % per annum.

TABLE 7. Self-radiolysis of some steroids labelled with tritium.

Compound	Specific activity mc/mM	Storage conditions	Activity concn. mc/ml	Storage Temp. °C	Storage Time months	Decomp. %	G(-M)
D(+)-Aldosterone-1,2-T	3680	Benzene : ethanol (9 : 1)	0.1	0	5	N.D.[a]	—
D(+)-Aldosterone-1,2-T	7800	Benzene : ethanol (4 : 1)	0.1	0	4	N.D.[a]	—
Cholesterol-7α-T	3450	Benzene	4	20	14	2	0.05
Cortisol-1,2-T	2000	Benzene : methanol (1 : 1)	1	20	13	15	0.7
Cortisol-1,2-T	30900	Benzene : methanol (1 : 1)	1	20	13	23	0.07
Oestradiol-6,7-T	500	Ethanol	1	—20	5	3	1.3
Oestradiol-6,7-T	29200	Ethanol	2	0	2	9	0.2
Oestradiol-6,7-T	8200	Ethanol	1	2	1	N.D.	—
Oestradiol-6,7-T-17β-Acetate	36300	Benzene : ethanol (9 : 1)	2	20	8	35	0.2
Pregnenolone-7α-T	454	Benzene	1	20	17	1	0.1
Pregnenolone-7α-T	2500	Benzene	1	20	17	1	0.03

[a] No "iso-aldosterone" was observed to form on storage under these conditions.

The storage of labelled organic acids at low chemical concentrations in solutions which are predominantly alcoholic can often result in the formation of esters, especially if additional acid is present. Hempel [20] observed this to occur on storage of tritiated phenylalanine, lysine, α-aminoadipic acid and leucine, in 80 % ethanol containing 0.1 *N* hydrochloric acid. It is inadvisable to have both free hydrochloric acid and high concentrations of ethanol present together, unless the solutions are kept frozen.

3. Analytical Problems in the Detection of Impurities in Labelled Compounds.

Consignments of radiochemicals from commercial suppliers are normally accompanied by a technical data sheet describing the purity of a particular compound and the methods which have been employed for the analyses. It should be remembered that no method of analysis is infallible and that

FIG. 1. Analysis of cholesterol-T.

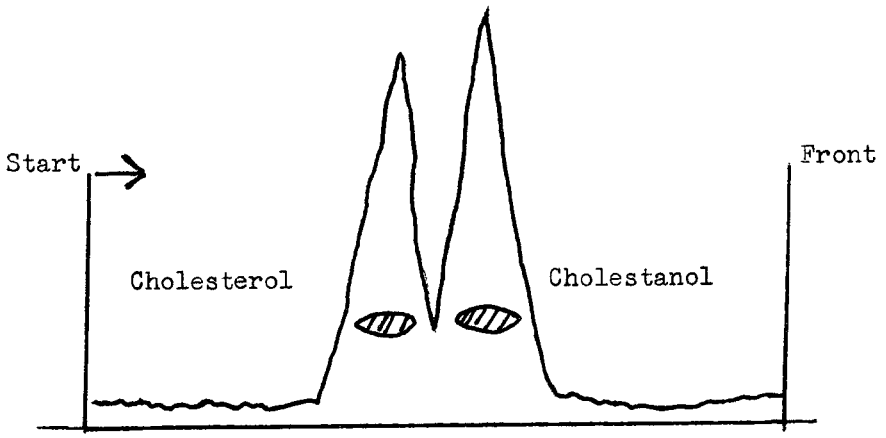
(1) By paper chromatography.
   (*a*) Descending elution by (100-120° C) petroleum ether on paper pre-equilibrated by 80 % methanol (this system separates double bond isomers).
   (*b*) Reserve phase on paper saturated with 10 % paraffin in ethoxyethanol : *n*-propanol : methanol : water (70 : 20 : 60 : 50) saturated with paraffin.
   (*c*) Reverse phase on paper saturated with 10 % paraffin in 84 % acetic acid (saturated with paraffin).
   (*d*) Reverse phase on paper saturated with 10 % phenoxyethanol in *n*-hexane saturated with phenoxy-ethanol.
(2) By thin-layer chromatography on silica gel G.
   (*a*) In cyclohexane : ethyl acetate (60 : 40) [R_f 0.53].
   (*b*) In methylene chloride : acetone (80 : 20) [R_f 0.67].
   (*c*) Reverse phase TLC on silica gel G layers pre-dipped in 10 % paraffin in ether, in 90 % acetic acid saturated with liquid paraffin [R_f O.2].

None of these systems separates cholesterol and cholestanol.

the more independent checks used for evaluating the purity, the nearer one gets to the "absolute" value. Reliance on a single chromatographic system is normally most unwise, and even much more evidence than this can often be misleading as is illustrated by the following example concerning a batch of tritiated cholesterol. In four paper chromatographic systems and three thin-layer systems (Fig. 1) one radioactive peak was obtained for the cholesterol-1α-T. Impregnation of the silica gel with silver nitrate (Fig. 2) followed by

FIG. 2. Analysis of cholesterol containing cholestanol.

Thin-layer chromatography on silver nitrate-silica gel in chloroform : acetone ((98 : 2). [30 grm Merck silica gel-G slurried with 7.5 grm silver nitrate dissolved in 60 ml water].



Double elution required

R_f[a] Cholesterol 0.53
Cholestanol 0.63

elution with chloroform: acetone demonstrated that the cholesterol-1α-T was impure and was contaminated with cholestanol-T.

Reverse isotope dilution analysis with cholesterol even with four or more recrystallisations failed to separate or show the presence of an impurity. It was necessary to prepare the 5,6-dibromocholesterol and recrystallise that several times to obtain the true radiochemical purity.

Reverse isotope dilution analysis of some deoxyadenosine which contained adenine as an impurity was another case in which recrystallisation was found to be insufficient to separate out the impurity.

Another case of chromatography failing to detect an impurity has recently been reported [21] in the case of some aldosterone-4-C14.

4. Effect of Impurities on the Tracer Use of Radiochemicals.

It is seldom possible to predict the effect of impurities (both chemical and radiochemical) on the results obtained from a tracer experiment using an impure radiochemical. It is made even more difficult because in only a few examples are the products of self-radiolysis of compounds on storage known. At present the only practical method is to isolate the impurities and to actually test these under the experimental conditions, as a type of control experiment. An example of this approach is described by Wand, Zeuthen and Evans [22] who isolated the self-radiolysis products of tritiated thymidine and studied their behaviour with synchronised cells of *Tetrahymena pyriformis*. Results showed that these decomposition products did not label DNA but were rapidly and efficiently incorporated into macromolecular structures in the cytoplasm. Treatment of the cells with DNase or RNase did not remove the labelling by these impurities. For comparison a sample of thymidine-T irradiated with gamma rays was also tested. The results of these authors demonstrate quite clearly how misleading results can be obtained, and suggest that non-specific labelling of the cytoplasm in the tracer use of thymidine labelled with tritium (or carbon-14) for cytological studies must be interpreted with caution and related to the purity of the radioactive thymidine used for the experiments.

In the use of L-histidine (*ring*-2-C14) to measure histidine decarboxylase activity Thunberg [23] had trouble due to the formation by self-decomposition of small amounts of histamine (*ring*-2-C14). Although the levels were extremely low they affected the assay, which involved measuring the histamine produced. Fortunately it was not difficult to remove this contaminant from the L-histidine, but he did find it necessary to do this every 2-3 weeks if the full sensitivity of the assay was to be maintained.

Recently Tait and co-workers [21] have demonstrated the potential source of inaccuracy in the measurement of aldosterone secretion rates due to the presence of labelled 17-*iso*aldosterone in labelled aldosterone used for this purpose. Not only does this impurity arise fairly readily both in the prepa-

ration of labelled aldosterone and in the preparation of a solution of that steroid suitable for injection, but the different handling by the body of these two compounds magnifies the effect of the impurity in the usual secretion rate determinations [24, 25]. At least it is now possible to state that very little, if any, of the "*iso*" compound is formed on storage of aldosterone-1,2-T in benzene solution.

It would be wrong to conclude from these foregoing examples that impurities will always be more trouble than their quantitative presence suggests. It has been pointed out [26] to one of us that although the overall decomposition rate of thiosemicarbazide-S35 is greater in methanol than when it is stored dry or in aqueous solution [1], yet the compound stored in methanolic solution behaves quite satisfactorily in steroid double isotope derivative analysis throughout its useful life. It seems likely that stored in this way formaldehyde is produced by the action of the radiation on the methanol and that this then reacts with the thiosemicarbazide; provided this reaction is not too extensive it appears not to interfere in the subsequent analytical procedure. In this and similar uses users may be well able to ignore the "recommended" storage conditions in order to gain convenience provided they check the labelled compound under the conditions of their use of it. Indeed, it could well be that on occasions a "worse" method of storage (as determined by the supplier using total impurity produced as his criteria) may be actually *better* for an individual in his particular application if the impurities formed are different from those in the "recommended" method of storage and they interfere less in his procedure.

The presence of radioactive molecules not labelled in the position specified for a given tracer compound can present a different kind of "impurity" problem. This problem is extremely unlikely to be met with in the use of carbon-14 compounds where the position of the carbon-14 atom(s) is known with certainty from the method used for preparing the labelled compound. However, because of the ready exchange of hydrogen atoms with tritium atoms under conditions used for the preparation of tritiated compounds [13], many "specifically" labelled tritiated compounds have as an "impurity" some radioactive molecules which are labelled in other positions. An example is the use of uridine-5-T as a specific precursor of RNA. A number of investigators have commented on the non-specificity of uridine-5-T as a tracer for RNA [27, 28]. When uridine-5-T is transformed into thymidine, the tritium label at the 5-position is lost. If the uridine-5-T contains any uridine-6-T then methylation does not displace all the tritium. Analysis of various preparations of uridine-5-T prepared by catalysed halogen-tritium replacement[13], has shown that the uridine-5-T may have up to 3 % of uridine-6-T present. This is just one of many possible examples and pin-points the importance of knowing the degree of specificity of labelling in "specifically" labelled compounds.

5. Additional Tables of Decomposition Rates.

(a) *Tritium compounds.*

As well as the additional data already presented in Tables 2, 4, 5, 6, 7, two further tables are included which deal with tritiated nucleotides (Table 8) and with the storage of a number of compounds in aqueous solution containing a few per cent of ethanol (Table 9).

Table 8. Self-radiolysis of nucleotides labelled with tritium.
All these compounds were stored in sterilised aqueous solutions at +2° C at a radioactive concentration of 1 mc/ml.

Compound	Specific activity mc/mM	Storage time months	Decomp. %	G(-M)
Adenosine-T(G)-5′-monophosphate, lithium salt	644	12	<5	<0.7
Cytidine-5-T-5′-monophosphate, ammonium salt	500	7	3	0.9
Cytidine-5-T-5′-monophosphate, ammonium salt	6750	7	3	0.07
Deoxycytidine-5-T-5′-monophosphate, ammonium salt	9200	5	35	1.0
Deoxyuridine-5-T-5′-monophosphate, ammonium salt	2650	4	20	2.4
Thymidine-(*methyl*-T)-5′-monophosphate, ammonium salt	2200	7	<5	<0.4
Uridine-5-T-5′-monophosphate, sodium salt	572	12	<3	<0.5
Uridine-5-T-5′-monophosphate, ammonium salt	6630	6	<3	<0.1

(b) *Carbon-14 compounds.*

Table 10 lists decomposition data for a number of compounds. For convenience they are grouped by classes of compounds as follows :

Amino-acids.
Aliphatic compounds.
Aromatic compounds.
Heterocyclic compounds (including nucleosides and nucleotides).
Carbohydrates.
Steroids.

Readers are reminded that because of the difficulty in many cases of estimating the fraction of its own energy absorbed this has been assumed to be unity for the purposes of calculating G(-M) values. However, with freeze-dried carbon-14 compounds it is possible to keep this fraction relatively low by storing it as spread out as possible.

TABLE 9. Self-radiolysis of some tritiated compounds in aqueous solution containing a few per cent ethanol.

Compound	Specific activity mc/mM	Storage conditions	Radioactive conc. mc/ml	Temp. °C	Storage time months	Decomp. %	G(-M)
DL-Adrenaline-7-T	1,400	Aqueous solution	1.0	+ 2	3.5	100	High
DL-Adrenaline-7-T	1,400	Aqueous solution containing 1 % ethanol	1.0	+ 2	3.5	20	5.0
DL-Adrenaline-7-T	1,400	Aqueous solution containing 0.1 % sodium formate	1.0	+ 2	3.5	100	High
Deoxycytidine-5-T	500	Aqueous solution containing 2 % ethanol	1.0	+ 2	7	2	0.6
Deoxycytidine-5-T	14,700	Aqueous solution containing 2 % ethanol	1.0	+ 2	5	N.D.	—
L-3(3,4-Dihydroxy-phenyl)alanine-(*ring*-2,5,6-T)	28,700	Aqueous solution containing 1 % ethanol	1.0	+ 2	3.5	5	0.06
DL-Phenylalanine-T(G)	2,000	Aqueous solution	1.0	—40	11	20	1.1
DL-Phenylalanine-T(G)	2,000	Aqueous solution containing 1 % ethanol	1.0	—40	11	12	0.7
Serotonin-T(G)*	5,650	Aqueous solution	1.1	+ 2	2.5	25	2.2
Serotonin-T(G)*	5,650	Aqueous solution containing 2 % ethanol	1.1	+ 2	2.5	5	0.4
L-Tyrosine-3,5-T	1,000	Aqueous solution containing 1 % ethanol	1.0	+ 2	3	N.D.	—
L-Tyrosine-3,5-T	42,300	Aqueous solution containing 1 % ethanol	1.0	+ 2	3	N.D.	—
Uracil-5,6-T	5,600	Aqueous solution containing 1 % ethanol	0.5	+ 2	3.5	2	0.1

* As creatinine sulphate complex.

TABLE 10. Self-decomposition of some compounds labelled with carbon-14.

Compound	Specific activity mc/mM	Storage conditions	Temp. °C	Storage time months	Decomp. %	G(-M)
Amino acids						
S-Adenosyl-methionine(*methyl*-^{14}C)	23.0	Aqueous solution pH 3 containing 2 % ethanol	—40	8	2	1.3
DL-Alanine-1-^{14}C	21.7	Freeze-dried solid	20	47	3	0.3
L-Alanine-^{14}C(U)	20.2	Freeze-dried solid	20	37	1	0.2
L-Alanine-^{14}C(U)	85	Aqueous solution containing 2 % ethanol	—40	34	N.D.	—
L-Arginine-^{14}C(U)	324	Aqueous solution containing 2 % ethanol	—40	3	N.D.	—
L-Asparagine-^{14}C(U)	102	Aqueous solution containing 2 % ethanol	—40	11	5	0.6
L-Asparagine-^{14}C(U)	102	Aqueous solution containing 2 % ethanol	—40	6	N.D.	—
L-Aspartic acid-^{14}C(U)	6.1	Freeze-dried solid	20	23	2	1.8
L-Aspartic acid-^{14}C(U)	106	Aqueous solution containing 2 % ethanol	—40	20	N.D.	—
L-Citrulline(*carbamyl*-^{14}C)	35.9	Freeze-dried solid	—40	13	5	1.3
DL-Cystine-3-^{14}C hydrochloride	39.5	Freeze-dried solid	20	25	1	0.1
L-Cystine-^{14}C(U)	324	Aqueous solution containing 2 % ethanol	—20	4	N.D.	—
Folic acid-2-^{14}C, potassium salt	31.4	Freeze-dried solid	—40	13	N.D.	—
L-Glutamic acid-^{14}C(U)	130	Aqueous solution containing 2 % ethanol	—40	24	N.D.	—
L-Glutamine-^{14}C(U)	32.2	Freeze-dried solid	—40	23	4	0.7
L-Glutamine-^{14}C(U)	38.8	Freeze-dried solid	—20	3	N.D.	—
Glycine-1-^{14}C	2.0	Aqueous solution, 10μc/ml	20	15	N.D.	—
Glycine-2-^{14}C	31.7	Freeze-dried solid	20	12	N.D.	—
Glycine-^{14}C(U)	8.1	Freeze-dried solid	20	20	N.D.	—
Glycine-^{14}C(U)	38	Aqueous solution containing 2 % ethanol	—40	12	N.D.	—
L-Histidine(*ring*-2-^{14}C)	41.5	Freeze-dried solid	—40	11	N.D.	—
DL-5-Hydroxytryptophan(*methylene*-^{14}C)	21.8	Freeze-dried solid	—40	47	3	0.3

TABLE 10. *(continued)*

Compound	Specific activity mc/mM	Storage conditions	Temp. °C	Storage time months	Decomp. %	G(-M)
L-Leucine-1-^{14}C	8.3	Freeze-dried solid	20	15	N.D.	—
L-*iso*-Leucine-^{14}C(U)	8.7	Freeze-dried solid	20	24	N.D.	—
DL-Lysine-1-^{14}C hydrochloride	15.5	Freeze-dried solid	20	25	4	1.3
L-Lysine-^{14}C(U) hydrochloride	7.5	Freeze-dried solid	20	14	1	1.2
L-Lysine-^{14}C(U) hydrochloride	324	Aqueous solution containing 2 % ethanol	—40	2	N.D.	—
L-Methionine(*methyl*-^{14}C)*	25.0	Freeze-dried solid under nitrogen	—20	13	N.D.	—
L-Methionine(*methyl*-^{14}C)*	56.8	Freeze-dried solid under nitrogen	—20	4	N.D.	—
DL-3-Phenyl(alanine-1-^{14}C)	44.2	Freeze-dried solid	20	12	N.D.	—
DL-3-Phenyl(alanine-2-^{14}C)	14.7	Freeze-dried solid	20	23	2	0.7
L-3-Phenylalanine-^{14}C(U)	7.0	Freeze-dried solid	20	12	N.D.	—
L-Proline-^{14}C(U)	8.2	Freeze-dried solid	20	23	1	0.7
L-Proline-^{14}C(U)	120	Aqueous solution containing 2 % ethanol	—40	11	N.D.	—
Sarcosine-1-^{14}C	8.1	Freeze-dried solid	20	83	N.D.	—
L-Serine-^{14}C(U)	87.4	Aqueous solution containing 2 % ethanol	—40	28	N.D.	—
L-Threonine-^{14}C(U)	5.5	Freeze-dried solid	20	27	2	1.7
L-Thyroxine-^{14}C[L-4(3,5-Diiodo-4-hydroxyphenoxy)-3,5-diiodo(phenyl-alanine-^{14}C(U)]	23.2	Freeze-dried solid	—20	3	2	3.5
DL-Tryptophan(*benzene ring*-^{14}C(U))	19.8	Freeze-dried solid	20	11	5	2.8
DL-Tryptophan(*methylene*-^{14}C)	32.9	Freeze-dried solid	20	11	N.D.	—
L-Tyrosine-^{14}C(U)	5.5	Freeze-dried solid	20	11	N.D.	—
L-Tyrosine-^{14}C(U)	225	Aqueous solution containing 2 % ethanol	—40	16	N.D.	—
DL-Valine-4-^{14}C	4.8	Freeze-dried solid	20	22	N.D.	—
L-Valine-^{14}C(U)	6.9	Freeze-dried solid	20	23	1	0,8

* See also Table 3.

TABLE 10 *(continued)*

Compound	Specific activity mc/mM	Storage conditions	Temp. °C	Storage time months	Decomp. %	G(-M)
Aliphatic compounds						
(Acetyl-1-^{14}C) choline chloride	6.4	Freeze-dried solid	—40	26	N.D.	—
Acetyl choline(*methyl*-^{14}C) chloride	10.4	Freeze-dried solid	—40	23	N.D.	—
Adipic acid-1,6-^{14}C	11.1	Freeze-dried solid	20	11	1	1.0
Bromoacetic acid-2-^{14}C	37.5	Liquid	20	11	3	0.9
Calcium DL-glycerate-1-^{14}C	70.2	Freeze-dried solid	—40	17	N.D.	—
Carbon tetrachloride-^{14}C	7.2	Liquid	20	35	N.D.	—
Chloracetic acid-2-^{14}C	20.6	Solid	0	12	15	8.2
Choline(*methyl*-^{14}C) chloride	32.0	Dried on paper	—80	11	N.D.	—
DL-Citric acid-1,5-^{14}C	15.4	Freeze-dried solid	—40	10	N.D.	—
Creatine-1-^{14}C	7.8	Freeze-dried solid	20	23	N.D.	—
n-Decane-1-^{14}C	3.3	Liquid	20	65	N.D.	—
Ethan-1-ol-2-amine-2-^{14}C hydrochloride	4.9	Freeze-dried solid	20	12	N.D.	—
Ethyl-1-^{14}C iodide	7.1	Liquid	20	17	8	8.5
Ethylene diamine-1,2-^{14}C dihydrochloride	4.8	Freeze-dried solid	20	59	N.D.	—
Ethylene diaminetetra(acetic-2-^{14}C) acid sodium salt	21.6	Freeze-dried solid	20	12	N.D.	—
Formaldehyde-^{14}C	13.5	Aqueous solution	20	12	19	16.1
Formic acid-^{14}C	21.8	Liquid	20	23	N.D.	—
Fumaric acid-1,4-^{14}C	12.0	Solid	20	12	N.D.	—
Fumaric acid-2,3-^{14}C	10.9	Solid	20	19	N.D.	—
Glycerol-2-^{14}C	8.0	Liquid	—40	11	N.D.	—
Glycerol-^{14}C(U)	14.3	Liquid	—40	11	N.D.	—
Glyceryl-1-^{14}C tripalmitate	12.5	Benzene solution	20	25	N.D.	—

TABLE 10 *(continued)*

Compound	Specific activity mc/mM	Storage conditions	Temp. °C	Storage time months	Decomp. %	G(-M)
n-Hexadecane-1-^{14}C	19.4	Benzene solution	20	12	N.D.	—
Iodoacetic acid-2-^{14}C	6.5	Solid	—40	17	N.D.	—
Lauric acid-^{1}-^{14}C	21.0	Benzene solution	20	36	N.D.	—
Linoleic acid-1-^{14}C	37.8	Benzene solution	20	11	5	1.5
Linoleic acid-^{14}C(U)	442	Benzene solution	20	3	100	High
Malathion-^{14}C[0,0-Dimethyl S-(1,2-di (ethoxycarbonyl) ethyl-1,2-^{14}C) phosphorodithioate]	2.2	Benzene solution	20	16	N.D.	—
Methyl bromoacetate-1-^{14}C	5.5	Liquid under nitrogen	20	11	9	19.2
Methyl bromoacetate-2-^{14}C	5.4	Liquid under nitrogen	20	5	N.D.	—
Methyl bromoacetate-2-^{14}C	40.5	Liquid under nitrogen	20	5	14	9.2
Methylamine-^{14}C hydrochloride	34.5	Freeze-dried solid	20	12	N.D.	—
Myristic acid-1-^{14}C	15.4	Benzene solution	20	24	N.D.	—
n-Octadecane-1-^{14}C	25.5	Benzene solution	20	24	N.D.	—
Palmitic acid-^{14}C(U)	495	Benzene solution	20	3	N.D.	—
Potassium cyanate-^{14}C	15.8	Freeze-dried solid	20	12	N.D.	—
iso-Propyl iodide-1,3-^{14}C	7.5	Liquid	20	23	1	0.7
Putrescine-^{14}C[Tetramethylene diamine-1,4-^{14}C dihydrochloride]	7.8	Freeze-dried solid	20	26	N.D.	—
Sodium *n*-butyrate-1-^{14}C	16.0	Freeze-dried solid	20	22	1	0.4
Sodium cyanoacetate-2-^{14}C	14.7	Freeze-dried solid	20	23	4.5	1.7
Sodium formate-^{14}C	10.8	Freeze-dried solid	0	14	N.D.	—
Sodium formate-^{14}C	42.8	Freeze-dried solid	0	8	2	0.7
Sodium glyoxalate-2-^{14}C	4.6	Freeze-dried solid	20	23	3	3.5

TABLE 10 *(continued)*

Compound	Specific activity mc/mM	Storage conditions	Temp. °C	Storage time months	Decomp. %	G(-M)
Sodium DL-3-hydroxybutyrate-3-^{14}C	5.7	Freeze-dried solid	—40	25	N.D.	—
Sodium DL-3-hydroxybutyrate-4-^{14}C	8.6	Freeze-dried solid	—40	51	N.D.	—
Sodium DL-lactate-1-^{14}C	25.6	Freeze-dried solid	—40	11	2.5	1.1
Sodium DL-lactate-2-^{14}C	35.6	Freeze-dried solid	—40	13	N.D.	—
Sodium L-lactate-^{14}C(U)	19.4	Freeze-dried solid	—20	16	N.D.	—
Sodium malonate-1-^{14}C	14.5	Freeze-dried solid	20	3	N.D.	—
Sodium propionate-1-^{14}C	10.2	Freeze-dried solid	20	23	2	1.1
Sodium propionate-2-^{14}C	9.9	Freeze-dried solid	20	11	1	1.2
Stearyl alcohol-1-^{14}C	16.0	Benzene solution	20	23	N.D.	—
Succinic acid-1,4-^{14}C	5.7	Solid	20	11	N.D.	—
Succinic acid-2,3-^{14}C	4.0	Solid	20	11	N.D.	—
Succinyl *bis*(choline(*methyl*-^{14}C) iodide)	8.6	Freeze-dried solid	20	31	N.D.	—
DL-Tartaric acid-1,4-^{14}C	5.1	Freeze-dried solid	20	23	N.D.	—
Thiourea-^{14}C	21.6	Solid	20	23	3	0.7
Trichloracetic acid-1-^{14}C	6.8	Solid	—40	12	2	3.0
Trichloracetic acid-2-^{14}C	10.5	Solid	—40	11	N.D.	—
Urea-^{14}C	15.4	Freeze-dried solid	20	25	N.D.	—
Urea-^{14}C	39	Freeze-dried solid	—40	22	N.D.	—
Aromatic compounds						
DL-Adrenaline(*carbinol*-^{14}C)DL-bitartrate	7.3	Freeze-dried solid	—20	9	N.D.	—
DL-Adrenaline(*carbinol*-^{14}C)DL-bitartrate	10.9	Freeze-dried solid	20	27	N.D.	—
DL-*nor*-Adrenaline(*carbinol*-^{14}C)DL-bitartrate	17.2	Freeze-dried solid	20	9	N.D.	—

TABLE 10 *(continued)*

Compound	Specific activity mc/mM	Storage conditions	Temp. °C	Storage time months	Decomp. %	G(-M)
Aldrin-^{14}C	81.0	Benzene solution	20	5	1	0.3
Aniline hydrogen sulphate-^{14}C(U)	37.0	Freeze-dried solid	20	23	N.D.	—
Benzaldehyde-(*carbonyl*-^{14}C)	4.1	Liquid under nitrogen	20	30	14	15.1
Benzene-^{14}C(U)	26.5	Liquid	20	12	N.D.	—
γ-Benzene hexachloride-^{14}C(U)	34.0	Benzene solution	20	20	N.D.	—
Benzoic acid(*carboxyl*-^{14}C)	23.3	Solid under nitrogen	20	23	N.D.	—
Benzoic acid(*ring*-^{14}C(U))	48.2	Solid	20	11	N.D.	—
Benzyl alcohol (*carbinol*-^{14}C)	3.0	Liquid	20	23	2	3.6
Dieldrin-^{14}C	70.4	Benzene solution	20	6	1	0.3
2,4-Dichlorophenoxy (acetic acid-1-^{14}C)	12.1	Solid	20	11	N.D.	—
9,10-Dimethyl-1,2-benzanthracene-9-^{14}C	9.3	Solid	20	8	1	1.7
9,10-Dimethyl-1,2-benzanthracene-9-^{14}C	9.3	Solid	20	32	6	2.5
2,4-Dinitrophenylhydrazine-^{14}C(U)	4.1	Solid	20	59	5	2.6
2-(Methyl-^{14}C)naphthalene	5.1	Solid	20	24	N.D.	—
1-Naphthol-1-^{14}C	2.3	Solid	20	23	N.D.	—
Neostigmine iodide-^{14}C[Tri(methyl-^{14}C) (N,N-dimethyl-*m*-carbamatophenyl) ammonium iodide]	5.3	Freeze-dried solid	—40	40	N.D.	—
Salicylic acid (*carboxyl*-^{14}C)	15.5	Solid	20	15	N.D.	—
Vitamin A (*carbinol*-^{14}C)*	2.8	Benzene solution containing antioxidant**	—20	19	8	19
Vitamin A (*carbinol*-^{14}C) acetate	2.9	Benzene solution containing antioxidant**	—20	30	12	17
Heterocyclic compounds						
Adenine-8-^{14}C	31.5	Freeze-dried solid	20	12	N.D.	—

* See also Table 2.
** Antioxidant was 0.05 % butylated hydroxy anisole + 0.05 % butylated hydroxy toluene.

TABLE 10 *(continued)*

Compound	Specific activity mc/mM	Storage conditions	Temp. °C	Storage time months	Decomp. %	G(-M)
S-Adenosyl-methionine(*methyl*-^{14}C)	23.0	Aqueous solution pH 3 containing 2 % ethanol	—40	8	2	1.3
Adenosine-8-^{14}C	28.4	Aqueous solution	—40	20	N.D.	—
Adenosine-8-^{14}C-5′-monophosphate, ammonium salt	21.4	Freeze-dried solid	—40	13	N.D.	—
Adenosine-^{14}C(U)-5′-monophosphate, ammonium salt	255	Sterile aqueous solution under vacuum	—40	13	3	0.1
Adenosine-8-^{14}C-5′-triphosphate, sodium salt	4.8	Freeze-dried solid	—20	7	2	7
Benzyl penicillin-^{14}C potassium[Potassium 6-phenyl(acet-1-^{14}C)-amidopenicillanate]	15.7	Freeze-dried solid	—40	17	2	0.9
D-Biotin(*carbonyl*-^{14}C)	31.5	Solid	20	11	N.D.	—
Cytidine-^{14}C(U)-5′-monophosphate, ammonium salt	193	Aqueous solution	—40	14	2	0.1
Cytosine-2-^{14}C sulphate	21.5	Freeze-dried solid	20	39	N.D.	—
Deoxyadenosine-^{14}C(U)	275	Aqueous solution	—40	6	N.D.	—
Deoxycytidine-^{14}C(U)	245	Aqueous solution	—40	7	N.D.	—
Deoxyguanosine-^{14}C(U)	272	Aqueous solution	—40	19	1	0.02
Diquat(*ethylene*-^{14}C)dibromide[N,N′-Ethylene-^{14}C(U)-2,2′-bipyridylium dibromide monohydrate]	10.6	Freeze-dried solid	20	14	N.D.	—
6-Furfurylaminopurine-8-^{14}C	16.5	Solid	0	12	1	0.6
Guanine sulphate-8-^{14}C	36.1	Solid	20	37	N.D.	—

TABLE 10 *(continued)*

Compound	Specific activity mc/mM	Storage conditions	Temp. °C	Storage time months	Decomp. %	G(-M)
5-Hydroxytryptamine-3′-^{14}C creatinine sulphate	39.6	Freeze-dried solid	—40	12	N.D.	—
Hypoxanthine-8-^{14}C	9.6	Solid	20	23	N.D.	—
Nicotinamide (*carbonyl* ^{14}C)	13.2	Freeze-dried solid	20	12	N.D.	—
Nicotinic acid (*carboxyl*-^{14}C)	27.9	Freeze-dried solid	20	23	N.D.	—
Nicotinic acid-6-^{14}C	26.2	Freeze-dried solid under nitrogen	20	4	N.D.	—
Orotic acid-6-^{14}C	44.5	Freeze-dried solid	20	11	N.D.	—
Paraquat(*methyl*-^{14}C)chloride [*bis*(N-methyl-^{14}C)-4,4′-bipyridylium chloride]	10.1	Freeze-dried solid	20	21	N.D.	—
Quinolinic acid-6-^{14}C	29.3	Freeze-dried solid under nitrogen	20	12	N.D.	—
2-Thiouracil-2-^{14}C	29.9	Freeze-dried solid	20	23	1	0.2
Thymidine-^{14}C(U)	250	Aqueous solution	—40	7	N.D.	—
Thymine-2-^{14}C	58.3	Freeze-dried solid	20	4	N.D.	—
Uracil-2-^{14}C	61	Freeze-dried solid	20	4	N.D.	—
Uric-acid-2-^{14}C	20.8	Solid	20	24	3	0.7
Uridine diphospo(glucose-^{14}C(U)), ammonium salt	76	Aqueous sodium phosphate buffer solution (0.05*M*, pH 6.4), containing 2 % ethanol	—20	10	N.D.	—
Uridine-4-^{14}C-5′-monophosphate, ammonium salt	16.6	Freeze-dried solid under nitrogen	—20	9	1	0.8
Uridine-^{14}C(U)-5′-monophosphate, ammonium salt	223	Aqueous solution	—40	12	1	0.05
Uridine-4-^{14}C-5′-triphosphate, ammonium salt	30.4	Aqueous solution containing 2 % ethanol	—20	7	2	1.2

TABLE 10 *(continued)*

Compound	Specific activity mc/mM	Storage conditions	Temp. °C	Storage time months	Decomp. %	G(-M)
*Carbohydrates**						
D-Arabinose-^{14}C(U)	3.9	Freeze-dried solid	—40	25	2	2.5
2-Deoxy-D-ribose-^{14}C(U)	1.7	Freeze-dried solid	—40	15	N.D.	—
Dulcitol-1-^{14}C	8.2	Freeze-dried solid	—20	5	N.D.	—
Erythritol-^{14}C(U)	2.3	Freeze-dried solid	—20	25	N.D.	—
D-Fructose-^{14}C(U)	86.2	Dried on paper	—40	14	N.D.	—
D-Galactosamine-1-^{14}C hydrochloride	3.8	Freeze-dried solid	—40	16	N.D.	—
D-Galactose-1-^{14}C	3.8	Freeze-dried solid	—40	30	N.D.	—
D-Galactose-1-^{14}C	35.4	Dried on paper	—40	11	1	0.3
D-Glucosamine-1-^{14}C hydrochloride	4.1	Freeze-dried solid	—40	15	3	6.0
D-Glucose-1-^{14}C	39.7	Dried on paper	—40	8	N.D.	—
D-Glucose-1-^{14}C	39.7	Aqueous solution containing 3 % ethanol	—40	6	N.D.	—
D-Glucose-1-^{14}C	57.5	Aqueous solution containing 3 % ethanol	—40	3	N.D.	—
D-Glucose-2-^{14}C	21.1	Dried on paper	—40	17	1	0.3
D-Glucose-2-^{14}C	33.4	Aqueous solution containing 3 % ethanol	—40	5	N.D.	—
D-Glucose-6-^{14}C	27.5	Aqueous solution containing 3 % ethanol	—40	6	N.D.	—
D-Glucose-6-^{14}C	35.2	Aqueous solution containing 3 % ethanol	—40	8	N.D.	—
D-Glucose-^{14}C(U)	194	Dried on paper	—40	12	N.D.	—
D-Glucose-^{14}C(U)	3.9	Aqueous solution	—40	10	5	15.8
D-Glucose-^{14}C(U)	65.7	Aqueous solution containing 3 % ethanol	—40	15	2	0.3
D-Glucose-^{14}C(U)	196	Aqueous solution containing 3 % ethanol	—40	7	N.D.	—
L-Glucose-1-^{14}C	2.9	Freeze-dried solid	—40	27	N.D.	—
D-Glucose-1-^{14}C-6-phosphate, disodium salt	3.0	Freeze-dried solid	—40	30	N.D.	—

* Nucleosides and nucleotides are listed under heterocyclic compounds.

TABLE 10 *(continued)*

Compound	Specific activity mc/mM	Storage conditions	Temp. °C	Storage time months	Decomp. %	G(-M)
D-Glucose-^{14}C(U)-6-phosphate, disodium salt	127	Dried on paper	—40	23	N.D.	—
Lactose-1-^{14}C	7.5	Freeze-dried solid	—40	11	N.D.	—
Maltose-^{14}C(U)	4.5	Freeze-dried solid	—40	10	N.D.	—
D-Mannose-1-^{14}C	3.5	Freeze-dried solid	—40	23	2	3.1
D-Mannose-1-^{14}C	31.2	Dried on paper	—40	36	N.D.	—
D-Mannose-^{14}C(U)	4.9	Freeze-dried solid	—40	35	2	1.4
Methyl (α-D-Gluco-^{14}C(U)) pyranoside	131	Dried on paper	—40	35	N.D.	—
D-Ribose-1-^{14}C	8.2	Freeze-dried solid	—40	11	N.D.	—
D-Ribose-1-^{14}C	26.8	Dried on paper	—40	22	1	0.2
D-Ribose-^{14}C(U)	2.0	Freeze-dried solid	—40	16	N.D.	—
Sorbitol-1-^{14}C[D-Glucitol-1-^{14}C]	3.2	Freeze-dried solid	—20	12	N.D.	—
L-Sorbose-^{14}C(U)	2.5	Freeze-dried solid	—40	12	N.D.	—
Steroids						
Cholesterol-4-^{14}C	55.8	Benzene solution	20	3	N.D.	—
Cholesteryl (linoleate-1-^{14}C)	16.0	Benzene solution	20	11	2	1.4
Cholesteryl-4-^{14}C linoleate	20.9	Benzene solution	20	23	N.D.	—
Cholesteryl (oleate-1-^{14}C)	10.4	Benzene solution	20	23	2	1.0
Cholesteryl-4-^{14}C palmitate	21.2	Benzene solution	20	22	2	0.5
Corticosterone-4-^{14}C	32.4	Benzene solution containing 2 % ethanol	20	14	N.D.	—
Cortisol-4-^{14}C*	30.4	Benzene solution containing 10 % ethanol	20	13	1	0.3
Cortisone-4-^{14}C	25.2	Benzene solution containing 10 % methanol	20	8	4	2.5
Cortisone-4-^{14}C	29.7	Benzene solution containing 10 % methanol	20	8	9	4.9

* See also Table 2.

TABLE 10. *(continued)*

Compound	Specific activity mc/mM	Storage conditions	Temp. °C	Storage time months	Decomp. %	G(-M)
Dehydroepiandrosterone-4-^{14}C	27.5	Benzene solution	20	23	N.D.	—
17-α-Hydroxyprogesterone-4-^{14}C	35.9	Benzene solution	20	36	N.D.	—
17-α-Hydroxyprogesterone-4-^{14}C caproate	38.3	Benzene solution	20	26	N.D.	—
Oestradiol-4-^{14}C	54.4	Benzene solution containing 5 % methanol	20	3	N.D.	—
Prednisolone-4-^{14}C	25.4	Benzene solution containing 5 % ethanol	20	3	3	4.9
Δ^5-Pregnenolone-4-^{14}C	24.0	Benzene solution	20	24	N.D.	—
Progesterone-4-^{14}C	36.1	Benzene solution	20	24	N.D.	—
Testosterone-4-^{14}C	29.2	Benzene solution	20	24	N.D.	—
19-*nor*-Testosterone-4-^{14}C	29.6	Benzene solution	20	11	1	0.4
19-*nor*-Testosterone-4-^{14}C	50.2	Benzene solution	20	4	N.D.	—

TABLE 11. Self-decomposition of some compounds labelled with sulphur-35

Compound	Specific activity mc/mM	Storage conditions	Temp. °C	Storage time weeks	Decomp. %	G(-M)
Captan-^{35}S [N-(Trichloromethylthio)-tetrahydro-phthalimide-^{35}S]	7	Solid *in vacuo* over P_2O_5	20	44	<4	<19
Captan-^{35}S	9	Solid *in vacuo* over P_2O_5	20	27	N.D.	—
Dibenzyl sulphoxide-^{35}S	5	Solid *in vacuo* over P_2O_5	20	29	N.D.	—
Dimethyl sulphoxide-^{35}S	23	Liquid	20	14	<4	<10
p-Iodobenzenesulphonyl chloride-^{35}S	160	Solid	—30	6	5	3.4
p-Iodobenzenesulphonyl chloride-^{35}S	175	Solid	—30	15	N.D.	—
p-Iodobenzenesulphonyl chloride-^{35}S	175	Solid	—30	56	11	2.1
L-Methionine-^{35}S	206	Aqueous solution, 5 mc/ml initially	—30	5	N.D.	—
L-Methionine-^{35}S	206	Sterile aqueous solution 5 mc/ml initially	20	4	1	0.7
L-Methionine-^{35}S	206	Sterile aqueous solution 5 mc/ml initially	20	10	5	1.8
L-Methionine-^{35}S	281	Aqueous solution 10.1 mc/ml initially	—30	25	6*	0.9
Mustard gas-^{35}S [*bis*-2-Chloroethyl sulphide-^{35}S]	50	Liquid	—30	12	N.D.	—
Mustard gas-^{35}S	280	Solution in diethyl ether 0.8 mg/ml	—30	30	8**	1.1
Mustard gas-^{35}S	280	Solution in diethyl ether, 0.8 mg/ml	—30	28	24	3.8
Parathion-^{35}S [0,0-Diethyl O-*p*-nitrophenyl phosphorothionate-^{35}S]	2	Liquid	20	5	4	250***
Phenyl *iso*thiocyanate-^{35}S	235	Acetonic solution, 23 mc/ml initially	—30	5	1	0.5
Phenyl *iso*thiocyanate-^{35}S	235	Acetonic solution, 7 mc/ml initially	—30	5	N.D.	—
Potassium thiocyanate-^{35}S	12	Solid *in vacuo* over P_2O_5	20	23	N.D.	—

* D-Methionine < 0.03 %.
** About one half of the impurity was thiodiglycol.
*** This value is liable to more than usual error in view of the low time, decomposition, and specific activity from which it is calculated.

Compound	Specific activity mc/mM	Storage conditions	Temp. °C	Storage time weeks	Decomp. %	G(-M)
Sodium cholesteryl sulphate-^{35}S*	5	Solid *in vacuo* over P_2O_5	20	17	N.D.	—
Sodium cholesteryl sulphate-^{35}S*	3.3	Solid *in vacuo* over P_2O_5	20	26	34	500
Sodium dehydro*epi*androsterone sulphate-^{35}S	5	Solid *in vacuo* over P_2O_5	20	22	<3	<26
Sodium ethanesulphonate-^{35}S	11	Solid *in vacuo* over P_2O_5	20	51	<4	<12
Sodium hexadecyl sulphate-^{35}S	5.5	Solid *in vacuo* over P_2O_5	20	25	<3	<22
Sodium octadecyl sulphate-^{35}S	3	Solid *in vacuo* over P_2O_5	20	45	<3	<33
Sodium pregnenolone sulphate-^{35}S	5	Solid *in vacuo* over P_2O_5	20	16	N.D.	—
Sodium testosterone sulphate-^{35}S	5	Solid *in vacuo* over P_2O_5	20	23	N.D.	—
Sodium tetradecyl sulphate-^{35}S	2.2	Solid *in vacuo* over P_2O_5	20	45	<3	<46
2-Sulphanilamido-3-methoxypyrazine-^{35}S	3.5	Solid *in vacuo* over P_2O_5	20	39	N.D.	—
Tetramethylthiuram disulphide-^{35}S	11.5	Solid *in vacuo* over P_2O_5	20	26	2	7
Thiopentone-^{35}S [5-Ethyl-5-(1-methyl-butyl)-2-thiobarbituric acid-^{35}S, sodium salt]	6	Solid *in vacuo* over P_2O_5	20	27	N.D.	—
Thiosemicarbazide-^{35}S	297	Solid *in vacuo* over P_2O_5	20	4	4	2.1
Thiosemicarbazide-^{35}S	367	Solid *in vacuo* over P_2O_5	20	11	2	0.4
Thiosemicarbazide-^{35}S	150	Solid *in vacuo* over P_2O_5	0	9	1	0.5
Thiosemicarbazide-^{35}S	150	Aqueous solution, 1 mg/ml	0	9	6	3.2
Thiosemicarbazide-^{35}S	150	Methanolic solution, 1 mg/ml	0	9	12	6.6
Thiouracil-^{35}S	2	Solid *in vacuo* over P_2O_5	20	16	N.D.	—
Thiouracil-^{35}S	8	Solid *in vacuo* over P_2O_5	20	44	<4	<17
Triethylamine-N-sulphonate-^{35}S	35	Solid *in vacuo* over P_2O_5	20	8	20	54
p-Toluene sulphonic acid-^{35}S	170	Solid	20	10	<4**	<2
p-Toluene sulphonic acid-^{35}S	210	Solid	—30	20	3	0.7

* See text.

**After storage 36 % of the activity became insoluble in ether, although it had the same radiochromatographic purity and infra-red spectrum as the soluble fraction.

(c) Sulphur-35 compounds.

Some additional results [29] on the storage of compounds labelled with sulphur-35 are given in Table 11. The discrepancy between the two results for sodium cholesteryl sulphate-S35 is of interest as the two batches were prepared by quite different methods. This is evidently another case of susceptibility to impurity, though it is worth noting that both batches were carefully recrystallised, and in fact the more stable batch had a significantly lower initial radiochemical purity.

Results of a more detailed study [29] on the self-decomposition of DL-cysteine-S35 are given in Table 12.

TABLE 12. Production of cystine from DL-cysteine-S35 hydrochloride on storage in aqueous solution.

Initial specific activity 39.5 mc/mM
Initial radioactive concentration 3.9 mc/ml
Solutions stored under nitrogen at 20°C

Time of storage Days	Cystine[a] present %	Cystine formed after 2nd day %	G(cystine)
0	1.9[b]	—	—
2	7.8[b]	—	—
15	9.4	1.6	13
33	13.5	5.7	21
70	14.6	6.8	13
126	18.5	10.7	14

[a] Determined polarographically.
[b] After the initial sample had been taken the solutions were sterilised by autoclaving and the bulk of the cystine produced in the first two days is due to this treatment.

(d) Selenium-75 compound.

L-Selenomethionine-Se75, having an initial specific activity of 1.0c/mM, exhibited no detectable decomposition or racemisation when stored for 4 months at 20° C and a radioactive concentration of 1 mc/ml, or when stored for a similar period at −30° C and 20 mc/ml [29].

(e) Phosphorus-32 compounds.

In our previous review [1] we described "DFP-P32" (di*iso*propylphosphoro-flouridate-P32) as having a high rate of self-decomposition. This was an error due to a faulty analytical method. Use of two alternative methods of analysis has enabled this figure to be revised, and from three series of expe-

riments [30] it is now possible to quote the initial rate of decomposition for this compound in propylene glycol as about 1 % per week. This is for an initial specific activity of about 80 mc/mM and a radioactive concentration of about 300 μc/ml. The impurity produced by sterilisation (2.5 Megarads) is now found to be less than 0.2 %.

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