Validity of Tritium Tracers. Stability of Tritium Atoms in Purines, Pyrimidines, Nucleosides and Nucleotides

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SUMMARY

The effect of pH and temperature on the stability of tritium atoms at various positions in purines, pyrimidines, nucleosides and nucleotides is discussed. The importance of recognizing several types of instability is stressed.

The formation of a hydrate on storing solutions of tritiated uridine and other results of the self-radiolysis of tritiated precursors of the nucleic acids are described. Alcohol solutions and low temperatures are recommended for minimising self-decomposition.

This paper draws attention to some possible artefacts and erroneous conclusions which may result from experimental data in the uses of tritiated precursors of the nucleic acids (DNA and RNA) in biochemical investigations.

The instability of the tritium *atoms* in the labelled compound and the instability of the *compound* on storage, are two causes of erroneous conclusions in experiments using tritium labelled compounds (and particularly purines, pyrimidines, nucleosides and nucleotides), which are not yet, according to our observations, sufficiently recognised.

Instability in the first sense means exchangeability of the tritium atoms under chemical or biological conditions, without other apparent decomposition, either before or during the experiment. Three kinds of tritium label can be distinguished. They are :

(*a*) a tritium label which is readily (often instantaneously) exchanged in hydroxylic solvents under neutral conditions. This is termed "labile" tritium and usually refers to tritium atoms attached to N, O or S atoms. Such compounds are of very limited value as tracers;

(*b*) a tritium label which is not exchanged under all conditions of pH and temperature, which does not result in decomposition of the compound,

and which requires the presence of a hydrogen transfer catalyst (chemical or biochemical) for exchange to occur in hydroxylic solvents. This is termed "non-labile" tritium;

(*c*) a less clearly defined tracer in which the tritium atoms are normally regarded as "non-labile" but which can be exchanged with the hydrogen atoms of a hydroxylic solvent under certain conditions of pH and temperature. This tritium is referred to as "acid-labile" or "alkali-labile" and the rate of exchange is temperature dependent.

Most of the difficulties in the uses of tritium tracers arise with compounds in this class (*c*), where the tritium atoms are acid or alkali-labile. The net effect is a change in the molar specific activity of the tracer compound being used, and may even result in a complete loss of the label.

Instability in the second sense means the decomposition of the compound by self-radiolysis, giving not only tritiated water as a possible impurity, but also tritium labelled organic impurities, the presence of which may completely invalidate the experiment.

A knowledge and understanding of these problems which can arise, enables the research worker to interpret the results of a tracer investigation with more confidence. We therefore present in this paper some relevant recent observations from our laboratory, further illustrated by other recent published work.

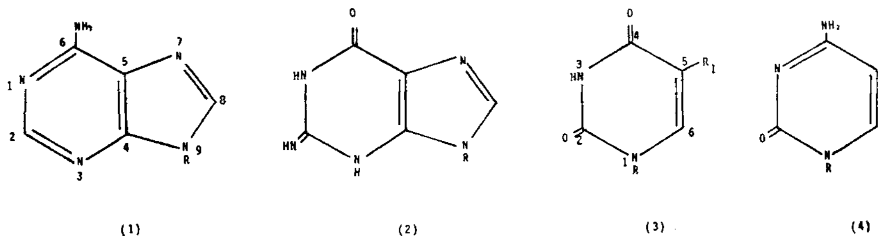
Commonly used tritiated precursors in nucleic acid studies include the purine bases, adenine and guanine; the pyrimidines uracil, cytosine, thymine and orotic acid; the nucleosides adenosine, guanosine, cytidine, uridine and the corresponding 2′-deoxy-D-ribonucleosides including thymidine; and the nucleoside 5′-mono-, di- and tri-phosphates.

A. Preparative methods and specificity of labelling.

Most labelled compound producers supply nucleic acid precursors in which the position of the tritium label is more or less certainly known, either from the method of preparation or by experimental verification. However, many of the methods used for the preparation of generally tritiated compounds yield tracers in which the specificity of the labelling is uncertain [1]. Research workers should always bear this point in mind, for it is usually best to use a tracer compound in which the specificity of the labelling is known so that any peculiar results can be more easily interpreted.

Catalysed exchange reactions [2, 3], halogen-tritium replacement [2, 4] and biochemical procedures [2] are the methods mostly used for the preparation of tritiated purines, pyrimidines, nucleosides and nucleotides. The actual method and the reaction conditions employed, often define the specificity of the labelling and determine the stability of the tritium atoms in the labelled molecules.

Nucleosides and nucleotides having the purine base moiety derived from adenine (1; R = H) contain tritium atoms in the 2- and/or 8-positions. Those labelled compounds containing the guanine (2; R = H) moiety can be labelled only in the 8-position. Tritiated compounds which have the pyrimidine base moiety, are labelled in the 5- and/or 6-positions, unless these positions are occupied by functional groups as in thymine (3; R = H; R_1 = Me). It is not usual for the labelling to be present in the sugar moiety unless the compound has been tritiated by metal catalysed exchange in a tritiated solvent [(2)] or by exposure to tritium gas [(2)]. In such cases the sugar moiety may contain 5-20 % of the tritium activity.



In tritiated thymidine (3; R_1 = Me; R = 2′-deoxy-D-ribose) the tritium atoms normally occupy the 6-position and/or are present in the 5-methyl group. In these positions the tritium atoms are quite stable under acid or alkaline conditions, even on heating to 100° C [(5)]. The stability of tritiated thymidine has been discussed in detail elsewhere [(2, 5)]; there are no special problems in the use of this compound as a tracer for DNA [(6)]. However, it is worth citing the investigations of Fink and Fink [(7)] concerning the relative retention of tritium and carbon-14 labels of nucleosides incorporated into nucleic acids of *Neurospora*, as an example of the importance of knowing the specificity of the labelling.

B. STABILITY OF THE TRITIUM ATOMS.

The stability of tritium atoms in molecules under biological conditions has been discussed [(2, 8)]. In this paper we are concerned with the loss of the tritium label by exchange with hydrogen atoms of the solution, which may occur as a result of the experimental conditions used, such as pH and temperature for example.

(i) *Compounds containing a pyrimidine base moiety.* Under neutral conditions of pH at +2° C the rate of exchange of tritium atoms from the 5- and 6-positions of the pyrimidine moiety of compounds stored in aqueous solution is slow. It is about 5 % after 1 year from the 5-position and less than 1 % per annum from the 6-position [(9)]. However, Spanner, Stanford and

Waterfield [9] have shown that even under neutral pH conditions, the autoclaving of tritiated pyrimidines in solution at 121° C (15 p. s. i.) for 120 minutes results in up to 15 % of the tritium being exchanged from the 5-position but less than 2 % from pyrimidines labelled in the 6-position.

Fink [10] showed that heating in acid solution exchanged all the tritium activity from the 5-position of the pyrimidine moiety. Thus uracil-5-T, uridine-5-T and cytidine-5-T all lose their tritium label when heated in *N*-sulphuric acid at 100° C during 9 days. Uracil-5-T exchanges its tritium atom about 5 times faster than uridine-5-T under these conditions [10]. Similar losses occur under alkaline conditions. Thus uridine-5-T exchanged 73 % of its tritium over 4 days, while cytidine-5,6-T lost all its tritium from the 5-position, in 2*N*-barium hydroxide at 100° C [10].

Thus, in the vigorous acid or alkaline hydrolysis of the tritiated nucleic acids, isotope losses by exchange can be expected if the tritium atom is in the 5-position of the pyrimidine moiety of the tracer compound used. We therefore recommend the use of precursors labelled (and shown to be labelled) in the 6-position of the pyrimidine moiety if acidic or basic conditions are used at temperatures exceeding 50° C.

(ii) *Compounds containing a purine base moiety.* In 1967 Shelton and Clark published [3] an elegant exchange method for the preparation of tritiated purines, purine nucleosides and nucleotides, labelled in the 8-position of the purine ring moiety. The method is a simple base catalysed exchange of tritium from tritiated water into the purine ring at 100° C. The exact mechanism of this basic exchange reaction is not known but some kinetic data have been published [3]. This procedure, and catalysed halogen-tritium replacement reactions, are used by many commercial suppliers of tritiated compounds, for labelling nucleic acid precursors.

Conversely it has been reported recently that tritium losses by exchange occur during the alkaline hydrolysis of RNA labelled with guanosine-8-T [11, 12]. Even at 37° C in 0.4 *N*-potassium hydroxide for 18 hours, up to 30 % tritium activity was reported lost by exchange from guanosine and guanosine monophosphate, while less than 15 % was lost from uridine and uridine monophosphate labelled in the 5-position [12].

Of course with labelled compounds containing the guanine ring moiety (2; R = H), only the 8-position of the purine ring can be labelled. However, with compounds which have the adenine ring (1; R = H) both the 2 and the 8 positions can carry the tritium label. We have therefore carried out some preliminary investigations on the relative stability of the tritium atoms in the 2 and 8 position of adenine under several conditions of pH and temperature The results are summarised in the Table I.

Losses of tritium atoms by exchange from the 8-position of adenine-2,8-T (prepared by platinum catalysed exchange in tritiated water [2]), adenosine-T(G) (mainly 2,8-T) and guanosine-8-T under almost neutral conditions at 121° C

TABLE 1. Exchange of tritium from adenine-T in solution [a].

Time hrs	Temperature °C	pH	Percent loss of tritium (forming THO)	
			Adenine-2-T	Adenine-8-T
0.3	121° [b]	1	< 2	< 5
24	37°	5.1	3	8
1	100°	7.4	6	48
3	121° [b]	7.4	20	97
24	37°	8	3	3 [c]
0.3	121° [b]	9-10	42 [d]	53

[a] Adenine 2-T was prepared by catalytic dehalogenation of 2-chloroadenine (cf. ref. 4) and adenine-8-T by base catalysed exchange in tritiated water (3). The molar specific activities were respectively 12 Ci/mmole. and 1.4 Ci/mmole and the radioactive concentration 2.2 mCi/g solution. Tritiated water was isolated by distillation of the solution *in vacuo* and measurements of the tritium activity made using a Nuclear Chicago Mark I liquid beta scintillation counter (cf. ref. 13).

[b] Autoclaved at 15 p.s.i.

[c] Van Dyke *et al.* (14) did not report any loss of the tritium atom from adenosine-8-T when an alkaline solution (1*N*-KOH) was incubated for 20 hours at 37° C.

[d] A further ~ 40 % is exchanged if the heating is repeated.

(autoclave) during 20 minutes, have been reported [9]. The losses were respectively 59, 54 and 98 percent. It is seen from the Table I that the tritium atoms in the 2 and the 8 positions of the purine ring are relatively stable on heating in acidic solutions, in agreement with other investigators [3, 12, 15]. For example, hydrolysis of RNA with 1 *N*-perchloric acid at 25° C for 20 hours resulted in no loss of tritium from the guanine-8-T moiety [11]. However, under strongly basic conditions, even the tritium atom in the 2-position of the purine moiety is exchanged, but less readily than the tritium atom at the 8-position.

The attention of research workers is drawn to these losses of tritium by exchange and the need to apply corrections especially in any quantitative investigations e.g. in the assay of enzyme activity [16, 17]. A double isotope experiment with the corresponding carbon-14 compounds enables such corrections to be made [12].

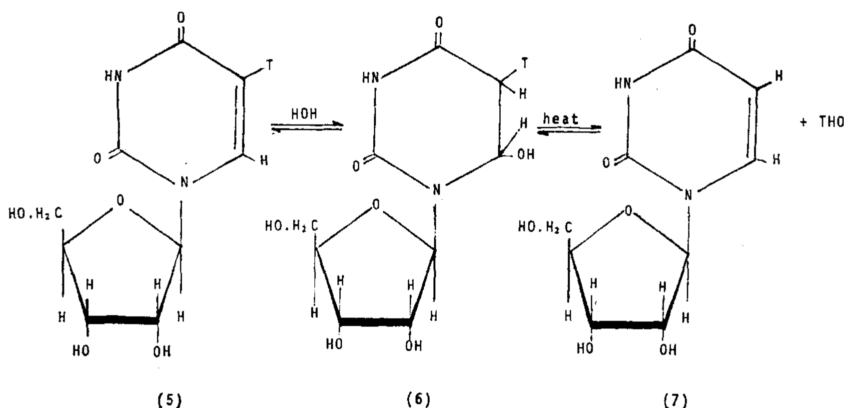
C. STABILITY OF TRITIATED NUCLEIC ACID PRECURSORS ON STORAGE.

The importance of radiochemical purity in tracer uses of tritiated compounds has been discussed in many publications including a number of reviews [1, 8, 18]. The presence of impurities can lead to false conclusions from

autoradiographs [19] and to spurious isotope effects [20] for example, and some special points are discussed in this paper.

One must always be aware of the possibility of some "labile" tritium (as THO) being formed during the storage of tritiated purines, pyrimidines, nucleosides and nucleotides in aqueous solution [1, 9]. This can arise by slow exchange either from the parent compound or from self-radiolysis products. Guanosine-8-T (at 4.9 Ci/mmole) for example, gave 13 % labile tritium on storage in solution (1 mCi/ml) at +2° C during 6 weeks, without detectable decomposition [9], whereas orotic acid-5-T (at 4.6 Ci/mmole) gave 66 % labile tritium on storage in solution (1 mCi/ml) during 32 weeks. Although only 5 % other radiochemical impurities were detected, analysis by ultraviolet light absorption spectrometry showed that 67-70 % of the orotic acid-5-T had decomposed. Note that paper and thin-layer chromatographic analysis will not detect tritiated water and although the compound may "appear" radiochemically pure by these methods, extensive decomposition may have occurred yielding tritiated water as the only radioactive product. The presence of tritiated water as an impurity, while easily removed by lyophilization, can result in some misleading conclusions in the uses of tritium labelled compounds, for example in the assay of enzymes by "tritium release" measurements [16].

Hydrate formation. An interesting phenomenon was observed during the storage of uridine-5-T at very high molar specific activity (23 Ci/mmole) in aqueous solution (1 mCi/ml) at +2° C. After 7 weeks the uridine-5-T (5) no longer exhibited the characteristic light absorption spectrum of uridine (max. 262 mμ) but showed only an inflexion at 235-237 mμ. Chromatographic analysis on paper demonstrated that the compound was 97-98 % radiochemically pure, and the amount of labile tritium present as tritiated water was 1.6 %. After heating the solution at 90° C for about 10 minutes, a redetermination of the UV absorption spectrum now showed the characteristic maximum of 262 mμ for uridine, while the amount of labile tritium present had increased to 2.5 %.



These results have led us to speculate on the formation of a hydrate (6), probably in quite a small yield, induced by the tritium beta radiation. The formation of thermally labile photohydrates of uridine by UV irradiation is well known [21–24]. On warming the solution containing the uridine-5-T hydrate to form uridine (7), an increase in the amount of labile tritium (as THO) is expected [22], as indeed was found.

Storage. Many tritiated compounds are best stored in a solvent (e.g. water and/or ethanol) at liquid nitrogen temperature (−196° C) [26]. However, it is not always convenient to store solutions at this temperature. At higher temperatures recommended conditions for the storage of tritiated purines, pyrimidines, nucleosides and nucleotides at high molar specific activity (above say 1 Ci/mmole) are given in the Table 2, together with *estimates* of their decomposition to produce radiochemical impurities (excluding THO) during one month. These estimates are based on recent results from our laboratories which are given in the Table 3, and on previously published data [18].

In general, the presence of alcohol as a radical scavenger minimises the rate of decomposition of the tritiated nucleic acid precursors in solution. Note however, that quite small amounts (0.5 %) of alcohol *may* inhibit the action of certain enzymes as observed for example in RNA polymerase studies [25].

TABLE 2. Storage conditions for tritiated 'nucleics'.

Compound class	Recommended storage conditions	Radioactive concentration mCi/ml	Temp. °C	Estimated decomposition % per month
Purines	Solid	—	−20	0.5-1
Purines	Aqueous solution	1.0	+ 2	1-2
Purines	Aqueous solution containing 2 % ethanol	1.0	+ 2	0.5-1
Pyrimidines	Aqueous solution	1.0	+ 2	1-5
Pyrimidines	Aqueous solution containing 2 % ethanol	1.0	+ 2	0.5-2
Nucleosides	Aqueous solution	1.0	+ 2	1-3
Nucleosides	Aqueous solution containing 2 % ethanol	1.0	+ 2	0.5-1
Nucleoside monophosphates ammonium salt	Aqueous solution	1.0	+ 2	0.5-2
	Aqueous solution containing 2 % ethanol	1.0	+ 2	0.5-1
	Ethanol/water (1 : 1)	1.0	−20	<0.5
Nucleoside di- and triphosphates ammonium salt	Ethanol/water (1 : 1)	1.0	−20	<0.5

TABLE 3. Self-radiolysis of tritiated 'nucleics'.

Compound	Specific activity mCi/mmole	Storage conditions	Concentration mCi/ml	Temperature °C	Time months	Decomposition %	G(—M) ([18])
Adenine-2,8-T	439	Solid	—	—40	11	N.D.	—
Adenine-2,8-T	2,900	Aqueous solution	1.0	+ 2	9	22	1.1
Guanine-8-T	750	Solid	—	—40	22	4	0.3
Cytosine-5,6-T	131	Aqueous solution	1.0	+ 2	10	N.D.	—
5-Methylcytosine-T(G)	3,050	Aqueous solution	1.0	+ 2	6	3	0.2
Thymine-(*methyl*-T)	14,000	Aqueous solution	1.0	+ 2	4	N.D.	—
Thymine-T(G)	3,528	Solid	—	—40	18	20	0.4
Thymine-T(G)	2,600	Aqueous solution	8.2	—40	11	20	0.9
Thymine-T(G)	12,200	Aqueous solution	1.0	+ 2	4.5	18	0.4
Uracil-5,6-T	7,900	Aqueous solution	1.0	+ 2	4.5	N.D.	—
Uracil-5-T	23,350	Aqueous solution	1.0	+ 2	4	3	0.05
Uracil-5-T	1,000	Aqueous solution	1.0	+ 2	11	1	0.1
Uracil-6-T	6,230	Aqueous solution	1.0	+ 2	5	2	0.1
Uracil-5,6-T	5,600	Aqueous solution containing 1 % ethanol	0.5	+ 2	3.5	2	0.1
Adenosine-T(G)	2,880	Aqueous solution	4.9	+ 2	4.5	14	1.3
Adenosine-T(G)	2,340	Aqueous solution	1.0	+ 2	6	N.D.	—
Adenosine-T(G)	2,900	Aqueous solution	1.0	+ 2	6	5	0.3
Cytidine-T(G)	2,500	Aqueous solution	1.0	+ 2	8	5	0.3
Cytidine-T(G)	4,540	Aqueous solution	1.0	+ 2	8	N.D.	—
Cytidine-5-T	15,000	Aqueous solution	2.2	+ 2	14	25	0.2
Cytidine-5-T	25,000	Aqueous solution	1.0	+ 2	17	15	0.1
Deoxyadenosine-T(G)	1,540	Aqueous solution	1.0	+ 2	8	<3[a]	<0.3
Deoxycytidine-5-T	8,300	Aqueous solution	1.0	+ 2	8	25	0.5
Deoxycytidine-5-T	14,700	Aqueous solution	1.0	+ 2	5	N.D.	—
Deoxycytidine-5-T	500	+ 2 % ethanol	1.0	+ 2	7	N.D.	—

TABLE 3. Self-radiolysis of tritiated 'nucleics'.

Compound	Specific activity mCi/mmole	Storage conditions	Concentration mCi/ml	Temperature °C	Time months	Decomposition %	G(—M) ([18])
Deoxycytidine-5-T	5,050	Aqueous solution	1.0	+ 2	3.5	20	2.9
Deoxyuridine-5-T	8,530	Aqueous solution	1.0	+ 2	6	N.D.	—
Deoxyuridine-6-T	21,400	Aqueous solution	1.0	+ 2	3	N.D.	—
Guanosine-T(G)	240	Solid	—	—20	20	7	1.6
Guanosine-T(G)	1,250	Solid	—	—20	6	N.D.	—
Guanosine-8-T	500	Aqueous solution	1.0	+ 2	8	N.D.	—
Guanosine-8-T	6,000	Aqueous solution	1.0	+ 2	5	5	0.2
Inosine-T(G)	1,050	Aqueous solution	1.0	+ 2	16	3^b	0.2
Thymidine-(*methyl*-T)	18,000	Ethanol/water (7 : 3)	5.0	+ 2	5	<2	<0.03
Thymidine-(2'-*deoxy-D-ribose*-5-T)	3,800	Aqueous solution	1.0	+ 2	3	N.D.	—
Thymidine-6-T(*n*)	36,600	Aqueous solution	1.0	+ 2	2	<2	<0.03
Uridine-T(G)	4,250	Aqueous solution	1.0	+ 2	3	<2	<0.2
Uridine-5-T	5,000	Aqueous solution	1.0	+ 2	8.5	25	0.8
Uridine-5-T	23,000	Aqueous solution	1.0	+ 2	2	2	0.05
Uridine-6-T	4,030	Aqueous solution	1.0	+ 2	7	12	0.5
Uridine-6-T	6,000	Aqueous solution	1.0	+ 2	5	13	0.5
Adenosine-T(G)-5'-monophosphate	644	Aqueous solution	1.0	+ 2	12	<5	<0.8
(lithium salt)	885	Aqueous solution	1.0	+ 2	8	8	1.2
Adenosine-8-T-3', 5'-cyclic monophosphate [c]	2,340	Ethanol/water (1 : 1)	1.0	+ 2	7	<2	<0.1
Cytidine-5-T-5'-	6,750	Aqueous solution	1.0	+ 2	7	3	0.1
monophosphate [c]	500	Ethanol/water (1 : 1)	1.0	+ 2	4	N.D.	—
	8,900	Ethanol/water (1 : 1)	1.0	+ 2	3	N.D.	—
Deoxycytidine-5-T-5'-	9,200	Aqueous solution	1.0	+ 2	5	35	1.1
monophosphate [c]	3,950	Aqueous solution + 2 % ethanol	1.0	+ 2	6	N.D.	—

TABLE 3. Self-radiolysis of tritiated 'nucleics'.

Compound	Specific activity mCi/mmole	Storage conditions	Concentration mCi/ml	Temperature °C	Time months	Decomposition %	G(—M) (18)
Deoxyuridine-5-T-5'-monophosphate [c]	2,650	Aqueous solution	1.0	+ 2	4	20	2,4
	2,830	Aqueous solution + 2 % ethanol	1.0	+ 2	4	N.D.	—
Guanosine-8-T-5'-monophosphate [c]	4,100	Ethanol/water (1 : 1)	1.0	+ 2	6	N.D.	—
Thymidine-(*methyl*-T)-5'-monophosphate [c]	2,200	Aqueous solution	1.0	+ 2	7	<5	<0.4
	7,540	Aqueous solution	1.0	+ 2	5	<5	<0.1
Uridine-5-T-5'-monophosphate [c]	572	Aqueous solution	1.0	+ 2	12	<3	<0.6
	6,630	Aqueous solution	1.0	+ 2	6	<3	<0.1
	7,320	Aqueous solution	1.0	+ 2	12	35	0.5
	11,250	Aqueous solution	1.0	+ 2	6	28	0.6
Uridine-5-T-5'-triphosphate sodium salt	1,860	Aqueous solution + 2 % ethanol	1.0	+ 2	10	23	1.6
	1,500	Ethanol/water (1 : 1)	1.0	—20	2	N.D.	—
	1,500	Ethanol/water (1 : 1)	1.0	+20	2 days	N.D.	—

N.D. = none detectable (less than 1 %); decomposition data does not include labile tritium as THO unless stated.

[a] 14 % labile tritium as THO.

[b] 20 % labile tritium as THO.

[c] Ammonium salt.

The tritiated nucleoside triphosphates are more sensitive to self-decomposition than the corresponding di- and monophosphates, and on storage in aqueous solutions the triphosphates decompose initially to a mixture of the diphosphate (mainly) and the monophosphate. The di- and mono-phosphates then decompose at a lower rate forming the nucleoside and eventually the free purine or pyrimidine base together with other self-radiolysis products.

Conclusions. Research workers are recommended to use, whenever possible, specifically labelled tritiated nucleic acid precursors as tracers. The tritium atoms are most stable in these compounds when the labelling is in the 2-position of the purine ring moiety or the 6-position of the pyrimidine ring. In any quantitative studies, if compounds labelled in other positions are used, a careful check must be made for any loss of tritium atoms by exchange. In general, aqueous ethanol is recommended as the solvent for the storage of tritiated "nucleics ".

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REFERENCES

1. Evans, E. A. — Tritium and its Compounds, Ch. 6, London, Butterworths, (1966).
2. Evans, E. A. — Tritium and its Compounds, Ch. 4, London, Butterworths (1966).
3. Shelton, K. R. and Clark, J. M. — *Biochemistry*, **6** : 2735 (1967).
4. Vaalburg, W., Van Zanten, B. and Buijze, C. — *Recueil*, **87** : 827 (1968).
5. Evans, E. A. and Stanford, F. G. — *Nature*, **199** : 762 (1963).
6. Cleaver, J. E. — Thymidine metabolism and cell kinetics, Amsterdam, North-Holland, (1967).
7. Fink, R. M. and Fink, K. — *J. Biol. Chem.*, **237** : 2889 (1962).
8. Evans, E. A. — Paper presented at the 9th Japan Conference on Radioisotopes, Tokyo 13-15 May, 1969. Abstracts, page 339 (Proceedings to be published).
9. Waterfield, W. R., Spanner, J. A. and Stanford, F. G. — *Nature*, **218** : 472 (1968).
10. Fink, R. M. — *Arch. Biochem. Biophys.*, **107** : 493 (1964).
11. Wilt, F. H. — *Analyt. Biochem.*, **27** : 186 (1969).
12. Manor, H., Goodman, D. and Stent, G. S. — *J. Mol. Biol.*, **39** : 1 (1969).
13. Turner, J. C. — *Int. J. Appl. Radiat. Isotopes*, **20** : 499 (1969).
14. Van Dyke, K., Szustkiewicz, C., Lantz, C. H. and Saxe, L. H. — *Biochem. Pharmacol.*, **18** : 1417 (1969).
15. Eidinoff, M. L. and Knoll, J. E. — *J. Amer. Chem. Soc.*, **75** : 1992 (1953).
16. Oldham, K. G. — *Radiochemical Methods of Enzyme Assay*, 34 (Radiochemical Centre, Amersham, 1968) (RCC Review Series No. 9).
17. Scholtissek, C. and Rott, R. — *J. Gen. Virol.*, **4** : 125 (1969).
18. Bayly, R. J. and Evans, E. A. — *Storage and Stability of Compounds Labelled with Radioisotopes.* (Radiochemical Centre, Amersham, 1968) (RCC Review Series No. 7).
19. Wand, M., Zeuthen, E. and Evans, E. A. — *Science*, **157** : 436 (1967).
20. Oldham, K. G. — *J. Labelled Compounds*, **4** : 127 (1968).

21. WITKOP, B. — *Photochem. Photobiol.*, **7** : 813 (1968).
22. CHAMBERS, R. W. — *J. Amer. Chem. Soc.*, **90** : 2192 (1968).
23. NOSSEN, S. S. and FRÖHOLM, L. O. — *Int. J. Appl. Radiat. Isotopes*, **20** : 363 (1969).
24. MATSUKAGE, A., KAWADE, Y. and FUKUTOME, H. — *Radiat. Res.*, **37** : 617 (1969).
25. STRAAT, P. A., Ts'o, P. O. P. and BOLLUM, F. J. — *J. Biol. Chem.*, **243** : 5000 (1968).
26. EVANS, E. A. — *Nature*, **209** : 169 (1966).