A NEW APPROACH TO SPECIFIC LABELLING OF ORGANIC COMPOUNDS WITH TRITIUM: CATALYSED EXCHANGE IN SOLUTION WITH TRITIUM GAS

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Received on May 24, 1974

SUMMARY

In a new approach to the specific labelling of organic compounds with tritium use is made of isotope exchange with tritium of certain specific hydrogen atoms in organic molecules when stirred with tritium gas in solution at room temperature in the presence of a metal hydrogen transfer catalyst. Specifically labelled tritiated compounds of high specific activity and radiochemical purity are obtained.

The method can be used for labelling a wide variety of organic compounds including purines, purine nucleosides and nucleotides, aromatic amines and amino acids, carbohydrates and steroids. Numerous examples are cited and possible mechanisms discussed.

INTRODUCTION

Isotope exchange reactions are widely used for the labelling of organic compounds with tritium[1], either by catalysed exchange in a tritiated solvent or by exposing the compound to tritium gas (Wilzbach method) in radiation catalysed isotope exchange procedures[2,3]. Both these general exchange methods have their limitations[1], in particular the non-specificity of the labelling.

During the preparation of specifically labelled compounds by catalysed reduction of halogen-containing intermediates in solution with tritium gas, some non-specific labelling is often observed to occur at the same time. An example is the preparation of [3',5',9-3H]folic acid by the catalytic reduction of 3',5'-dibromo-folic acid[4]. Numerous other examples have been noted (e.g. [5,6-3H]-uridine from 6-bromouridine and [2,8-3H]adenine from 2-chloroadenine[5]) but were not investigated in detail, until now. As the amount of exchange in these "non-specific" positions is often fairly high, it was thought possible that some tritium compounds might be prepared at high specific activity by simply exposing the parent compound to the catalytic tritiation conditions.

This paper describes the results obtained over a period of several years from 1968, when representative members of the various classes of organic compounds, e.g. amino acids, carbohydrates, nucleic acid precursors, steroids etc. were treated in this way.

There have been reports[6-10] of a similar mild tritium exchange method, but the molar specific activities obtained have been rather low (usually less than 100 mCi/mmol) and a limited number of compounds tested.

A recent report[11] describes the use of this technique of "gas/solution" exchange for the preparation of deuterium labelled oestrogens.

GENERAL PROCEDURE

In 1962 Evans[12] showed that in the presence of a platinum or palladium catalyst, tritium gas and water at pH <7 (ie in acidic conditions) undergo rapid exchange. The exchange was found to be at least 15 times slower in alkaline solutions. Thus, many tritium labelled compounds are now prepared at high molar

specific activity by catalysed reduction with tritium gas in alkaline solution. So far, such high specific activity labelling has been unsuccessful in aqueous acidic solutions. However, it has been shown[5] that weak organic acids such as glacial acetic acid can be used as solvent under certain catalytic conditions which favour rapid labelling of the substrate and a slow exchange labelling of the solvent.

In the labelling method we now describe it is necessary to use aqueous solutions of pH 7 (and above), glacial acetic acid or, for some compounds, neutral organic solvents such as ethyl acetate.

The method consists of stirring the organic compound (10-50 mg) with the catalyst (50-250 mg) in the solvent (1-5 ml) under tritium gas (10-25 curies) for periods of 5 minutes to 20 hours. In our investigations phosphate buffer of the required pH was normally used as the solvent in order to maintain the pH of the aqueous solution above pH 7. Acetic acid was used only for the exchanges of benzylic hydrogen atoms (see later). Many of the common supported catalysts have proved satisfactory. However, the palladium oxide on barium sulphate catalyst described by Kuhn and Hass[13] has been found most generally useful in the labelling of the compounds so far tested.

Following the exchange reaction, the catalyst is filtered off and readily labile tritium removed in vacuo (by successive evaporations of a protic solvent). The product is then purified by standard procedures, e.g. paper, thin-layer and column chromatography.

RESULTS AND DISCUSSION

A wide range of compounds was studied and those which labelled successfully

(ie to give clean products with a specific activity in excess of 500 mCi/mmol) fell into three main classes, A, carbohydrates, B, benzylic compounds (those compounds containing hydrogen atoms in benzylic positions) and C, nucleics (compounds containing a purine nucleus). As the site of tritiation is different in each of these cases, the results are discussed initially under the three headings.

A. Carbohydrates

D-Glucose was chosen as a typical carbohydrate. Using 0.1M phosphate buffer, pH 7 and $PdO/BaSO_4$ catalyst it was found that within the period of one hour a radiochemically pure tritium labelled D-glucose at high specific activity was produced. Oxidation[14] to D-gluconic acid resulted in the loss of more than 99% of the tritium (under which conditions D-[2-3H]glucose retained all its activity), thus showing that the product was D-[1-3H]glucose, i.e. was specifically labelled at C_1.

The exchange reaction was then studied in more detail to establish optimum conditions for the gas/solution exchange. It was found that good yields and high specific activities could be obtained using D-glucose (0.12 mmol) with $PdO/BaSO_4$ catalyst (100 mg) in pH 7 phosphate buffer and 10 curies of tritium gas with a reaction time of two hours. Under these conditions a radiochemically pure product could be obtained in good yield and high specific activity (see Table 1). The yield (and specific activity) could be increased somewhat with a longer reaction time (30% in 4 hours, 40% in 16 hours). However, an increase in reaction time also resulted in the formation of radioactive impurities (up to 3%).

To see if the yield or specific activity could be increased using a non-aqueous solvent, N,N-dimethylformamide was used instead of the phosphate buffer.

TABLE 1 CARBOHYDRATES

Compound	Crude material yield (mCi)	Crude material radiochemical purity (%)	Specific activity of pure product (mCi/mmol)	Specificity of labelling (% in C_1)
D-Erythrose	2190	92	18,300	-
L-Arabinose	1130	>95	10,000	>99[c]
D-Lyxose	908	95	10,100	-
D-Ribose	1840	>95	15,300	-
D-Xylose	741	98	7,130	-
2-Deoxy-D-ribose	925	97	15,000	-
D-Fructose	120	~50	750	98[d]
D-Galactose	554	96	6,160	-
D-Glucose	470	>98	3,900	>99[c]
D-Mannose	1140	76[a]	8,700	-
2-Deoxy-D-galactose	1715	99	14,300	-
2-Deoxy-D-glucose	1160	>97	10,800	98[e]
L-Fucose	150	>98	1,000	>99[c]
L-Rhamnose	240	>95	1,950	-
N-Acetyl-D-glucosamine	423	>95	4,400	98[f]
D-Glucosamine hydrochloride	440	>90	3,940	97[f]
D-Glucose-1-phosphate	15.5	0	-	-
Methyl-α-D-glucoside	14.5	<10	120	-
Methyl-β-D-glucoside	12.4	<10	100	-
3-O-Methyl-D-glucose	354	>99	3,450	>98[e]
Sorbitol	6.4	~20	-	-
Lactose	357	>98	3,000	>98[g]
Maltose	135	90	1,100	98[c]
Sucrose	54	0[b]	-	-
Dextran	50	71	1,920	-

Carbohydrate (0.12 mmol), $PdO/BaSO_4$ catalyst (100 mg), 0.1M sodium phosphate buffer pH 7 (0.5 ml), water (1.5ml) stirred under tritium gas (10 Ci) for 2 hours.

a) most of impurity was D-[3H]mannitol; b) no [3H]glucose, [3H]fructose or [3H] sucrose present; c) see ref. 14; d) see ref. 15; e) see ref. 16; f) see ref. 17; g) hydrolysis shows >98% of activity in D-glucose moiety.

The result was disappointing; a yield of only 16 mCi of crude product consisting of five components was obtained.

The effect of batch to batch variation in the catalyst was next investigated. Quite large differences in yield and specific activity were noted with different batches of catalyst, the specific activity of the tritiated glucose varying from 2-5 curies/mmol. The radiochemical purity of the product, however, was high in all cases. It was also found that storage of the catalyst for several months resulted in decreased radioactive yields.

A range of carbohydrates was then studied, in order to identify which structural features were necessary for tritium labelling by this new exchange method. The particular examples were chosen to study the effect of chain length and ring size; the effect of substituents at C_1, C_2 and C_6 and the effect of the sugar being part of a dimer or polymer. The results are summarised in Table 1.

The general conclusions are as follows:(a) Any reducing sugar can be labelled by the new isotope exchange method to give a relatively pure product, in high yield and at high specific activity. In all cases where the specificity of labelling was studied, the labelling was found to be exclusively at C_1. (b) Non-reducing sugars (except for D-fructose) give low radioactive yields and the crude product usually consists of several components. As would be expected, dextran, maltose and lactose could be labelled ([3H]lactose, exclusively in the glucose moiety) whereas sucrose could not. Thus, the gas/solution method has potential application for the analysis of polysaccharides which have reducing end-groups.

If the carbohydrates in Table 1 are arranged in order of increasing molar specific activity it is found that (with two major exceptions) the order falls roughly into the following groups of carbohydrates: ketose, 6-deoxyhexose, 2-

aminohexose, hexose, pentose, 2-deoxyhexose, 2-deoxypentose, tetrose. This arrangement is found to be that of increasing reactivity (for example, in the formation of glycosides[18]). The exceptions are D-mannose and D-ribose, each of which appear more reactive than the other carbohydrates in the respective groups of hexoses and pentoses. It is precisely these two sugars which have been well established as possessing anomalous high reactivity, see for example, the work of Reeves[19].

Since it seemed likely that an acyclic form of the sugar, possibly the free aldehyde form, was involved in the gas/solution exchange, several derivatives were examined under the exchange conditions to test this hypothesis. The α- and β-methylpyranosides of D-glucose, which possess a fixed pyranose ring form and cannot open, but are otherwise of identical configuration to D-glucose, gave no tritiated products from the exchange. Similarly, α-D-glucose-1-phosphate gave no radioactive product. Sucrose, a non-reducing disaccharide of D-glucose and D-fructose, which cannot ring open, likewise gave no active product. Sorbitol gave little product, thus indicating the need for a keto-/aldehydo-group for tritium exchange to be possible.

Overend et al[20] calculated for various carbohydrates a function "B" which was a measure of the ease of transformation from the cyclic to reducible form, appropriately weighted to allow for the back reaction. If a graph is drawn of the molar specific activity of the tritiated carbohydrates formed by the gas/solution exchange against Overend's log B value a straight line passing through the origin is obtained. This strongly suggests that similar factors are influencing the gas/solution exchange as are operative in Overend's polarography studies, ie these factors are affecting the transformation from cyclic to reducible (for polarographic wave measurements) or to acyclic form (for gas/solution exchange).

In many of the exchange reactions the amount of labile tritium formed in the solution was measured. It was found with the carbohydrates that for a given length of reaction time the amount of labile tritium formed was fairly constant and not related to the structure of the carbohydrate. Also, the same amount of labile tritium was produced regardless whether or not the carbohydrate was labelled.

B. Benzylic Compounds

The gas/solution exchange technique was next applied to amino acids. Those not containing an aromatic or pseudo aromatic system did not label readily, for example, L-alanine gave only 10 mCi of a crude product which consisted of several components. On the other hand, L-3-phenylalanine was found to give a labelled product at fairly high specific activity (750 mCi/mmol). Using a higher catalyst to compound ratio, a product at 6.7 curies/mmol was later obtained (see Table 2). Degradation (alkaline permanganate oxidation) showed that less than 2% of the activity was in the benzene ring and labilisation of tritium from the 2-position *via* the azlactone[21] showed less than 6% of the activity at C_2 of the side-chain. A reverse isotope dilution analysis as L-phenylalanine showed the product to be 100% pure. Thus, using the new gas/solution exchange L-phenylalanine was labelled specifically (>92%) at the benzylic position, without loss of optical purity.

Other aromatic amino acids and other compounds containing benzylic protons were found to label readily (see Table 2). In general, the compounds were not chosen to demonstrate slight structural differences which might throw light on a possible exchange mechanism but rather because of their interest for research applications. However, various interesting observations can be made.

1. The tritiation is specific at the benzylic position(s) unless the benzene ring is activated and the exchange is performed in aqueous alkaline solution, when aromatic substitution can also take place. This has been demonstrated by the

TABLE 2 BENZYLIC COMPOUNDS

Compound	Weight (mg)	Catalyst† (mg)	Solvent Volume (ml)	Solvent pH	Tritium (Ci)	Reaction time (hours)	Crude material Radiochemical purity (%)	Crude material Yield (mCi)	Specific activity (mCi/mmol)
d-Amphetamine sulphate	37	100	2[d]	8.6	25	3	>95	1000*	10,000
L-3,4-Dihydroxyphenylalanine	30	250	5[c]	7	25	1	70	114*	3,100
L-3,4-Dihydroxyphenyl(2-methyl)alanine	30	250	5[c]	7	25	1	70	125*	790
3,4-Dihydroxyphenylethylamine	12	100	1.5[c]	8.6	25	1	-	160*	4,500
3,4-Dihydroxyphenylethylamine	10	100[a]	1.5[d]	8.6	25	1	-	111*	4,400
Folic acid	25	150[a]	4[e]	>14	25	1	-	304*	30,000
Folic acid	25	150	4[c]	8	25	1	<10	209	-
Glycyl-L-phenylalanine	30	250	5[c]	7	25	1	90	2620	-
l-Noradrenaline	21	100	2[c]	7	10	1	70	15	-
Oestriol	5	5[b]	1.5[g]	-	10	2	86	470*	40,000
Oestriol	5	5[b]	2[f]	-	10	2	83	171*	12,000
Oestriol-16α-(β-D-glucuronide)	5	5[b]	1.5[g]	-	10	2	86	210*	32,000
Oestetrol	4	5[b]	1.5[g]	-	10	2	93	300*	46,000
L-Phenylalanine	10	40	2[c]	7	25	1	80	68	750
L-Phenylalanine	22	250	5[c]	7	25	1	90	835	6,700
L-Phenylalanine	10	20[b]	2[g]	-	10	1	74	52	~700
L-Phenylalanylglycine	30	250	5[c]	7	25	1	90	932	7,600
Tyramine	20	100	2[c]	7	25	2	70	182	1,450

† $PdO/BaSO_4$ * yield of purified material

(a) 10% palladium on calcium carbonate (b) 10% palladium on charcoal (c) 0.2M phosphate (d) 0.2M phosphate
(e) 4% aqueous KOH (f) ethylacetate (g) glacial acetic acid.

following experiments. Degradation of [3H]amphetamine sulphate showed that 99% of the radioactivity was in the side-chain; triton NMR spectroscopy[22] showed that all the radioactivity in [3H]oestriol was at the 6 and 9 positions; labilisation of tritium from the aromatic ring by heating with acid showed up to 10% of the radioactivity in [3H]dopamine and [3H]tyramine was in the ring; alkaline permanganate oxidation of [3H]folic acid (exchange performed in 4% potassium hydroxide solution) showed[4] that only 58% of the activity was in the benzylic (9) position.

2. The stereochemistry is preserved unless the benzylic position is an asymmetric centre. Thus, L-phenylalanine, L-3,4-dihydroxyphenylalanine (L-dopa), L-3,4-dihydroxyphenyl(2-methyl)alanine (α-methyl-L-dopa) and L-phenylalanylglycine (hydrolysed and the optical purity of the L-phenylalanine determined) were tritiated without loss of optical purity, whereas l-[3H]noradrenaline showed the presence of 27% of the d-isomer. The oestrogens were obtained with a crude radiochemical purity of 83-93%. The 7-17% of impurity, which may have been due to isomerisation at the 9-position, was easily separated by chromatography and removed from the purified product.

3. Exchange predominantly into the benzylic position(s) can best be effected in basic aqueous solution or in glacial acetic acid. Many of the water soluble compounds are unstable in solutions of high pH and therefore the tritium exchange reaction was performed in buffered solution at pH 7-8.6. Under these conditions specific activities of 0.7-10 curies/mmol could be achieved. That 10% $Pd/CaCO_3$ could be used instead of the $PdO/BaSO_4$ catalyst was shown with dopamine where a similar yield and specific activity of the tritiated product was given. Surprisingly, folic acid gave poor results in phosphate buffer pH 8 but gave a good yield of tritiated product at high specific activity when the exchange was performed under "dehalogenation" conditions, that is, in the 4% potassium

hydroxide solution used as solvent for the catalytic dehalogenation of 3',5'-dibromofolic acid. Glacial acetic acid proved a better solvent than ethyl acetate for the labelling of oestrogens, when high specific activities were obtained. With phenylalanine, however, glacial acetic acid and aqueous solutions gave similar rather low specific activities. As would be expected for an exchange reaction, a higher catalyst:substrate ratio was found to give higher specific activities.

4. In general, the more stable the compound in solution the higher is the radiochemical purity of the crude product of the reaction. Thus, the catecholamines gave a crude product of approximately 70% radiochemical purity, whereas the more stable amine, amphetamine, gave an almost radiochemically pure product direct from the exchange reaction.

C. Nucleics

Using the conditions employed for the carbohydrates, adenine nucleotides gave labelled products at high specific activity but guanine nucleotides under the same conditions gave products at relatively low specific activity. In the latter cases large quantities of labile tritium, as tritiated water, were also formed.

The specific activity of the product was then investigated as a function of reaction time and experiments with inosine-5'-monophosphate showed that the specific activity rose to a maximum and then decreased (see Table 3). The time required for exchange to give significant specific activities was much shorter than that required for the carbohydrates and benzylic compounds. Similar results were found with guanine nucleotides. However, the rate of exchange of adenine nucleotides was much slower. Investigations with adenosine-5'-monophosphate and adenosine-5'-triphosphate indicated that the optimum time for exchange was about one hour and that specific activities

TABLE 3 NUCLEICS

Compound	Weight (mg)	Catalyst (mg)	Solvent+ Volume (ml)	Solvent+ pH	Tritium (Ci)	Reaction time (mins)	Crude material Radiochemical purity (%)	Crude material Yield (mCi)	Specific activity (mCi/mmol)
Adenine arabinoside	10	50	2	8.2	10	240		97•	5,600
Adenosine	30	50	1.5	8.2	10	120		675•	12,000
Adenosine-3',5'-cyclic phosphate	10	50	2.5	10	10	60	90	600	25,000
Adenosine-5'-monophosphate	25	125	2.5	10	10	960	>95	295	11,000
Adenosine-5'-triphosphate	10	100	2.5	10	25	60		349•	20,000
Deoxyadenosine	30	50	1.5‡	8.2	25	210		1,180•	17,000
Deoxyadenosine-5'-triphosphate	10	50	2.5	10	10	60		256•	18,100
N^6,O^2-Dibutyryladenosine-3',5'-cyclic phosphate	10	100	1.5	10	10	60		344•	19,500
Guanosine	30	50	1.5	8	10	120		15•	420
Guanosine-3',5'-cyclic phosphate	10	40	2	8	10	20	95	94	880
Guanosine-5'-triphosphate	10	40	2	8.2	10	30	~80	28	1,500
Inosine-5'-monophosphate	25	125	2.5	8	10	5	98	45	1,200
Inosine-5'-monophosphate	25	125	2.5	8	10	20	98	195	4,900
Inosine-5'-monophosphate	25	125	2.5	8	10	60	>95	174	3,900
Inosine-5'-triphosphate	25	125	2.5	8	10	20	~80	178	2,700
Nicotinamide	10	50	2	8	10	120	~15	2,300	-
Nicotinamide adenine dinucleotide	10	50	2	8	10	120		1,350	-
Puromycin	15	50	4	8.2	10	120		43•	3,740
Puromycin aminonucleoside	10	50	1.5	8.2	10	120		188•	13,700
Thymidine	10	50	1.2	8	10	60	~40	379	~5,000
Thymine	10	50	1.2	8	10	60	~90	581	~7,000
Uracil	10	50	1.2	8	10	60	~86	870	~9,000
Uridine	10	50	1.2	8	10	60	~10	623	~2,500

Catalyst: $Pd/BaSO_4$ + 0.1M phosphate buffer ‡dilute ammonia • yield of purified material

approaching the theoretical maximum (29 Ci/mmol) for the exchange of one hydrogen atom with tritium could be attained.

Hydrolysis of tritiated adenosine labelled by the gas/solution exchange showed (using paper chromatography) that the label was in the adenine moiety; the ribose being unlabelled, as would be expected from the results already obtained with the carbohydrate derivatives. Under the hydrolysis conditions there was no significant formation of labile tritium as tritiated water. Hence, the label in purine nucleosides and by inference, purine nucleotides is in the purine moiety. In the case of guanine derivatives the only possible position of exchange is the 8-position. Treatment of the tritiated adenine nucleotides under conditions where exchange of tritium occurs only from the 8-position[23] indicated that adenine nucleotides were labelled specifically in the 8-position. This finding has also been confirmed by triton NMR[22].

Investigations of the radiochemical purity of purine containing nucleotides indicated that exchange into monophosphates occurred with only a small amount of chemical decomposition; the radiochemical purity of compounds direct from the tritium exchange reaction being, in general, greater than 95%. Similar results were found with nucleoside diphosphates. In the case of triphosphates, some decomposition (or hydrolysis) was observed to occur but the radiochemical purity of the product was usually in excess of 80%, the main impurity being the diphosphate. The crude product could then be purified without noticeable loss of any of the tritium label.

The gas/solution exchange was then applied to other compounds containing a purine function. It was found (Table 3) that with compounds such as puromycin and its aminonucleoside, where there were no easily reducible groups, a fairly pure product at high specific activity could be produced. However, it appeared

that the readily reducible nicotinamide in nicotinamide adenine dinucleotide had been hydrogenated. This was further demonstrated by performing the exchange reaction with nicotinamide itself when a high yield of a reduced (non-u.v. absorbing) product was obtained.

The interest in tritium labelled polynucleotides, especially DNA and RNA, prompted an investigation of the effect of the gas/solution exchange on the other major nucleic acid components, uridine and thymidine. As long as these were not reduced then the mild tritium exchange procedure could be employed for the preparation of high specific activity tritium labelled polynucleotides which contained adenine and guanine residues. Unfortunately, and somewhat surprisingly, it was discovered that both uridine and thymidine were reduced under the exchange conditions, uridine to a greater extent than thymidine. In both cases, it was shown chromatographically that the main component of the reaction mixture was the dihydro-compound. Thus, the new exchange reaction has only limited use in the polynucleotide field - for the labelling of polynucleotides containing only adenine and guanine residues. As might be expected from the use of a short reaction time[24], the bases thymine and uracil were not reduced under the same tritium exchange conditions, but were, in fact, obtained tritium labelled in a fairly pure form at reasonably high specific activity.

In many of the exchange reactions the amount of labile tritium found in the solution was measured. Much more labile tritium was formed with the purines than with the carbohydrates, mentioned earlier. The amount varied with the structure of the purine and increased greatly with time, as shown in Table 4. Similarly, under identical conditions L-histidine gave a high yield (918 mCi) of a fairly pure product (greater than 94% radiochemical purity; specific activity about 13 curies/mmol), but when the same tritium exchange was repeated for a longer period of time (2 hours) the yield of tritiated histidine dropped

TABLE 4 NUCLEOSIDE MONOPHOSPHATES

Compound	Weight as salt (mg)	Yield (mCi)	Specific activity (Ci/mmol)	Labile tritium (Ci)
Adenosine-5'-monophosphate	9.46	226	12.6	2.85
Guanosine-5'-monophosphate	9.55	19.5	1.34	4.07
Inosine-5'-monophosphate	13.04	343	16.7	4.45
Xanthine-5'-monophosphate	9.8	206	11.1	3.29

Nucleoside monophosphate (0.0175 mmol as determined by ultra-violet spectroscopy), $PdO/BaSO_4$ catalyst (50 mg), 0.1M sodium phosphate buffer pH 8 (2.5 ml), stirred under tritium gas (10 Ci) for 30 minutes.

to 6 mCi and the amount of labile tritium in solution increased from 2 curies to 4 curies. Thus, it appears that the imidazole system,as present in histidine, reacts under the exchange conditions as an activated purine.

MECHANISM

The molar specific activities of the compounds labelled by the new gas/solution exchange procedure are much higher than could be explained by a theory involving exchange with the tritiated water formed by reduction of the noble metal oxide catalyst or that formed by exchange of the tritium gas with the solvent.

All the positions which are readily labelled are near centres of high electron density ie on (or in one case adjacent to) a carbon atom which has a ketonic function (carbohydrates); on a carbon atom adjacent to a benzene ring (benzylic); at the 8-position of the purine nucleus (nucleics). The

preferential labelling by exchange reactions of purines at the 8-position[25] and aromatic compounds in the side chain[26] has been reported by other workers. Thus, it would appear that the compound is bound to the catalyst at this point of high electron density. Since tritium gas is also adsorbed to the catalyst exchange can take place (with a certain degree of stereospecificity, as shown by l-noradrenaline and the oestrogens) and then the tritiated compound and tritium-hydrogen gas are desorbed (analysis of the gas above the exchange solution at the end of a two hour reaction using D-glucose showed the isotopic abundance of the tritium gas to have been lowered considerably). This type of exchange is presumably by a free radical mechanism. However, further detailed investigations are needed to determine whether this is so or whether the mechanism could involve an ionic transition state.

Apparently, there is also a second, competing reaction in which tritium labelled compounds adsorbed to the catalyst exchange their tritium atoms for hydrogen atoms from the solvent, the radioactivity appearing as tritiated water. This is most noticeable with the purines (and the imidazole, histidine) and occurs to a greater extent in the guanosine and inosine series of compounds than with the adenosine analogues, thus following the known pattern[27, 28] of lability of the hydrogen in the 8-position of purines in aqueous solutions which contain no metal catalysts.

It was shown that the presence of catalyst accelerated the labilisation of tritium from the 8-position of the purines. In a series of experiments with different purine nucleotides the general method was followed until after the tritium gas had been removed at the end of the exchange reaction. The solution was stirred *in vacuo* for one hour and then the catalyst and labile tritium were removed following the usual methods. In all cases this resulted in most of the tritium being labilised from the labelled compound into the solvent. Thus, tritium is

being exchanged at a much faster rate than occurs without a catalyst present[23, 29], ie a catalysed labilisation of tritium.

The mechanism of this second exchange reaction might also be free radical, although an ionic ylide form of intermediate as discussed by Elvidge et al[28] has more appeal.

These mechanisms would help to account for the asymptotic rise in specific activity with time observed with the carbohydrates and benzylic compounds where labilisation of tritium from the relatively stable positions is very slow, compared with the rise and (eventual) fall in specific activity encountered with the purines, where labilisation is found to be rapid.

CONCLUSIONS

A wide variety of organic compounds of interest as tracers in biological research can now be prepared specifically labelled with tritium by a catalysed isotope exchange reaction in solution using tritium gas. This new approach to labelling with tritium has many advantages over other exchange labelling methods. The technique can also be used, of course, for specific labelling with deuterium.

ACKNOWLEDGEMENTS

The authors thank Dr. J.R. Catch for his interest in this work and members of the Tritium Section of the Organic Department, The Radiochemical Centre, for their technical assistance. The permission of Dr. W.P. Grove (Managing Director, The Radiochemical Centre) and the U.K.A.E.A. to publish this paper, is gratefully acknowledged.

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