Russian Chemical Reviews **72** (5) 423 – 446 (2003) © 2003 Russian Academy of Sciences and Turpion Ltd

DOI 10.1070/RC2003v072n05ABEH000781

Solid-phase method for tritium labelling of biologically active compounds

V P Shevchenko, I Yu Nagaev, N F Myasoedov

Contents

I.	Introduction	423
II.	Key information on hydrogen spillover	423
III.	Solid-phase reactions in the synthesis of tritium-labelled preparations	425
IV.	Characteristics of solid-phase hydrogenolysis by tritium	440
V.	Conclusion	444

Abstract. The solid-phase method for conducting hydrogenolysis, hydrogenation and isotope exchange reactions is described. The potential of the solid-phase method for tritium labelling of various organic compounds including lipids, nucleotides, amino acids, peptides, *etc*. is demonstrated. The influence of the reaction conditions on the yield and on the molar radioactivity of the labelled specimens is considered. It is shown that the results obtained can be interpreted by the dependence of tritium spillover on the nature of the initial compound and other factors. The bibliography includes 158 references.

I. Introduction

Organic compounds labelled by tritium (a radioactive isotope of hydrogen) are widely used in research in chemistry and biology, in medicine and agriculture. The range of such compounds manufactured by various companies comprises hundreds of names and is being constantly extended and updated. Among labelled compounds, physiologically active substances are especially abundant, first of all, because of their use in biology and medicine. The structures of labelled products needed for various purposes become more complicated with time. Therefore, labelling procedures have to be modernised not only to prepare each of the main classes of organic compounds but also to introduce a label into various compounds of the same class.

The steadfast attention to the problem of synthesis of tritium-labelled compounds is due to the fact that tritium has an appropriate half-life (12.26 years) and high molar radioactivity. High-performance liquid chromatography allows one to isolate specimens with a radiochemical purity of 96% – 98% even in the case where highly rigorous conditions are used for labelling such that the yield of the labelled compound is only several percent. Specific binding to receptors of tritium-labelled preparations is virtually free from isotope effects; this the accounts for high sensitivity of this method and permits accurate determination of the rate constants for binding. Tritium-labelled compounds are also used in pharmacokinetic studies to determine the target organ in which a drug is mainly accumulated and the rates of excretion of this drug from the organism. Tritium compounds are also needed to study metabolism and substrate specificity of enzymes and to search for new efficient enzyme inhibitors. Recalling also the fact that tritium-labelled specimens are at least 10 times less expensive than ^{14}C-labelled ones, one can understand why every aspect of related to the preparation of tritium isotopomers of biologically active compounds arouses considerable interest.

The methods for introduction of a tritium label can be divided into two main groups, namely, various versions of isotope exchange and chemical methods (usually, tritiation, dehalogenation, selective tritiation and dehalogenation).

This review considers the solid-phase method for tritium labelling. In view of the specific nature of solid-phase (without a solvent) synthesis, hydrogenation and isotope exchange are considered as particular cases of hydrogenolysis.[1] According to this method, an organic substance is applied on a support (sorbent) where a metal catalyst has been applied preliminarily, and this system is heated under a tritium gas atmosphere. Solid-phase hydrogenolysis proved to be the process of choice for introducing a tritium label into organic compounds. Using these reactions, tritium-labelled lipids, peptides, proteins, nucleic acids, carbohydrates, glycoproteins, nucleoproteins, lipoproteins, glycolipids, alkaloids, terpenoids and many other classes of biologically active compounds have been obtained rather easily.

Plentiful data on the reactions of various compounds applied on a support with activated hydrogen species formed on the metal catalysts have been reported. The enhanced mobility of activated hydrogen on inert supports has been discovered.[2] The flow of activated hydrogen species from one phase to another has been called hydrogen spillover. It is hydrogen (tritium) spillover that determines the possibility of solid-phase hydrogenolysis. Therefore, before considering the factual information on solid-phase reactions involved in tritium labelling of organic compounds, we give an account of the main data concerning the spillover of hydrogen, which would be useful for understanding the main features and the mechanism of hydrogenolysis.

II. Key information on hydrogen spillover

In most cases, solid-phase reactions are carried out under rigorous conditions, namely, at temperatures above 200 °C (usually, at 300, 500 °C or higher). For the majority of biologically active substances, the use of high temperatures is inadmissible; these substances are studied at lower temperatures. The regularities of the occurrence of solid-phase reactions established in low- and high-temperature experiments are different. Therefore, the data

V P Shevchenko, I Yu Nagaev, N F Myasoedov Institute of Molecular Genetics, Russian Academy of Sciences, pl. Kurchatova 2, 123182 Moscow, Russian Federation. Fax (7-095) 196 02 21.
Tel. (7-095) 196 02 12 (V P Shevchenko, I Yu Nagaev).
Tel. (7-095) 196 00 01. E-mail: img@glas.apc.org (N F Myasoedov)

Received 15 October 2002
Uspekhi Khimii **72** (5) 471 – 497 (2003); translated by Z P Bobkova

available from the literature concerning the nature of activated hydrogen species and the conclusions concerning processes related to hydrogen spillover on supports (high-temperature experiments) require critical analysis to be applied to the introduction of tritium into biologically active compounds (low-temperature experiments) .

The activity of metal catalysts in the isotope exchange decreases in the series Pt > Pd > Ni.[3] The chemisorption of H_2 usually occurs at 200–300 °C.[4] The products of dissociation of molecular hydrogen on platinum migrate to the support in which a dynamic equilibrium is established between the atomic and protic forms of hydrogen.[5,6] The number of active species, for example, for silica gel, amounts to $\sim 10^{12}$ g^{-1} (see Refs 7–9). It was found that silica gel bearing activated hydrogen species is able to catalyse hydrogenation of alkenes. Under these conditions, hydrogenation mainly takes place on the sites generated owing to hydrogen spillover from the gas phase.[7–11]

Hydroxy groups, oxides or carbon can act as hydrogen acceptors.[12] For example, a study by FT IR spectroscopy has shown the occurrence of isotope exchange between deuterium and hydrogen of the OH groups on Y zeolite (ZeY).[13] It has also been shown [14] that the OH groups of the support exchange with tritium under hydrogenation conditions. The reaction between the spillover species and the CH_3O fragments of the substrate molecules has been found to give OH groups and CH_4.[15,16]

The destruction products formed in reactions can serve as carriers of the active hydrogen species from the initiator to the acceptor.[17–20]

The diffusion coefficients of hydrogen are determined using various methods including temperature-programmed desorption [21] and adsorption [22] methods, electron microscopy [23] and NMR.[24,25] The diffusion over the surface of the support can occur over a distance of up to 90–200 nm from the metal catalyst (platinum) particles.[24,25]

A mathematical model of spillover has been considered in several publications.[26–28] This model assumes that the activated species are formed on the source metal and the support conveys these species to the substrate and back. One-, two- and three-dimensional diffusion kinetic models for hydrogenolysis of a supported organic substrate have been constructed.[28] The models take into account the concentration gradient of the spillover hydrogen and poisoning of the catalyst during the reaction. It was shown that hydrogenation is almost localised in the reaction areas around the crystallites of the metal catalyst and, hence, there exists some ratio of the catalyst, the support and the substrate optimal for this particular reaction. Data supporting both the atomic and protic natures of the spillover species can be found in the literature. Currently, most researchers hold the opinion that the spillover involves hydrogen atoms.[18,29,30] It was found [18] that the rate of disappearance of the EPR signal from perylene corresponds to the rate of absorption of H_2 by the Pt/ZeY system. It is noteworthy that direct EPR monitoring of atomic hydrogen on most supports is possible only at temperatures below −170 °C. Thorough analysis of the shape of the peak and the chemical shift in the ^1H NMR spectra has shown [30] that hydrogen migrates over the SiO_2 surface as $H^{\bullet}$ radicals. The adsorption sites for these radicals may be represented by SiO or Si groups.

It has been shown by EPR that tetravalent cerium is reduced to trivalent on the ZeY + Pt(black) system in the presence of hydrogen.[31] Using *N-tert*-butyl-α-phenylnitrone or 2,4,6-tri-*tert*-butylnitrobenzene, which served as traps for hydrogen, weakly bound hydrogen atoms participating in benzene hydrogenation have been detected.[32]

It has been found that the exchange processes of $^2H_2$ with the OH groups of semiconductor supports do not differ much for *n*- and *p*-type semiconductors with applied platinum;[33] thus it can be concluded that spillover involves uncharged species.

The first step of spillover is homolytic cleavage of the H—H bond on the metal, regardless of whether hydrogen atoms or protons participate in the spillover. The rate-limiting step is either the surface diffusion on the support or the transfer of particles from the catalyst to the support. Since the activation energies of these elementary steps are different, the limiting step of spillover may change upon temperature variation.[7] The prevalent opinion is that hydrogen spillover is limited by diffusion over the surface of the support.[6]

The hypothesis according to which spillover proceeds by a proton mechanism has also been reported.[11,34,35] Using reduction with temperature programming and IR spectroscopy, it was shown that, first, hydrogen quickly dissociates to atoms and then forms H^+ ions, which diffuse into the substrate bulk and slowly react with oxygen atoms of the support material.[36] In a number of studies,[37–39] the increase in the electrical conductivity of TiO_2 in a hydrogen atmosphere was attributed to proton diffusion to the titanium dioxide. The electrical conductivity of TiO_2 attached to platinum is the first to increase and then this also takes place for TiO_2 without platinum.

The proton mechanism of spillover is also indicated by the correlation between the spillover rate and the proton affinity of the promoter,[11,34] which can solvate the diffusing proton: water > methanol > ethanol > n-propanol > n-butanol. In the presence of these promoters, the spillover rate does not depend on the surface area of the applied platinum, whereas in the presence of, for example, acetic acid, which has a low proton affinity, this dependence does exist. In the latter case, the reaction rate can be determined by the rate of proton solvation.

Even those who support the atomic nature of hydrogen spillover have to admit [40] that no unambiguous spectroscopic data indicating that $H^{\bullet}$ act as activated hydrogen species have yet been obtained. In some cases, experimental evidence for both mechanisms is found for the same catalyst systems under the same reaction conditions.[40] Depending on the type of testing or the particular spectroscopic technique used, either atomic hydrogen or protons are identified. The model proposed by Roland *et al.*[40] describes the spillover as the formation of an electron donor on the surface of the support. In this consideration, weakly chemisorbed hydrogen is regarded as atomic, while strongly chemisorbed hydrogen is taken as protons. Quantitative data obtained by spectroscopic methods do not allow one to identify unequivocally the nature of the activated species in the solid-phase catalytic reactions.

Tritium can also react with a substrate in either atomic or ionic form. However, the results of the hypothetical interactions of the electrons generated in parallel with the formation of protons with either gaseous hydrogen or with the substrate molecules have not been considered in the literature. The latter process could also be interpreted as a specific case of electrochemical reaction. Electrons are free to migrate over the crystallite of the metal catalyst or over the support (for example, graphite). However, if the substrate is an insulator, the electron conductivity may prove to be negligibly low.

It is worth mentioning that the conductivity in the metal crystallite decreases as the temperature rises, because electron scattering is enhanced upon disordering of the motion of atoms caused by their thermal excitation. Metalloids usually have positive temperature coefficients of conductivity.[41] The interaction with electrons might be responsible for the disappearance of the EPR signal of perylene adsorbed on the Pt/ZeY system [18] and for the reduction of tetravalent cerium to trivalent cerium.[31] It was shown above [6–10] that hydrogenation can be accomplished by gaseous hydrogen; this may be related to the presence of electrons arising upon the spillover of protons.

Research into the reactions of various organic molecules with atomic tritium formed upon heating of tritium gas to 2000 K on a tungsten filament has shown [42] that, even when the target is cooled with liquid nitrogen, the degree of replacement of hydrogen in the molecule of an organic compound does not exceed 10%. In this case, the chemical and radiochemical yields of the exchange were exceptionally low compared to the yields of solid-phase reactions. Indeed, even at room temperature, atomic hydrogen reduces

many metal oxides, reacts with sulfur, nitrogen and phosphorus, and also reacts with oxygen to give hydrogen peroxide; in addition, the hydrogen atoms easily recombine. The reactions involving radicals produce a variety of side products. Thus, if the label is introduced *via* a reaction with atomic tritium, the yields of labelled biologically active compounds would be very low. At temperatures used to carry out solid-phase reactions, atomic tritium generated in the reaction of tritium gas with the catalyst is converted into molecular tritium, which does not play any important role in the synthesis of labelled preparations. This seems to account for the stability of organic compounds in these reactions.

Probably, noticeable participation of atomic tritium in reactions could be expected at higher temperatures, which are not normally used in solid-phase labelling. Indeed, only at high temperatures can radical elimination as well as hydrogenation and dehalogenation involving atomic tritium compete with energetically more favourable recombination of tritium atoms.

It is of interest to analyse the data concerning the reactions of radicals with the substrate under conditions more appropriate for the investigation of these systems.[43] For example, it was found that radicals are more prone to undergo elimination than addition reactions.[43] This is due to the fact that the elimination

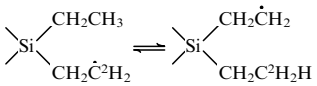
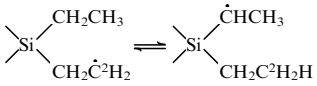
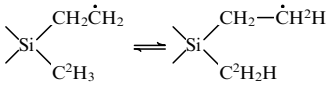
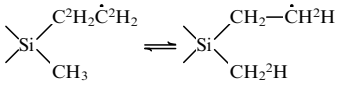
$$H^{\bullet} + RX \longrightarrow HX + R^{\bullet}$$

involves a linear reaction centre, which ensures the lowest activation barrier. During the addition

$$H^{\bullet} + CH_2{=}CHY \longrightarrow HCH_2C^{\bullet}HY,$$

the reaction centre is a triangle; this, undoubtedly, increases the activation energy of this reaction. The intramolecular transfer of hydrogen atoms was studied by EPR (although, at low temperatures), and the kinetics of free-radical intramolecular transfer of protium and deuterium atoms was first studied by direct spectral methods using hydrocarbon groups grafted to the solid surface (silica gel) (Table 1).[44]

Table 1. Rate constants for free-radical intramolecular transfer of protium and deuterium atoms.[44]

Process	T /K	$10^{-5}k$ /s^{-1}
	246	66 ± 7
	295	12 ± 1
	373	5 ± 0.5
	373	20 ± 2

It was shown that the radical centre can migrate; therefore, different polymerisation, isomerisation, *etc.* products can be formed not only at the sites of the initial formation of radicals.

It is of interest to analyse the regularities involved in homolytic and electrophilic substitution of tritium for hydrogen in aromatic compounds[45] in order to compare them with the regularities of isotope exchange. For electrophilic substitution, where H^+ acts as the electrophilic species, *ortho*-, *para*- and *meta*-directors are distinguished in the molecule (Table 2). The proton addition is the slow step in this reaction. For example, on treatment of aromatic compounds with a mixture of trifluoroacetic acid and heavy water, the rate of deuterium exchange was found to vary in parallel with the equilibrium degree of protonation.

Table 2. Rate constants for deuterium inclusion (rel.u.) into different positions of the aromatic ring with different substituents.

Position of the substituent	Rate of inclusion for the substituent			
	CH_3	CH_3O	Cl	I
ortho	83	23 000	0.035	0.043
meta	1.9	0.25	0.002	0.003
para	83	55 000	0.160	0.112

Let us compare electrophilic alkylation of aromatic compounds with the corresponding radical reactions. Methylation of toluene and ethylation of bromo- and fluorobenzene by treatment with $R^+[Al_2Cl_7]^-$ proceed as typical electrophilic substitution reactions (Table 3). Homolytic methylation gives a different ratio of substitution products.

Table 3. Inclusion (%) of the CH_3 and C_2H_5 groups into *ortho*-, *meta*- and *para*-positions during alkylation of aromatic compounds.

Process	Inclusion position		
	ortho	*meta*	*para*
Methylation of			
toluene [a]	50 – 60	10 – 14	34 – 26
fluorobenzene [b]	57	37	6
bromobenzene [b]	67	23	10
Ethylation of			
fluorobenzene [a]	43	14	43
bromobenzene [a]	24	22	54

[a] Electrophilic substitution. [b] Homolytic methylation.

As will be shown below, this type of specificity is also observed during solid-phase hydrogenolysis by tritium. It is evident that this type of process specificity cannot be interpreted when considering only the source or the mechanism of transport of activated species to the substrate. However, since these species have different properties (a radical and an ion), by investigating the reaction products and/or distribution of a label in them, one can draw conclusions in favour of a particular mechanism of hydrogen spillover.

III. Solid-phase reactions in the synthesis of tritium-labelled preparations

Solid-phase reactions proved to be fairly efficient for the introduction of a tritium label into organic compounds. When selective methods are used, the problem of optimisation of the conditions of introduction becomes especially topical. Highly labelled materials can be prepared in solid-phase processes such as selective isotope exchange, selective hydrogenation of unsaturated carbon – carbon and heteroatom – carbon bonds and selective reductive dehalogenation of one of the several halogen atoms or reductive dehalogenation for organic molecules containing labile unsaturated fragments.

It should be mentioned that the solid-phase reactions listed above are accompanied by side processes, which considerably influence the radiochemical characteristics of labelled specimens. For example, when substances applied on a catalyst are treated with tritium gas, the final molar radioactivity of the products depends appreciably on the isotope effects. The destruction of organic compounds not only hampers purification of labelled materials but also results in isotope dilution of tritium. Evidently, the reactions of organic compounds with the resulting protium –

tritium mixture decrease the degree of inclusion of tritium into the molecules of these compounds. A model has been proposed [46] that can be used to explain the influence of different factors on the degree of inclusion of tritium into organic molecules.

1. Solid-phase isotope exchange

Isotope exchange is the method of choice for introducing a tritium label, as it does not require the synthesis of precursors. In addition, isotope exchange allows one to prepare a labelled specimen using very small amounts (5–10 mg) of biologically active substances, which are often scarce and expensive. This method is inapplicable only when the starting compound is totally destroyed under experimental conditions.

The solid-phase catalytic isotope exchange is a complex process affected by numerous factors related to the properties of both organic compounds and the catalysts. For example, the efficiency of solid-phase isotope exchange depends appreciably on the melting point of the organic compound. In view of this fact, all compounds can be divided into two groups. The first one includes substances that behave like liquids at the chosen reaction temperature (in this case, the molecules can migrate over the support and with respect to one another); the second group comprises compounds that exist as crystals during the reaction (in this case, direct contact with the catalyst active sites is impossible and the degree of isotope exchange mainly depends on the efficiency of tritium spillover). Naturally, the regularities of insertion of the label into compounds of the first and second groups are dissimilar. Lipid-related compounds usually have low melting points, while nucleotides, nucleosides, amino acids, peptides and their derivatives have high melting points. Therefore, it is expedient to consider the regularities of labelling of compounds with different properties by this procedure and then to present data on the radiochemical characteristics of labelled specimens obtained under the optimal conditions for each class of compound.

a. Study of the dependence of the degree of solid-phase isotope exchange on the nature of organic compounds

Let us consider the results of experimental studies of the influence of the nature of organic compounds on the kinetics of their labelling and the dependence of the molar radioactivity of the resulting preparations on the reaction temperature.

Experiments on introducing a label into crystalline L-valine and L-alanine showed that the degree of isotope exchange smoothly increases with an increase in the temperature and the kinetic curves reach a plateau.[47, 48]

A similar situation is observed in introduction of the label into adenine, guanine, xanthine, hypoxanthine (Table 4), deazaguanine and 3-indolylacetic acid.[51]

Table 4. Relative molar radioactivity of adenine, guanine, xanthine and hypoxanthine (5% $Pd/CaCO_3$, $\tau = 60$ min) at various temperatures.[49, 50]

T /°C	RMR (%)			
	adenine	guanine	xanthine	hypoxanthine
20	1.4	1.3	0.8	1.7
120	1.8	1.7	2.4	–
140	16.8	6.5	20.9	13.1
150	34.7	16.7	50.0	–
160	54.7	25.7	67.4	16.7
180	100	71.9	96.5	63.0
200	–	91	100	100
220	–	100	–	98.8

Note. Here and in other tables, τ is the experiment duration; RMR is the relative molar radioactivity; the highest molar radioactivity attained in this series of experiments was taken to be 100%.

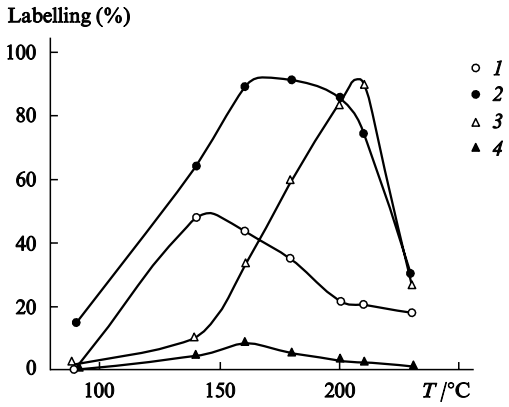


Figure 1. Degree of labelling of the substrate *vs.* the temperature of isotope exchange.[52]
Reaction conditions: $\tau = 15$ min; 5% Pt/C : hexadecane, 10 : 1 (*1*); 5% Pt/C : 6-ketopalmitic acid, 10 : 1 (*2*); 5% Pt/C : methyl stearate, 10 : 1 (*3*); 5% Pd/C : cetyl alcohol, 6 : 1 (*4*).

The kinetics of variation of the molar radioactivity of purine bases and amino acids are similar: the kinetic curves and the curves for the temperature dependence of the degree of labelling reach a plateau.

The curves obtained for low-melting compounds of lipid nature look quite different (Fig. 1). Similar types of dependence are also found in the case of other catalysts ($Pd/BaSO_4$, Rh/C),[52] which differ in the ability to initiate the isotope exchange and in the temperature at which the highest molar radioactivity is attained. The appearance of maxima on these dependences can be explained in the following way. The molecules of, for example, a low-melting fatty acid, unlike those of high-melting compounds, can create a certain structured system around the active sites of the catalyst;[53] a layer of chemisorbed molecules functioning as ligands appears on the catalyst surface.[54, 55] These systems are more stable against poisoning and deactivation. In view of the foregoing, the processes taking place on the catalyst surface during the solid-phase isotope exchange between tritium gas and low-melting compounds can be described by the following scheme. First, the adsorbed molecules of the substance undergo a redistribution on the catalyst surface to give ordered systems involving some of the substrate molecules and the catalyst active sites. The greater part of the molecules moves freely and comes in contact with these ordered structures. Therefore, the rate and the degree of exchange during labelling of such substances are higher than those in the case of high-melting compounds.[52, 53] As the temperature and process duration increase, the degree of isotope exchange becomes higher but the rate of side processes also increases. Starting from certain temperatures, the structured chemisorbed layer is destroyed; this stops the isotope exchange and induces decomposition of, first, the fraction of the specimen with the highest degree of labelling.[53] This accounts for the appearance of maxima in the plots for the dependence of the molar radioactivity of fatty acids on the temperature and the duration of isotope exchange.[52, 56]

This type of regularity has been observed when introducing the label into hexadecane, 6-ketopalmitic acid, methyl stearate and cetyl alcohol (see Fig. 1); the positions of the maxima and the patterns of temperature dependences of the molar radioactivity can be substantially different for different compounds.

All attempts to explain the appearance of the maxima by other reasons (for example, by high content of bound hydrogen in the activated carbon,[57] sublimation of a part of the compound from the catalyst surface, a decrease in the catalytic activity of the metal after thermal treatment [58, 59]) have not been confirmed experimentally.

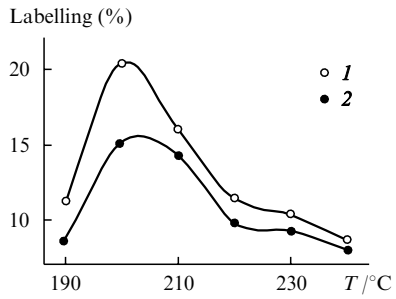


Figure 2. Degree of labelling of stearic acid *vs*. the temperature of isotope exchange (10% $Pd/BaSO_4$); (*1*) standard catalyst; (*2*) catalyst pre-calcined at 240 °C and 0.1 Pa.

Upon degassing and thermal deactivation of the catalyst, the degree of labelling of, for example, stearic acid really decreases but the general extremal pattern of the temperature dependence does not change (Fig. 2).[60] The proposed interpretation of the pattern of these curves would be confirmed if the maxima disappeared in the case of merely mechanical application of stearic acid onto the sorbent mixed with the standard catalyst. Indeed, with such application, the formation of the structured system of stearic acid molecules around the active sites of the catalyst would be appreciably hampered due to the short reaction time, and, hence, the degree of inclusion of tritium would be more uniform for all molecules of organic acid. Therefore, this modification of the conditions of the solid-phase reaction should entail some decrease in the degree of isotope exchange.

An experiment of this type has been carried out:[60] a methanol solution of stearic acid was mixed with the Alusorb or Silasorb sorbent in 1 : 10 ratio, the solvent was removed, and the residue was dried. Then the support – substance mixture was thoroughly triturated in a mortar together with the catalyst and the reactions with the aliquot portions of this mixture were carried out at different temperatures (Table 5). As can be seen from the data given in the Table, with an increase in temperature, the molar radioactivity of the specimen first increases, then decreases and subsequently varies insignificantly.

Table 5. Relative molar radioactivity of labelled stearic acid at various temperatures of isotope exchange (5% $Pd/BaSO_4$, catalyst : substance ratio is 20 : 1, τ = 15 min) on the catalyst without a support and using an additional support.[60]

T /°C	RMR (%)		
	no support	Alusorb	Silasorb
190	42	46	47
200	71	76	80
210	82	81	82
220	100	79	81
230	48	75	84
240	36	82	83

Thus, the degree of labelling depends substantially on the aggregative state of the substrate under the solid-phase reaction conditions.[52, 61]

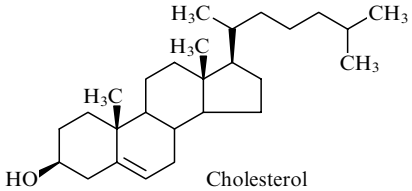
A study of the dependence of the degree of labelling on the length of the carbon chain in homologues of fatty acids whose melting points are markedly lower than the temperature at which the isotope exchange is carried out showed a linear decrease in the molar radioactivity with a decrease in the chain length. Hence, the isotope exchange for this type of substance is a probability process. Some other features were noted. For example, the temperatures corresponding to the maximum degree of isotope replacement (T_{max}) were different for different homologues. For example, the T_{max} values for lauric (m.p. = 44 °C), myristic (m.p. = 58 °C), palmitic (m.p. = 64 °C) and stearic (m.p. = 70 °C) acids were 190, 200, 205 – 210 and 210 °C, respectively.[52, 62]

Apparently, for representatives of one homologous series (for example, for fatty acids), T_{max} depends on the temperature of phase transition (melting). At a definite temperature, diffusion of these molecules increases so sharply that the efficiency of isotope exchange reaches a maximum and does not depend on the efficiency of hydrogen spillover. The conducting of these reactions at higher temperatures results only in activation of side processes.

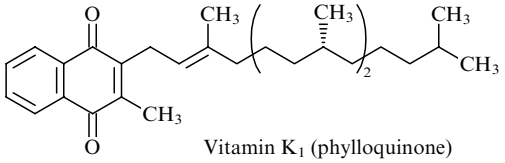
However, for high-melting sodium salts of acetic, propionic, and butyric acids, the molar radioactivity did not show this type of unambiguous dependence on the number of carbon atoms.[63] Evidently, the accessibility of the substance bulk during the isotope exchange reaction differs appreciably depending on whether the substance occurs on the support surface in the solid (crystalline) or in the liquid state.

b. Introduction of a tritium label into lipophilic compounds and into low-molecular-mass bioregulators by solid-phase isotope exchange

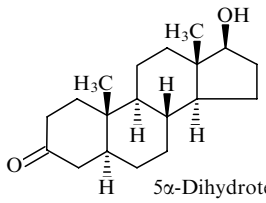
Compounds of lipid nature vary appreciably in thermal stability and in stability against hydrogenolysis; they can be either polar or non-polar and either aliphatic or aromatic. Examples of such compounds are given below.



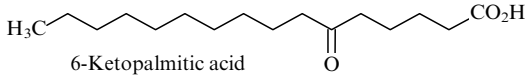
Cholesterol



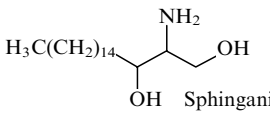
Vitamin K_1 (phylloquinone)



5α-Dihydrotestosterone

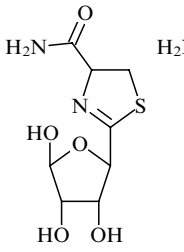


6-Ketopalmitic acid

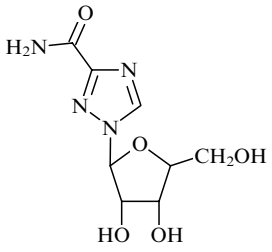


Sphinganine

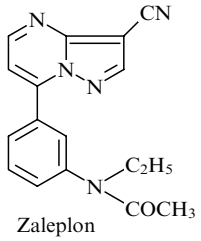
In this section, in addition to lipophilic compounds, we also consider other substances used in pharmacology. For example,



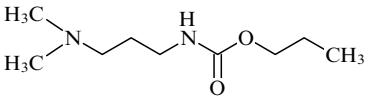
Tiazofurin



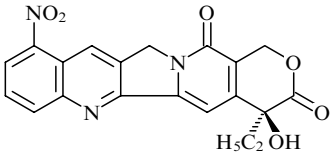
Ribavirin



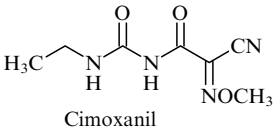
Zaleplon



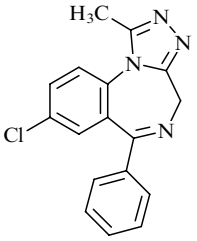
Propamocarb



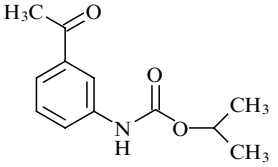
9-Nitrocamptothecin



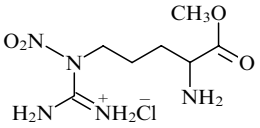
Cimoxanil



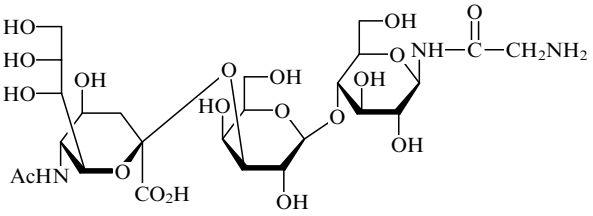
Alprazolam



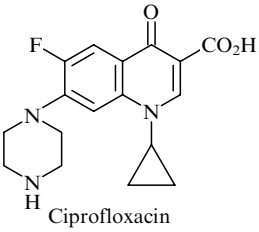
Isopropyl *N*-(*m*-acetylphenyl)-carbamate



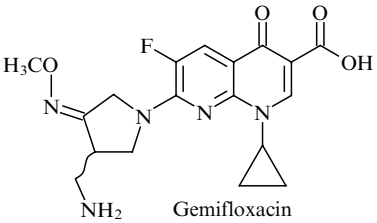
N-Nitroarginine methyl ester hydrochloride



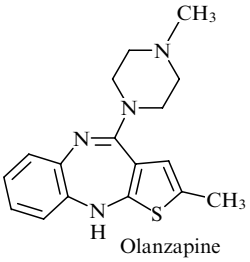
Derivative of the trisaccharide sialyllactose



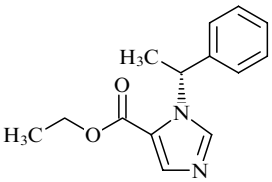
Ciprofloxacin



Gemifloxacin



Olanzapine



Etomidate

As a rule, these compounds are polyfunctional and contain fragments that are unstable under solid-phase reaction conditions. Many of these substances (called, for convenience, pharmaceuticals) possess hydrophobic properties, like compounds of a lipid nature.

To optimise the conditions of labelling in compounds of this type, the degree of isotope exchange has been studied as a function of the following variables: temperature (in the 60–240 °C region), the catalyst : substance ratio, the reaction time (in the range of 5–60 min), the nature of the catalyst (5% Pt/C, 5% Pd/C, 5% Rh/C, 5% $Pd/BaSO_4$) and some others.[52, 56, 61–71] For example, it was found that the best conditions for introducing a label into thermally stable isobutyric acid are as follows: 5% Pd/C, 30 min, 170 °C (Table 6).[63] Under more rigorous conditions, the yield of the labelled product decreased.

Table 6. Molar radioactivity (MR) of labelled sodium isobutyrate obtained with solid-phase isotope exchange carried out under various conditions.[63]

Reaction conditions			MR /PBq mol^{-1}
catalyst	τ /min	T /°C	
5% $Pd/BaSO_4$	60	210	5.26
	60	190	3.85
	60	170	2.78
5% Pd/C	60	210	5.04
	60	190	5.44
	60	170	5.26
	30	170	5.30
	20	170	5.22
	15	170	4.85
	5	170	4.11

Study of the influence of the structure of compounds having similar physicochemical properties on the molar radioactivity of labelled specimens has shown[63] that the probability of inclusion of tritium, for example, in the methyl group of the isobutyric, propionic and butyric acids, is more than three times higher than the probability of its inclusion into methylene groups (Table 7). The reasons for this difference are considered below.

Table 7. Molar radioactivity of labelled organic acids prepared by solid-phase isotope exchange (5% Pd/C, T = 200 °C).[63]

Compound[a]	MR /PBq mol^{-1}	Amount of inserted label	
		per hydrogen atom	per methyl group
Acetic acid	1.20	0.40	1.20
Propionic acid	3.18	0.64	2.62
Butyric acid	3.74	0.53	2.62
Isobutyric acid	5.56	0.79	2.64

[a] The corresponding sodium salts were used.

By solid-phase reactions carried out under the optimal conditions selected by the optimisation technique, labelled specimens with various structures have been prepared (Tables 8–10).[50, 52, 53, 62, 65, 70–74]

Table 8. Molar radioactivity of aliphatic compounds.

Compound	MR /PBq mol^{-1} (see [a])	Ref.
6-Ketopalmitic acid	27.5–27.8	52
Methyl stearate	38.0–40.0	62
Cetyl alcohol	5.0–5.5	52
Hexadecane	17.0–17.5	62
Cholesterol	0.5–1.0	71
5α-Dihydrotestosterone	0.52–0.56	53
5α-Dihydrotestosterone enanthate	1.30–1.50	53
Sphinganine	7.78	69

[a] The molar radioactivity of the specimen caused by introduction of one tritium atom is 1.08 PBq mol^{-1}.

Table 9. Molar radioactivity of aromatic compounds (palladium catalyst, $\tau = 15$ min).

Compound	T /°C	MR /PBq mol^{-1}	Yield (%)	Ref.
3-(3-Pyridyl)phenyl-isoxazole	60	0.0004	25	64
Alprazolam	175	0.15	4	74
Camptothecin	190	0.96	7	70
Tiazofurin	155	0.67	55	74
Ribavirin	160	1.11	80	74
Isopropyl-*N*-(*m*-acetyl-phenyl)carbamate	140	1.32	30	73
Gemifloxacin	180	0.34	5	53
Olanzapine	200	0.63	10	62
Zaleplon	180	0.19	2	62

Table 10. Conditions of tritium labelling of low-molecular-weight bioregulators.

Compound	Reaction conditions		MR [a]	Yield (%)	Ref.
	catalyst	T /°C			
4-Ethyl-2,6,7-trioxa-1-phosphabicyclo-[2.2.2]octane 1-oxide	5% Pt/C	170	1.15	14	50
Sodium salt of *N*-acetylneuraminic acid α-methyl-glycoside	5% Pd/$CaCO_3$	200	0.46	25	76
Derivative of the trisaccharide sialyllactose	5% Pd/$CaCO_3$	160	0.32	15	76
Propamocarb	5% Pd/$BaSO_4$	135	0.56	58	73
Cimoxanil	5% Pd/$CaCO_3$	90	0.006	30	73

[a] Expressed in PBq mol^{-1}.

It has been noted above that the nature of the tritium active species can be traced most clearly in reactions with aromatic compounds. The susceptibility to electrophilic substitution is known [45, 72] to be different for different aromatic systems. It depends on the structure of the compound and on the presence of substituents; this was demonstrated, for example, by measuring the rate constants for the isotope exchange during the preparation of [8-^{3}H]nucleotides of the adenine series. The data reported by Sidorov and Myasoedov [75] allow one to suggest that if the activated hydrogen species that react with an organic compound are represented by positively charged tritium ions, the degree of labelling should decrease in the sequence ribavirin, tiazofurin, zaleplon and alprazolam. Indeed, it was shown [53, 74] that ribavirin was labelled to a 7–8 times higher degree than alprazolam. Apparently, this is due to the fact that the highest probability of the electrophilic substitution is expected for the triazole fragment of ribavirin. Therefore, ribavirin should exhibit the highest molar radioactivity over the given series (see Table 9). Due to the presence of the sulfur atom in the thiazole fragment, tiazofurin is substantially deactivated and the electrophilic substitution is less efficient.

Not every substance withstands the conditions of the solid-phase isotope exchange to the same extent. The chemical yield of hexadecane and cetyl alcohol after the exchange is 80%, whereas labelled compounds containing free amines, neuraminic acid and so on are formed in less than 20% yields.[61, 69, 76] The stabilities of the specimens can be sharply different even in the case of compounds with similar structures (see Table 10). For example, the yield of the sodium salt of *N*-acetylneuraminic acid α-methyl-glycoside at 200 °C is almost twice as high as that of the derivative of the trisaccharide sialyllactose at 160 °C. It was shown [76] that the degree of inclusion of the label into the sodium salt of *N*-acetylneuraminic acid α-methylglycoside increases ~4-fold as the temperature rises from 160 to 200 °C (Table 11). If the derivative of the trisaccharide sialyllactose did not decompose at 200 °C, it would be possible to obtain a labelled specimen with a molar radioactivity of > 1 PBq mol^{-1}.

Table 11. Relative molar radioactivity of the labelled sodium salt of *N*-acetylneuraminic acid α-methylglycoside (the ratio catalyst : substance is 25 : 1) for different conditions of solid-phase isotope exchange.

Reaction conditions			Yield (%)	RMR (%)
catalyst	T /°C	τ /min		
5% Pd/$BaSO_4$	140	40	92	13
	180	5	75	39
5% Pd/$CaCO_3$	140	40	95	16
	160	25	91	22
	180	5	74	46
	200	5	25	86
	220	5	8	100

The large scatter in the yields of labelled specimens is also typical of aromatic compounds. The compounds containing triazole, pyrazole and thiazole fragments are more stable during the solid-phase method of labelling than isoxazole derivatives; however, the yields of these compounds under the conditions optimal from the standpoint of higher molar radioactivity were 2 to 80% (see Table 9).

Thus, if the compound is stable, the solid-phase procedure gives labelled specimens with a very high molar radioactivity. For introducing a label into labile compounds, it is necessary to use modified procedures. There are two approaches for increasing the yield of such compounds: (1) organic compounds are applied onto an additional inert support and are mixed mechanically with the catalyst; (2) the catalyst is modified prior to the reaction.

c. The introduction of the tritium label into components of nucleic acids by solid-phase isotope exchange

The best catalyst for introducing a tritium label into purine bases is 5% Pd/$CaCO_3$ (Table 12). The dependence of the molar radioactivity on the reaction temperature for these compounds shows a gradual increase to 140 °C and a sharp growth in the range of 150–200 °C (see Table 4).[49] Apparently, the increase is due to the

Table 12. Molar radioactivity of the specimens obtained by tritium labelling of purine bases under various conditions.

Compound	Reaction conditions [a]		MR (see [b])	Yield (%)	Ref.
	catalyst	T /°C			
Kinetin	Pd/$BaSO_4$	140	5.56	12.5	49
6-Benzyl-aminopurine	Pd/$CaCO_3$	190	5.74	13	77
Guanine	Pd/$CaCO_3$	200–210	0.83	75	75
Xanthine	Pd/$CaCO_3$	200–210	0.93	75	75
Hypoxanthine	Pd/$CaCO_3$	200–210	1.90	67	75
Adenosine	Pd/$CaCO_3$	200–210	0.89	93	75
2′-Deoxy-guanosine	Pd/$CaCO_3$	200–210	1.56	17	75

[a] In all experiments, $\tau = 30$ min. [b] Expressed in PBq mol^{-1}.

fact that tritium atoms start to play a noticeable role in the isotope exchange, instead of the cations.

The introduction of a label into components of nucleic acids with different stabilities has been reported [75, 78–80] (Table 13). It can be seen from the data presented in the table that high degrees of replacement of protium by tritium were attained in compounds with a rather simple structure. In the case of polymeric derivatives or compounds containing labile fragments, labelling had to be performed at relatively low temperatures, which substantially influenced the molar radioactivities of the labelled preparations. The major side products obtained according to this procedure were those resulting from hydrogenation, isomerisation and destruction processes.

Table 13. Molar radioactivity of purine and pyrimidine compounds and their derivatives prepared by solid-phase isotope exchange.[50, 78–80]

Compound	T /°C	MR [a]	Compound	T /°C	MR [a]
Adenine	170	2.01	2′,3′-Dideoxy-	120	2.44
Thymine	170	4.30	thymidine		
Uracil	170	1.82	3′-Azidothymidine	120	2.33
Benzyladenine	190	6.67	phosphonate		
Deazaguanine	220	0.44	Acyclovir	120	2.07
7-Thia-8-oxo-	170	0.01	phosphonate		
guanosine			2′,5′-ApApAp	200	2.15
Theophylline	160	0.92	3′,5′-ApA	110	0.34
Furfuryladenine	160	5.93	3′,5′-ApApA	110	1.85
Guanosine	210	2.56	3′,5′-pApApAp	110	1.00
2′-Deoxyguanosine	200	1.30	Poly-A (7-8S)	180	0.38 [b]
3′-Azidothymidine	120	1.11	Poly-U (12S)	180	0.44 [b]
Acyclovir	210	4.63	RNA 15S	180	0.45 [b]

[a] Expressed in PBq mol^{-1}. [b] Specific activity /TBq g^{-1}.

To increase the molar radioactivity of labile compounds, additional supports were used; this made it possible to carry out the reaction at higher temperatures (Table 14).[51] It can be seen from the data given in the table that an increase in the catalyst : compound ratio entails an increase in the degree of tritium inclusion with a simultaneous decrease in the yield of the labelled specimen. With the use of an additional support and a mixture of 5% Pd/$CaCO_3$ with indol-3-ylacetic acid supported on Al_2O_3, the yield of the desired product increased to 12.5%. According to ^3H NMR data, the distribution of the label at 200 °C is uniform over all the stable positions of tritium in indol-3-ylacetic acid, which is indicative of a considerable contribution of atomic tritium to the labelling process.

Table 14. Relative molar radioactivity of labelled indol-3-ylacetic acid obtained by labelling under various conditions.[51]

Reaction conditions				RMR (%)	Yield (%)
I [a]	II [b]	III [c]	T /°C		
Catalyst: 5% Pd/$CaCO_3$					
5 : 1	–	–	150	17.1	1.9
10 : 1	–	–	150	40.0	0.7
20 : 1	–	–	150	53.3	0.4
50 : 1	–		150	66.7	0.2
5 : 1	Al_2O_3	10 : 1	200	100	12.5
Catalyst: 5% Pd/$BaSO_4$					
5 : 1	$CaCO_3$	10 : 1	200	84.4	3.2
5 : 1	$CaCO_3$	50 : 1	200	–	–
5 : 1	Al_2O_3	10 : 1	200	84.0	10.2
5 : 1	Al_2O_3	50 : 1	200	44.0	6.0
Catalyst: 5% Pd/Al_2O_3					
5 : 1	Al_2O_3	10 : 1	200	55.6	5.3
5 : 1	Al_2O_3	50 : 1	200	56.0	1.9

[a] The catalyst : substance ratio /mg μmol^{-1}. [b] Additional support. [c] The additional support : substance ratio /mg mg^{-1}.

The proportion of tritium in purine bases depends on the nature of the initial compound.[79] As can be seen from the data listed in Table 15, the presence of the phosphate group decreases the content of tritium in the purine moiety of the molecule. Possible interpretations of this finding are discussed below.

Table 15. Molar radioactivity and fraction of tritium (%) in the specimens obtained by labelling of purine bases.

Compound	MR /PBq mol^{-1}	Fraction of tritium
Adenosine	4.44	48
Guanosine	2.56	30
Acyclovir	4.78	14
AMP	0.46	12
ATP	0.46	13
NAD	4.10	1.3
Coenzyme A	0.07	89

Note. Here and in other tables, AMP is adenosine 5′-monophosphate, ATP is adenosine 5′-triphosphate, NAD is nicotinamide dinucleotide.

d. The introduction of the tritium label into amino acids and peptides by solid-phase isotope exchange

Many amino acids are thermally more stable than nucleic acids. Data on the solid-phase introduction of the tritium label into some amino acids have been reported (Table 16).[81] At 220 °C, the distribution of the label at the valine carbon atoms ($\alpha : \beta : \gamma$) is described by a 1 : 1 : 6 ratio (τ = 20 min, the 5% Pd/C : compound ratio is 10 : 1). The distribution of the label (%) in [2,3-^{3}H]alanine under the same conditions is given by [81]

Compound	$n = 0$	$n = 1$	$n = 2$	$n = 3$
$CH_n{}^3H_{3-n}C^3HNH_2CO_2H$	3	15	22	5
$CH_n{}^3H_{3-n}CHNH_2CO_2H$	5	17	23	7

At high temperatures, the degree of isotope replacement is of the same order of magnitude at any atom of the amino acid.

Other data have been obtained at lower temperatures.[82] The distribution of tritium in the (*R*)-4-hydroxy-[G-^{3}H]-L-proline molecule was studied. This compound was synthesised by heating (150 °C) for 60 min of a mixture of 5% Rh/Al_2O_3 with the activated Norit carbon impregnated with an aqueous solution of amino acid and then freeze-dried (the catalyst : Norit : (*R*)-4-hydroxy[G-^{3}H]-L-proline ratio was 10 : 50 : 1). The ratio of the activation energy (E_a) of hydrogen exchange at a given carbon

Table 16. Molar radioactivity, yield and optical purity of labelled amino acids prepared by solid-phase isotope exchange (200 °C, 60 min).[86]

Compound	MR /PBq mol^{-1}	Yield (%)	Optical purity (%)
Glycine	1.8	90	–
Serine	1.4	50	75
Alanine	3.2	65	82
Valine	6.5	85	94
Leucine	7.9	70	85
Isoleucine	8.1	75	88
Histidine	4.5	20	50
Tryptophane	3.6	30	70

atom to the activation energy (E) of hydrogen exchange in methane is as follows:

Position of the label	3β	3α	5α	5β	2	4
$E_a : E$	0.946	0.856	0.653	0.794	0.852	0.924
Content of the label (%)	1	6	59	21	9	4

When the solid-phase isotope exchange is carried out under relatively mild conditions, the following tendency can be traced: the lower the activation energy for the exchange at a given carbon atom, the higher the degree of replacement of protium by tritium.

In the case of amino acids, the use of an additional support also promoted an increase in the molar radioactivity (Table 17).[48] Activated carbon, alumina and calcium carbonate are used most often. This procedure made it possible to obtain labelled compounds having various stabilities with a high molar radioactivity.

Table 17. Molar radioactivity of biotin and amino acids prepared by solid phase isotope exchange using various additional supports.[48]

Compound	Reaction conditions				MR /PBq mol^{-1}
	I [a]	II [b]	III [c]	T/°C	
Catalyst: 5% Pd/$CaCO_3$					
Biotin	10 : 1	Al_2O_3	10 : 1	200	1.6
L-Phenylalanine	16 : 1	Al_2O_3	5 : 1	200	3.03
Catalyst: 5% Rh/Al_2O_3					
L-Methionine	10 : 1	Al_2O_3	10 : 1	200	3.30
Catalyst: 5% Pd/$BaSO_4$					
L-Histidine	10 : 1	$CaCO_3$	10 : 1	220	4.60

[a] The catalyst : substance ratio /mg μmol^{-1}. [b] Additional support. [c] The additional support : substance ratio /mg mg^{-1}.

The influence of the same additional support, in particular, carbon, prepared by different procedures on the degree of labelling has been studied[83] (Table 18). In an experiment, phenylalanine was applied on the Norit carbon or on fibrous carbon in a ratio of 1 : 10. The resulting blends were mechanically mixed with 5% Pd/C in 1 : 1 ratio. After treatment with tritium (T = 130 °C, τ = 25 min), the yield and the molar radioactivity of labelled phenylalanine were determined.

It was found that with the use of 5% Pd/C, the yield was as low as 1% and with a mixture of 5% Pd/C with the Norit carbon, the yield was even lower, 0.1%. A higher yield and a higher molar radioactivity (at 140 °C) were attained by using fibrous carbon.

Table 18. Yield and relative molar radioactivity of labelled phenylalanine obtained by solid-phase catalytic isotope exchange under various conditions (τ = 25 min, T = 130 °C).[83]

Reaction conditions		Yield (%)	RMR (%)
catalyst	support		
5% Pd/C	–	1	–
	Norit carbon	< 0.1	–
	fibrous carbon	16	100
5% Pd/$CaCO_3$	Norit carbon	2	29
	fibrous carbon	39	44
5% PdO/ Al_2O_3	Norit carbon	1.5	17
	fibrous carbon	41	35
5% PdO/ $BaSO_4$	Norit carbon	2	22
	fibrous carbon	35	47

Similar results were obtained upon mixing fibrous carbon–phenylalanine systems with 5% Pd/$CaCO_3$, 5% PdO/$BaSO_4$ and 5% PdO/Al_2O_3 (see Table 18). When fibrous carbon was used, the yields of phenylalanine were 15–20 times and the molar radioactivities were 1.5–2 times as high as those obtained in the presence of the activated Norit carbon, which is used most often in these reactions.

This result was attributed[83] to the fact that fibrous carbon consists of very thin graphite layers (30–500 Å thick) laid to form highly ordered structures;[84] therefore, it adsorbs approximately an order of magnitude more hydrogen than the usual carbon. In addition, the amounts of hydrogen adsorbed on and desorbed from graphite nanofibres at room temperature were substantially different, indicating chemisorption of hydrogen on carbon. This was confirmed by temperature-programmed desorption.[84] Hence, fibrous carbon as a support has certain advantages over other materials. A higher saturation by tritium molecules of the whole bulk of the reaction mixture should be favourable for the efficiency of its spillover and should lead to a higher concentration of the activated species of tritium directly in the reaction area.

The higher yield of labelled organic compounds makes it possible to obtain a plausible yield of labelled preparations at higher temperatures (35–45 °C higher than in the case of the standard procedure). This, in turn, makes it possible to increase substantially the molar radioactivity of the material; for example, in the case of phenylalanine, this value increases ~3-fold.[83]

The method according to which a mixture of the substance with the catalyst is treated before the reaction by transition metal salts also proved to be efficient for increasing the molar radioactivity of thermally unstable preparations. For example, rhodium salts were used in the labelling of amino acids.[85] As noted above, the molar radioactivity of labelled compounds can be increased by creating conditions for more efficient hydrogen spillover under which isotope exchange takes place throughout the whole bulk of the substrate or by increasing the thermal stability of the substance (in this case, the label can be introduced at a higher temperature). In order to elucidate the role of rhodium trichloride during labelling, it was used to impregnate the catalyst and the support both separately and jointly.

It can be seen from the data presented in Table 19 that the key role of $RhCl_3$ is not to stabilise the amino acid by complex formation but rather to decrease the destructive action of the activated tritium species on the substrate. When the catalyst was treated with rhodium trichloride and then mixed mechanically with the support (fibrous carbon or Al_2O_3) on which phenylalanine had been applied, the yield of the labelled product increased 3–6-fold. On treatment of the support with a solution with $RhCl_3$ and phenylalanine followed by freeze-drying of the resulting mixture and subsequent mechanical mixing with the catalyst, the yield of the labelled product increased only 2–4-fold. In both experiments, molar radioactivities of the same order were obtained. This fact indicates that in the case of using the conven-

Table 19. Yield and relative molar radioactivity of labelled phenylalanine obtained with different ways of modification of reaction ingredients by rhodium trichloride (τ = 20 min, T = 190 °C, the ratio support : phenylalanine is 10 : 1, catalyst : support is 1 : 1, phenylalanine : $RhCl_3$ is 1 : 1).

Catalyst	Support	Yield (%)	RMR (%)
5% PdO/$BaSO_4$	fibrous carbon	4.2	100
	fibrous carbon/$RhCl_3$	14.8	18
	the same	20.5	21
		31.1	9
5% PdO/$BaSO_4$/$RhCl_3$	–	1.5	48
5% PdO/$BaSO_4$	Al_2O_3	8.9	83
	Al_2O_3, $RhCl_3$	17.2	44
5% PdO/$BaSO_4$/$RhCl_3$	Al_2O_3	30.4	42

tional procedure (an aqueous solution of amino acid and $RhCl_3$ is freeze-dried with the catalyst), $RhCl_3$ not only stabilises labile compounds but, simultaneously, it modifies the initiator of hydrogen spillover. Apparently, co-precipitation of rhodium salts on palladium with subsequent heating under an atmosphere of tritium gas gives rise to bimetallic active sites, which acquire new properties of both an adsorbent and an initiator of hydrogen spillover. Phenomena of this type have been considered in detail [86] in a study of the influence of technetium on the efficiency of solid-phase isotope exchange.

Comparison of the catalytic activity of mono- (Pd or Pt) and bimetallic systems (Pd–Tc or Pt–Tc) showed a non-additive increase in the catalytic activity (synergism) in the case of bimetallic catalysts with respect to the activity of metal catalysts used separately under the same conditions.[87] Some researchers explain the synergistic effect by the fact that hydrogen is activated on one type of active sites and the reactions proceed on another type of active sites.[88–91] This model assumes that the spillover of hydrogen between these sites is enhanced. Other researchers believe that the synergism is mainly related to the interaction of the atoms of one metal catalyst with the other.[92–102] In this case, hydrogen becomes activated and reacts in the same phase, *i.e.*, the activated hydrogen species migrate from one metal atom to the other within the bimetallic active site.

In solid-phase experiments, the degree of labelling of amino acids was lower in the presence of bimetallic catalysts than in the presence of monometallic catalysts.[86] This implies that no activation of tritium spillover takes place on the bimetallic catalysts, and the activated tritium species merely migrate from one metal atom to the other within the common catalytic site. Indeed, the capacity for the sorption of hydrogen is lower for bimetallic catalysts than for monometallic ones,[87] which is usually related to the decrease in the efficiency of hydrogen spillover.

Thus, the increase in the stability of amino acids resulting from catalyst modification by rhodium salts is mainly due to the fact that the reaction of the molecules of these compounds with activated tritium species proceeds to a lesser extent on bimetallic catalysts than on monometallic ones. Therefore, in the case of solid-phase labelling in the presence of metal catalysts, the degree of decomposition of the initial substance decreases after treatment of the catalysts with rhodium salts, which allows the isotope exchange to be carried out at higher temperatures. Indeed, without rhodium salts, raising the temperature by 60 °C (a 5% PdO/Al_2O_3 : amino acid ratio of 10 : 1, τ = 30 min, a tritium pressure of 350 hPa) resulted in a ~20-fold decrease in the yield of glycine and a 5-fold decrease in the yield of ω-aminocaproic acid.[85]

T /°C	160	180	200	220
Yield of glycine (%)	66	21	5	3
Yield of ω-aminocaproic acid (%)	4.8	1.8	1.2	0.9

When the catalyst was treated with rhodium trichloride, the decrease in the yield of labelled amino acids with temperature rise was found to be markedly retarded (Table 20). This made it possible to introduce the label into thermally unstable ω-aminocaproic acid at 180 °C (molar radioactivity of the product was 0.85 PBq mol^{-1}, yield 45%).[85]

Evidently, the degree of isotope exchange can also be increased by creating conditions for more efficient interaction of the activated tritium species with substrate molecules.

The tritium labelling of high-melting compounds can be enhanced using 4-dimethylaminopyridine (Table 21).[85] Many reactions involving protons can proceed only in the presence of this compound.[103] Apparently, after co-precipitation with the substrate, this molecule can facilitate the diffusion of tritium cations into the substance bulk and thus increase the probability of isotope exchange. The data presented in Table 21 confirm the legitimacy of this hypothesis. However, it is too early to draw more definite conclusions. Evidently, solid-phase reactions of this type can be carried out only at relatively low temperatures; otherwise, tritium would be inserted into 4-dimethylaminopyridine, which would reduce the molar radioactivity of the labelled specimen and would markedly complicate isolation and purification of the target product.

Table 20. Yield and relative molar radioactivity of labelled amino acids obtained by solid-phase isotope exchange under various conditions (the 5% $Pd/CaCO_3$: $RhCl_3$ ratio is 8 : 1).[85]

Compound	τ /min	T /°C	Yield (%)	RMR (%)
Glycine	20	200	83	30
	30	210	75	35
	60	210	73	37
	120	210	71	40
	20	240	45	70
	10	260	30	93
ω-Aminocaproic acid	15	170	42	80
	15	200	14	100

Table 21. Yield and relative molar radioactivity of phenylalanine obtained with various methods of application of 4-dimethylaminopyridine (τ = 20 min, the 5% $Pd/CaCO_3$: substance ratio is 10 : 1, the phenylalanine : 4-dimethylaminopyridine ratio is 1 : 1, T = 160 °C).

Reaction conditions	Yield (%)	RMR (%)
Catalyst – phenylalanine mixture	31.4	38
Catalyst – phenylalanine mixture treated with 4-dimethylaminopyridine	20.0	56
Catalyst – 4-dimethylaminopyridine mixture treated with phenylalanine	14.4	85
Catalyst treated with a solution of phenylalanine and 4-dimethylaminopyridine	10.4	100

As in the case of compounds of a lipid nature, the replacement of protium by tritium is usually higher for the methyl groups of amino acids than for the methylene groups or than the isotope exchange at a tertiary carbon atom (Table 22).[104]

Table 22. Relative reactivity of amino acids in solid-phase isotope exchange and tritium distribution in labelled compounds (T = 150 °C).[104]

Compound	Relative reactivity	Fraction (%) of the label at carbon atoms with different degrees of substitution		
		RCH_3	RR^1CH_2	RR^1R^2CH
Alanine	0.52	96	–	4
Asparagine	0.75	–	60	40
Aspartic acid	0.25	–	60	40
Threonine	0.76	73	9	18
Leucine	1.04	86	5	9
Isoleucine	0.95	86	5	9
Valine	1.0	92	–	8

Data on the label distribution in aromatic amino acids and on the relative reactivities of these substrates are summarised in Table 23.[104] It can be seen that the highest degree of isotope exchange is found for histidine, the major amount of the label being concentrated in the aromatic ring. The same is observed for other aromatic amino acids. The degree of isotope substitution depending on the nature of the amino acid varies in the sequence:[104] basic amino acids > aliphatic amino acids without ionic groups > acidic amino acids.

Table 23. Relative reactivities of aromatic and heteroaromatic amino acids and serotonin in the solid-phase isotope exchange and tritium distribution in labelled compounds (T = 150 °C).[104]

Compound	Relative reactivity [a]	Fraction (%) of the label at different positions		
		α-position	β-position	ring
Histidine	1.10	20	4	76
Phenylalanine	0.77	13	40	47
Tryptophan	0.96	11	24	65
Thyroxin	0.72	11	35	54
Serotonin	1.2	35	7	58

[a] Valine was used as the reference.

Labelling of peptides is often carried out using procedures of liquid-state hydrogenation and dehalogenation of their precursors prepared by multistage synthetic protocols. In most cases, this is done using derivatives whose molecules include dehydroproline, iodophenylalanine or diiodotyrosine.[105] The molar radioactivity of the specimens obtained by this method was 0.77 – 2.35 PBq mol^{-1}, depending on the precursor.

The synthesis of peptides is usually carried out either in solution or in the solid state. The synthesis of precursors is a very cumbersome and expensive stage. Therefore, solid-phase isotope exchange between the tritium gas and peptide usually proves to be more efficient.

Peptides are more labile compounds than amino acids. In addition, there exists the problem of racemisation of amino acid residues at the α-position during the labelling. Therefore, labelled peptides are obtained by conducting reactions at relatively low temperatures (Table 24).[106] The distribution of the label in the amino acid residues in these peptides was approximately equal but hydrogen at the α-carbon atoms of amino acids proved to be the most reactive in the isotope exchange with tritium. For example, the peptide Met-Gly-His-Phe-Pro-Gly-Pro contained after labelling (140 °C), on average, 1.44 tritium atoms, and 88% of them were in the α-positions of amino acid residues. At 180 °C, 4.29 tritium atoms were incorporated in this peptide, and only 76% of tritium occurred in the α-position of the amino acid residues.[106] Thus, the same trend is observed: the higher the reaction temperature, the more uniform the distribution of the label (Table 25).

The influence of the peptide structure on the distribution of the label among amino acid residues was studied using model

Table 24. Molar radioactivity of tritium-labelled peptides (T = 140 °C, labelling was carried out using an additional support or the rhodium complex with the peptide).[106]

Peptide	MR /PBq mol^{-1}
Tyr-D-Ala-Gly-Phe-Leu-Arg	1.68
Thr-Lys-Pro-Arg-Pro-Gly-Pro	1.96
Met-Gly-His-Phe-Pro-Gly-Pro	1.74
Tyr-Ala-Gly-Phe-Tyr-Pro	1.63
Gly-Leu-Leu-Asn-Leu-Lys	1.40
Tyr-Pro-Arg	1.44
Tyr-D-Ala-Gly-Phe-Gly(OH)	1.78
Gly-Asn-NH_2	1.42
Tyr-Pro-Pro-Gly-Gly-Ser-Lys-Val-Ile-Leu-Phe	2.33
Tyr-Ala-Gly-Phe-Leu	1.67
Tyr-Gly-Gly-Phe-Leu-Arg	1.11
Tyr-Gly-Gly-Phe-Leu	0.89
Met-Glu-His-Phe-Pro-Gly-Pro	1.69

Table 25. Distribution of tritium in the labelled His-Gly-Gly tripeptide at various temperatures.[104]

T /°C	MR /PBq mol^{-1}	His	Gly(I)	Gly(II)
135	1.85	86 / 17.2	5 / 2.5	9 / 4.5
150	4.07	72 / 14.4	12 / 6	16 / 8
170	6.30	63 / 12.6	16 / 8	21 / 10.5

Note. The fraction of the label in the given amino acid (%) is indicated above the bar and the fraction of the label in the same amino acid per hydrogen atom (%) is below the bar.

Table 26. Distribution of tritium in labelled tripeptides (T = 150°C).[104]

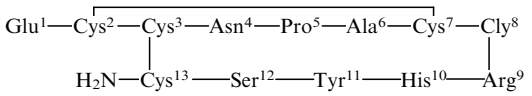
Peptide	MR /PBq mol^{-1}	Yield (%)	Label distribution (%)		
			His (or Val)	Gly(I)	Gly(II)
His-Gly-Gly	4.07	40	72	12	16
Gly-His-Gly	2.41	25	62	30	8
Gly-Gly-His	2.59	12	57	29	14
Val-Gly-Gly	2.59	45	72	10	18
Gly-Val-Gly	1.41	30	13	59	28
Gly-Gly-Val	1.74	32	6	79	15

tripeptides.[104] It can be seen from the data listed in Table 26 that at 150 °C, the label is inserted non-uniformly. For example, the amounts of tritium in Gly(I) and Gly(II) in the Gly-Gly-Val peptide differ by a factor of ~5. When tritium is introduced in identical amino acid residues, the label is mainly found in terminal amino acids; in the case where both residues are terminal, the label is inserted predominantly into the amino acid residue located at the N-end of the peptide. The patterns of label distribution in a free amino acid molecule and in the same molecule incorporated in a peptide are similar. At 180 °C, the molar radioactivity of Val-Gly-Gly reaches 7.70 PBq mol^{-1}. The overall replacement of protium by tritium amounts to 70%. It should be noted that racemisation did not exceed 1%, which corresponds to the accuracy of the experiment.[104]

Interesting results have been obtained during the introduction of a tritium label into dalargin (Tyr-D-Ala-Gly-Phe-Leu-Arg), a synthetic analogue of the endogenous opioid peptide [Leu^5]enkephalin (Tyr-Gly-Gly-Phe-Leu), which is used as a drug in gastroenterology.[107] In conformity with the temperature dependence of the degree of isotope substitution, the isotope label was incorporated in all amino acid fragments of dalargin (a 5% Rh/Al_2O_3 : peptide : Al_2O_3 ratio of 2 : 1 : 20). At 140 °C, higher reactivity was found for alanine and glycine residues, while at 190 °C, the fraction of tritium that entered the aromatic residues of phenylalanine and tyrosine increased. A temperature of 160 °C and storage in an atmosphere of tritium gas for 40 min were found to be the conditions of choice for labelling, the molar radioactivity of the labelled peptide being 1.93 PBq mol^{-1}. This peptide fully retained the biological activity, which was estimated as the ability to bind to the opioid receptors of the rat brain.

During labelling of more complex amino acid sequences, it may happen that one fragment of the molecule shields another fragment from the reaction with active tritium species. In this case, the distribution of the label along the amino acid chain is no longer uniform.[108]

For example, the distribution of the label in α-conotoxin G1



(the 5% Rh/Al_2O_3 : peptide : Al_2O_3 ratio was 5 : 2 : 50, T = 140 °C, τ = 20 min, the molar radioactivity of the labelled preparation was 1.30 PBq mol^{-1}) was as follows:

Amino acid fragment	Glu^1	Cys^2	Cys^3	Cys^7	Gly^8	Arg^9	His^{10}	Tyr^{11}	Ser^{12}	Cys^{13}
Fraction of the tritium label (%)	3.33	6.80	8.93	7.80	2.61	1.64	50.56	4.08	3.55	8.92

The Asn^4-Pro^5-Ala^6 fragment contained less than 2% of the label. Apparently, the low reactivity of these residues is related to the spatial structure of this peptide. The pattern of distribution of tritium in the peptide chain appears to be governed by not only the capacity for isotope exchange but also by the accessibility of the particular amino acid fragment for the activated species of tritium and by conformational factors related to the possibility of formation of the S_Ei transition state (see below). Indeed, according to ^1H NMR data, the Asn^4-Pro^5-Ala^6-Cys^7 fragment, unlike the rest of the molecule of this peptide, has a rigid conformation.[109] The extremely high degree of isotope exchange at the C(2) atom in the histidine residue can be explained by strong interaction of its imidazole ring with the arginine fragment.[109, 110]

The native character of the labelled peptide was proved not only by chromatography and spectroscopy but also by full retention of the biological activity determined, for example, from its binding to the nicotinic acetylcholine receptor.

Approaches for introducing a label into proteins have been developed (Table 27).[28, 104]

Table 27. Preparation conditions and radiochemical and biological characteristics of tritium-labelled proteins.

Proteins	T /°C	Yield (%)	Specific radioactivity /mCi mg^{-1}	Biological activity (%)
Collagenase	80	25	25	70
Collagen	60	10	8	–
Lichenase	60	40	28	80
β-Glucosidase	20	55	7	86
	40	40	20	75
	60	30	70	28
	80	10	200	12

The proteolytic activity of labelled collagenase was measured relative to azocasein and was found to be >70% of the initial activity. The labelled collagen retained the solubility, stability against trypsin and the ability to form fibrils in solution accessible for hydrolysis by collagenase of microorganisms and invertebrates. The temperature dependences of the chemical yield, molar radioactivity and biological activity were studied taking β-glucosidase as an example. As expected, with temperature rise, the yield and the biological activity diminished, while the molar radioactivity increased. The distribution of the label between the amino acid residues in β-glucosidase is reflected in Table 28.[104] Tritium is incorporated in each of the amino acid fragments but the contributions they make to the total radioactivity of the protein are substantially different.

The foregoing suggests that there are at least three temperature regions in performing solid-phase reactions characterised by different features of the inclusion of the label. The borders of these regions are not rigidly fixed, as they vary depending on the reaction conditions and the nature of the substrate. Apparently, this pattern of variation of the efficiency of tritium inclusion into organic compounds can be explained in the following way. In the first temperature region (~130–150 °C), the isotope exchange involves predominantly the tritium cations. In the second region (~150–220 °C), both the tritium cations and the tritium atoms participate in the reactions. In the third region (>220 °C), labelling is mainly accomplished by atomic tritium.

Table 28. Distribution of the tritium label among the amino acid residues in β-glucosidase (T = 40 °C, τ = 60 min).

Amino acid residue	Molar fraction of the residue	Fraction of radioactivity (%)	Amino acid residue	Molar fraction of the residue	Fraction of radioactivity (%)
Asp	3.0	2.2	Cys	0.1	0.1
Thr	10.2	5.9	Met	4.1	4.2
Ser	3.8	2.0	Ile	4.7	2.5
Glu	4.0	1.5	Leu	9.3	7.4
Pro	0.3	0.1	Tyr	3.8	6.7
Gly	11.1	2.4	Phe	5.0	13.4
Ala	9.9	5.2	Lys	8.1	20.7
Val	11.7	5.6	His	2.1	7.4
Trp	3.6	8.9	Arg	5.2	5.4

2. Solid-phase hydrogenation by tritium

Heterogeneous catalytic hydrogenation is yet another method for preparing tritium-labelled compounds. Solid-phase hydrogenation is carried out by the same procedures as the solid-phase isotope exchange. The solid-phase hydrogenation reactions possess a number of features inherent in the isotope exchange reactions, but they also have some distinctive features. Hydrogenation of mono- and disubstituted carbon – carbon double bonds proceeds even at room temperature, while unsaturated carbon – heteroatom bonds are usually hydrogenated under more rigorous conditions (Table 29).[53, 62, 63, 68, 111]

Receptor or analytical investigations require labelled preparations with molar radioactivities much higher than those normally obtained by hydrogenation of double bonds

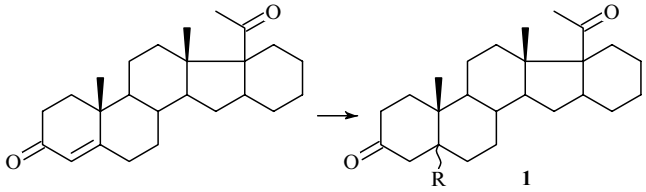
Table 29. Molar radioactivity and yields of labelled compounds obtained by tritium labelling using solid-phase hydrogenation.[53, 62, 63, 68]

Starting compound	Reaction conditions			MR [a]	Yield (%) [b]
	catalyst	T /°C	τ /min		
Allyl alcohol	5% $Pd/BaSO_4$	20	30	2.20	76
But-3-en-1-ol	5% $Pd/BaSO_4$	20	30	1.95	85
11-Cyanoundecanoic acid	5% Rh/C	80	180	2.05	51
2,2,6,6-Tetramethyl-4-oxopiperidine	5% Rh/C	80	180	4.80	57
2,2,6,6-Tetramethyl-4-hydroxyiminopiperidine	5% Rh/C	100	180	4.80	20
Benzylamine	5% Rh/Al_2O_3	60	180	7.78	45
(1*R*,2*R*)-1-Amino-2-(4-methylpiperidinylmethyl)cyclohex-4-ene	5% Pd	120	15	1.15	2
Methacrylic acid	5% Pd/C	170	20	5.63	80

[a] Expressed in PBq mol^{-1}. [b] The yield of a reduced or hydrogenated product.

(1.85 – 2.04 PBq mol^{-1} per bond). In this case, the solid-phase hydrogenation procedure is more efficient than the standard liquid-phase procedure. This is due to the fact that hydrogenation carried out with heating is accompanied by isotope exchange (see Table 29).

For example, hydrogenation of methacrylic acid by treatment with tritium gas on the 5% Pd/C catalyst at 170 °C gave a labelled specimen with a molar radioactivity ~3 times as high as could be expected upon the reduction of the double bond.[63] In addition, by using solid-phase hydrogenation, one can increase the yield of the labelled specimen (with respect to the liquid-phase method). This situation is often observed when hydrogenation affords isomeric products as, for example, in the reduction of 16α,17α-cyclohexanopregn-4-ene-3,20-dione.



1

R = ◄H (5β,16α,17α-cyclohexanopregnane-3,20-dione),
····H (5α,16α,17α-cyclohexanopregnane-3,20-dione).

The reaction gives rise to saturated steroids, namely, the 5α- and 5β,16α,17α-cyclohexanopregnane-3,20-dione isomers, in a ratio of 1 : 14. Only several percent of the 5α-isomer could be isolated from the reaction mixture; preparative amounts of this compound (a 1 : 3 ratio in the reaction mixture) have been obtained by using solid-phase hydrogenation.[112] This difference can be explained by the fact that the liquid-phase hydrogenation proceeds in the active sites of the catalyst, while in the solid-phase process, atoms of the metal catalyst generate activated tritium species, which react with a steroid molecule. Evidently, when the steroid is adsorbed on the catalyst active sites during hydrogenation in solution, the influence of the steric factors is maximum; however, if the active species of tritium attack the double bonds of the substrate molecule adsorbed on a support, the formation of 5α-isomers is more likely.

The dependence of the 5α- to 5β-isomer ratio on the reaction temperature can be followed using the data of Table 30, which presents also the amounts of isomers in the reaction mixture obtained from 1 mg of the substance introduced in the reaction.

For the solid-phase hydrogenation of unsaturated carbon–heteroatom bonds, thorough selection of the labelling conditions is required. For example, the nitrile of glycolic acid was used as the precursor in the synthesis of labelled ethanolamine. This precursor was reduced in the presence of 5% Pt/C, 5% Pd/C, 5% Rh/Al_2O_3 and 5% Rh/$CaCO_3$.[113–115] The 5% Rh/C catalyst (Table 31), a temperature of 100 °C and a reaction time of 20 min were found to be the best conditions as regards the synthesis of a highly labelled specimen (1.04 – 1.48 PBq mol^{-1}). When palladium catalysts are used, the labelled specimen is formed in 10% – 20% yield and has a molar radioactivity of 0.15 – 0.22 PBq mol^{-1} (see Ref. 114). This rather low yield can be explained by assuming that dehydration, deamination and other reactions take place in parallel on the metal catalysts.[61,69]

Table 30. Ratio (z), amounts (m_1, m_2) and molar radioactivities of the 5α- and 5β-isomers of compound **1** in the reaction mixture at various temperatures.

T /°C	z	m_1 /mg mg^{-1}	m_2 /mg mg^{-1}	MR [a] (rel.u.)
80	1 : 3	0.16	0.46	1.04
100	1 : 2.7	0.17	0.45	1.27
110	1 : 2.1	0.20	0.41	1.49
140	1 : 1.8	0.15	0.28	2.84
170	1 : 1.5	0.08	0.13	–

[a] The radioactivity of saturated steroids obtained by the liquid-phase method.

Table 31. Relative molar radioactivities of the specimen prepared by the reduction of glycolic acid nitrile (catalyst 5% Rh/C, the catalyst : substance ratio is 5 : 1) at different reaction temperatures and durations.

T /°C	RMR (%) (see [a])	τ /min	RMR (%) (see [b])
20	11	5	26
50	31	10	50
70	55	15	80
90	67	20	100
100	100	30	92
120	85	40	78
140	66	50	57
160	33	60	29
180	13	75	16
190	6	90	11
		120	9
		150	6

[a] Reaction time 20 min. [b] Reaction temperature 100 °C.

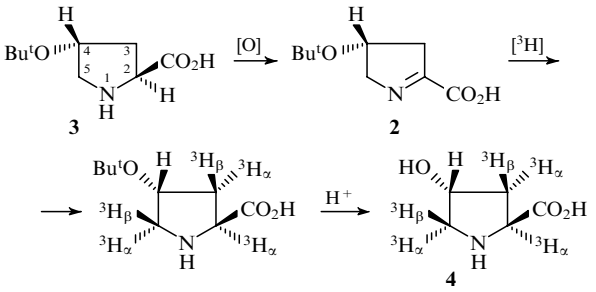
As an example of solid-phase reduction of the keto group, one can consider the synthesis of [2-^{3}H]inositol from *scyllo*-inosose (pentahydroxycyclohexanone) and 2,2,6,6-tetramethyl-4-hydroxypiperidine from 2,2,6,6-tetramethyl-4-oxopiperidine.[62,113] To select the optimal conditions for the reduction of *scyllo*-inosose, the 5% Pd/$CaCO_3$, 5% Rh/Al_2O_3, 5% Pd/$BaSO_4$, 5% Pt/C and 5% Pd/C catalysts were tested. The best results were attained with the rhodium catalyst (the catalyst : substrate ratio was 10 : 1) and with heating to 150 °C for 15 min (the molar radioactivity of the labelled specimen was 0.56 PBq mol^{-1} and the yield was 25%). 2,2,6,6-Tetramethyl-4-oxopiperidine was hydrogenated on 5% Rh/C to give 2,2,6,6-tetramethyl-4-hydroxypiperidine (see Table 29).

The molar radioactivities attained upon labelling in the former and in the latter case differ by a factor of ~9, as the efficiency of isotope exchange depends appreciably on the structure of the compound. In the former case, the keto–enol tautomerism of *scyllo*-inosose is hampered and the label is inserted only *via* ketone hydrogenation. In the latter case, all four α-hydrogen atoms have time to be replaced before the keto group is reduced. This appears to be a weighty reason supporting the formation of $^3H^+$ as the activated species in the spillover.

Apart from the reduction of the C≡N fragment to $C^3H_2NH_2$ and the C=O fragment to $C^3(H)OH$, data on hydrogenation of C=NOH to C^3HNH_2 have been reported. For example, reduction of 2,2,6,6-tetramethyl-4-hydroxyiminopiperidine to 2,2,6,6-tetramethyl-4-aminopiperidine has been described;[62] in this case, the isotope exchange is no less intensive than in the case of reduction of 2,2,6,6-tetramethyl-4-oxopiperidine (see Table 29).

In order to have an apprehension of the mechanism of solid-phase hydrogenation, Zolotarev *et al.* studied [116] the reduction of (*R*)-4-*tert*-butoxy-4,5-dihydro-3*H*-pyrrole-2-carboxylic acid (**2**) prepared from amino acid **3**.

Compound **2** was applied onto alumina, dried and mechanically mixed with 5% Pd/$BaSO_4$. The mixture was subjected to freeze-drying (at a catalyst : Al_2O_3 : substrate ratio of 10 : 20 : 1, T = 100 °C, τ = 60 min). After the reaction, the *tert*-butyl protection was removed by treating the reaction mixture with hydrochloric acid and the product was purified by the procedure described previously.[117–119] The yield of (*R*)-4-hydroxy[G-^{3}H]-L-proline (**4**) was 23% and the molar radioactivity was 1.85 PBq mol^{-1}. The isotopomer was isolated in a yield of 4%. The distribution of the label was determined by ^{3}H NMR spectroscopy.



The distribution of the label in proline **4**, obtained by hydrogenation of compound **2** by tritium gas at 100 °C, was as follows:

Position of the label	3β	3α	5α	5β	2	4
Content of the label (%)	29	20	0	35	16	0

4,5-Dihydro-3*H*-pyrrole-2-carboxylic acid was used as the reference compound. The distribution of the label in [G-^{3}H]proline (the molar radioactivity was 2.07 PBq mol^{-1}) obtained on hydrogenation of 4,5-dihydro-3*H*-pyrrole-2-carboxylic acid at 100 °C is given by

Position of the label	3β	3α	5	2	4
Content of the label (%)	34	34	6	26	0

Apparently, the first stage of hydrogenation of compound **2** involves the capture of tritium cations by nitrogen and oxygen atoms; as a consequence, the mobility of hydrogen at the third and fifth carbon atoms substantially increases and the carboxy group acquires the ability to occupy the *trans*-position relative to the 4-*tert*-butoxy group. Therefore, hydrogenation of the initial double bond can be accompanied by the addition of tritium into the 2-, 3- and 5-positions. The attack by tritium of the 5-position occurs necessarily from the β-H side; the attack of the 3-position is governed, on the one hand, by shielding of the β-H atom by the carboxy group and, on the other hand, by shielding of the α-H atom by the 4-*tert*-butoxy group. Thus, the contents of tritium in the α- and β-positions at C(3) should be comparable. Indeed, the data presented here on the distribution of the label show that the label is missing from the 5α-position of proline **4**, while the ratio of the degrees of labelling at C(3) is 29 : 20 in favour of the 3β-position. This may be due to the fact that the label in the 2-position of proline **4** appears, in the researchers' opinion,[116] upon the intramolecular transfer of tritium from the 3α-position of the molecule. If hydrogenation of 4,5-dihydro-3*H*-pyrrole-2-carboxylic acid gives a racemate, no steric restrictions are involved in the labelling of the C(3) atom, and the major portion of tritium is found at the 3-position of the molecule of this amino acid. For the same reason, the content of the label at the 2-position of the proline molecule increases and the probability of tritium insertion into the 5-position decreases ~6-fold.

If the first stage of the solid-phase hydrogenation is the reaction of the substrate with the tritium cations, it is reasonable to assume that the addition of the tritium cations to the molecules of unsaturated amino acid gives two options for the subsequent transformations: (1) capture of an electron to give a radical, which undergoes recombination with atomic tritium; (2) hydrogenation involving tritium gas on the sites arising due to the spillover.[7–11] The observed steric effects may be indicative of a predominant direct participation of tritium gas in the hydrogenation.

As an example, Fig. 3 shows the curves for the time variation of the yield of the labelled amino acid obtained by the reduction of 2-benzoylamino-3,3-dimethylacrylic acid to give *N*-benzoyl[^{3}H]-valine (2.74 PBq mol^{-1}) at different temperatures and different pressures of tritium gas.[81] It can be seen that with an increase in temperature, duration and pressure of tritium gas, the hydrogenation rate increases, but below 100 °C, the reaction does not proceed to completion,[81] although even at 60 °C, isotope equilibration of virtually all the labile hydrogen atoms (attached to nitrogen, oxygen, sulfur and other atoms) takes place as a result of

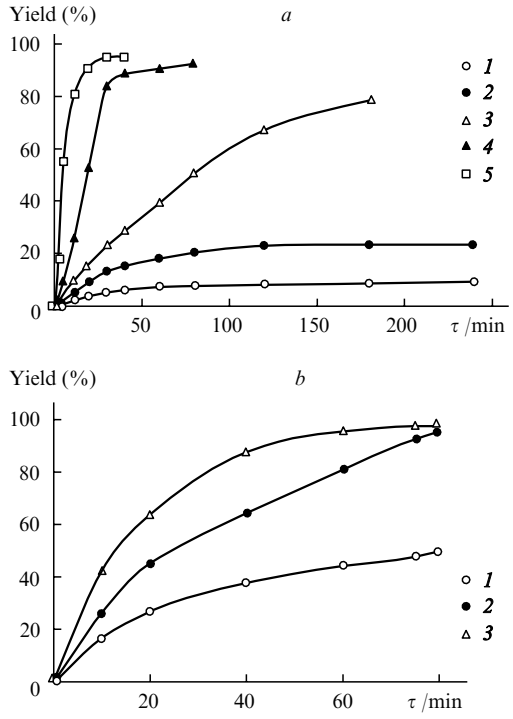


Figure 3. Yield of the labelled product of solid-phase hydrogenation of 2-benzoylamino-3,3-dimethylacrylic acid (P = 333 hPa, 5% Pd/$BaSO_4$ as the catalyst) *vs.* reaction time at different temperatures (*a*) and different pressures (*b*).
a: T /°C: (*1*) 60, (*2*) 80, (*3*) 100, (*4*) 120, (*5*) 140;
b: P /hPa: (*1*) 50, (*2*) 150, (*3*) 250; T = 120 °C.

tritium spillover. Apparently, this is due to the fact that at these temperatures, the metal catalyst is rapidly saturated with hydrogen and deactivated (see Section IV).

The activation energy for hydrogenation of 2-benzoylamino-3,3-dimethylacrylic acid (46.09 kJ mol^{-1}) is close to the activation energy for the isotope exchange between valine and tritium gas (50.28 kJ mol^{-1}).[81,120] In the researchers' opinion, this attests that both reactions are limited by the same step of tritium inclusion. These values are in good agreement with the well-known activation energy of the solid-phase hydrogenolysis on platinum metals.[7] It can be asserted that this step is not isotope exchange because the activation energies for the isotope exchange and hydrogenation depend appreciably on the nature of the substrate and differ even for different fragments of the same molecule. The assumption that a process related to the formation of active tritium species and to the spillover of these species to the reaction area is the limiting step seems doubtful. It has been shown[81] that even at 20 °C, the active hydrogen atoms in the 2-benzoylamino-3,3-dimethylacrylic acid molecule are replaced by tritium over a period of 20 min. Therefore, the most plausible assumption is that the activation energies presented above are due to the fact that the tritium cation has to approach a carbon atom of the organic compound for the formation of the transition state.

The solid-phase hydrogenation technique can be used to reduce uridine derivatives. For example, the reduction of 5-hydroxymethyl-2′-deoxyuridine 5′-monophosphate (7% Pd/$BaSO_4$, T = 170 °C, τ = 30 min) yielded thymidine 5′-monophosphate with a molar radioactivity of 2.54 PBq mol^{-1} in 10% yield.[121] It is of interest that the use of hydrogenation to prepare high-labelled purine and pyrimidine compounds gave no advantages over isotope exchange.[78] For example, the reduction of 5-formyluracil and 5-hydroxymethyluracil to thymine gave labelled preparations with molar radioactivities of 3.15 and 4.07 PBq mol^{-1}, respectively,[80,122,123] while labelled thymine

obtained by isotope exchange had a molar radioactivity of 4.26 PBq mol^{-1}. In the case of reduction of 5-hydroxymethyldeoxyuridine to thymidine, the molar radioactivity was even lower (2.82 PBq mol^{-1}).[124]

3. Solid-phase dehalogenation

Catalytic dehalogenation is the best known hydrogenolysis reaction. If tritium gas is used as the reagent, dehalogenation gives rise to tritium-containing compounds.

Usually, aromatic compounds are dehalogenated by a liquid-phase procedure.[105] This affords labelled specimens with a molar radioactivity of 0.7–0.9 PBq mol^{-1}. However, the liquid-phase approach cannot be used, as a rule, for labelling of saturated halogen-containing compounds under conditions acceptable for work with tritium gas.

The solid-phase catalytic dehalogenation was first employed to introduce a tritium label into uracil.[1] Characteristic features of this reaction as applied to tritium labelling of nucleic acid components have been studied in detail.[125]

The advantages of the solid-phase approach have been demonstrated in relation to the dehalogenation of aliphatic compounds.[60] 11-Chloroundecanoic acid was chosen as the model compound for working out the conditions for the labelling of aliphatic compounds by dehalogenation. The dependence of the degree of labelling of both 11-chloroundecanoic acid and undecanoic acid formed in the reaction on the nature of the catalyst and the reaction temperature was studied (Fig. 4). The molar radioactivity of 11-chloroundecanoic acid reached 6.30 PBq mol^{-1}, while that of the undecanoic acid formed from it was 4.20 PBq mol^{-1}. For comparison, debromination of 3-bromopropionic acid (10% Pd/C, 1 M NaOH, τ = 4 h, P = 905 hPa) furnishes [3-^{3}H]propionic acid with a molar radioactivity of 0.72 PBq mol^{-1} (see Ref. 126).

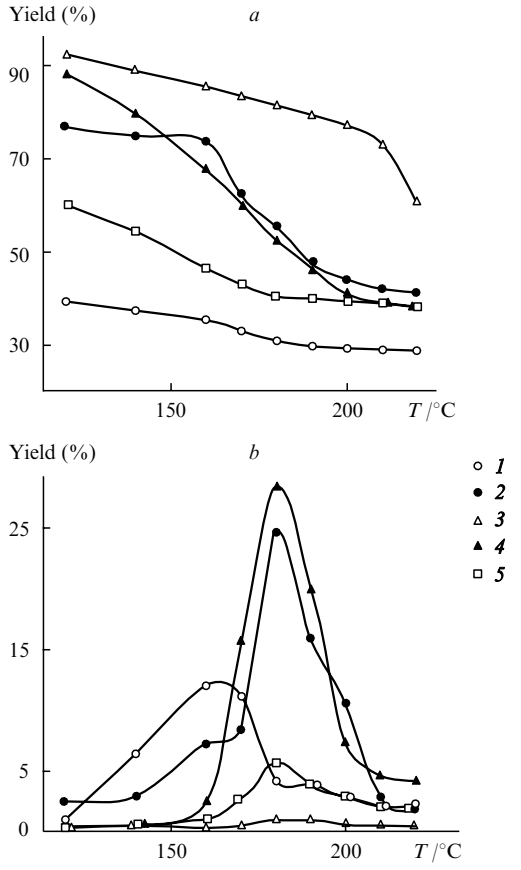


Figure 4. Yield of labelled 11-chloroundecanoic acid (*a*) and undecanoic acid produced from it (*b*) *vs.* temperature and the catalyst nature (the catalyst : acid ratio is 5 : 1, τ = 20 min).
(*1*) 5% Pt/C; (*2*) 10% Pd/C; (*3*) 5% Pd/C; (*4*) 10% Pd/$BaSO_4$; (*5*) 5% Rh/C.

It can be seen from the above data that under the optimal conditions (10% Pd/$BaSO_4$, T = 180 °C), the yield of undecanoic acid reached 25%–30%. If the goal was to label the starting compound, the reaction was carried out at 170 °C and the yield of the chlorine-containing precursor was 50%–60% (see Fig. 4). It was found that the isotope exchange in halogen-containing saturated acids proceeded much more readily than dehalogenation. Apparently, this is due to the fact that halogens are nucleophiles and can be removed from the organic molecule under these conditions only as hydrogen halides. Therefore, the solid-phase dehalogenation of aliphatic compounds at relatively low temperatures is less probable than isotope exchange. At high temperatures, a radical mechanism of dehalogenation is most likely.

The introduction of a label *via* solid-phase dehalogenation has been studied in detail for bromo-substituted components of nucleic acids (Table 32).[75, 127]

Table 32. Molar radioactivities of labelled heteroaromatic compounds prepared by solid-phase debromination (5% Pd/C, T = 100 °C, τ = 120 min; the position of tritium corresponds to the bromine position in the precursor).

Compound	MR /PBq mol^{-1}	Compound	MR /PBq mol^{-1}
[5-^{3}H]Uracil	0.60	[8-^{3}H]Guanosine 5′-monophosphate	0.19
[8-^{3}H]Guanine	0.46		
[5-^{3}H]Cytidine	0.60	[5-^{3}H]Uridine 5′-monophosphate	0.10
[8-^{3}H]Guanosine	0.25		
[8-^{3}H]Adenosine	0.39	[5-^{3}H]Cytidine 5′-monophosphate	0.22
[5-^{3}H]Uridine	0.43		
[5-^{3}H]2′-Deoxyuridine	0.68	[8-^{3}H]2′-Deoxyadenine 5′-monophosphate	0.08
[5-^{3}H]2′-Deoxycytidine[8-^{3}H]	1.22	[8-^{3}H]2′-Deoxyguanosine 5′-monophosphate	0.06
2′-Deoxyadenine	0.61		
[8-^{3}H]Adenosine 5′-monophosphate	0.18	[5-^{3}H]2′-Deoxycytidine 5′-monophosphate	0.06

It has been shown[127] that the degree of dehalogenation increases with an increase in the palladium content on carbon or with an increase in the catalyst to substance ratio. This is due to the fact that hydrogen bromide deactivating the catalyst is formed as a side product. When a certain palladium : halogen-containing precursor ratio was reached, the yield increased to 85%–90% and then remained constant. Therefore, the notion of catalytic capacity of the catalyst was introduced for these reactions. This value is defined as the number of moles of the halogen derivative which can be converted into the debrominated compound by a particular amount (weighed portion) of the palladium catalyst. During the study, it was found that these compounds cannot migrate over the support surface; therefore, the catalytic capacity depends on the reaction temperature and the nature of the initial halogen derivative.

Solid-phase dehalogenation of aromatic compounds is less efficient than dehalogenation in solution (Table 33). In the latter case, the experimental procedure is simpler and better reproducible, the reaction products can be more easily purified and often have better radiochemical parameters. Therefore, study of the solid-phase dehalogenation of aromatic compounds makes sense if it is necessary to label a halogen-substituted biologically active compound with retention of its native structure. For example, alprazolam was converted into a labelled preparation with a molar radioactivity of 0.15 PBq mol^{-1} without loss of chlorine even at 175 °C,[128] *i.e.*, selective isotope exchange was carried out for an

Table 33. Molar radioactivities of labelled aromatic compounds obtained by debromination in solution (the position of tritium corresponds to the bromine position in the precursor).

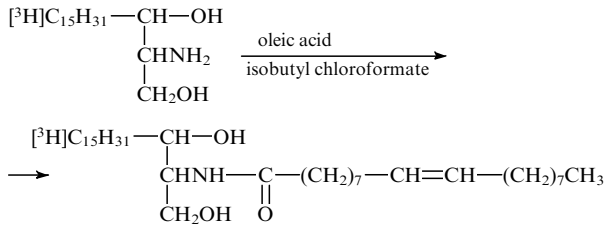
Compound	MR /PBq mol^{-1}	Compound	MR /PBq mol^{-1}
[8-^{3}H]Adenosine	0.36	[8-^{3}H]GMP	0.41
[8-^{3}H]Guanosine	0.47	[5-^{3}H]Cytidine	0.64
[5-^{3}H]Uracil	0.67	[2-^{3}H]Adenine	0.93
[5-^{3}H]Uridine	0.78	[2,8-^{3}H]Adenine	1.85
[5-^{3}H]2′-Deoxy-uridine	0.79	[5-^{3}H]Cytosine	1.11
		[8-^{3}H]ATP	0.63
[8-^{3}H]AMP	0.23	[8-^{3}H]GTP	0.41
[5-^{3}H]UMP	0.14	[5-^{3}H]UTP	0.63
[5-^{3}H]CMP	0.04	[5-^{3}H]CTP	0.78

Note. The following designations are used: UMP is uridine 5′-monophosphate, CMP is cytidine 5′-monophosphate, GMP is guanosine 5′-monophosphate, GTP is guanosine 5′-triphosphate, UTP is uridine 5′-triphosphate, CTP is cytidine 5′-triphosphate.

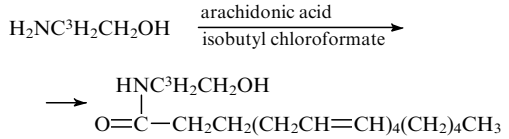
organic molecule containing fragments labile under a tritium gas atmosphere.

4. Preparation of tritium-containing compounds on the basis of reagents labelled by the solid-phase method

In some cases, it proves to be more difficult to introduce a label into a biologically active compound than into one of its fragments. In these cases, the label is introduced into this fragment and then the structure of the molecule is reconstituted. For example, *N*-oleoyldihydrosphingosine with a high molar radioactivity was synthesised in two stages. First, dihydrosphingosine was labelled by a solid-phase procedure;[69] then the product was condensed with oleic acid by the method of mixed anhydrides.

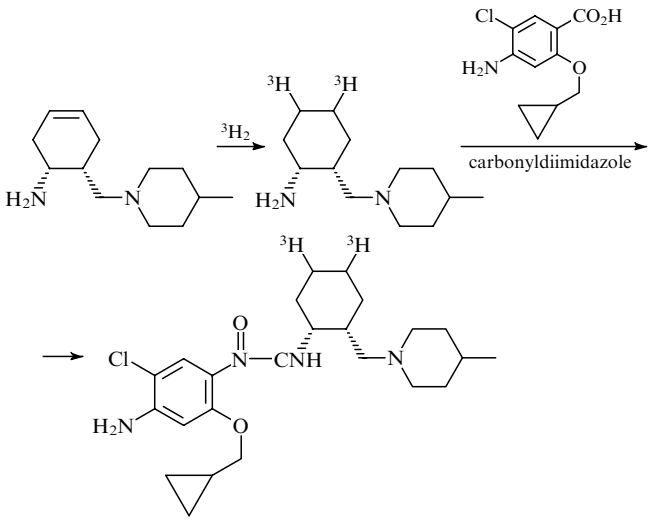


2-Hydroxyethylamides of eicosapolyenoic acids were synthesised in a similar way. Evidently, it is impossible to introduce any significant amounts of tritium into the final compound without affecting the double bonds of polyunsaturated fatty acids. Therefore, solid-phase tritiation of glycolic acid nitrile was used to obtain aminoethanol,[114] which was then condensed with a fatty acid by the method of mixed anhydrides.



The unlabelled reagent was taken in a tenfold excess.

The condensing agents used in this type of reactions include carbonyldiimidazole, carbonylditriazole and carbodiimides. For example, carbonyldiimidazole was used to prepare labelled (1*R*,2*R*)-*N*-[2-(4-methylpiperidylmethyl)cyclohexyl]-4-amino-5-chloro-2-cyclopropylmethoxybenzamide.[74]



Acetylation of amines by non-labelled reagents presents no problem. Reactions using acetic anhydride, acetyl chloride, *etc.* give high yields of amides. However, synthesis of labelled analogues of these compounds presents a number of difficulties, which make these procedures unattractive. The main difficulty is high volatility of these substances. Work with such compounds (unlike non-volatile compounds) can be carried out only in a specially equipped laboratory. In addition, extensive use of these reagents is hampered by their high cost. Sodium acetate can be labelled by a direct reaction, as it is non-volatile (m.p. = 324 °C);[61] therefore, this compound makes a convenient reagent for the preparation of labelled amides. The method consists of mixing of solutions of labelled sodium acetate [129] and amine hydrochloride; this gives labelled acetic acid and sodium chloride. After addition of the condensing agent, the tube is sealed and heated. When the reaction has been completed, the contents of the tube are frozen and refrozen to separate non-volatile products, the residue is dissolved in methanol, and the solution is acidified by hydrochloric acid and concentrated to remove traces of labelled acetic acid. Dicyclohexylcarbodiimide and carbonylditriazole were used as condensing agents. The best results were attained with carbonylditriazole (the yield of the labelled preparation reached 15%).

5. The selectivity problems in the solid-phase hydrogenolysis by tritium

It has been shown above that molecules containing halogen atoms can be labelled using selective isotope exchange. As has already been noted, labelling of alprazolam made it possible to obtain a labelled specimen with a molar radioactivity of 0.15 PBq mol^{-1} (see Ref. 128).

This Section considers approaches designed for solid-phase labelling either by isotope exchange when the substrate molecules contain readily reducible fragments (for example, nitro groups or double bonds) or by selective dehalogenation and hydrogenation (Fig. 5, Table 34).[130, 131] The selective hydrogenation can involve

Table 34. Molar radioactivities of labile compounds labelled in the presence of heterogeneous platinum group metal catalysts.[131]

Compound	*T* /°C	MR /PBq mol^{-1}
9-Nitrocamptothecin	190	0.59
Dimethomorpholine	160	0.24
Thyroxin	180	0.25
Methyl ester of *N*-nitroarginine	180	0.85
Etomidate	160	0.86
Vitamin K_1	130	2.3

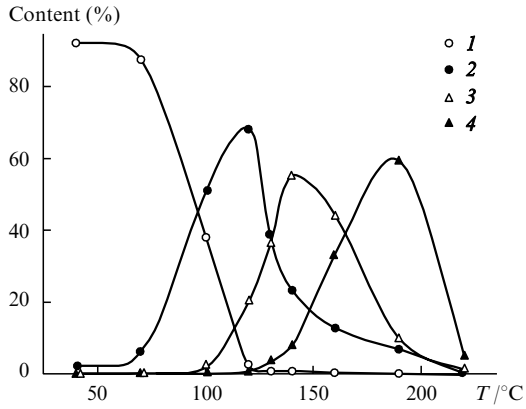
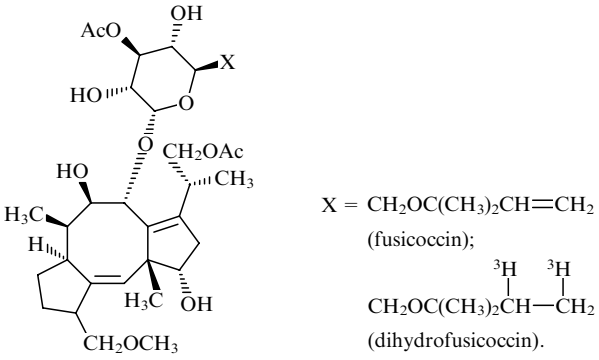
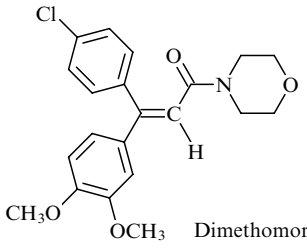


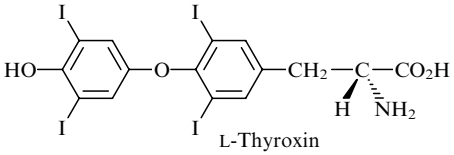
Figure 5. Temperature dependence of the content of hydrogenation products of fusicoccin in the reaction mixture.[130]
(*1*) Fusicoccin, (*2*) dihydrofusicoccin (DHF); (*3*) [$^3H_4$]tetrahydrofusicoccin (THF); (*4*) [$^3H_6$]hexahydrofusicoccin (HHF).

either hydrogenation of one or several double bonds or hydrogenation of one aromatic fragment in the presence of several other such fragments.

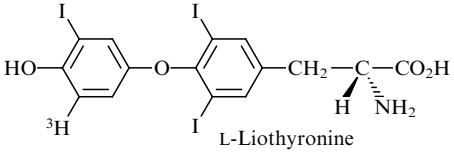




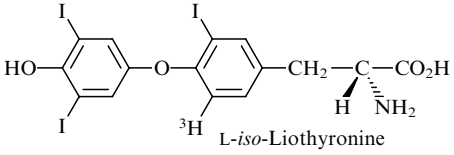
Dimethomorpholine



L-Thyroxin



L-Liothyronine



L-*iso*-Liothyronine

For compounds containing nitro groups, isotope exchange was carried out at temperatures of 180–190 °C and for compounds containing double bonds, at 100–160 °C.

As noted above, stability of a biologically active compound can be increased by using rhodium trichloride, an additional support or a partially deactivated catalyst. For example, in the case of thyroxin and vitamin K_1, palladium catalysts deactivated by lead diacetate were used;[130] rhodium trichloride was employed for dimethomorpholine[73] and an additional support, for etomidate.[131]

For selecting the optimal conditions for labelling compounds with double carbon–carbon bonds, a special study has been carried out to establish the temperatures of reduction of double bonds with different degrees of substitution.[130]

Fusicoccin was chosen as the model compound to elucidate the conditions of labelling by selective solid-phase hydrogenation of one double bond,[132–134] because this compound contains mono-, tri- and tetra-substituted double bonds. Selective solid-phase hydrogenation was studied in the temperature range of 40–220 °C, the reaction time and the pressure of tritium gas being also varied (see Fig. 5, Table 35). The selective hydrogenation of a monosubstituted double bond starts even at 40 °C and proceeds efficiently at 100 °C.

Table 35. Dependence of the content of major hydrogenation products of fusicoccin in the reaction mixture on the pressure of tritium gas and the reaction time (T = 100 °C, the Lindlar catalyst : substance ratio is 10 : 1).[129]

Reaction conditions		Content (%)		
P /hPa	τ /min	fusicoccin	[$^3H_2$]DGP	[$^3H_4$]TGP
210	20	38	51	2
280	20	32	57	2
350	20	13	60	3
350	60	6	69	4

An increase in the pressure of tritium gas and the reaction time affect to a lesser extent the process selectivity.[130] If the goal of the experiment is to obtain the highest yield of dihydrofusicoccin, the reaction should be carried out at 130 °C (see Fig. 5).

The results were verified during labelling of vitamin K_1 (Table 36).[130] The presented data show that trisubstituted double bonds withstood temperatures in the 120–140 °C range; therefore, labelling of vitamin K_1 was carried out at 130 °C (see Table 34).

Table 36. Content of vitamin K_1 in the reaction mixture and degree of isotope exchange at different temperatures of tritium labelling (the Lindlar catalyst : substance ratio is 10 : 1, P = 333 hPa, τ = 20 min).[130]

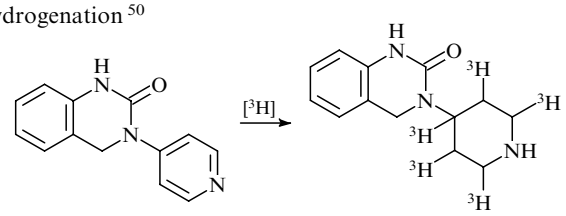
T /°C	Content (%)	RMR (%)	Radiochemical purity (%) (see [a])
80	99	9	95
100	68	16	64
120	60	52	21
140	16	100	8

[a] Radioactivity of the label in the substance divided by the radioactivity of the reaction mixture.

Thyroxin was used as the halogen-containing compound to study the possibility of labelling by selective dehalogenation. At high temperatures (180–200 °C), liothyronine and *iso*-liothyronine could be isolated from the reaction mixture in a pure state. At 180 °C (τ = 10 min), the yields of these compounds were 3.2% and 0.4%, and at 200 °C (τ = 5 min), they were 5.3% and 1.3%, respectively. The molar radioactivity of the dehalogenated products reached 0.7 PBq mol^{-1} (see Ref. 130).

Research into solid-phase hydrogenation and dehalogenation is usually necessary for studying the stability of a particular labile fragment in the molecule corresponding to a given class of compounds and to carry out solid-phase isotope exchange with retention of the native state of the initial compound as, for example, in the case of labelling of vitamin K_1.

An absolutely different situation occurs in working out the conditions of selective hydrogenation of one aromatic fragment in the presence of other aromatic fragments (for example, the pyridine and benzene rings). Hydrogenation of aromatic compounds in solution is known to occur only at very low pH.[68, 135] However this results in high isotope dilution of the labelled specimen. For example, in the synthesis of cyclohexylmethylamine from benzylamine using the liquid-phase hydrogenation procedure, the molar radioactivity of the labelled preparation was 1.66 PBq mol^{-1}, while the solid-phase hydrogenation procedure gave a specimen with a molar radioactivity of 7.78 PBq mol^{-1} (see Ref. 68). In this case, the advantage of the solid-phase approach is obvious. Apparently, the nature of the activated tritium species is largely responsible for whether or not the process can, in principle, be selective. If these species are cations, the process selectivity would be high (the pyridine fragment is protonated much more efficiently than the benzene fragment). The aromatic structure of the pyridine fragment is violated and hydrogenation is thus facilitated. Thus in the case of selective hydrogenation [50]



the labelled molecule contained five tritium atoms (6.44 PBq mol^{-1}). The content of this product in the reaction mixture was 7 times as high as the total content of all other substances, *i.e.*, the process was highly selective. This indicates that tritium cations play an active role in the hydrogen spillover. In other words, the specificity of solid-phase reactions may be largely responsible for the relative stability of organic compounds labile under the conditions of hydrogenolysis. Therefore, below we consider the features of solid-phase reactions that influence not only the retention of the native structure of the initial compounds (including chirality) but the distribution of the label in the molecules.

IV. Characteristics of solid-phase hydrogenolysis by tritium

A leading specialist in the synthesis of tritium-labelled organic compounds, Evans, has noted [136] that labelling methods in which organic compounds are kept under a tritium atmosphere with process initiation by a Tesla discharge or by UV or γ-radiation or gas exposure of a material applied on a heterogeneous catalyst are nothing but various modifications of the Wilzbach method. Many researchers still adhere to this point of view.[137] However, back in the 1960s, it was found that the label distribution in the aromatic ring obtained using heterogeneous catalysts differs appreciably from that obtained using other procedures (Tables 37, 38).[138, 139]

It can be seen from these data that electrophilic substitution predominates in the replacement of hydrogen in the aromatic ring by tritium using heterogeneous catalysts, whereas in other cases, radical substitution is the prevailing process. A similar tendency has been noted in another publication.[140] For example, upon labelling of a peptide with N-terminal tyrosine supported on a heterogeneous catalyst at 135 °C, the amount of the label inserted in the *ortho*-position relative to the hydroxy group of tyrosine was ~20 times as high as that in the *meta*-position.

Table 37. Distribution of the label (%) in toluene introduced by the Wilzbach method and by its modifications.[138]

Label position	No activating factor (Wilzbach method)	Activation method		
		γ-radiation	Tesla discharge	UV radiation
ortho	56.5	54.7	39.7	49.8
meta	29.3	29.3	40.8	33.5
para	14.2	16.0	19.5	16.7

Table 38. Distribution of the label in benzoic and salicylic acids introduced by the Wilzbach method (I) and by treatment with tritium gas of the acids applied on the heterogeneous catalyst (II).[139]

Acid	Content of the label (%) in the position		
	ortho	*meta*	*para*
Benzoic	58.3 / 5.0	21.3 / 72.5	20.4 / 22.5
Salicylic	39.0 [a] / 16.9 [a]	48.4 [a] / 31.7 [a]	13.6 [a] / 51.4 [a]

Note. The value indicated above the bar was obtained using method I and that below the bar was obtained using method II. [a] Relative to the hydroxy group.

Thus, the mechanism of reactions between tritium gas and a substance applied on a heterogeneous catalyst differs from that involved in labelling by the Wilzbach method.

Usually, this is related to the processes taking place when catalysts are saturated with hydrogen.[141–143] According to a view proposed and substantiated on the basis of a study of the valence state of hydrogen in intermetallic hydrides,[141] hydride dispersion can be considered as being due to a redox process giving rise to hydride ions $H^{\delta'-}$, which takes place when the hydrogen concentration (c_H) in the solid hydride is higher than a critical concentration $c_{H_{cr}}$. The $c_{H_{cr}}$ value is determined by the redox potential of the reaction

$$H_2 + MM \longrightarrow H^{\delta+} + MM^{\delta-} \longrightarrow H^{\delta'-} + MM^{\delta'+},$$

where MM is the metallic matrix of the catalyst.

According to this model, hydrogen chemisorbed on the active sites of the catalyst surface donates some of its s-electrons to the conduction band of the metal. Therefore, at low c_H values, the behaviour of absorbed hydrogen is described satisfactorily by the Ubbelode model,[142] which implies high diffusional mobility of $H^{\delta+}$ and an increase (or, at least no decrease) in the electrical conductivity of the hydride phase with respect to that of the initial metal. The $c_{H_{cr}}$ value corresponds to a concentration of the oxidant ($H^{\delta+}$) at which these species oxidise the metallic matrix and become electron density acceptors, being converted into bulky and low-mobility $H^{\delta-}$ ions, and thus deplete the conduction band of the metal. It is evident that at higher degrees of filling of voids by $H^{\delta-}$ ions having a relatively great effective volume, the electron deficiency on the metal atoms that form the metal sublattice in the crystal structure of the catalyst should increase, which may restrict the reversible adsorption of hydrogen. This sharply decreases the concentration of activated species (tritium, in the case of preparation of labelled substances) able to enter into exchange reactions, and hence arrests almost completely the isotope exchange. The $c_{H_{cr}}$ value may not be attained for a long period when hydrogen spillover takes place.

The causes of the transfer of activated species from the metal crystallite to the support are not discussed here in detail, as this has been the subject of numerous publications (see, for example, Refs 144–148) which evaluate how this process is affected by adsorp-

tion of molecular hydrogen, extensive rearrangement of the sorbent surface, relaxation of disordered structures and other factors. The migration of a proton over the oxygen-containing surface of the support follows a relay mechanism. The activation energy of this transfer equals 20.1 kJ mol^{-1} (see Ref. 149). Study of spillover over the support surface revealed both electronic and ionic conduction.[35] Therefore, the observed process, unlike those involved in the Wilzbach method, can be described more reliably in terms of the 'capacitor model' proposed by Shevchenko *et al.*[150] According to this model, the $^3H^+$ ions and the electrons formed in the reaction of tritium gas with the catalyst migrate separately over the surface. The support surface acts as a sort of multilayer capacitor. Depending on the experimental conditions, interaction of tritium cations with electrons (discharge of the neighbouring oppositely charged zones) takes place with one or another probability, giving rise to tritium atoms, which rapidly recombine and pass to the gas phase. When the flux of $^3H^+$ ions and electrons reaches the substrate, isotope exchange, hydrogenation, dehalogenation and other reactions occur efficiently. If the substrate contains easily protonated groups, the degree of labelling should markedly diminish. It is evident that at higher temperatures, the probability of interaction between neighbouring oppositely charged zones would increase; therefore, the contribution of the reactions of atomic tritium to the processes resulting in the formation of the labelled compound would also increase. Thus, if the substrate contains readily protonated groups, the molar radioactivity of the labelled product should markedly increase with temperature rise (the neutral species of atomic tritium would react more efficiently with these compounds). As an example, one can consider the above-mentioned preparation of labelled peptide with N-terminal tyrosine. Thus at 150 °C, the degree of labelling of the *ortho*-position relative to the hydroxy group of tyrosine was only 3 times higher than that of the *meta*-position and the molar radioactivity increased 1.9-fold.[140]

The distribution of the label in aromatic amino acids is especially useful for elucidation of the nature of the activated tritium species. This can be illustrated using the distribution of the label in aromatic rings of tyrosine and serotonin as examples. The contents (%) of the label in the aromatic rings (the total amount of the label in the ring was taken to be 100%) were as follows:[104]

Position	*ortho*	*meta*
Tyrosine	78	22
Serotonin	85	15

As noted in the previous Section, the distribution of the label corresponding to that expected in terms of the electrophilic substitution model can serve as strong evidence supporting the assumption that the tritium cation is the activated species involved in the hydrogen spillover.

The published data provide grounds for two conclusions: (1) inclusion of the label follows predominantly the electrophilic substitution mechanism; (2) the lower the reaction temperature, the higher the predominance of electrophilic substitution. The 'capacitor model' concept is useful for explaining the roles of particular factors in the preparation of tritium-labelled specimens and eliminates many contradictions arising in the study of the theoretical aspects of this problem.

For example, the capacitor model can account for the high yields of labelled specimens obtained *via* solid-phase reactions. This is due to the fact that the participation of atomic tritium in the labelling is insignificant, as the radical detachment of hydrogen followed by recombination of the resulting radical is a more intricate process than the mere recombination of atomic tritium.[151–153]

As noted above, the mechanism of tritium labelling influences the distribution of the label in organic molecules. For instance, a ^3H NMR study (alanine supported on carbon was labelled in the presence of platinum group catalysts) has shown[140] that at 130 °C, the rate of tritium inclusion to the third carbon atom is 7.5 times as high as that to the second carbon atom. In the 70–155 °C range, the rate constant for isotope exchange changed by two orders of magnitude (the activation energy was equal to 62.8 kJ mol^{-1}). At 30 °C, the isotope label was selectively inserted into the methyl group of alanine, whereas, at 190 °C, the label was uniformly distributed throughout the alanine molecules. The activation energy of the isotope exchange in the methyl group of alanine was 58.7 kJ mol^{-1} and that for the alanine methine group was 107.7 kJ mol^{-1}. This sharp difference can be explained in the following way. The amino group is readily protonated, which hampers the reaction of $^3H^+$ with the carbon atom of the methine group of alanine. The charge that appears on nitrogen induces migration of the electron density in the methyl group (hyperconjugation[45]). As a result, the deficit of electron density on the carbon atom at the 2-position is compensated, the methyl-group hydrogen atoms become more active, and the activation energy of isotope exchange at the third carbon atom decreases. At higher temperatures (190 °C), radical elimination reactions become more significant. Therefore, the labelling efficiency at the 2-position sharply increases, which is responsible for the different label distribution.

The validity of this approach is confirmed by the distribution of the label in some other amino acids (Table 39). Only in the case of serine and methionine was the C(2) position labelled to a higher degree. Apparently, this is due to the fact that oxygen and sulfur, like amino groups, are easily protonated and the carboxy group located near the amino group makes the isotope exchange at C(2) prevailing.

Table 39. Distribution of the label (%) in amino acids applied on heterogeneous catalysts and treated with tritium gas at 150 °C.[104]

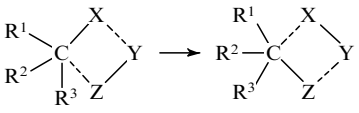
Amino acid	Content of the label at the atoms				
	C(2)	C(3)	C(4)	C(5)	C(6)
Alanine	4	96			
Asparagine	40	60			
Aspartic acid	40	60			
Arginine	19	23	14	44	
Proline	9	6	4	81	
Methionine	75		4	21	
Serine	70	30			
Threonine	18	9	73		
Leucine	4	5	5	86	
Lysine	31	10	12	18	29
Valine	4	4	92		

The possibility of introducing a label into organic substances susceptible to hydrolysis is also largely determined by the mechanism of this process. It is known that liquid-phase reactions proceed in the active sites of the catalyst. These sites adsorb, first of all, unsaturated fragments of molecules and, hence, hydrogenolysis and hydrogenation are the predominant types of reactions. Under solid-phase conditions, labelling is due to reactions of molecules of the substance with activated tritium. Apparently, reduction of the $N-CHR-C_6H_5$ and $O-CHR-C_6H_5$ fragments to toluene or its derivatives or reduction of the $-NO_2$, $=N-OR$ groups to amino groups, *etc.*, would be hampered under solid-phase reaction conditions, as nitrogen and oxygen atoms are prone to be protonated and isotope exchange can be carried out selectively without disturbing the native structures of biologically active products. An important feature of solid-phase hydrogenolysis is the retention of the initial structure of compounds including chirality. Now we consider the reasons for this fact.

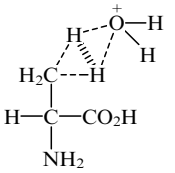
When labelling biologically active compounds, researchers pay considerable attention to the problem of preserving the

optical activity. Only the invariability of the native conformation of compounds can ensure the retention of the initial biological properties of the substrate.

Research into characteristic features of electrophilic substitution in the series of aliphatic compounds [45] basically ended in the 1960s. Bimolecular (S_E2), monomolecular (S_E1) and intramolecular (S_Ei) mechanisms of electrophilic substitution were found. Of special interest for our purposes are data concerning the S_Ei mechanism because it is obvious that upon substitution according to this mechanism, the stereochemical configuration does not change.



The results of calculation of the spatial repulsion forces based on the putative structures of the transition state were consistent with experimental results.[45] The mechanism of the solid-phase isotope exchange has been studied by quantum-chemical calculations.[154] The water molecule was taken as Y. The results of Dewar AM1 semiempirical calculations of fragments of the potential energy surface for the interaction of alanine carbon atoms with the hydroxonium ion were refined by Hartree – Fock *ab initio* calculations in the 3-21G and 6-31G basis sets using the GAMESS program. According to the results obtained, the activation energy of isotope exchange decreases due to an additional interaction of hydrogen atoms, *i.e.*, this transition state is additionally stabilised by a bond between the exchanging hydrogen ions. In the transition state, tritium attacks the C – H bond from the same side as where the atom being replaced is located, *i.e.*, the S_Ei mechanism is executed. Thus, if this isotope exchange takes place at a chiral carbon atom, the native configuration of the initial compound will be retained. The transition state involved in the S_Ei mechanism of electrophilic aliphatic substitution can be represented as follows:[154]



Direct evidence for the assumption that the active species of hydrogen react with the substrate without modifying its crystal lattice was obtained in the 1980s.[155] Experiments were carried out with 4-isopropyl-3-methylphenol. This compound is not optically active in solution but it spontaneously forms chiral structures (dextro- and levorotatory crystals) upon crystallisation. The powder obtained from one sort of 4-isopropyl-3-methylphenol crystals was triturated with 5% Pt/C and kept for 95 min at 80 °C under a hydrogen atmosphere. The reaction product proved to be optically active.

At higher temperatures where the contribution of atomic tritium to the isotope exchange becomes substantial, the absence of racemisation can be explained by the fact that the activation energy for radical elimination reactions is markedly lower than that for the electrophilic isotope exchange (Table 40). Apparently, in this case, the retention of chirality is due to the fact that the rate of recombination of the resulting radicals is incomparable with the rates of radical formation or, all the more, conformational changes.

The possibility of preparing labelled compounds containing trisubstituted unsaturated carbon – carbon bonds depends also on the nature of activated tritium. It is known [45] that the transfer of $^3H^+$ is the rate-determining step of hydrogenation and that the carbenium ion ($R' - H^3HC - C^+H - CH_2R$) is formed as the intermediate product. This intermediate is reduced to give a saturated compound. Thus, the presence of an easily protonated fragment which has acquired a charge near a C = C bond decreases the probability of protonation of this double bond and thus this bond is additionally strengthened. The formation of carbenium ions during the solid-phase hydrogenation is indirectly confirmed by the possibility of double bond migration ($R' - H^3HC - CH = CHR$). This process is detected most easily if R = OH. In this case, migration of the double bond gives an aldehyde stable under the reaction conditions. For example, in hydrogenation of allyl alcohol at room temperature under solid-phase conditions, the yield of aldehyde was ~10% – 20%.[156] Thus, by solid-phase reactions, it is possible to carry out selective isotope exchange and obtain unsaturated labelled preparations.

Table 40. Kinetic parameters of the radical elimination and addition reactions.[152]

Reaction	E_{e0} /kJ mol^{-1}	$10^{11}r_e$ /m	$(r^{\neq}/r_e)_0$	ΔH /kJ mol^{-1}
$H^{\bullet} + RH \rightarrow H_2 + R^{\bullet}$	54.6	3.762	0.48	94.5
$H^{\bullet} + CH_2 = CHX \rightarrow$ $\rightarrow CH_3C^{\bullet}HX$	101.6	4.563	0.41	227.6
$H^{\bullet} + HOR \rightarrow$ $\rightarrow H_2 + RO^{\bullet}$	38.3	2.812	0.47	33.5
$H^{\bullet} + O = CR^1R^2 \rightarrow$ $\rightarrow HOC^{\bullet}R^1R^2$	102.9	4.401	0.38	149.7

Note. The following designations are used: r_e is the distance equal to the sum of vibration amplitudes of atoms of the reacting bonds in the transition state; r^+ is the parameter characterising the position of the transition state on the section r_e; the $(r^+/r_e)_0$ ratio characterises the position of the transition state for $\Delta H = 0$.

If the reaction is carried out at a higher temperature, one should bear in mind that double bonds may be reduced by atomic tritium. Comparison of the kinetics of radical addition and elimination reactions has shown that hydrogen elimination always proceeds more easily than hydrogen addition to a double bond. This is due to different geometries of the reaction centre and to stronger repulsion in the non-linear centre, which is formed upon the reaction of activated hydrogen with the double bond (see Table 40).[43] Another important parameter is the force constant of the bond being attacked, which is always higher for a multiple bond than for a single bond. Moreover, the activation energy of the addition reaction depends more appreciably on the enthalpy change than the activation energy of elimination. As a result, the major process is recombination of atomic tritium, resulting from coupling of tritium cations with electrons, which increases the stability of unsaturated compounds under these conditions.

Thus, if the organic molecule contains easily protonated fragments, they are the first to react with activated tritium species. If the protonated fragments in the molecule are labile, this may give a series of specific side products. In the labelling of compounds whose melting points are higher than the temperature used to conduct the isotope exchange, the degree of isotope exchange would mainly depend on the efficiency of tritium spillover in the bulk of the organic substance applied on a catalyst. If protonated fragments or charged groups present initially in the molecule prevent electrophilic substitution or tritium spillover to the bulk of the organic compound, the final molar radioactivity of the labelled specimen would substantially diminish.

Indeed, it was noticed long ago [136] that the exchange of hydrogen isotopes occurs, first of all, in hydroxy, carboxy, amino and other functional groups. When tritium labelling by solid-phase isotope exchange is carried out for amines [68] (benzylamine, spermine, ethylenediamine and its derivatives) (Table 41), a series of deaminated products are formed; in the case of spermine, it was impossible to isolate the labelled specimen from the reaction mixture.

Table 41. Molar radioactivity and yield of tritium-labelled amines (5% Pd/C, $T = 190$ °C, $\tau = 15$ min).[53,61,74]

Compound	MR /PBq mol^{-1}	Yield (%)
Ethylenediamine	0.13	50
Piperazine	0.32	23
1,4-Diazabicyclo[2.2.2]octane	0.46	15

In the labelling of high-melting compounds, the physicochemical properties of the starting compound exert a considerable influence on the final molar radioactivity of the labelled compound. For example, whereas in methyl stearate molecules (m.p. = 39 °C) hydrogen is replaced almost completely by tritium, in the case of sodium acetate (m.p. = 324 °C), according to ^{3}H NMR spectroscopy,[68] only some of the molecules occur in the areas where isotope exchange proceeds efficiently.

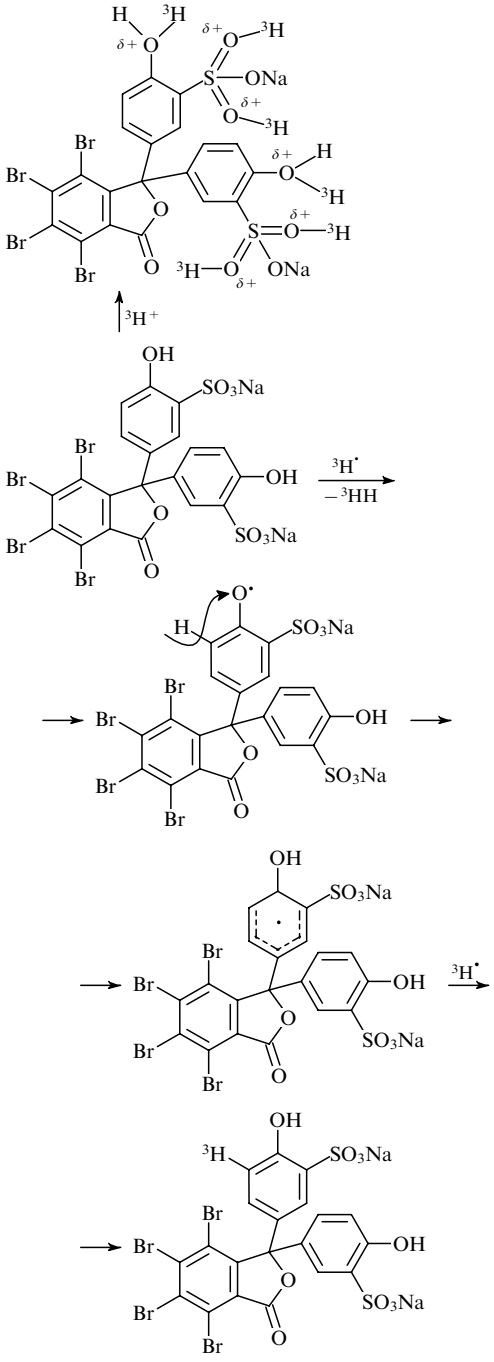
Analysis of the ^{3}H NMR spectra of labelled sodium acetate has shown that labelled molecules comprise 74% triply substituted, 24% doubly substituted and 2% monosubstituted isotopomers, *i.e.*, the molar radioactivity should be ~3 PBq mol^{-1}. However, in reality, the molar radioactivity of [^{3}H]CH_3CO_2Na was 1.1–1.3 PBq mol^{-1}. The reason is that the ^{3}H NMR technique allows one to determine only the ratio of different tritium-containing isotopomers in sodium acetate molecules. The amount of the unlabelled initial substance remaining in the reaction mixture is not detected by ^{3}H NMR spectroscopy. Therefore, the discrepancy between the results obtained by ^{3}H NMR and by radioactivity measurements is due to the fact that ~60% of sodium acetate has not entered into the isotope exchange, which markedly decreases the final molar radioactivity of the labelled preparation. Indeed, as shown by micrographic studies,[157] solid-phase hydrogenolysis of another high-melting compound, namely, disodium *trans*-epoxysuccinate (m.p. = 262 °C), is not accompanied by a noticeable diffusion of this compound over the surface of the palladium catalyst.

Thus, if the melting point of the compound is comparable with or lower than the temperature at which the isotope exchange is carried out, displacement of substrate molecules relative to one another is possible; each molecule can be subjected to the action of activated tritium with the same probability,[60] and the effect of the tritium spillover to the bulk of the given substance on the final molar radioactivity of the labelled specimen is moderate. This can be illustrated by the following data on the molar radioactivity obtained upon solid-phase tritium labelling of fatty acids:[29]

Acid	Stearic	Palmitic	Myristic	Lauric
MR /PBq mol^{-1}	13.0–13.5	12.5–12.9	12.0–12.1	11.0–11.5

To confirm the statement that solid-phase reactions can involve both tritium cations and atomic tritium whose contributions to the final molar radioactivity of the labelled specimen depend on the nature of the initial compound, it was necessary to choose a model compound that, on the one hand, could serve as a sort of trap for tritium cations and, on the other hand, would be sufficiently thermally stable to ensure favourable conditions for isotope exchange involving atomic tritium.

A series of compounds have been tested,[53] but they proved to be either good substrates for tritium cations or insufficiently thermally stable. Sulfobromophthalein satisfied the above-outlined conditions to the highest extent.[158] The synthetic route to the tritium-labelled sulfobromophthalein is presented below:



This compound contains easily protonated groups (including sulfo groups), which may give rise to a substantial positive charge; in addition, it withstood heating to 280–290 °C in the presence of a catalyst in an atmosphere of tritium gas. The label was introduced in the presence of palladium supported on barium sulfate, calcium carbonate, carbon or alumina. The catalyst : substance ratio was 10 : 1 and the temperature range was 150–290 °C. At 150–180 °C, the yield of the specimen was almost quantitative; at 180–220 °C, it gradually decreased to 60%; at 250 °C, to 53%; at 270 °C, to 46%; and at 285 °C, to 34%. Even when the reaction

was carried out at 210 °C for 0.5 h, the molar radioactivity of the labelled specimen did not exceed 0.08 PBq mol^{-1}. Only at temperatures higher than 285 °C did the radioactivity reach 0.5–0.6 PBq mol^{-1}. The fact that the aromatic compound containing four bromine atoms can be isolated in 30%–50% yield after the reaction with tritium gas at these high temperatures attests to the lack of interaction between tritium cations and protonated sulfobromophthalein molecules.

The observed phenomenon supports the view that labelling proceeds in this case by the mechanism of radical elimination of hydrogen with subsequent recombination of the resulting radical. Indeed, the reaction of atomic tritium with the phenolic fragment of the sulfobromophthalein yields the aryloxy radical, which then undergoes recombination with the abstraction of hydrogen from the *ortho*-position. This gives a radical which can be stabilised upon conjugation with the radical centre delocalised over three positions of the benzene ring.[45] This is followed by the reaction with atomic tritium to give the labelled specimen. It is clear that the spillover of electrically neutral atomic tritium into the bulk of the substrate crystallite would take place even if the molecules of the substance contain easily protonated groups.

Thus, labelling in solid-phase reactions involves both tritium cations and tritium atoms. The molar radioactivity of labelled specimens at relatively low temperatures (usually below 180 °C) is mainly determined by reactions of the organic compound with tritium cations. The degree of isotope exchange depends mainly on the efficiency of tritium spillover to the bulk of the organic compound applied on the catalyst. At higher temperatures, the contribution of atomic tritium in the labelling increases.

V. Conclusion

This review covers the results of synthesis of labelled specimens using solid-phase hydrogenolysis reactions including hydrogenation and isotope exchange with tritium. The regularities of these reactions were studied, in particular, the effects of the nature of the substrate and the catalyst, the pressure of tritium gas, the process duration, temperature, *etc*. on the yield and molar radioactivity of the resulting specimens. Examples of preparation of labelled biologically active compounds are cited.

It was found that at temperatures below 180 °C used most often for solid-phase labelling, the key radiochemical parameters of labelled specimens (yield, molar radioactivity, tritium distribution) are governed by the reaction of $^3H^+$ with the substrate. At higher temperatures, the concentration of atomic tritium increases. In this case, especially for substrates containing easily protonated groups, the molar radioactivity of the product should markedly increase (non-charged species of atomic tritium react more efficiently with these compounds).

The service life of the catalyst depends on the time it takes to reach a certain threshold concentration of hydrogen in the crystallites of the metal catalyst, $c_{H_{cr}}$. The time required to reach this value is dictated by the relationship between the rate of saturation of metal crystallites with tritium gas and the efficiency of its spillover. The efficiency of tritium spillover as applied to the synthesis of labelled organic compounds is determined by the time period tritium exists as a system of cations and electrons solvated on the support surface and in the substrate bulk.

If the goal is to prepare a labelled specimen in a high yield and with the distribution of the label corresponding most closely to that obtained upon electrophilic substitution, the solid-phase reaction should be carried out at the lowest temperature that would ensure the required molar radioactivity. The reaction time is determined by the period of catalyst deactivation. If the aim is to obtain the labelled specimen with the highest possible molar radioactivity, the reaction is carried out in the temperature range in which the required [^{3}H]-labelled compound can be isolated in a yield of at least 5%–10%. The reaction time is usually 5–15 min.

Judging by the data reported in the literature, a promising expedient is to combine various catalysts (palladium–rhodium or palladium–technetium mixed catalyst), supports (separate application of the catalyst and the substrate followed by mechanical mixing) or substrates (consecutive application of several substrates). These approaches increase the yield of the labelled specimen and in some cases, the synthesis of such substances would be otherwise impossible.

References

1. USSR P. 243 621; *Byull. Izobret*. (39) 159 (1976)
2. V V Rozanov, O V Krylov *Usp. Khim*. **66** 117 (1997) [*Russ. Chem. Rev*. **66** 107 (1997)]
3. Kh M Minachev, R V Dmitriev, N G Rassamakhina, A A Dergachev *Dokl. Akad. Nauk SSSR* **291** 393 (1986) [a]
4. J E Benson, H W Kohn, M Boudart *J. Catal*. **5** 307 (1966)
5. F Roessner, A Hagen, R V Dmitriev, in *Catalysis by Microporous Materials* Vol. 94 (Amsterdam: Elsevier, 1995) p. 472
6. F Roessner, U Roland, T Braunschweig *J. Chem. Soc., Faraday Trans*. **91** 1539 (1995)
7. W C Conner, G M Pajonk, S J Teichner *Adv. Catal*. **34** 1 (1986)
8. G C Bond, in *Spillover of Adsorbed Specier* (Eds G M Pajonk, S J Teichner, J E Germain) (Amsterdam: Elsevier, 1983) p. 1
9. M Lacroix, G M Pajonk, S J Teichner *Bull. Soc. Chim. Fr*. 265 (1981)
10. S J Teichner, in *New Aspects of Spillover Effect in Catalysis* (Amsterdam: Elsevier, 1993) p. 27
11. M Lacroix, G M Pajonk, S J Teichner *Bull. Soc. Chim. Fr*. 258 (1981)
12. R V Levy, M Boudart *Ind. Chim. Belg*. **38** 506 (1973)
13. U Roland, R Salzer, S Stolle, in *Zeolites and Related Microporous Materials: State of the Art 1994* Vol. 84 (Amsterdam: Elsevier, 1994) p. 1231
14. J A Altham, G Webb *J. Catal*. **18** 133 (1970)
15. D Bianchi, M Lacroix, G M Pajonk, S J Teichner *J. Catal*. **68** 411 (1981)
16. D Bianchi, M Lacroix, G M Pajonk, S J Teichner *J. Catal*. **59** 467 (1979)
17. M Boudart, A M Aldag, M A Vannice *J. Catal*. **18** 46 (1970)
18. L E Hunt *J. Catal*. **23** 93 (1971)
19. W C Neikam, R A Vannice *J. Catal*. **20** 260 (1971)
20. W C Neikam, R A Vannice *J. Catal*. **27** 207 (1972)
21. R Kramer, M Andre *J. Catal*. **58** 287 (1979)
22. P A Sermon, G C Bond *J. Chem. Soc., Faraday Trans*. **72** 730 (1976)
23. T Fleisch, R Abermann *J. Catal*. **50** 268 (1977)
24. T C Sheng, I D Gay *J. Catal*. **71** 119 (1981)
25. R F Taylor, M M Silva-Crawford, B C Gerstein *J. Catal*. **62** 401 (1980)
26. R K Edvinsson, R R Hudgins, P L Silveston, in *New Aspects of Spillover Effect in Catalysis* (Amsterdam: Elsevier, 1993) p. 229
27. Y W Nam, P L Silveston, in *New Aspects of Spillover Effect in Catalysis* (Amsterdam: Elsevier, 1993) p. 235
28. A V Filikov, Candidate Thesis in Chemical Science, Institute of Molecular Genetics, Russian Academy of Sciences, Moscow, 1985
29. R V Dmitriev, K H Steinberg, A N Detjuk, F Hoffmann, H Bremer, Kh M Minachev *J. Catal*. **65** 105 (1980)
30. D H Lentz, W C Connor, J P Fraissard *J. Catal*. **117** 281 (1989)
31. W C Neikam, M A Vannice, in *Proceedings of the 5th International Congress on Catalysis* Vol. 1 (Amsterdam: Elsevier, 1973) p. 609
32. A F Carley, H A Edwards, B Mile, M W Roberts, C C Rowlands, T E Hanlock, S D Jackson *J. Chem. Soc., Faraday Trans*. **90** 3341 (1994)
33. Kh M Minachev, R V Dmitriev, K Kh Shtainberg, A N Detyuk, Kh Bremer *Izv. Akad. Nauk SSSR, Ser. Khim*. 2682 (1978) [b]
34. R V Levy, M Boudart *J. Catal*. **32** 304 (1974)
35. E Keren, A Soffer *J. Catal*. **50** 43 (1977)
36. M Misono, H Igarashi, K Katamura, T Okuhara, N Mizuno, in *New Aspects of Spillover Effect in Catalysis* (Amsterdam: Elsevier, 1993) p. 105
37. J Herrman, P Pichat, in *Spillover of Adsorbed Specier* (Eds G M Pajonk, S J Teichner, J E Germain) (Amsterdam: Elsevier, 1983) p. 77

38. Th Braunschweig, U Roland, H Winkler, in *New Aspects of Spillover Effect in Catalysis* (Amsterdam: Elsevier, 1993) p. 183
39. U Roland, R Salzer, Th Braunschweig, F Roessner, H Winkler *J. Chem. Soc., Faraday Trans.* **91** 1091 (1995)
40. U Roland, Th Braunschweig, F Roessner *J. Mol. Catal. A: Chem.* **127** 61 (1997)
41. L Pauling *General Chemistry* (San-Francisco: W H Freeman and Co., 1970)
42. E Kikuchi, T Matsuda, in *New Aspects of Spillover Effect in Catalysis* (Amsterdam: Elsevier, 1993) p. 53
43. E T Denisov *Usp. Khim.* **69** 166 (2000) [*Russ. Chem. Rev.* **69** 153 (2000)]
44. V A Radtsig, S N Kostritsa *Izv. Akad. Nauk, Ser. Khim.* 1468 (1997) [b]
45. C K Ingold *Structure and Mechanism in Organic Chemistry* (2nd Ed.) (London: Cornell University Press, 1969)
46. D A Knyazev, N F Myasoedov, A V Bochkarev *Usp. Khim.* **61** 384 (1992) [*Russ. Chem. Rev.* **61** 204 (1992)]
47. Yu A Zolotarev, V S Kozik, D A Zaitsev, E M Dorokhova, N F Myasoedov *Dokl. Akad. Nauk SSSR* **308** 1146 (1989) [b]
48. Yu A Zolotarev, E V Laskerov, V S Kozik, E M Dorokhova, S G Rozenberg, Yu A Borisov, N F Myasoedov *Izv. Akad. Nauk, Ser. Khim.* 757 (1997) [b]
49. G V Sidorov, N F Myasoedov *Radiokhimiya* **37** 270 (1995) [c]
50. V P Shevchenko, I Yu Nagaev, N F Myasoedov *Radiokhimiya* **44** 72 (2002) [c]
51. O V Lushkina, G V Sidorov, N F Myasoedov *Bioorg. Khim.* **19** 117 (1993) [d]
52. V P Shevchenko, S V Faradzheva, I Yu Nagaev, N F Myasoedov *Radiokhimiya* **40** 84 (1998)
53. I Yu Nagaev, V P Shevchenko, N F Myasoedov *Radiokhimiya* **41** 289 (1999) [c]
54. K Saito, A Nakamura, H Takey, B Wang *J. Am. Chem. Soc.* **112** 58 (1980)
55. P N Rylander, in *Catalytic Hydrogenation in Organic Synthesis* (New York: Akademic Press, 1979) p. 31
56. N F Myasoedov *J. Labelled Compd. Radiopharm.* **33** 391 (1993)
57. A Ozaki *Isotopic Studies of Heterogeneous Catalysis* (New York: Academic Press, 1977)
58. Ya E Geguzin *Fizika Spekaniya* (The Physics of Agglomeration) (Moscow: Nauka, 1967)
59. A A Lopatkin, Zh V Strel'nikova, V P Lebedev *Vestn. Mosk. Univ., Ser. 2, Khim.* 255 (1956)
60. S V Faradzheva, Candidate Thesis in Chemical Sciences, Moscow State University, Moscow, 1990
61. V P Shevchenko, I Yu Nagaev, A V Potapova, N F Myasoedov *Radiokhimiya* **36** 440 (1994) [c]
62. V P Shevchenko, Doctoral Thesis in Chemical Sciences, Institute of Molecular Genetics, Russian Academy of Sciences, Moscow, 1992
63. V P Shevchenko, I Yu Nagaev, N F Myasoedov, A V Kamernitskii, I S Levina, L E Kulikova *Radiokhimiya* **42** 176 (2000) [c]
64. V P Shevchenko, I Yu Nagaev, A V Potapova, N F Myasoedov *Radiokhimiya* **35** 132 (1993) [c]
65. G P Akulov, Ju L Kaminski, N A Korsakova, B K Kudelin *J. Labelled Compd. Radiopharm.* **31** 227 (1992)
66. G P Akulov, E V Snetkova, Ju L Kaminski, B K Kudelin, V L Efimova *J. Labelled Compd. Radiopharm.* **29** 1351 (1991)
67. V P Shevchenko, I Yu Nagaev, N F Myasoedov *Radiokhimiya* **41** 80 (1999) [c]
68. V P Shevchenko, I Yu Nagaev, N F Myasoedov *Radiokhimiya* **36** 445 (1994) [c]
69. V P Shevchenko, I Yu Nagaev, A V Potapova, N F Myasoedov *Radiokhimiya* **37** 265 (1995) [c]
70. V P Shevchenko, I Yu Nagaev, N F Myasoedov *Radiokhimiya* **40** 75 (1998) [c]
71. V P Shevchenko, I Yu Nagaev, N F Myasoedov *Radiokhimiya* **35** 106 (1993) [c]
72. J L Wong, J H Keek Jr *J. Chem. Soc., Chem. Commun.* **4** 125 (1975)
73. V P Shevchenko, I Yu Nagaev, N F Myasoedov *Radiokhimiya* **41** 526 (1999) [c]
74. V P Shevchenko, I Yu Nagaev, N F Myasoedov *Radiokhimiya* **40** 79 (1998) [c]
75. G V Sidorov, N F Myasoedov *Usp. Khim.* **68** 254 (1999) [*Russ. Chem. Rev.* **68** 229 (1999)]
76. V P Shevchenko, I Yu Nagaev, N F Myasoedov, N V Bovin *Radiokhimiya* **45** 2 (2003) [c]
77. O V Lushkina, G V Sidorov, N F Myasoedov *Bioorg. Khim.* **19** 113 (1993) [d]
78. G V Sidorov, N F Myasoedov, V I Mishin, V N Kramerov *Zh. Prikl. Khim.* **72** 2017 (1999) [e]
79. G V Sidorov, Doctoral Thesis in Chemical Sciences, Institute of Molecular Genetics, Russian Academy of Sciences, Moscow, 1999
80. G V Sidorov, N F Myasoedov *J. Labelled Compd. Radiopharm.* **34** 353 (1994)
81. Yu A Zolotarev, V S Kozic, D A Zaitsev, E M Dorokhova, N F Myasoedov *J. Labelled Compd. Radiopharm.* **29** 507 (1991)
82. Yu A Zolotarev, Yu A Borisov *Izv. Akad. Nauk, Ser. Khim.* 1056 (1999) [b]
83. V P Shevchenko, I Yu Nagaev, K V Shevchenko, N F Myasoedov *Radiokhimiya* **44** 537 (2002) [c]
84. A Chambers, C Park, R T K Baker, N M Rodriguez *J. Phys. Chem. B* **102** 4253 (1998)
85. V P Shevchenko, I Yu Nagaev, N F Myasoedov *Radiokhimiya* **45** 1 (2003) [c]
86. V P Shevchenko, I Yu Nagaev, N F Myasoedov, N N Popova, G N Pirogova *Radiokhimiya* **44** 533 (2002) [c]
87. N N Popova, Candidate Thesis in Chemical Sciences, Institute of Physical Chemistry, Russian Academy of Sciences, Moscow, 1992
88. B Delmon *Bull. Soc. Chim. Belg.* **88** 979 (1979)
89. B Delmon *React. Kinet. Catal. Lett.* **13** 203 (1980)
90. B Delmon *React. Kinet. Catal. Lett.* **22** 1 (1983)
91. M Karroua, H Matralis, P Grange *J. Catal.* **133** 371 (1993)
92. H Topsoe, B S Clausen, F E Massoth *Hydrotreating Catalysts. Science and Technology* (Berlin: Springer, 1996)
93. S I Kim, S I Woo *Appl. Catal.* **74** 109 (1991)
94. S I Kondrat'ev, S B Nikishenko, G V Antoshin, A A Slinkin, E A Fedorovskaya, Kh M Minachev *Kinet. Katal.* **25** 1168 (1984) [f]
95. M W J Craje, V H J de Beer, A M van der Kraan *Appl. Catal.* **68** 57 (1991)
96. R Candia, B S Clausen, J Bartholdy, N-Y Topsoe, B Lengeler, H Topsoe, in *Proceedings of the 8th International Congress on Catalysis* Vol. 1 (Weinheim: Verlag Chemie , 1984) p. 375
97. M J Ledoux, G Maire, S Hantzer, O Michaux, in *Proceedings of the 9th International Congress on Catalysis* Vol. 1 (Ottawa: Chemical Institute of Canada, 1988) p. 74
98. H Topsoe, B S Clausen, R Candia, S Moing *J. Catal.* **68** 435 (1981)
99. R Candia, B S Clausen, H Topsoe *J. Catal.* **77** 364 (1982)
100. S M A M Bouwens, F B M van Zon, M P van Dijk *J. Catal.* **146** 375 (1994)
101. O Sorensen, B S Clausen, R Candia, H Topsoe *Appl. Catal.* **13** 363 (1985)
102. B Scheffer, E M van Oeri, P Arnoldy, V H J de Beer, J A Moulijn *Appl. Catal.* **25** 301 (1986)
103. W Seglich, G Hoefle *Angew. Chem., Int. Ed. Engl.* **8** 981 (1969)
104. Yu A Zolotarev, Doctoral Thesis in Chemical Sciences, Institute of Molecular Genetics, Russian Academy of Sciences, Moscow, 1998
105. C Tömböly, R Dixit, I Lengyel, A Borsodi, G Tóth *J. Labelled Compd. Radiopharm.* **44** 355 (2001)
106. D A Zaitsev, Yu A Zolotarev, N F Myasoedov *Dokl. Akad. Nauk SSSR* **313** 619 (1990) [a]
107. Yu A Zolotarev, A K Dadayan, B V Vas'kovskii, N V Kost, S K Garanin, V P Makarenkova, N F Myasoedov *Bioorg. Khim.* **26** 512 (2000) [d]
108. Yu A Zolotarev, E V Bocharov, A K Dadayan, I E Kasheverov, M N Zhmak, I V Maslennikov, Yu A Borisov, A S Arsen'ev, N F Myasoedov, V I Tsetlin *Bioorg. Khim.* **26** 587 (2000) [d]
109. I V Maslennikov, A G Sobol, K V Gladky, A A Lugovskoy, A G Ostrovsky, V I Tsetlin, V T Ivanov, A S Arseniev *Eur. J. Biochem.* **254** 238 (1998)
110. L W Guddat, J A Martin, L Shan, A B Edmundson, W R Gray *Biochemistry* **35** 11329 (1996)

111. V N Kramerov, Candidate Thesis in Chemical Sciences, Research-and-Production Union State Institute of Applied Chemistry, Leningrad, 1987
112. V P Shevchenko, I Yu Nagaev, A V Potapova, N F Myasoedov, A V Kamernitsky, I S Levina, L E Kulikova, A N Smirnov *J. Labelled Compd. Radiopharm.* **41** 919 (1998)
113. V P Shevchenko, I Yu Nagaev, N F Myasoedov, in *Vserossiiskaya Konferentsiya '50 Let Proizvodstva i Primeneniya Izotopov v Rossii', (Tezisy Dokladov) Obninsk, 1998* [The All-Russian Conference '50 Year of Production and Application of Isotopes in Russia' (Abstracts of Reports) Obninsk, 1998] p. 33
114. S I Rogov, V P Shevchenko, I Yu Nagaev, N F Myasoedov, V V Bezuglov, M Yu Bobrov, V M Fedoseev *Radiokhimiya* **39** 458 (1997) [c]
115. H W Cook, W E M Lands *Can. J. Biochem.* **53** 1220 (1975)
116. Yu A Zolotarev, E V Laskerov, V S Kozik, E M Dorokhova, S G Rozenberg, Yu A Borisov, N F Myasoedov *Izv. Akad. Nauk, Ser. Khim.* 1820 (1997) [a]
117. N F Myasoedov, Yu A Zolotarev, V I Penkina, O V Petrenik, in *I Vsesoyuznoe Soveshchanie po Probleme 'Poluchenie i Vydelenie Radioaktivnykh Izotopov' (Tezisy Dokladov)* [The First All-Union Meeting on the Problem 'Synthesis and Isolation of Radioactive Isotopes' (Abstracts of Reports)] (Tashkent: FAN, 1980) p. 162
118. Yu A Zolotarev, V S Kozic, E M Dorokhova, N F Myasoedov *J. Labelled Compd. Radiopharm.* **29** 997 (1991)
119. Yu A Zolotarev, N F Myasoedov, V I Penkina, O V Petrenik, V A Davankov *J. Chromatogr.* **207** 231 (1981)
120. Yu A Zolotarev, V S Kozic, E M Dorokhova, D A Zaitsev, N F Myasoedov *J. Radioanal. Nucl. Chem.* **162** 3 (1992)
121. G V Sidorov, N F Myasoedov *Bioorg. Khim.* **22** 291 (1996) [d]
122. G V Sidorov, N F Myasoedov *J. Radioanal. Nucl. Chem.* **121** 461 (1988)
123. E K Ivan'kova, G V Sidorov, N F Myasoedov *Radiokhimiya* **23** 296 (1981) [c]
124. G V Sidorov, N F Myasoedov, O V Lushkina, in *Proceedings of International Symposium on Synthesis and Applications of Isotopically Labelled Compounds* (Amsterdam: Elsevier, 1992) p. 460
125. N S Marchenkov, Candidate Thesis in Chemical Sciences, I V Kurchatov Institute of Atomic Energy, Moscow, 1974
126. G F Taylor, K K Foarde, T D Webber, J A Kepler *J. Labelled Compd. Radiopharm.* **44** 165 (2001)
127. N F Myasoedov, N S Marchenkov, K S Mikhailov, in *Organicheskie Soedineniya, Mechennye Radioaktivnymi Izotopami (Sbornik Dokladov)* [Organic Compounds Labelled by Radioactive Isotopes (Abstracts of Reports)] (Prague: Czechoslovak Committee on Atomic Energy, 1977) p. 275
128. V P Shevchenko, I Yu Nagaev, A V Potapova, N F Myasoedov *Radiokhimiya* **40** 70 (1998) [c]
129. V P Shevchenko, I Yu Nagaev, N F Myasoedov, in *3-ya Rossiiskaya Konferentsiya po Radiokhimii 'Radiokhimiya 2000' (Tezisy dokladov)* [The Third Russian Conference on Radiochemistry 'Radiokhimiya 2000' (Abstracts of Reports)] (St Petersburg: LPM-Kontakt, 2000) p. 255
130. V P Shevchenko, I Yu Nagaev, N F Myasoedov *Radiokhimiya* **44** 349 (2002) [c]
131. V P Shevchenko, I Yu Nagaev, N F Myasoedov *Radiokhimiya* **44** 353 (2002) [c]
132. V L Sadovskaya, L Yu Rakitin, I I Grishina, L M Krasnopol'skaya, G S Muromtsev, V P Shevchenko *Bioorg. Khim.* **16** 1407 (1990) [d]
133. V P Shevchenko, A V Potapova, N F Myasoedov *Radiokhimiya* **31** 75 (1989) [c]
134. V P Shevchenko, I Yu Nagaev, N F Myasoedov *Radiokhimiya* **31** 90 (1989) [c]
135. R E Lutz *J. Med. Chem.* **11** 273 (1968)
136. E A Evans *Tritium and Its Compounds* (London: Butterworth, 1966)
137. M Cabell, in *Isotopes in the Physical and Biochemical Sciences* Vol. 1 (Eds E Buncel, J R Jones) (New York: Elsevier, 1991) p. 71
138. H J Ache, W Herr, A Thiemann, in *Proceedings of the IAEA Symposium of the Chemical Effect and Nuclear Transformations, Vienna, 1961* Vol. 2, p. 111
139. T Meshi, Y Sato *Bull. Chem. Soc. Jpn.* **37** 683 (1964)
140. Yu A Zolotarev, Yu A Borisov, N F Myasoedov, in *Proceedings of Symposium: Synthesis and Application of Isotopically Labelled Compounds* (Eds J R Heys, D G Melillo) (Philadelphia: Elsevier, 1997) p. 653
141. K N Semenenko, V V Burnasheva, N A Yakovleva, E A Ganich *Izv. Akad. Nauk, Ser. Khim.* 214 (1998) [b]
142. A R Ubbelode *J. Chem. Soc.* 1143 (1950)
143. T R Gibb, in *Progress in Inorganic Chemistry* Vol. 3 (London, New York: Interscience, 1962) p. 315
144. N E Lyubashina, N N Savvin, I A Myasnikov *Dokl. Akad. Nauk SSSR* **268** 1434 (1983) [a]
145. J R Anderson *Structure of Metallic Catalysts* (New York: Academic Press, 1975)
146. S Katsuhiro *J. Chem. Soc. Jpn., Chem. Ind. Chem.* 35 (1975)
147. Yu N Rufov, in *Problemy Kinetiki i Kataliza* (The Problems of Kinetics and Catalysis) (Moscow: Nauka, 1975) Vol. 16, p. 212
148. S A Zav'yalov, I A Myasnikov, E E Gutman *Dokl. Akad. Nauk SSSR* **236** 375 (1977)
149. A B Yaroslavtsev *Usp. Khim.* **63** 449 (1994) [*Russ. Chem. Rev.* **63** 429 (1994)]
150. V P Shevchenko, I Yu Nagaev, N F Myasoedov, A B Susan, in *The Synthesis and Applications of Isotopes and Isotopically Labelled Compounds* (Dresden: Wiley, 2000) p. 59
151. E T Denisov *Mendeleev Commun.* 1 (1992)
152. E T Denisov *Usp. Khim.* **66** 953 (1997) [*Russ. Chem. Rev.* **66** 859 (1997)]
153. E T Denisov *Kinet. Katal.* **35** 671 (1994) [f]
154. E V Laskatelev, Candidate Thesis in Chemical Sciences, Institute of Organoelement Compounds, Moscow, 1997
155. R Lamartine, R Perrin *Stud. Surf. Catal.* **17** 251 (1983)
156. V V Salov, V P Shevchenko, I Yu Nagaev, O M Petrukhin, N F Myasoedov *Radiokhimiya* **29** 662 (1987) [c]
157. T Kitamura, T Harada *J. Mol. Catal.* **148** 197 (1999)
158. V P Shevchenko, I Yu Nagaev, N F Myasoedov *Radiokhimiya* **43** 367 (2001) [c]

[a] — *Dokl. Chem. (Engl. Transl.)*
[b] — *Russ. Chem. Bull., Int. Ed. (Engl. Transl.)*
[c] — *Radiochemistry (Engl. Transl.)*
[d] — *Russ. J. Bioorg. Chem. (Engl. Transl.)*
[e] — *Russ. J. Appl. Chem. (Engl. Transl.)*
[f] — *Kinet. Catal. (Engl. Transl.)*