Journal of Labelled Compounds — April-June — Vol. II, No. 2

Studies on the tritium labelling of some local anaesthetics and amino acids by the microwave discharge modification of the Wilzbach technique

T. GOSZTONYI[1] AND N. WALDE
AB Atomenergi, Studsvik, Nyköping, Sweden
Received on 8 March 1966

SUMMARY

Some local anaesthetics and amino acids were labelled with tritium by the microwave discharge modification of the Wilzbach technique.

The following substances were investigated: local anaesthetics: carbocaine, citanest, l-cocaine, tetracaine and xylocaine; amino acids: glycine, DL-α-alanine, DL-Leucine, DL-phenylalanine and DL-tryptophane.

The range of microwave energy used was 4,000 to 270,000 Wsec.

The range of specific activities obtained was from 0.2 to 29.4 mC/mM; and for the most used substance, DL-leucine, from 0.4 to 27.2 mC/mM.

The effects of some reaction parameters on the labelling were investigated. Results obtained are discussed.

INTRODUCTION

Several modifications of the original [1, 2] Wilzbach technique are known in the literature. Some of them use an external energy source instead of the radiation energy of tritium, such as different types of electric discharges [3, 4, 5, 6, 7, 8, 9, 11], γ-radiation [4], or UV-irradiation [6,10], while others use catalysts [12, 13] or treat the organic compound adsorbed on charcoal with tritium [14,15]. The aim of these modifications was to obtain higher specific activities and/or to decrease the amount of by-products formed in the reaction.

The modification of the Wilzbach technique using microwave discharge has been initiated by Westermark, *et al* [11] and elaborated by Ghanem and

[1]Present address: National Atomic Energy Commission, Institute of Isotopes, Budapest, Hungary.

Westermark [6], and Ghanem [16]. They found that not only could the amount of tritium used and the reaction time be decreased by this method but that by using the « bent section technique » [6], the formation of large amounts of by-products due to the discharge could also be avoided.

It seemed advantageous therefore to do a more detailed study on this method using representatives of various groups of organic compounds as targets, i.e. local anaesthetics, amino acids, steroids and nucleobases. In this paper the results obtained by the labelling of local anaesthetics and amino acids are reported.

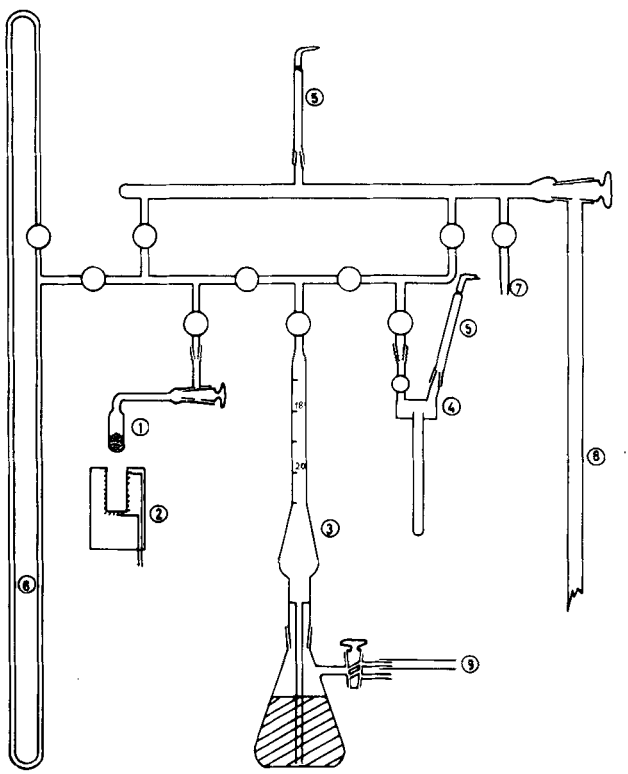


FIG. 1. — Apparatus for tritium handling.

1. Vessel for pyroforic uranium.
2. Electric furnace.
3. Toepler-pump.
4. Reaction vessel.
5. Pirani gauges.
6. Mercury manometer.
7. Connection for gas admittance.
8. Main vacuum line.
9. Connection to vacuum pump.

EXPERIMENTAL

Apparatus

A vacuum line suitable for all kinds of work with tritium gas has been built. Its schematic drawing is shown in Figure 1. Gauges of Pirani type served for measuring the pressure in both the main vacuum line and the reaction vessel. An LKB-Autovac gauge system (Type 3294 B) has been used together with a Philips PR 2210 A/21 recorder, so that the pressure during the reaction could be read at any time or recorded continuously. Tritium gas has been stored bonded to activated uranium powder prepared by an already described method [17].

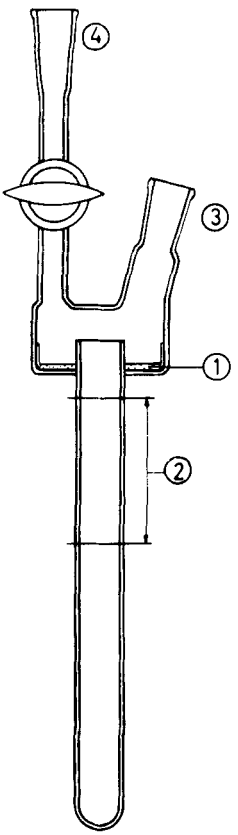


FIG. 2. — Reaction vessel.

1. Substance to be labelled.
2. Discharge zone.
3. B10 joint for Pirani gauge.
4. B10 joint to the vacuum line.

Figure 2 shows the drawing of the reaction vessel used, the volume of which (35.38 ml) has been calibrated manometrically. The rather high dead volume of the reaction vessel ensured a suitable pressure belonging to a given tritium activity. It can be seen that the discharge zone is well separated from the compound to be labelled.

Microwaves were produced in a " Radarmed 12 T 201 " generator (Robert Bosch Elektronik GmbH, Berlin — Wilmersdorf), which operated nominally at 2450 $\pm$ 50 MHz. The generator had a continuous power output control between 20 and 200 W. The radiator of the instrument was replaced by a resonance cavity. This cavity was a slight modification of that reported in [11], namely that the steel ball in the tuning device was replaced by a hollow teflon cylinder which offered a wider range tuning capacity. This is advantageous because all factors which influence the resonance frequency, i.e. dimensions of glass discharge tube, gas pressure, etc., can be varied within a wider range.

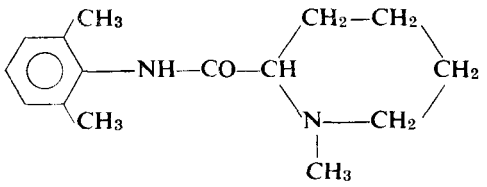
Procedure

The substance to be labelled was placed in the reaction vessel as shown in Figure2. In most of the experiments the substances were treated in powder form, while in others a crystalline film was produced by dissolving the compound in a suitable solvent and evaporating the solvent in vacuo in a rotating evaporator. In some cases, supports (glasswool, charcoal) were added to the solution before evaporating the solvent. Subsequently the reaction vessel was attached to the vacuum line and the whole system was thoroughly degassed until a constant pressure of 10^{-3} mm Hg or less was reached. Tritium was then admitted up to a suitable pressure and the reaction vessel was dismounted from the vacuum line and put into the resonance cavity. Microwave discharge was performed, during which the pressure change was continuously recorded. After that the reaction vessel was reconnected to the vacuum line, the remaining tritium was pumped off and the substance was purified.

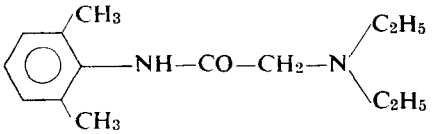
Materials

The following target materials were used:

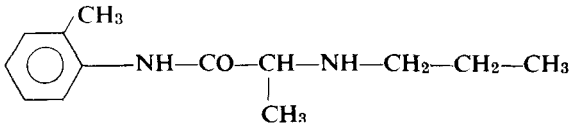
a) Local anaesthetics: carbocaine (I), xylocaine (II), citanest (III), tetracaine (IV), procaine (V) and l-cocaine (VI). Compounds I-V were kindly supplied by Astra AB (Södertälje) as hydrochlorides, while l-cocaine-HCl was a commercial product.



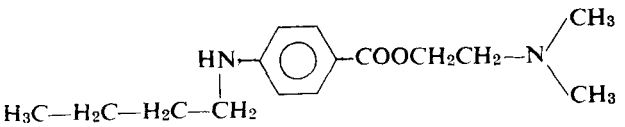
(I)



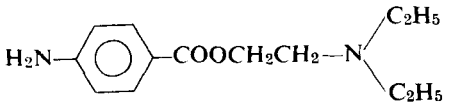
(II)



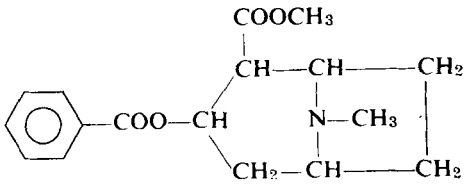
(III)



(IV)



(V)



(VI)

All of the hydrochlorides were converted into their free base and exposed to tritium in this form.

b) Amino acids: glycine, DL-α-alanine, DL-leucine, DL-phenylalanine and DL-tryptophane; all of these were commercial products of the best available quality.

Purification and purity control

a) Local anaesthetics

Labile tritium was removed by dissolving the compound in about 25 ml of methanol and evaporating the solvent in vacuo. This was repeated three times. The final residue was then dissolved in about 2 ml of methanol and applied to four half-sheets of Whatman No. 3 paper. A descending chromatography in *n*-butanol saturated with 1N acetic acid was then carried out. A thin strip of each of the paper sheets was scanned in a Packard M 7200 radiochromatogram scanner and spots were located by either a fluorescein or a Dragendorff spray-reagent. Most of the radioactivity usually appeared in impurities. The zones containing the labelled compound were cut into small pieces and eluted three times with hot methanol. After evaporating the solvent in vacuo, the residue was redissolved in methanol, the solution was decolorized with charcoal if necessary and an ethereal solution of anhydrous oxalic acid or an ethereal HCl solution was added. Oxalates or hydrochlorides were precipitated and then recrystallized three times from methanol-ether.

Radiochemical purity was checked by paper chromatography in *n*-butanol saturated with 1N acetic acid using the scanner mentioned above. All of the local anaesthetics investigated were radiochemically pure after the purification described above, except carbocaine, which contained a part of radioactivity not bonded to carbocaine.

b) Amino acids

The products after tritiation were dissolved in the minimal amount of water and were brought to the top of a column of Dowex-50-W-X8 ion exchange resin (H^+-form). (18.5 mm ∅, 14.5 cm long, 100-200 mesh). The column was washed with one liter of water. Amino acids were eluted with 250 ml of 12.5% NH_4OH. After evaporating the solvent in vacuo, the residue was redissolved in water, decolorized with charcoal, filtered and evaporated to dryness. Removal of labile tritium before purification is unnecessary.

Radiochemical purities were checked by paper chromatography in *n*-butanol saturated with 1N acetic acid or *n*-butanol-methanol-water (10:10:5) systems, and by thin layer chromatography on Silicagel-G layers (without activation) using the butanol-acetic acid-water (4:1:1) solvent system. After scanning the chromatograms, spots were located with ninhydrin spray reagent.

Radioactivity measurements

Radioactivity measurements were carried out in a Tricarb liquid scintillation spectrometer (Model 3314) using a toluene-alcoholic PPO-POPOP solution (3 g PPO and 0.3 g POPOP in 1 litre of toluene and 0.8 litre of absolute alcohol) and external standard for quenching correction.

Results and discussions

Reaction conditions were chosen arbitrarily. In general 100 mg of the target material was exposed to 80 mC of tritium gas which corresponded to a pressure of 0.7 mm Hg. The exposure time was usually determined by the pressure change during the reaction. When the pressure had dropped to a constant value, the reaction was stopped. In cases when only a very slow pressure decrease or even increase took place, however, the reaction was stopped after an interval chosen arbitrarily. As for the differences in pressure changes in the case of the different compounds, no adequate explanation can yet be given. It should also be mentioned that different reaction conditions could have an influence on the pressure change in the case of the same compound, as shown in Figure 3.

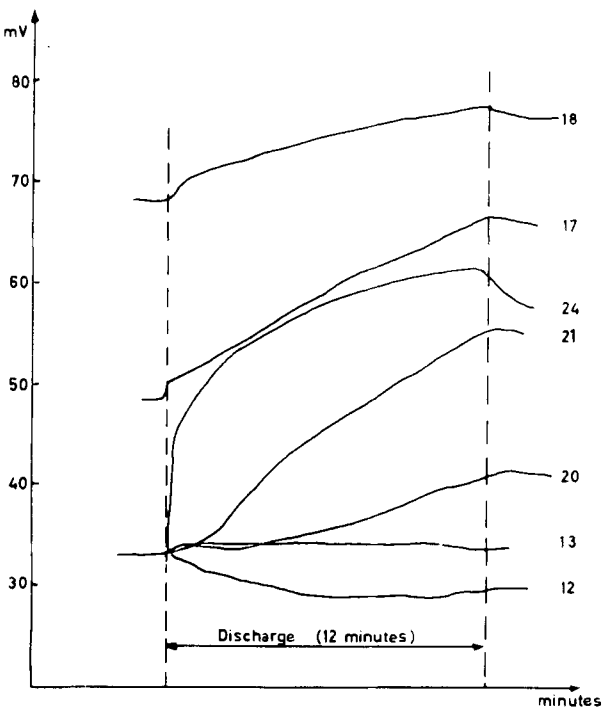


Fig. 3. — Effect of reaction conditions on the pressure change in labelling of DL-leucine. Figures refer to experiment numbers.

The labelling of local anaesthetics using this method has already been published [16]. In our work they have been used only for checking the apparatus. Results obtained in our experiments are summarized in Table 1. Specific activities and tritium fixation yields are somewhat higher than those reported

TABLE 1. Labelling of local anaesthetics.

Experiment No.	Compound		Tritium		Microwave			Spec. act.		T_2 fixed		Physical state during exposure	Compound isolated as
	Name	Weight mg	Pressure mm Hg	mC	W	Wsec $\times 10^{-3}$	Time min.	μC/mg	mC/mM	%	mC/Wsec $\times 10^5$		
1	Carbocaine	100	0.7	80	150	277	36	5.4	1.5	0.75	0.2	adsorbed on glasswool	HCl-salt
6	Carbocaine	100	0.7	80	150	256	28.5	30.4	9.9	5.2	1.6	crystalline film	oxalate
2	Carbocaine	105	0.7	80	150	270	30	2.6	0.7	0.36	0.1	adsorbed on charcoal	HCl-salt
3	Tetracaine	104	0.7	80	150	92	9	8.9	3.2	1.5	1.3	adsorbed on glasswool	oxalate
4	Xylocaine	108	0.7	80	150	157	16.8	10.3	3.3	1.9	1.0	adsorbed on glasswool	oxalate
5	Citanest	90	0.7	80	150	17	1.8	66.4	20.6	10.5	49	oil	oxalate
7	Procaine	90	0.7	80	150	95	10.6	4.9	1.6	0.75	0.6	oil	oxalate
11	l-Cocaine	96	0.7	80	150	135	15	86.6	29.4	11.6	6.9	crystalline film	HCl-salt

TABLE 2. Labelling of amino acids.

Experiment No.	Compound		Tritium		Microwave			Spec. act.		T_2 fixed		Recovery* %	Physical state during exposure
	Name	Weight mg	Pressure mm Hg	mC	W	Wsec $\times 10^{-3}$	Time min.	μC/mg	mC/mM	%	mC/Wsec $\times 10^{5}$		
26	Glycine	100	0.7	80	150	54	6	7.9	0.6	1.0	1.5	60	Powder
28	DL-Alanine	100	0.7	80	150	54	6	17.6	1.6	2.2	3.3	83	Powder
13	DL-Leucine	100	0.7	80	150	108	12	81.8	10.7	10.2	7.5	72	Powder
29	DL-Phenylalanine	100	0.7	80	150	54	6	72.4	11.9	9.0	13.5	76	Powder
30	DL-Tryptophane	100	0.7	80	150	4	0.5	71.0	14.5	8.9	177	64	Powder

* Recovery means the percentage of substance recovered after purification.

TABLE 3. Effect of supports on labelling of DL-leucine and glycine.

Experiment No.	Compound		Tritium		Microwave			Spec. act.		T_2 fixed		Recovery %	Physical state during labelling	Spec. activity factor
	Name	Weight mg	Pressure mm Hg	mC	W	Wsec $\times 10^{-3}$	Time min.	μC/mg	mC/mM	%	mC/Wsec $\times 10^{5}$			
13	Leucine	100	0.7	80	150	108	12	81.8	10.7	10.2	7.5	72	Powder	1.00
12	Leucine	105	0.7	80	150	108	12	78.9	10.3	10.3	7.7	70	Adsorbed on glasswool	0.96
15	Leucine	100	0.7	80	150	108	12	33.7	4.4	4.2	3.1	54	Mixed with 100 mg of 10% Pt-charcoal	0.41
24	Leucine	100	0.7	80	150	108	12	3.0	0.4	0.4	0.3	65	Mixed with 120 mg of Pt-black	0.04
26	Glycine	100	0.7	80	150	54	6	7.9	0.6	1.0	1.5	60	Powder	1.00
27	Glycine	100	0.7	80	150	54	6	2.9	0.2	0.36	0.5	59	Mixed with 100 mg of Pt-black	0.33

TABLE 4. Effect of amount of target substance on labelling of DL-leucine.

Experiment No.	Weight mg	Tritium		Microwave			Spec. act.		T_2 fixed		Recovery %	Physical stated uring labelling	Spec. activity factor
		Pressure mm Hg	mC	W	Wsec $\times 10^{-3}$	Time min.	μC/mg	mC/mM	%	mC/Wsec $\times 10^{5}$			
13	100	0.7	80	150	108	12	81.8	10.7	10.2	7.5	72	Powder	1.00
19	50	0.7	80	150	108	12	81.9	10.7	5.1	3.7	75	Powder	1.00
33	25	0.7	80	150	108	12	134.5	17.6	4.2	3.1	80	Powder	1.64
38	13	0.7	80	150	108	12	122.0	16.0	2.0	1.5	70	Powder	1.50

by Ghanem [16]. This could be due to differences in reaction conditions. There are indications that charcoal and glasswool supports have disadvantageous effects upon the labelling of carbocaine. Furthermore it should be mentioned that there seems to be no relation between chemical constitution and specific activity or tritium fixation values (compounds I-III are acylated anilines, while IV-VI are benzoic acid esters).

Results obtained in the labelling of amino acids are summarized in table 2. As could be expected, glycine showed only a low incorporation, compared with leucine, phenylalanine and tryptophane. The high specific activity of tryptophane after the short exposure time which was determined by the rapid pressure drop during the reaction is rather remarkable. As a comparison it should be mentioned that in earlier experiments [18] DL-leucine was labelled by « ordinary » Wilzbach exposure (188 mg leucine, 32 C tritium, 2 weeks) and 1.1 mC/mg (143 mC/mM) specific activity was obtained which means a tritium fixation of only 0.6%, though the specific activity was 13.5 times higher.

Subsequently the effect of some reaction parameters on the labelling results has been investigated. DL-leucine has been chosen as a model compound, because it could be easily purified and rather high specific activity could be obtained in the labelling experiments. Reaction conditions in experiment No. 13 were chosen as « standard » conditions.

At first the effect of various supports was investigated. Results are shown in Table 3. It can be seen that the addition of glasswool as adsorbent has practically no effect on specific activity, while the addition of catalysts has a definite decreasing effect. That is surprising when compared with the results of Meshi, *et al.* [13] and Cardinaud, *et al.* [9]. Both of these authors have found a positive catalytic effect of hydrogenation catalysts in ordinary Wilzbach labelling [13] and in labelling by high frequency electric discharge [9]. As an explanation of this contradiction one could suggest that there might be a significant difference between the labelling mechanism in our experiments and that of ordinary Wilzbach or high frequency electric discharge labelling. It seems to be probable that tritium atoms playing the main part in microwave labelling [6, 19] undergo a recombination on the active surface of catalysts before being able to react with the target molecules. This suggestion, however, must be proved by further experiments.

Experiments performed with different amounts of the target substance (Table 4) served as an attempt to obtain some information about the effect of the magnitude of the surface area. No special pretreatment (pulverization etc.) has been done, however. The substance was distributed on the bottom and side walls of the reaction vessel as evenly as possible. These conditions do not allow exact quantitative conclusions to be made but one can say that decreasing the amount of target substance (i.e. increasing the specific surface area) can result in a higher specific activity, as could be expected. It has to be mentioned, however, that the geometry of a given apparatus has a large influence on the results and it cannot be predicted which part of the reaction vessel could be reached by the reactive

TABLE 5. Effect of tritium amount on labelling of DL-leucine.

Experiment No.	Weight mg	Tritium		Microwave			Spec. act.		T_2 fixed		Recovery %	Physical state during labelling	Spec. activity factor
		Pressure mm Hg	mC	W	Wsec $\times 10^{-3}$	Time min.	μC/mg	mC/mM	%	mC/Wsec $\times 10^{5}$			
13	100	0.7	80	150	108	12	81.8	10.7	10.2	7.5	72	Powder	1.00
17	102	1.4	160	150	108	12	164.6	21.6	10.1	15.5	74	Powder	2.01
18	100	2.8	320	150	108	12	207.8	27.2	6.5	19.2	93	Powder	2.54
34	101	8.0	960	150	108	12	104	13.6	1.1	9.6	58	Powder	1.27

TABLE 6. Effect of power of microwave energy on labelling of DL-leucine.

Experiment No.	Weight mg	Tritium		Microwave			Spec. act.		T_2 fixed		Recovery %	Physical state during labelling	Spec. activity factor
		Pressure mm Hg	mC	W	Wsec $\times 10^{-3}$	Time min.	μC/mg	mC/mM	%	mC/Wsec $\times 10^{5}$			
20	98	0.7	80	50	36	12	51.9	6.8	6.3	14.1	64	Powder	0.63
21	99	0.7	80	100	72	12	67.9	8.9	8.4	9.3	62	Powder	0.83
13	100	0.7	80	150	108	12	81.8	10.7	10.2	7.5	72	Powder	1.00
25	100	0.7	80	200	144	12	110.0	14.4	13.7	7.6	64	Powder	1.34

species of tritium. So the results of these series of experiments are only informative.

Labelling results as functions of tritium amount used are summarized in Table 5. It can be seen that within a rather narrow pressure interval increasing the tritium pressure causes an increase in specific activities obtained. Further increase of the initial tritium pressure has a disadvantageous effect. As an explanation of this one can suggest that the mean free path of tritium atoms is considerably shorter at higher pressures, i.e. recombination of the atoms takes place before collision leading to labelled products. This fact could mean a limitation of the applicability of the method if high specific activities are required.

The effect of the power of the external energy source on labelling results is shown in Table 6. From these data it can be seen that increasing the microwave power results in an increase in specific activities, which is roughly proportional to the square roots of the power increase [6].

Ghanem [16] found that changes in power input had no effect on the pressure change during the reaction. Our results have shown, however, that specific activities obtained have no relationship with the pressure change. According to this fact the pressure change, which sometimes has an effect on the exposure time, cannot serve as a measure of the extent of the reaction.

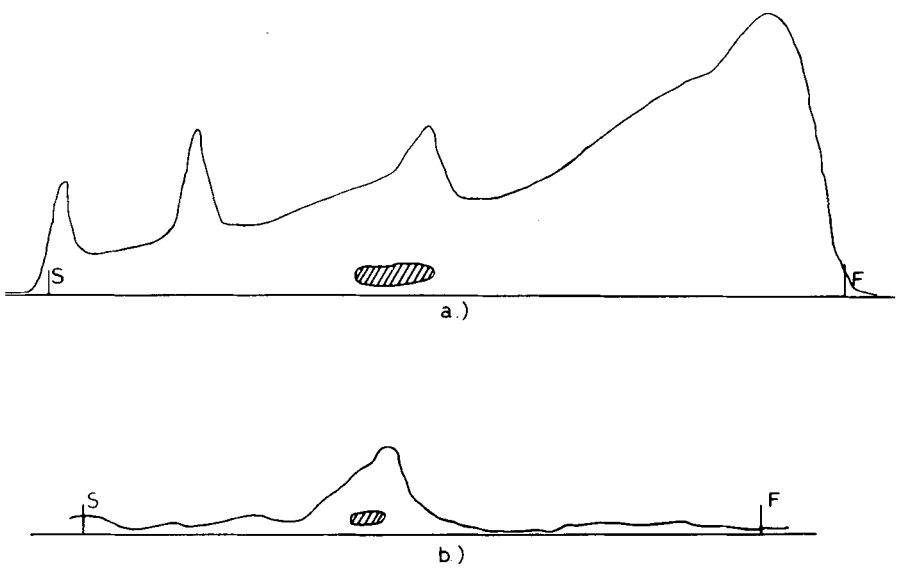


FIG. 4. — Radiopaperchromatogram of $-^3H$ procaine : *a*) Unpurified product, after removing labile tritium; *b*) Purified product (oxalate).
Solvent : *n*-Butanol saturated with 1N acetic acid. Paper: Whatman No. 1.

TABLE 7. Effect of exposure time on labelling of DL-leucine.

Experiment No.	Weight mg	Tritium		Microwave			Spec. act.		T_2 fixed		Recovery %	Physical state during labelling	Spec. activity factor
		Pressure mm Hg	mC	W	Wsec $\times 10^{-3}$	Time min.	μC/mg	mC/mM	%	mC/Wsec $\times 10^{5}$			
13	100	0.7	80	150	108	12	81.8	10.7	10.2	7.5	72	Powder	1.00
22	100	0.7	80	150	54	6	77.1	10.1	9.6	14.3	57	Powder	0.94
23	100	0.7	80	150	27	3	96.0	12.6	12.0	35.5	91	Powder	1.17
35	95	0.7	80	150	9	1	52.6	6.9	6.2	55.5	64	Powder	0.64
36	105	0.7	80	150	4.5	0.5	166.0	21.7	21.8	387.3	65	Powder	2.02

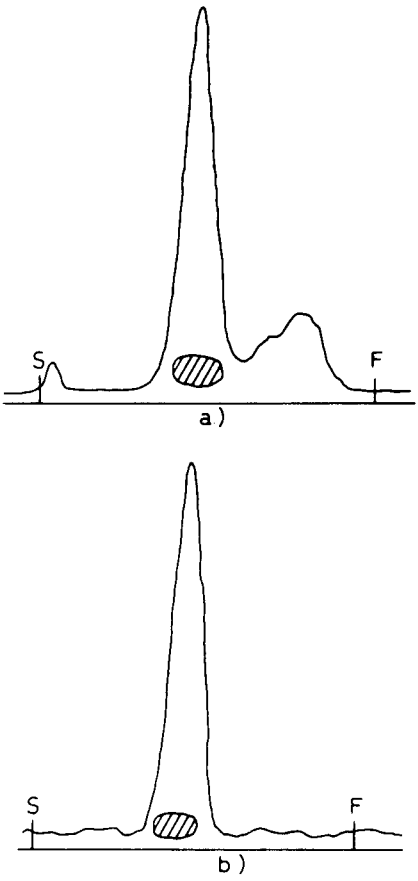


FIG. 5. — Radiothinlayerchromatogram of $-^3H$ DL-leucine: *a*) Unpurified product, including labile tritium; *b*) Purified product.
Solvent : *n*-Butanol-acetic/acid-water (4:1:1). Thin layer: Silicagel G, without activation.

The results of studying the effect of exposure time are given in Table 7. It can be seen that between 3 and 12 minutes there is practically no difference in specific activities obtained. The comparatively short reaction time in experiment No. 36 showed a twofold increase in specific activity. We also obtained a high specific activity in the case of tryptophane (Table 2, Experiment No. 30) with the same short exposure time. It seems to be probable that the labelling reaction is a matter of rather short time (i.e. several tens of seconds) and additional exposure decreases the specific activity due to secondary reactions and/or radiolysis.

Radiochemical purity of the labelled compounds usually was satisfactory after one chromatography purification step. Figure 4 and Figure 5 show radio-

chromatograms as examples of procaine and leucine, respectively, before and after purification. Though procaine (and also the other local anaesthetics) contained a lot of impurities after labelling, the unpurified products of amino acids were relatively purer. Recovery values in cases of amino acids are also rather high. The lower extent of decomposition during the labelling reaction promises a possibility of labelling more sensitive compounds and biological samples by this method as already published in the case of blood serum albumin [6].

Conclusion

During our experiments the labelling of organic compounds was investigated from a preparative point of view. Specific activities in the 10 mC/mM range were obtained in the cases of several local anaesthetics and amino acids by using 100 mg of the target material, 100 mC of tritium and several minutes reaction time. In general 1-10% of the tritium used has been fixed in the organic compound, but sometimes an even higher fixation percentage could be obtained. The mC/Wsec fixation yields were usually in the 10^{-4}-10^{-5} range, although the efficiency of yielding the exciting input is not known. Preliminary experiments using steroids and nucleobases as targets gave similar results. These facts showed that the method is applicable over a wide range provided not too high specific activities are required. It is to be stressed that all of the results are valid under the given geometrical conditions and changing these could mean a change in the results.

Acknowledgements

The authors are indebted to Professor T. Westermark and Mr. H. Lindroth (Div. of Nuclear Chemistry at the Royal Institute of Technology, Stockholm), for helpful discussions and valuable suggestions. The authors' thanks are due, also to Dr. B. Enander (Div. of Microwave Engineering at the Royal Institute of Technology, Stockholm) and Mr. H. Lindroth for the design and tuning of the resonance cavity and to Mr. H. Tovedahl (AB Atomenergi, Studsvik) for the radioactivity measurements. Mr. Tamas Gosztonyi is grateful to the International Atomic Energy Agency for a fellowship received from them.

REFERENCES

1. Wilzbach, K.E. — *J. Am. Chem. Soc.*, **79**, 1013 (1957).
2. As review see: Wenzel, M. and Schulze, P.E. — *Tritium Markierung*. Walter de Gruyter and Co., Berlin 1962.
3. Dorfman, L.M. and Wilzbach, K.E. — *J. Phys. Chem.*, **63** : 799 (1959).
4. Lemmon, R.M., Tolbert, B.M., Stromeier, W. and Whitemore, I.M. — *Science*, **129** : 1740 (1959).
5. Jackson, F.L., Kittinger, G.W. and Krause, F.P. — *Nucleonics*, **18** : No. 8, 102 (1960).

6. GHANEM, N. A. and WESTERMARK, T. — *Radioisotopes in the Physical Science and Industry*, International Atomic Energy Agency, Vienna 1962, Vol. III, pp. 43-67.
7. ACHE, H.J., HERR, W. and THIEMANN, A. — *Tritium in the Physical and Biological Sciences*, International Atomic Energy Agency, Vienna 1962, Vol. II, pp. 21-36.
8. SATO, Y., MESHI, T., TAKAHASHI, T. and SUGIMOTO, N. — *Jap. J. Pharm. and Chem.*, **32** : 317 (1960).
9. CARDINAUD, R., GROSSE, N. and FROMAGEOT, P. — *Preparation and Biomedical Application of Labeled Molecules*, Eur-2200e. Brussels, 1964, p. 23.
10. CACACE, F., GUARINO, A. and MONTEFINALE, G. — *Nature*, **189** : 54 (1961).
11. WESTERMARK, T., LINDROTH, H. and ENANDER, B. — *Intern. J. Appl. Rad. and Isotopes*, **7** : 331 (1960).
12. ACHE, H.J., THIEMANN, A. and HERR, W. — *Angew. Chem.*, **73** : 707 (1961).
13. MESHI, T. and TAKAHASHI, T. — *Bull. Chem. Soc. Japan*, **35** : 1510 (1962).
14. WENZEL, M., WOLLENBERG, H. and SCHULZE, P. E. — *Tritium in the Physical and Biological Sciences*, International Atomic Energy Agency, Vienna. 1962. Vol. II, pp. 37-46.
15. MAURER, R., WENZEL, M. and KARLSON, P. — *Nature*, **202** : 896 (1964).
16. GHANEM, N. A. — *Paper presented at the 3rd UN International Conference on the Peaceful Uses of Atomic Energy*, Geneve (1964) (A/Conf.28/P/458).
17. *Inorganic Isotopic Syntheses*. (Ed. R. H. Herber) W. A. Benjamin, Inc. New York, 1962. pp. 15-18.
18. GOSZTONYI T. and MÁRTON J. — To be published.
19. WESTERMARK, T. — Private communication.