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K. BEREI,
L. VASÁROS

# RECENT ADVANCES IN THE ORGANIC CHEMISTRY OF ASTATINE

Hungarian Academy of Sciences
CENTRAL
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INSTITUTE FOR
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B U D A P E S T

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# RECENT ADVANCES IN THE ORGANIC CHEMISTRY OF ASTATINE

KLARA BEREI and LÁSZLÓ VASÁROS

KFKI Atomic Energy Research Institute,
Budapest, Hungary

**ABSTRACT**

This is the preprint of a chapter submitted to The Chemistry of Functional Groups, Supplement D2, edited by S. Patai and Z. Rappoport. as a follow-up study of a previous chapter in the same series. It surveys the last decade investigations on the chemical behaviour of astatine, on preparation, identification and on some physicochemical properties of its organic compounds. A brief account is given also on the biomedical applications of $^{211}$At-labelled species.

The literature search was completed in November 1993.

# CONTENTS

# I. INTRODUCTION

The decade that has elapsed since the previous survey on the organic chemistry of astatine was published in this series[1] has seen a considerable increase of knowledge. The most important results that have been achieved are those concerning the preparation and identification of astatine labelled organic compounds utilizable for biomedical purposes and those from studying their chemical and biochemical properties. Much less has been done on systematically determining the physicochemical properties of fundamental organic astatine compounds. However, observations made on the stability of some organic astatine compounds in a biological environment both *in vitro* and *in vivo* represent an important contribution to the knowledge on their properties.

By virtue of the considerable amount of experimental data collected so far on the chemistry of astatine, the first Astatine volume of the Gmelin Handbook of Inorganic Chemistry[2] was published in 1985. In 1987 an international Astatine Workshop was included in the programme of the European Nuclear Medicine Congress[3]. The material of this Workshop (complemented with additional papers) was published in a special issue of Radiochimica Acta[4] in 1989.

The methods of synthesis have essentially followed the same pattern described previously[1] such as astatination of organic compounds *via* homogeneous and heterogeneous halogen exchange, decomposition of diazonium salts, electrophilic substitution and reactions of recoil astatine atoms *in situ*. Some new areas have also been explored, e.g. addition reactions, electrophilic substitution *via* demetalation of organotin and organothallium compounds, or applying crown ethers as phase transfer catalysts for nucleophilic halogen exchange.

A separate chapter is devoted to the possibilities of biomedical applications of $^{211}$At-labelled species because of their increased role in astatine research.

## II. PHYSICAL AND CHEMICAL PROPERTIES OF ASTATINE

So far as the physical and chemical properties of astatine itself are concerned not too many new data have been published in the last 10-12 years, however, a more thorough investigation of the literature has presented quite a few values of physicochemical — mainly thermodynamic — properties of At ions, At atom and the hypothetical $At_2$ molecule so far not commonly known (see Table 1).

It might be of interest to recall here the time-of-flight mass spectrometric investigation by Appelman and coworkers[14] from the sixties which allowed direct identification of $At^+$ ion — among other astatine species — owing to the extreme sensitivity of the method.

Consideration of compounds containing two or more astatine atoms in one molecule is purely theoretical in spite of the fact that two research groups reported detecting the $At_2$ molecule. Merinis and coworkers[15] assumed deposition of $At_2$ among AtCl, AtBr, AtI under thermochromatographic conditions on the wall of a quartz ampoule. Otozai and Takahashi[16] reported that they had identified $At_2$ by gas-liquid chromatography (GLC) and had also determined its boiling temperature (503 K) by extrapolation from the gas chromatographic behaviour. These findings are, however, regarded as erroneous[17-23] because of following considerations:

a) In typical chemical experiments astatine is present in $10^{-10}$-$10^{-15}$ mol $l^{-1}$ concentration which virtually excludes collision of two At atoms before meeting atoms or molecules of other species present in much higher concentration ($\geq 10^{-6}$ mol $l^{-1}$).

b) The heat of formation estimated for the $At_2$ molecule by extrapolation is a considerably lower value than that for the astatohalogenides or for the C—At bond in any organic compound of astatine. This means that under normal experimental conditions the thermodynamic factors would favour splitting of $At_2$ and the formation of another molecule with only one At atom in it.

TABLE 1. Some physicochemical properties of astatine ions, atom and molecule (calculated values)

| | Polarizability, $cm^3 \times 10^{24}$ | | | | Ref. |
|---|---|---|---|---|---|
| $At^-$ | 8.3 | | | | 5 |
| At | 5.8 | | | | 6 |
| | $\Delta H_f^\circ$ | $\Delta G_f^\circ$ | $S^\circ$ | $C_p^\circ$ | |
| | kJ $mol^{-1}$ | | J $mol^{-1}$ $K^{-1}$ | | |
| $At^-$ | | | | | |
| gaseous | -178.66 | -202.47 | 175.4 | 20.79 | 7 |
| hydrated | -8.4 | -7.9 | 125.5 | | 8 |
| crystal | | | 67.78 | | 9 |
| $At^+$ | 1004 | | | | 8 |
| $At^{2+}$ | 2761 | | | | 8 |
| At | | | | | |
| gaseous | 97.24 | 59.58 | 186.98 | 20.79 | 7 |
| crystal | | | 60.67 | | 8 |
| $At_2$ | | | | | |
| gaseous | 83.68 | 40.15 | 267.36 | 37.07 | 7 |
| crystal | | | 121.34 | 54.39 | 7 |
| Solvent | $\Delta H_{solv}$ | | $\Delta S$ | $\Delta C_p$ | |
| | kJ $mol^{-1}$ | | J $mol^{-1}$ $K^{-1}$ | | |
| $At^-$ | | | | | |
| $H_2O$ | 128.4[10] | | -107.95[9] | -66.94[13] | |
| MeOH | 322.4[11] | | -120.5[12] | | |
| EtOH | 297.3[11] | | -135.14[12] | | |
| n-PrOH | 288.9[11] | | | | |

In this respect it is remarkable that even with the time of flight mass spectrometry no $At_2$ has been able to be detected[14].

Another controversial issue is the chemical form of astatine in neutral solutions. On the basis of extensive studies on extractability and complex formation, Visser[23] has come to far reaching conclusions concerning the chemical and oxidation state of astatine species in neutral aqueous solutions. Rejecting the previously accepted assumption of the "At(0)"-form attached to the traces of organic impurities Visser suggests that astatine in neutral media is present as AtX, where X may be any monovalent anion (e.g. $HSO_4^-$, $NO_2^-$, $ClO_4^-$, etc.) depending on the system. This astatine species can easily be extracted by organic solvents (e.g. $CCl_4$, $C_6H_6$, n-$C_7H_{16}$), may form either anion or cation complexes, react with the SH-groups of proteins, replace the chloromercury group, can be reduced to $At^-$, and in general shows a "phenol-like" behaviour. Accordingly, a simpler redox potential scheme is suggested[23] for astatine, viz.

$$\overset{0.35\ V}{\longrightarrow}\ \overset{0.85\ V}{\longrightarrow}\ \overset{1.5\ V}{\longrightarrow}$$
$$At^- \quad At^+ \quad At^{3+} \quad At^{5+} \qquad (1)$$

than that postulated earlier by Appelman[20].

Despite Visser's convincing results and argumentation, ready reactions of astatine with organic species cannot be excluded.

# III. SYNTHESIS AND IDENTIFICATION OF ORGANIC COMPOUNDS

A natural selection of methods of synthesis seems to have been taking place during the last decade, at the same time as the widening range of organic astatine compounds prepared. Besides some basic representatives of different classes of compounds such as: aliphatic and alicylic, aromatic, heterocyclic derivatives and astatosteroids, the main emphasis has been laid on preparing biologically important species:[$^{211}$At]astatine-labelled amino acids, proteins, even monoclonal antibodies as well as drugs or other complex biomolecules which are capable of selective accumulation within specific tissues of living organisms (see Section V).

Astatination by means of nucleophilic halogen exchange, occasionally with the help of catalysts, and electrophilic replacement *via* demetalation seem to have become the preferred techniques. Short synthesis and separation time together with the possibility of carrier free preparation of labelled compounds are especially important factors bearing in mind the short half-life of astatine isotopes and the requirement of high specific activity for chemical and biomedical investigations.

## A. Halogen Exchange

Astatination of aromatic compounds has been achieved in homogeneous solutions or in heterogeneous systems by melting the reacting components. In the latter case, catalysts may be applied that promote the exchange reaction.

### *1. Homogeneous halogen exchange*

Nucleophilic halogen substitution in solutions of halobenzenes containing aliphatic amines has been used by Norseyev and coworkers[24-27] to produce astatobenzene and its substituted derivatives.

An aqueous solution of $At^-$ — obtained by reduction of astatine with hydrazine hydrate — is first evaporated to dryness and the residue dissolved by n-butylamine (BA), diethylamine (DEA) or triethylamine (TEA). The absence of any other oxidation form of astatine is proved by paper chromatography. In that no appreciable replacement could be observed in the absence of amines, a two step reaction mechanism has been assumed — described by equations (2) and (3) — the latter being the rate determining process. This assumption is based on the fact that, according to the kinetic analysis, the value of activation energy for the halogen exchange is not influenced by the character of the leaving halogen but strongly depends on the chemical nature of amine (see the data in Table 2) reflecting the influence of the steric factors on the rate of the second step of the replacement reaction[24]:

$$\left[C_6H_5-\overset{\overset{R_1}{|}}{\underset{\underset{R_3}{|}}{N}}-R_2\right]^+ X^- + At^- \longrightarrow C_6H_5At + \overset{\overset{R_1}{|}}{\underset{\underset{R_3}{|}}{N}}-R_2 + X^- \qquad (2)$$

$$C_6H_5X + \overset{\overset{R_1}{|}}{\underset{\underset{R_3}{|}}{N}}-R_2 \rightleftharpoons \left[C_6H_5-\overset{\overset{R_1}{|}}{\underset{\underset{R_3}{|}}{N}}-R_2\right]^+ X^- \qquad (3)$$

where X = Cl, Br, I;

$R_1$, $R_2$, $R_3$ = H, or $C_2H_5$ or $C_4H_9$.

Yields for chlorine, bromine and iodine replacement and the reaction time required under optimal experimental conditions are given in Table 3.

The same procedure has been utilized in preparing substituted astatobenzenes: m- and p-$AtC_6H_4X$ (X = $CH_3$, F, Cl, $NO_2$) by means of bromine replacement from corresponding substituted bromobenzenes[26]. No isomerization of disubsituted benzene derivatives could be observed.

TABLE 2. Activation energy for homogeneous halogen exchange by astatine in halobenzenes

| System | $E_{act.}$ kJ $mol^{-1}$ |
|---|---|
| $At^- + C_6H_5Br$ + TEA | 111.7 |
| $At^- + C_6H_5Br$ + DEA | 21.8 |
| $At^- + C_6H_5Br$ + BA | 17.2 |
| $At^- + C_6H_5Cl$ + BA | 17.6 |
| $At^- + C_6H_5I$ + BA | 17.2 |

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TABLE 3. Yields and reaction time for homogeneous halogen exchange by astatine from halobenzenes[25]

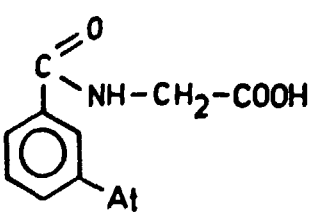
| Halobenzene | Yield, % | Reaction time, min |
|---|---|---|
| $C_6H_5Cl$ | 73 ± 2 | 60 |
| $C_6H_5Br$ | 85 ± 2 | 20 |
| $C_6H_5I$ | 99 ± 1 | 10 |

The isomers of $AtC_6H_4CF_3$ have been synthesized by the same team *via* nucleophilic chlorine replacement from the $ClC_6H_4CF_3$ isomers. The yields (30-45%) are much lower than in the case of chlorine replacement in chlorobenzene but no isomerization of the original compound could be observed thereby allowing determination by GLC of some physicochemical properties (see Section IV) of the products obtained for the first time[28].

In order to prepare allylastatide, a typical unsaturated astatine compound by homogeneous halogen exchange from allylbromide, the astatine solution must previously be treated with a very effective reducing agent: sodium formaldehyde sulphoxilate (rongalite).Under such conditions the yield of ~70% can be achieved but the product readily decomposes. Nevertheless, this new astatine compound has been identified and its boiling temperature determined by GLC[29] (see Section IV).

*2. Heterogeneous halogen exchange*

The melt method[30] widely used for heterogeneous halogen exchange was applied by Shiue and colleagues[31] to synthesize m-astatobenzoic acid, methyl m-astatobenzoate and m-astatohippuric acid (*1*). Using the bromine derivatives of these compounds as starting materials yields of about 60% could be achieved in about one hour carrying out the procedure in a sealed tube at high temperature (≥200 °C).



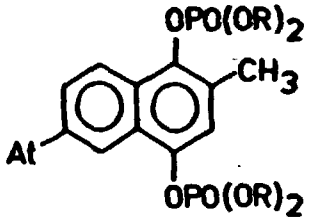

(*1*)

Norseyev and coworkers[32] obtained similar results when preparing the three isomers of astatobenzoic acid from the corresponding Cl, Br or I analogues using essentially the same technique. Investigating the influence of temperature as well as that of the composition of reaction mixture these authors have found that under optimal conditions the exchange reaction takes place within a few minutes with yields depending on the character of the leaving halogen and on its relative position to the COOH group as shown in Table 4.

TABLE 4. Yields of astatobenzoic acid isomers prepared by heterogeneous halogen exchange from chloro-, bromo- and iodobenzoic acids[32].

| Substrate | Yield, % | | |
|---|---|---|---|
| | ortho | meta | para |
| $ClC_6H_4COOH$ | 54 | 10 | 41 |
| $BrC_6H_4COOH$ | 68 | 49 | 32 |
| $IC_6H_4COOH$ | 66 | 61 | 39 |

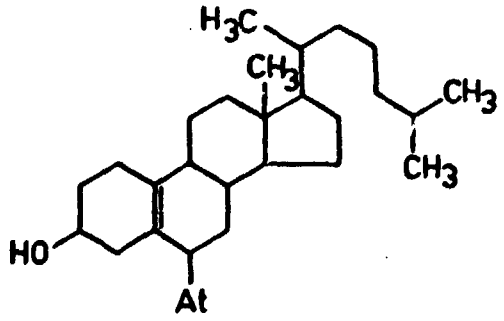
Brown[33,34] slightly modified the melt method to prepare an astatinated antitumour drug: 6-[$^{211}$At]astato-2-methyl-1,4-naphthoquinol bis (dihydrogenphosphate salt), often mentioned as 6-$^{211}$At-MNDP (**2**) from its iodo analogue 6-I-MNDP in a vacuum at 170 °C within ~ 10 minutes. Radiochemical yields of 40-50% can be achieved, the astatinated product is separated from the excess At$^-$ by ion chromatography and identified by thin layer chromatography (TLC). In this procedure the astatinated product (**2**) is obtained together with its iodo analogue which fact is not disturbing — rather the contrary since this happens to be advantageous for biological studies (see section V.C).



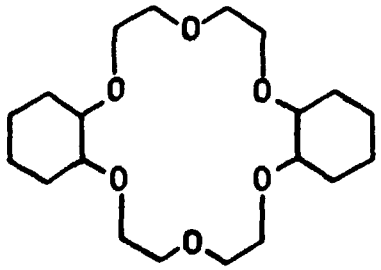

(2)

Heterogeneous halogen exchange at a lower temperature (50-60 °C) has been used for synthesis of the three isomers of astatonitrobenzene from the isomers of bromo-nitrobenzene with the yield of ~70%. The products were identified by GLC and HPLC[35].

In some cases crown ethers[36] have been utilized as phase transfer catalysts enhancing the halogen exchange under mild conditions, e.g. at relatively low temperature. This is especially crucial for labelling high molecular weight compounds with low thermal stability. Liu and his colleagues[37] succeeded in preparing 6-astatomethyl-19-norcholest-5(10)-en-3β-ol (3) from its iodo analogue at 70 °C applying dicyclohexyl-[18]-crown-6 (4) as a catalyst. The reaction took place within 10 minutes and the product was separated and

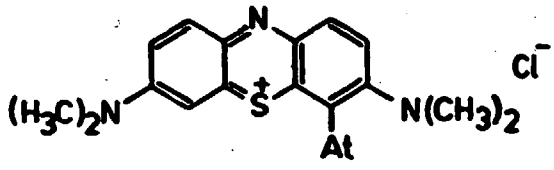



(3)




(4)

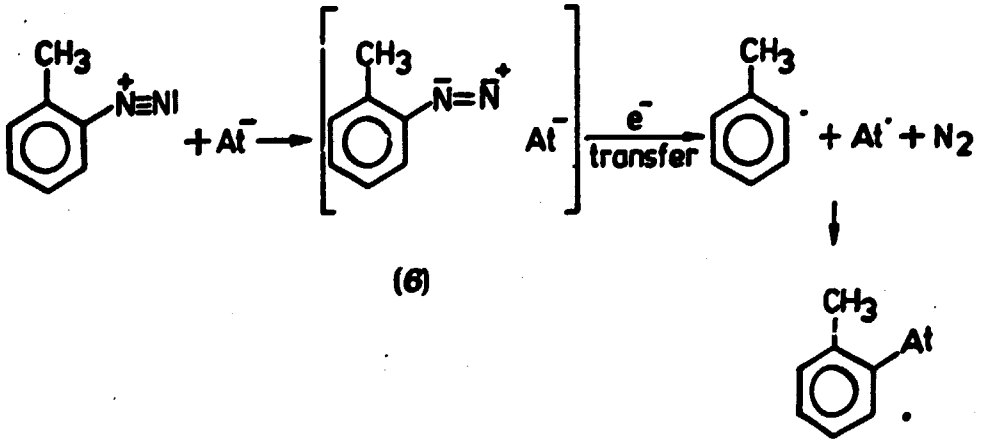
identified by TLC. The same catalyst was used by Brown and his team[38,39] to produce astatinated derivatives of methylene blue (MTB) from the iodo analogues at 100 °C within a few minutes with a yield of ~70%. 4-astato-MTB (5) was subsequently investigated for its biological distribution as a potential anti-melanoma agent (see Section V.C).



(5)

**B. Decomposition of Diazonium Salts**

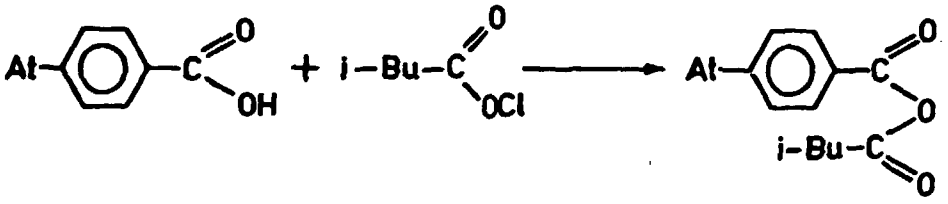
Astatination through diazo intermediates has attracted much less interest in the last ten years or so than earlier. Only one further attempt was made by Visser and Diemer[40] to study the mechanism of this process. Using p-aminobenzoic acid and p-toluidine (para isomers were employed to avoid the interference of steric effects) as starting materials for diazotation, they observed a high yield (~75%) for p-astatobenzoic acid and a low yield (~10%) for p-astatotoluene. This finding suggests a radical reaction channel (see equation 4) for decomposition of the aromatic diazonium salt where the electron withdrawing COOH group destabilizes the (6) cation and thus enhances the diazotation process, whereas the electron donating $CH_3$ group acts in the opposite way[41].



(6) (4)

All other investigations in this field were directed towards conjugating astatine with proteins. In most cases p-astatobenzoic acid (PAtBA) is produced first *via* diazotation; PAtBA is subsequently bound to the $NH_2$-group of protein by means of acylation with mixed anhydrides[42-44].

The most thorough investigation in this area was that of Harrison and Royle[43] on astatination of rabbit immunoglobulin (IgG) which could be used in animal experiments. PAtBA was produced with >90% radiochemical yield, and a reproducible overall yield of >30% for labelled protein was obtained with negligible deastatination of the latter *in vivo*. Such promising results could be achieved most probably due to the fact that the PAtBA was prepared without an iodine carrier and that the micro amounts of the product were purified from the macro amounts of contaminants by HPLC. To bind PAtBA to the protein acylation with mixed anhydride was used. Preparation of mixed anhydride (7) according to equation(5) could be carried out in about 20 minutes at ~0 °C.

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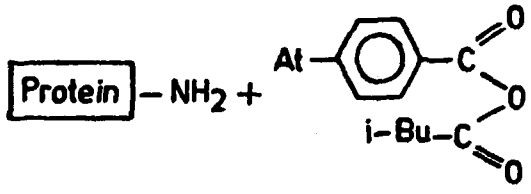
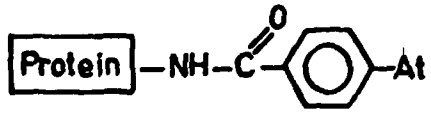


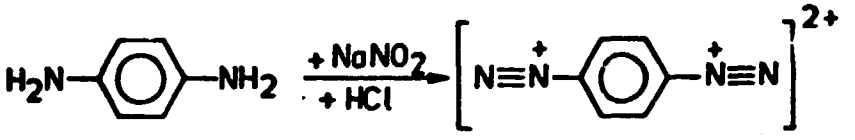

(6)




For astatination rabbit IgG protein is dissolved in borate buffer (pH = 9.3) and then added to **7** (see equation 6); the procedure takes about 1 hour at ~15 °C. The astatinated protein is separated from non-conjugated materials by gel filtration and eluted from the column by phosphate buffered saline.

Wunderlich and his colleagues[45,46] and Reimann[47,48] suggested a much faster procedure to incorporate astatine into proteins. They start with

p-phenyldiamine to produce bis diazonium salt by diazotation, in accordance with equation (7). One diazonium group of this compound is subsequently used for binding the astatine atom (equation 8), the other to conjugate the astatinated aromatic compound to the protein (equation 9) under non-oxydizing mild conditions. Although in commonly used dilute acidic solutions both mono and bis diazonium salt of phenyldiamine are formed and both products may be astatinated, advantage can be taken of the fact that only one of them, namely the p-astatophenyl diazonium salt (8), will participate in the reaction with protein.

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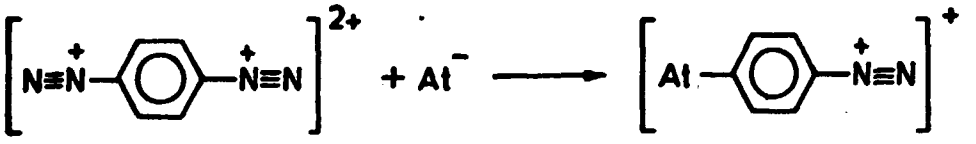


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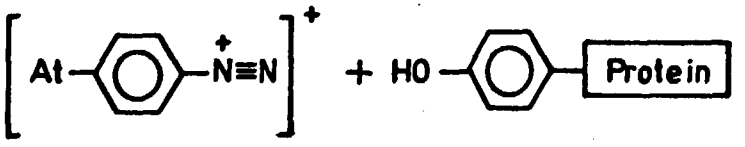


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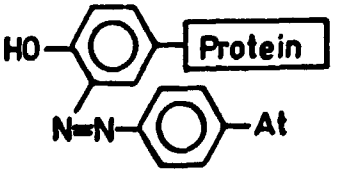


While trying to elaborate optimal conditions for labelling the proteins with astatine the authors[45-48] realized that the second and the third step (equations 8 and 9) can be carried out simultaneously thereby decreasing the synthesis time. Accordingly the whole procedure including also the separation of the labelled protein (human IgG) by gel filtration takes ~90 minutes; overall yield for astatination of ~50% could be achieved.

### C. Direct Electrophilic Replacement and Addition

Hydrogen substitution in benzene and its derivatives is observed only if the astatine is dissolved in concentrated acetic acid in the presence of $H_2Cr_2O_7$ or $HClO_4$ as oxidizing agents, at temperatures above 80 °C. Sharply decreasing yields for this reaction with increasing atomic number of the halogen in halobenzenes have been obtained[27,49,50]. Astatohalobenzenes were identified by GLC, astatophenols by HPLC. The data in Table 5 show that the OH group has the activating effect whereas halogens have the deactivating effect for hydrogen replacement in the aromatic ring.

TABLE 5. Yields of astatobenzene and substituted astatobenzene isomers for electrophilic hydrogen replacement in equimolar mixtures of $C_6H_6$ - $C_6H_5X$

| | Radiochemical yield, % | | | |
|---|---|---|---|---|
| X | $C_6H_5At$ | o-$AtC_6H_4X$ | m-$AtC_6H_4X$ | p-$AtC_6H_4X$ |
| OH | 14.0 | 16.3 | - | 48.0 |
| F | 64.9 | 2.3 | 0.5 | 6.5 |
| Cl | 66.5 | 1.9 | 0.6 | 4.3 |
| Br | 73.3 | - | - | - |
| I | 70.0 | - | - | - |

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To evaluate the effect of the subsituent more accurately a competition study with equimolar mixtures of $C_6H_6$ - $C_6H_5X$ (X = OH, F, Cl) was carried out by Norseyev and colleagues[50]. Quantitative evaluation of intermolecular competition for hydrogen replacement was performed on the basis of the

Hammett relationship applying the yields of replacement products instead of reaction rate constants, according to equation (10):

$$\lg(R_X/R_0) = \rho \cdot \omega \qquad (10)$$

where $R_0$ and $R_X$ are the yields of astatobenzene and substituted astatobenzenes, respectively;

$\rho$ is the reaction constant;

$\omega$ is the substituent constant for X[51].

Linear dependence has been obtained for the experimental points (see Figure 1.) giving a reaction constant $\rho = -3.4$ which confirms the electrophilic character of astatine species replacing hydrogen in the above mixtures. The authors[50] suggest protonated hypoastatite $[AcOHAt]^+$ to be responsible for the substitution in these systems.

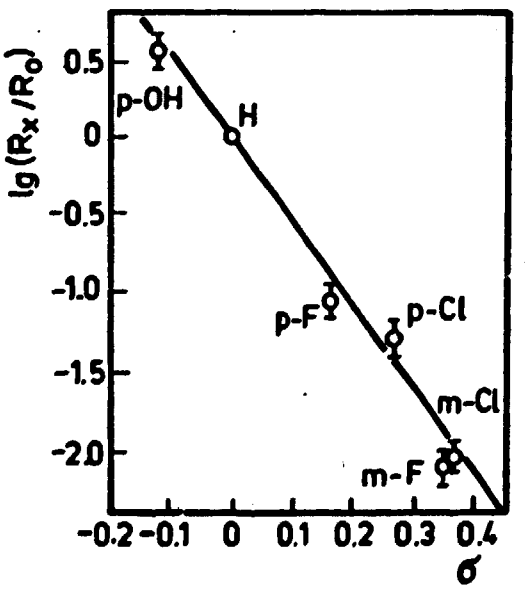



FIG. 1. Hammett plot for electrophilic hydrogen replacement in equimolar mixtures of benzene with halobenzenes and phenol. (Reproduced from Yu. V. Norseyev, D. D. Nhan, V. A. Khalkin, N. Q. Huan and L. Vasáros, *J. Radioanal. Nucl. Chem., Lett.* **95**, 137 (1985) by permission of Akadémiai Kiadó.)

In heterogeneous systems comprising an organic compound and an acidic aqueous solution (phase ratio: organic/aqueous = 1/100) protonated hypoastatous acid $[H_2OAt]^+$ (9) is formed which at 190 °C reacts with benzene and — to a decreasing extent — with halobenzenes forming hydrogen replacement products[27,52], as can be seen from the data in Table 6. It has been

TABLE 6. Yield and isomer distribution for electrophilic hydrogen replacement by astatine in heterogeneous mixtures of benzene and halobenzenes with $HClO_4$

| | | Isomer distribution (ortho + meta + para) = 100% | | |
|---|---|---|---|---|
| Substrate | Yield% | ortho | meta | para |
| $C_6H_6$ | 89.1 | | | |
| $C_6H_5F$ | 89.4 | 25.0 | 5.0 | 70.0 |
| $C_6H_5Cl$ | 72.0 | 30.0 | 20.2 | 49.8 |
| $C_6H_5Br$ | 8.1 | 33.2 | 21.0 | 45.8 |
| $C_6H_5I$ | 2.5 | 30.0 | 26.8 | 43.2 |

Reproduced from L. Vasáros, Yu. V. Norseyev, D. D. Nhan and V. A. Khalkin, *Radiochem. Radioanal. Lett.*, **54**, 239 (1982) by permission of Akadémiai Kiadó.

found that the absence of the oxidizing agent $Cr_2O_7^{2-}$ does not influence the outcome of the replacement. Apparently the acidity of $H_2SO_4$ or $HClO_4$ present in 0.05 - 0.1 mol $l^{-1}$ concentration is enough to keep hypoastatous acid in protonated state in accordance to equation (11):

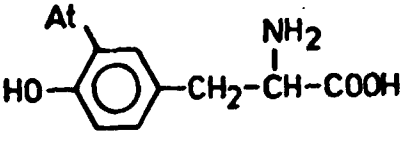
$$HOAt + H_3O^+ \rightleftharpoons [H_2OAt]^+ + H_2O \qquad (11)$$

(9)

$$[H_2OAt]^+ \rightleftharpoons At^+ + H_2O \qquad (12)$$

It has been suggested[52] that in the pre-equilibrium step of astatination **9** undergoes heterolytic fission (equation 12) forming the most reactive $At^+$ ion, which is responsible for hydrogen replacement in the aromatic ring.

The electrophilic character of astatine in aqueous acidic solutions has been taken advantage of to prepare carrier-free astatotyrosine (***10***) an important compound for biomedical investigations[53]. An optimal yield of ~90% was able to be obtained if aqueous solution of astatine together with tyrosine dissolved in a mixture of perchloric and acetic acid was heated in sealed ampoules to 150-160 °C for about 30 minutes. If the temperature is increased to above 170 °C it results in partial decomposition of the product. Astatotyrosine is separated from the inorganic components by ion exchange chromatography and identified by paper chromatography.




(***10***)

The assumed equilibrium between hypoastatous acid and its protonated form in aqueous solutions — influenced by the $H^+$-ion concentration — offered a possibility to study the electrophilic addition of astatine to the olefinic bond of ethylene forming ethylene astatohydrin (***11***).

$$AtCH_2CH_2OH$$

(***11***)

Synthesis of ***11*** was carried out in aqueous solutions containing different amounts of $HClO_4$ or $H_2SO_4$ at 0 °C, to avoid significant thermal decomposition of the product[27,54]. The newly prepared compound has been identified by liquid extraction at 0 °C and subsequent GLC analysis, taking into account its thermal instability.

The pH dependence of addition shows an almost linear drop of the yield in the pH = 0.3-3.0 region (see Figure 2) that may be caused by the decreasing concentration of strongly electrophilic $[H_2OAt]^+$ in accordance with equation (11). At higher pH values the much less effective electrophilic agent HOAt may be responsible for the addition reaction which is reflected in lower, slowly decreasing yields.

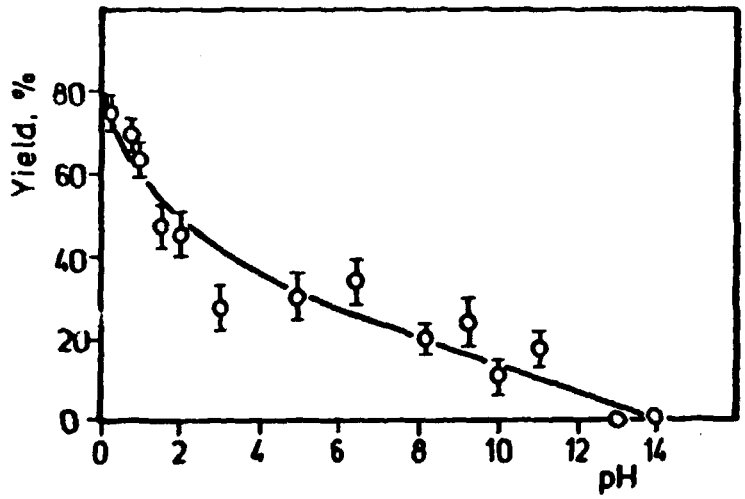



FIG. 2. pH dependence of ethylene astatohydrin yield as a result of electrophilic addition. (Reproduced from L. Vasáros, K. Berei and Yu. V. Norseyev, *Radiochim. Acta*, **49**, 119 (1989) by permission of R. Oldenburg Verlag.)

A successful attempt by Yi and coworkers[44] from the Sichuan University to label proteins with $^{211}At$ in a nearly neutral aqueous solution containing $H_2O_2$ oxidizing agent should be mentioned. Though the mechanism of the reaction as well as the nature of the protein — astatine bond remained uncertain, up to 96% of $^{211}At$ activity could be coupled to bovine serum albumin under mild conditions, viz. by incubation of the protein with sodium astatide dissolved in acetate buffer (pH = 5.6) at room temperature for about 30 minutes. The astatinated protein could be separated from non-conjugated components by gel filtration.

Visser and colleagues[55] have given an essentially different interpretation for the electrophilic astatination of proteins from that accepted earlier. Carrying out an extensive and systematic study on this subject they observed that high

yield and stable labelling could easily be obtained in 10 minutes with proteins containing unsubstituted SH groups, e.g. with bovine serum albumin, haemoglobin and β-lactoglobulin (see data in Table 7). On the other hand, proteins without such groups as cytochr ne-C and lysozyme could be astatinated only by applying $H_2O_2$, leading to a low yield in a time consuming procedure; and this label was easily destroyed by a number of reagents, even by contact with the Sephadex column used for separating the labelled products. In contrast, the $^{211}$At label of SH-containing proteins remained stable against denaturating agents such as urea, $(NH_4)_2SO_4$ or phenol, at room temperature for 24 hours.

TABLE 7. Electrophilic astatination of proteins[55]

| Protein | Method | Yield, % |
|---|---|---|
| Bovine serum albumin | $H_2O_2$ | 80-90 |
| Bovine serum albumin | $NaNO_3$ | 40-60 |
| Haemoglobin | $H_2O_2$ | 80-90 |
| Haemoglobin | $NaNO_3$ | 40-60 |
| β-lactoglobulin | $H_2O_2$ | 70-90 |
| β-lactoglobulin | $NaNO_3$ | 40-60 |
| Cytochrome-C | $H_2O_2$ | 1-35 |
| Lysozyme | $H_2O_2$ | 1-25 |

The fact that proteins containing free SH-groups could be labelled with fairly high yields even in the absence of oxidizing agents has been interpreted by asssuming that astatine of zero valency state (the so called "$At^0$") is an electrophilic agent strong enough to replace the hydrogen of the SH-group, thereby forming an S—At bond. The experimental finding that this bond seems

to be more stable than the S—I bond in analogous proteins was explained by the more expressed metallic character of astatine[55].

The necessity of $H_2O_2$ for the astatination of proteins containing no SH-group was accounted for by supposing that astatine species in a higher valency state — probably $At^{3+}$ — participate in this labelling process and possibly form a complex with one or another atom or group of proteins (e.g. O, N, S—S bridge, thioether or sulphoxide group). This would explain the effect of reagents which may destroy the weak complex bond.

### D. Electrophilic Astatination *via* Demetalation

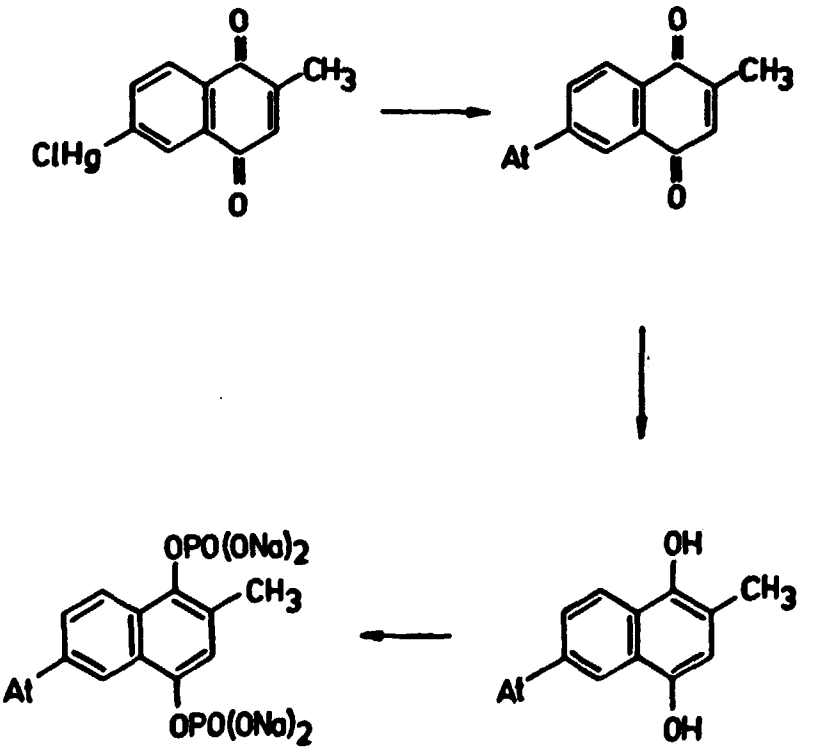
In contrast to direct electrophilic substitution in which the astatine attacks one of the C—H bonds, astatination through demetalation can be used to label a substrate at a preferred site in a regiospecific manner not affecting other sensitive sites of the molecule. Since a C—M bond is more sensitive to electrophilic attack than C—H, higher yields can be obtained in short reaction times under milder experimental conditions[56]. Organometallic compounds of mercury, thallium and tin have been used so far for astatination *via* demetalation.

#### *1. Organomercury compounds*

Chloromercury derivatives of aromatic compounds can be prepared by a number of indirect reaction routes, thereby allowing astatination *via* demercuration of a wide variety of compounds under mild experimental conditions.

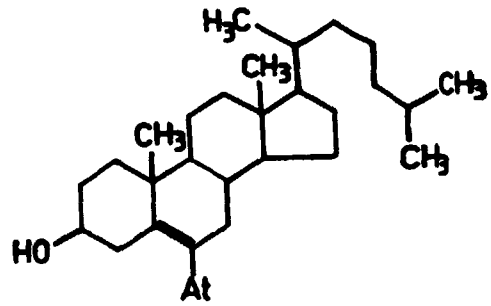
Brown[57] developed a five-step procedure for synthesizing 6-$^{211}$At-MNDP (**2**): the first step was to prepare the 6-chloromercury derivative from 2-methyl-1,4-naphtoquinone, this was followed by astatination through demercuration on site 6, reduction of the quinone to the quinol intermedier, then phosphorylation and, finally, hydrolysis (see equation 13). The astatinated

product together with its iodinated analogue was separated by ion exchange chromatography and identified by TLC. The procedure took more than 7 hours — about one half-life time of the $^{211}$At isotope — thereby reducing the 60% overall radiochemical yield to ~ 30%. The author, therefore, preferred the much simpler and shorter method of heterogeneous halogen exchange (see Section III.A.2) for routine synthesis of the astatinated drug **2**.

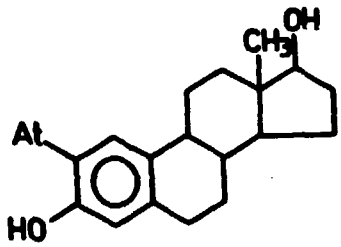



(13)

More promising seems to be the astatination through demercuration of steroids providing 6-astatocholesterol[58,59] (**12**) with ~95% yield as well as a mixture of 2-astatoestradiol (**13**), 4-astatoestradiol and 2-astato-4-iodoestradiol with 55%, 19%, and 18% yields, respectively[58]. Chloromercurated steroids are allowed to react with At in $H_2SO_4$, in the presence of $KI_3$, for 1 hour. The products are purified and identified by TLC.

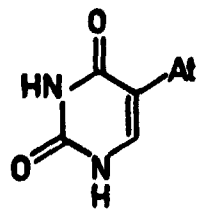


(12)



(13)

Visser and coworkers[58] have also reported the preparation of several astatopyrimidines: 5-astatouracil (*14*), 5-astatouridine (*15*), 5-astato-2-deoxyuridine (*16*), 5-astatocytosine (*17*), 5-astatocytidine (*18*) and 5-astato-2-deoxycytidine (*19*) as well as some of their nucleosides and nucleotides, including ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), in high yields under mild conditions similar to those described for steroids. The yields obtained for different products and the methods of their purification are listed in Table 8. For identification of all products, except RNA and DNA, TLC and electrophoresis are used.



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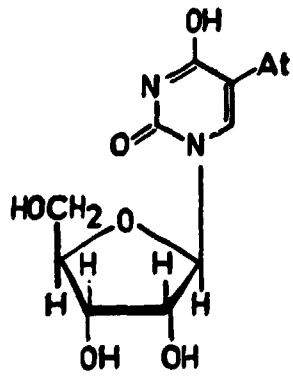
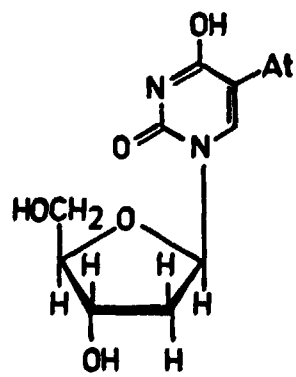
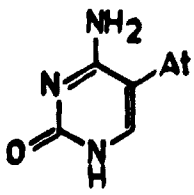


(15)



(16)



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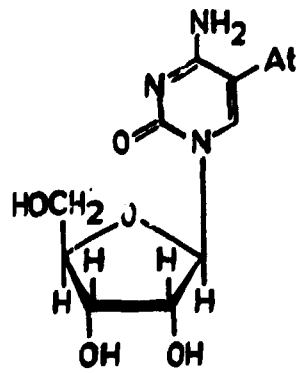
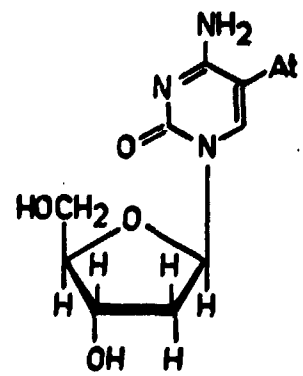


(18)



(19)

TABLE 8. Yields for astatination of some pyrimidines, their nucleosides and nucleotides *via* demercuration and methods of their purification[58]

| Product[a] | Yield, % | Purification[b] |
|---|---|---|
| 5-At-uracil | 80-90% | TLC |
| 5-At-uridine | 75-85% | TLC, PEP |
| 5-At-deoxyuridine | 80-90% | TLC, PEP |
| 5-At-UMP | 75-85% | TLC, PEP |
| 5-At-UTP | 65-75% | PEP, Extr. |
| 5-At-cytosine | 80-90% | PEP |
| 5-At-cytidine | 70-80% | TLC, PEP |
| 5-At-CMP | 75-85% | TLC, PEP |
| 5-At-dCMP | 70-80% | TLC, PEP |
| At-RNA | 92-98% | GF |
| At-DNA | 91-94% | GF |

[a]UMP=uridinemonophosphate; UTP=uridinetriphosphate;
CMP=cytidinemonophosphate; dCMP=deoxycytidinemonophosphate
[b]*TLC=thin layer chromatography; PEP paper chromatography;*
*Extr=extraction; GF=gel filtration*

*2. Organothallium compounds*

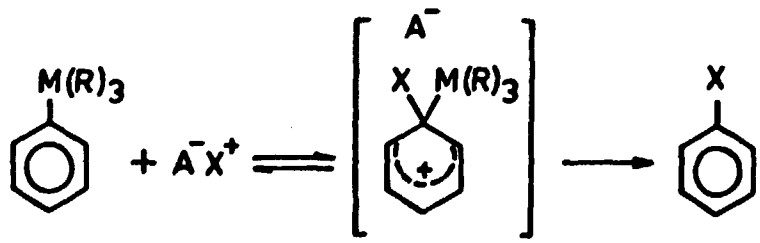
Electrophilic demetalation of thallium trifluoroacetate derivatives was utilized by Visser and Diemer[60] to synthesize o-astatobenzoic acid and p-astatoanisole. The organometallic compounds are forming by reaction of benzoic acid and of anisole with thallium trifluoroacetate at room temperature, in the dark. Astatination was carried out in dilute $H_2SO_4$, in the presence of

KI within 30 minutes. Yields of 70-90% were obtained; the astatinated products were separated by extraction and identified by TLC or — in the case of o-astatobenzoic acid — on the basis of its $pK_a$ value determined by extraction with heptane.

A serious disadvantage is that the organic compounds of thallium form under strong oxidative conditions and this very much limits the group of compounds which can be astatinated using this method.

### 3. *Organotin compounds*

Elements of Group IV of the Periodic System have been used in the last decade as organometallic precursors for aryl halogenide syntheses[56]. The mechanism of electrophilic demetalation of these precursors can be demonstrated by the simplified scheme shown in equation (14). The positive inductive effect of the alkyl substituents promotes the formation of intermediate (*20*) by increasing the electron density at the *ipso* carbon atom.




(14)

(*20*)

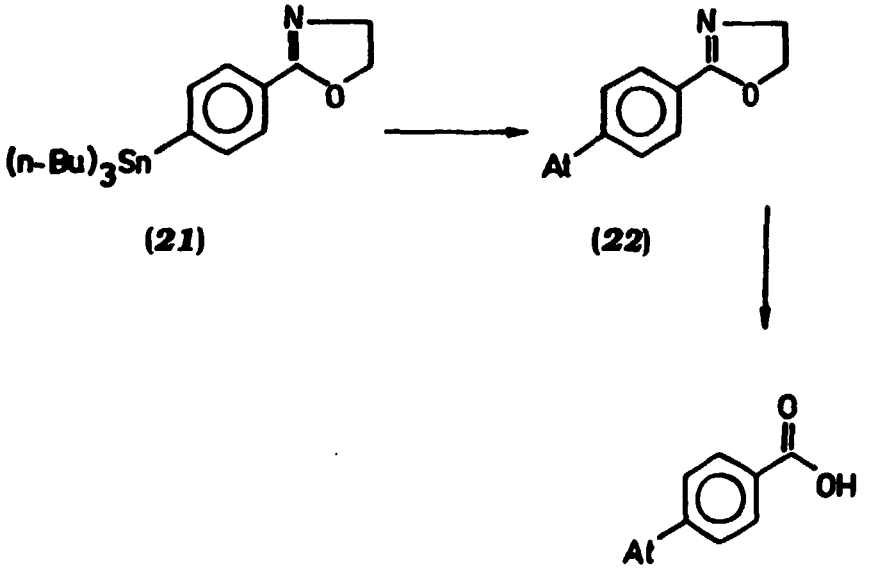
where $A^-X^+$ = halonium ion;

M = metal of Group IV;

R = alkyl substituents

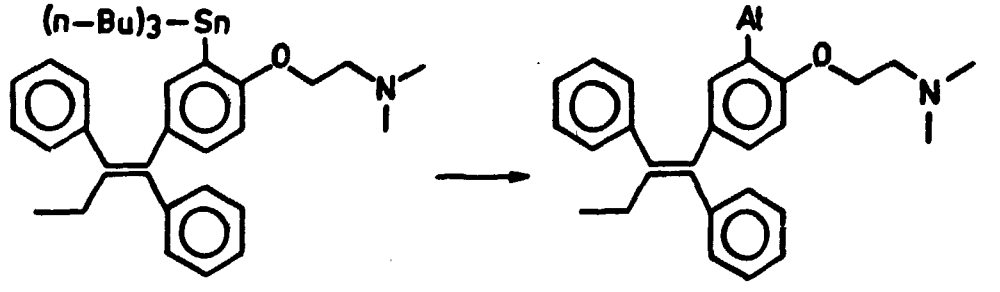
Milius and coworkers[61] were the first to employ the organometallic compounds of Sn(IV) for preparing p-astatobenzoic acid and 3-astatotamoxifen by electrophilic destannylation in accordance with equations (15) and (16). The tri-n-butylstannyl derivative of benzoic acid oxazoline (*21*) and that of

tamoxifen (**23**) were obtained using routine procedures known from the literature[62]. Astatination was carried out in sealed vials in a buffer solution (pH = 7.0), in presence of an iodide carrier and of $H_2O_2$ oxidizing agent. After a few minutes the reaction was quenched by addition of a reducing agent ($NaHSO_3$). To obtain p-astatobenzoic acid from the p-astatobenzoic acid oxazoline (**22**) the solution was treated with dilute HCl and subsequently with dilute NaOH at 100 °C.




(**21**) (**22**) (15)

A somewhat simpler procedure was followed to prepare 3-astatotamoxifen (**24**) since in this case the acid and base treatment is not needed:




(**23**) (**24**) (16)

Isolation and identification of astatinated products — together with their iodinated carriers — are performed by HPLC or TLC. The HPLC radiochromatogram of **24** and the chromatogram of its inactive iodo analogue

can be seen in Figure 3. In the presence of iodine, radiochemical yields of 80% and 60% could be achieved for p-astatobenzoic acid and astatotamoxifen, respectively. Without using an iodinated carrier, however, the yield did not exceed 0.5-1%.

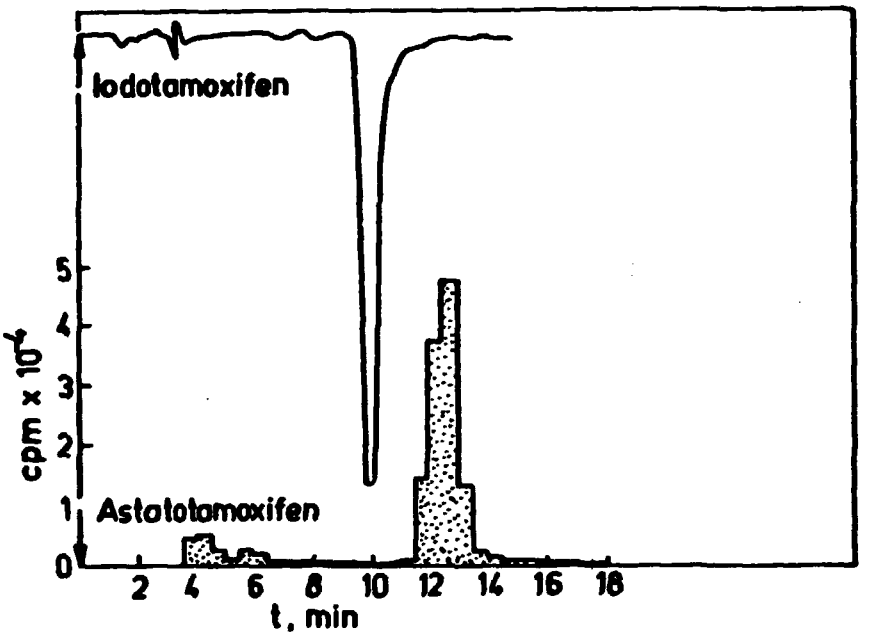
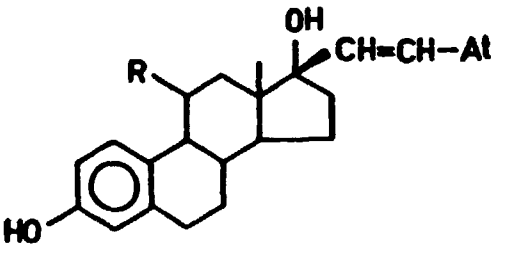



FIG. 3. HPLC chromatogram of 3-[$^{211}$At]astatotamoxifen and 3-iodotamoxifen. (Reproduced from R. A. Milius, W. H. McLaughlin, R. M. Lambrecht, A. P. Wolf, J. J. Carroll, S. J. Adelstein and W. D. Bloomer, *Appl. Radiat. Isot., Artic.*, **37**, 799 (1986) by permission of Pergamon Press.)

Pillai and colleagues[63] improved the method described above to such an extent that they obtained astatinated vinylsteroid hormones: 17-α-astatovinylestradiol and its 11-metoxy derivative (**25**) under carrier-free conditions with yields of ~80%. In the presence of iodine carrier the yield could be increased up to ~90%. The products were separated from the reaction mixture by extraction, purified and identified by HPLC.

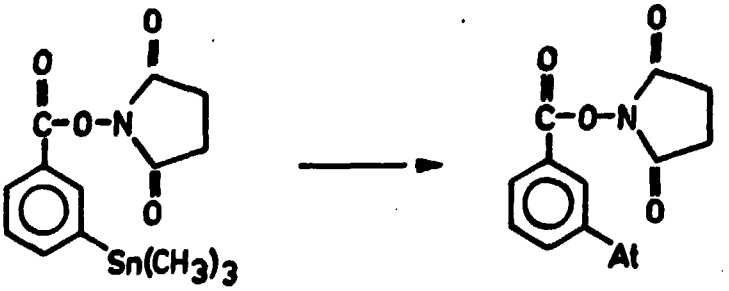



(25)

where R = H or $OCH_3$

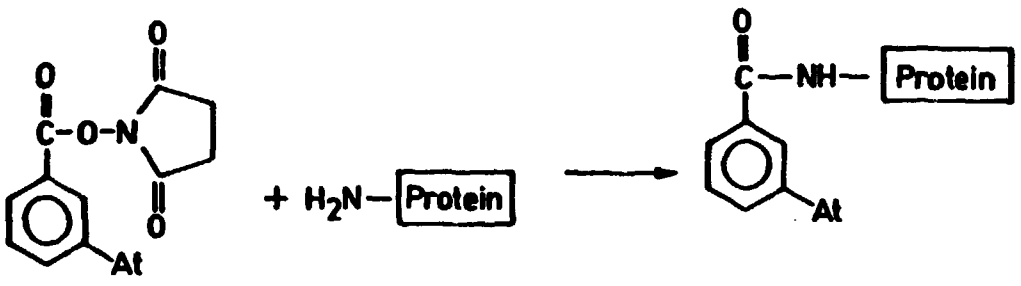
An important conclusion drawn from the last two studies is that with the help of trialkylstannyl derivatives astatine can easily be built into compounds with aromatic ring or with olefinic bond. This enables the method to be applicable for labelling a wide variety of biologically active species, drugs or proteins.

Zalutsky and his research group[64-68] have implemented this possibility. They prepared N-succinimidyl-3-astatobenzoate (27) by means of destannylation of its trimethylstannyl derivative (26) — in accordance with equation (17) — and used 27 to couple the $^{211}$At-containing aromatic ring to proteins, monoclonal antibodies and their fragments suitable for biological studies. Results from systematic studies have shown that the best conditions for astatination can be achieved if the astatine is dissolved in $CHCl_3$ and to this solution 26 is added together with a mild oxidizing agent: t-butyl hydroperoxide. The reaction at 25 °C is accomplished in 15 minutes. Yields of 90-95% can be obtained. HPLC ensures a fast separation of the astatinated product and its coupling to proteins (equation 18) can be accomplished in another 15 minutes with 50-65% yield, by means of incubation of the two components in borate buffer (pH = 8.5) at 4 °C.




(17)

(26) (27)




(18)

Separation of astatinated proteins from compounds of lower molecular weight is carried out by gel filtration. A typical elution curve for $^{211}$At-labelled goat IgG separation is shown in Figure 4.

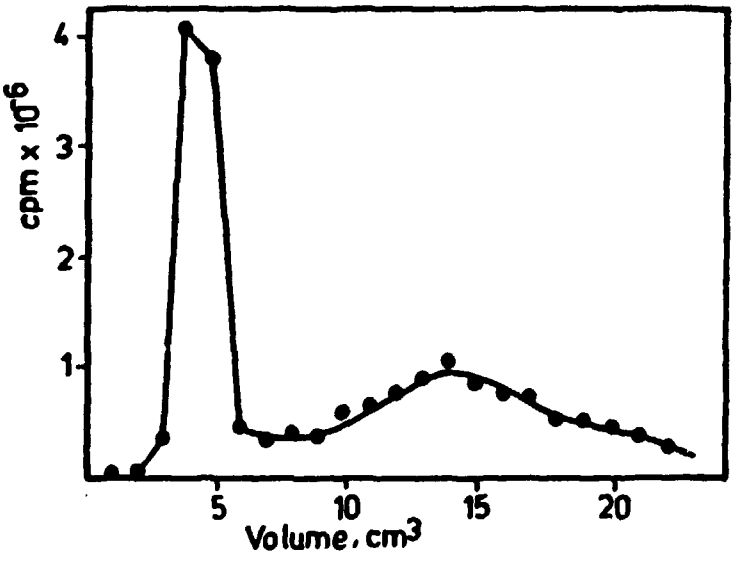



FIG. 4. Elution curve of $^{211}$At-labelled goat IgG from a Sephadex G-25 gel filtration column. (Reproduced from M. R. Zalutsky and A. S. Narula, *Appl. Radiat. Isot., Artic.*, **39**, 227 (1988) by permission of Pergamon Press.)

Wilbur and coworkers[69,70] made use of a slightly modified procedure to prepare the para isomer of **27** from the N-succinimidyl-4-tri-n-butylstannylbenzoate and subsequently coupled the astatinated aromatic ring to a monoclonal antibody/fragment with an overall radiochemical yield of 39-61%.

**E. Reactions of Recoil Astatine**

Reactions of recoil astatine — formed in nuclear transformation $^{211}Rn(EC)^{211}At$ — are not best suited for synthesizing any specific compound. Atoms originating in nuclear processes generally have an excess of kinetic, excitation and possibly ionization energy, and may therefore take part in several different reactions simultaneously. This results in a variety of labelled products (see Section IV.B.6 in Ref.[1]). For instance, recoil astatination of nitrobenzene leads to the formation of astatobenzene *via* $NO_2$-group replacement as well as to that of the three astatonitrobenzene isomers by H replacement[35]. Investigations in this field have been directed towards seeking the main characteristics of astatine in energetic state and the rules governing its chemical reactions[27,71].

The halogen character of unsolvated recoil astatine reacting in organic systems is confirmed by the linear dependence of the halogen replacement yields on the bond energy of X (F, Cl, Br, I) in monohalobenzenes both in liquid and in the gas phase, as can be seen from Figure 5. The same dependence was observed for the other recoil halogens replacing halogen atoms in halobenzenes and the phenomenon was explained by formation of a short-lived excited intermediate in the course of high energy replacement[72].

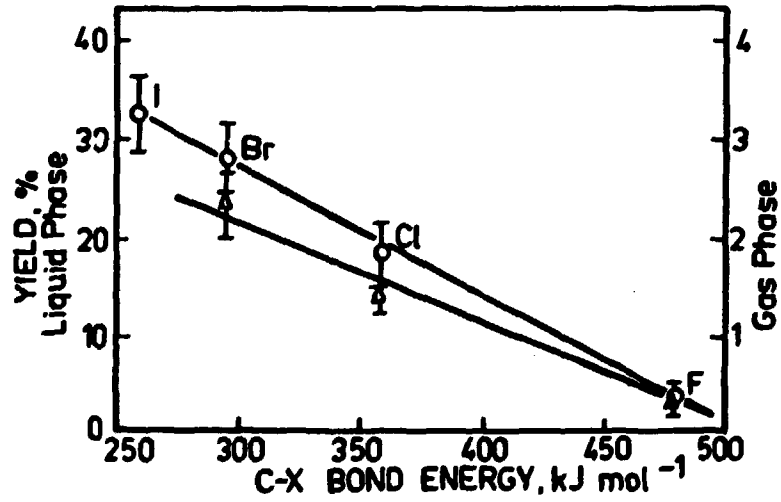



FIG. 5. Dependence of halogen (X) replacement by recoil $^{211}At$ on the C—X bond energy in halobenzenes. (Reproduced from L. Vasáros, K. Berei and Yu. V. Norseyev, *Radiochim. Acta*, **49**, 119 (1989) by permission of R. Oldenburg Verlag.)
(o - liquid, Δ-gas phase)

The influence of the second substituent on the chlorine replacement by recoil $^{211}$At has been studied in equimolar mixtures of $C_6H_5Cl$ with m- and p-$XC_6H_4Cl$ (X = F, Cl, Br, I, $CH_3$, $NO_2$)[73]. The results were interpreted on the basis of the linear free energy relationship[74] expressed by Hammett equation (see equation 10 in Section III.C). In Figure 6 the data show a slight but measurable effect of the second substituent on the chlorine replacement which is almost identical to that observed for recoil $^{38}$Cl and $^{125}$I replacing chlorine in analogous systems[73].

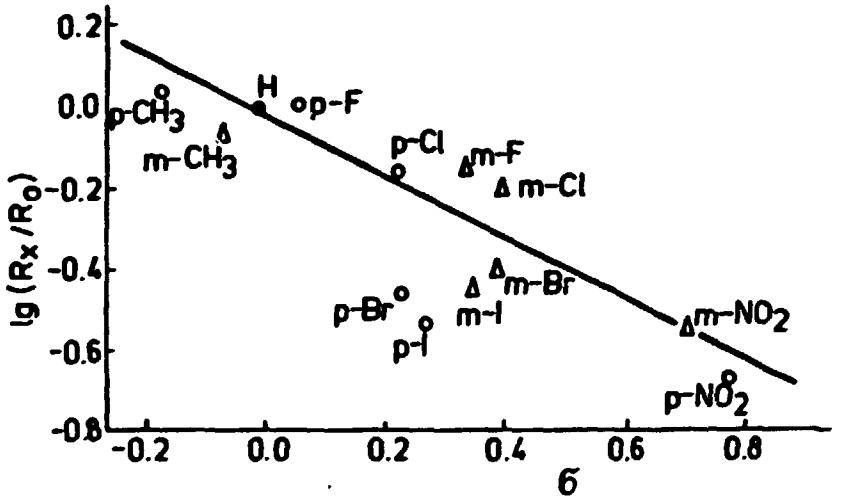



Fig. 6. Hammett plot for Cl replacement by recoil $^{211}$At in equimolar mixtures of chlorobenzene with substituted chlorobenzenes. (Reproduced from L. Vasáros, K. Berei and Yu. V. Norseyev, *Radiochim. Acta*, **49**, 119 (1989) by permission of R. Oldenburg Verlag.)

The values of reaction constants ($\rho$ = –0.5 - –0.7) obtained for recoil atoms in the above mentioned reactions are about one order of magnitude lower than those observed for thermal halogenation ($\rho$ = –3 - –12). This fact demonstrates that energetic replacement is indeed different from thermal processes. On the other hand the negative sign of the reaction constants indicates the electrophilic nature of recoil halogens — including $^{211}$At — taking part in these reactions.

# IV. PHYSICOCHEMICAL PROPERTIES OF ORGANIC COMPOUNDS

Extrapolation is still the main way of establishing the characteristic properties of organic astatine compounds. This extrapolation — either by calculation or on the basis of chromatographic behaviour — takes advantage of the values measured for analogous compounds of other halogens (see Section V in Ref.[1]). Much less new information has been gained from direct measuring the properties — such as thermal decomposition, solubility, etc. — of the astatine compounds themselves. Since many of the new astatine compounds have been prepared with the aim of using them for biomedical purposes, their stability under conditions which are similar to those in biological systems has also been studied.

Norseyev and Nefedov[75] used an experimental parameter — the so called "effective" atomic number, Z' — derived from the gas chromatographic behaviour of halogen compounds to make a rough assessment of some of the basic properties for a number of aliphatic astatine compounds. Table 9 provides the values of boiling temperature for these compounds.

More precise values for selected physicochemical constants have been established by using the gas chromatographic retention indices of simple aromatic astatine compounds: astatobenzene[76-79], astatotoluenes[77-79], astatohalobenzenes[77,78] and astatonitrobenzenes[35].

The dissociation energy of C—At bond in some aromatic compounds has been determined experimentally by measuring their thermal decomposition[80]. These data are summarized in Table 10. Boiling temperature and the heat of vapourization for the $AtC_6H_4CF_3$ isomers[28] as well as the boiling temperature for $AtCH_2CH{=}CH_2$[29], determined by extrapolation on the basis of their gas chomatographic behaviour, are shown in Table 11. The dissociation constants ($pK_a$) for astatoacetic and astatobenzoic acid have been determined by Norseyev and coworkers[32] using extraction and free solution electrophoresis. These

TABLE 9. Extrapolated boiling temperature values for some aliphatic astatine compounds[75]

| Compound | Tb, °C | Compound | Tb, °C |
|---|---|---|---|
| $CH_2AtF$ | 90 | $CH_2FCHAtF$ | 98 |
| $CH_2AtCl$ | 137 | $CH_2FCHAtCl$ | 143 |
| $CH_2AtBr$ | 168 | $CH_2FCHAtBr$ | 165 |
| $CH_2AtI$ | 208 | $CH_2FCHAtI$ | 197 |
| | | $CH_2ClCHAtF$ | 143 |
| $CHAtClF$ | 104 | $CH_2ClCHAtCl$ | 190 |
| $CHAtBrF$ | 133 | $CH_2ClCHAtBr$ | 214 |
| $CHAtFI$ | 170 | $CH_2ClCHAtI$ | 245 |
| $CHAtCl_2$ | 156 | $CH_2BrCHAtF$ | 165 |
| $CHAtClBr$ | 185 | $CH_2BrCHAtCl$ | 214 |
| $CHAtClI$ | 224 | $CH_2BrCHAtBr$ | 237 |
| $CHAtBr_2$ | 215 | $CH_2BrCHAtI$ | 268 |
| $CHAtBrI$ | 254 | $CH_2ICHAtF$ | 197 |
| $CHAtI_2$ | 292 | $CH_2ICHAtCl$ | 245 |
| | | $CH_2ICHAtBr$ | 268 |
| $CH_2FCH_2At$ | 117 | $CH_2ICHAtI$ | 300 |
| $CH_2ClCH_2At$ | 158 | | |
| $CH_2BrCH_2At$ | 182 | $CHF_2CHAtCl$ | 133 |
| $CH_2ICH_2At$ | 212 | $CHF_2CHAtBr$ | 155 |
| | | $CHF_2CHAtI$ | 185 |
| $CHF_2CH_2At$ | 100 | $CHCl_2CHAtCl$ | 225 |
| $CHFClCH_2At$ | 143 | $CHCl_2CHAtBr$ | 250 |
| $CHFBrCH_2At$ | 165 | $CHCl_2CHAtI$ | 280 |
| $CHFICH_2At$ | 197 | $CHBr_2CHAtBr$ | 300 |
| $CHCl_2CH_2At$ | 190 | $CHBr_2CHAtI$ | 330 |
| $CHClBrCH_2At$ | 214 | $CHI_2CHAtI$ | 390 |
| $CHClICH_2At$ | 245 | | |
| $CHBr_2CH_2At$ | 237 | 1-At-2-$CH_3C_3H_6$ | 140 |
| $CHBrICH_2At$ | 268 | 2-At-2-$CH_3C_3H_6$ | 123 |
| $CHI_2CH_2At$ | 300 | 1-At-2-$CH_3C_4H_8$ | 166 |
| | | $CH_2OHCH_2At$ | 195 |
| | | $CH_2$=$CHAt$ | 83 |
| | | $CH_2$=$CHCH_2At$ | 124 |

TABLE 10. Dissociation energy of C—At bond in some benzene derivatives[80]

| Compound | | $D_{C-At.}$, kJ mol$^{-1}$ |
|---|---|---|
| $C_6H_5At$ | | 187 ± 20 |
| $AtC_6H_4CH_3$ | ortho | 181 ± 10 |
| | meta | 181 ± 9 |
| | para | 182 ± 10 |
| $AtC_6H_4CF_3$ | ortho | 177 ± 9 |
| | meta | 177 ± 9 |
| | para | 176 ± 9 |
| $AtC_6H_4F$ | ortho | 180 ± 9 |
| | meta | 180 ± 9 |
| | para | 180 ± 8 |
| $AtC_6H_4Cl$ | ortho | 174 ± 9 |
| | meta | 175 ± 9 |
| | para | 180 ± 9 |
| $AtC_6H_4Br$ | ortho | 176 ± 8 |
| | meta | 177 ± 7 |
| | para | 178 ± 9 |

TABLE 11. Some thermochemical constants for the $AtC_6H_4CF_3$ isomers and for allylastatide

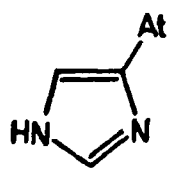
| Compound | | $T_b$, °C | $\Delta H_{vap}$, kJ mol$^{-1}$ | Ref. |
|---|---|---|---|---|
| $AtC_6H_4CF_3$ | ortho | 212 | 44.5 | 28 |
| | meta | 205 | 43.4 | |
| | para | 208 | 42.6 | |
| $AtCH_2CH{=}CH_2$ | | 129 | | 29 |
| | | 126 | | 75 |

values do not differ essentially from those established by Visser and colleagues[81] a number of years ago (see Table 14 in Ref.[1]).

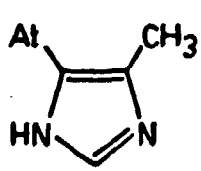
Those properties of organic astatine compounds applicable for biomedical studies have been investigated with special emphasis on the stability of the C—At bond under changing temperature and pH conditions, in the presence of oxidizing or reducing agents as well as — in some cases — *in vivo*.

Visser and colleagues[58] demonstrated the stability of 6-astatocholesterol (***12***) in dilute EtOH solutions at temperatures up to 70 °C as well as during incubation with oxidizing ($H_2O_2$) or reducing ($NaHSO_3$) reagents at room temperature. Essentially the same result has been obtained by Liu and his coworkers[59] who found ***12*** to be stable up to 80 °C. Astatoestradiols are less stable. Although no deastatination was observed in acidic media (pH = 1-7) at room temperature, in neutral solutions at 50 °C more than 75% of astatine was lost from the labelled compound in 20 hours. Higher pH and the presence of oxidizing agents caused increased decomposition[58].

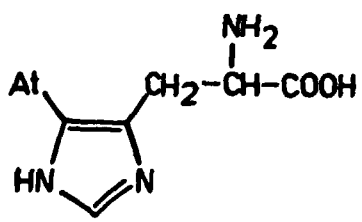
Imidazole derivatives: 4-astatoimidazole (***28***), 5-astato-4-methylimidazole (***29***) and 5-astatohistidine (***30***) do not decompose to any great extent at ambient temperature in the pH = 1-14 range for about 15 hours. Above 80 °C or on the addition of reducing or oxidizing species rapid deastatination takes place[82].




(***28***)




(***29***)




(***30***)

5-Astatouracil (***14***), 5-astatouridine (***15***) as well as 5-astatocytosine (***17***) and 5-astatocytidine (***18***) are stable at room temperature in the pH = 1-11.5 range over a period of 20 hours. At 50 °C and at pH = 11.5 about 30% decomposition is observed in 20 hours. Oxidizing as well as reducing reagents enhance this process for ***17*** and ***18*** but do not affect the stability of ***14***, and ***15***[58].

Astatinated nucleotides, and the mono and triphosphate of ***15*** have been found to be sensitive to hydrolysis suffering ~50% dephosphorilation in 20 hours at pH = 1 and T = 50 °C. The monophosphate of ***18*** behaves in a similar way also showing a slow decomposition of the sugar — pyrimidine bond. The monophosphate of ***19*** is even less stable: within 20 hours in acidic solutions at 50 °C it decomposes entirely into its components. Astatinated DNA and RNA are stable in the pH = 2-11.5 range at room temperature. In neutral solutions even the addition of oxidizing or reducing species in small amounts do not cause any decomposition. If the temperature is increased to 50 °C, it induces slow deastatination of about 15-20% in 20 hours[58].

Milius and coworkers[61] have found that the C—At bond of p-astatobenzoic acid is stable in hot acidic and basic solutions as well as in the presence of moderate reducing or oxidizing agents.

## V. BIOMEDICAL APPLICATIONS

By virtue of the advantageous nuclear characteristics of $^{211}$At for specific cell killing within a limited volume with relatively little damage to the neighbouring tissues, this isotope has been considered as a candidate for cancer therapy[83-86]. The mean linear energy transfer (LET) of its emission is 98.84 keV $\mu m^{-1}$ in water[87] which is very close to the average value of the optimal dose (100 keV $\mu m^{-1}$) for endoradiotherapeutic purposes (see Figure 7).

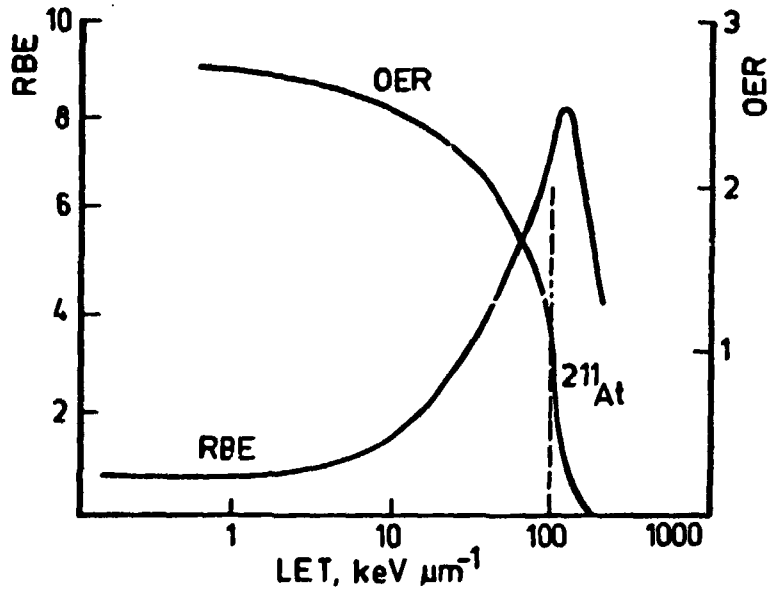



FIG. 7. Dependence of relative biological effect (RBE) and of oxygen enhancement ratio (OER) on the linear energy transfer (LET) of radiation. (Reproduced from I. Brown, *Appl. Radiat. Isot.*, **37**, 789 (1986) by permission of Pergamon Press.)

Furthermore, the sterilizing effect of α particles is not significantly influenced by the reduced oxygen tension expected in many tumours (hypoxic cells). Cell damage of such intense ionizing radiation is lethal due to non-rejoining double strand breaks in DNA[88]. The relative biological effect (RBE) of α particles originating from $^{211}$At decay has been determined as four times higher than

that of $^{60}Co$ γ radiation in human leukaemia cells[89]. RBE values ranging from 1 to 15 compared to lower LET γ and β radiations have been obtained for a variety of tissues *in vitro* and *in vivo*[84], in most cases this value lies between 2 and 5.

From the biomedical viewpoint the main consideration prior to administering this α emitter is the possible damage to healthy tissues. Obviously the more selective and localized is the accumulation of $^{211}At$-labelled compound in the tumour bearing tissues ("tumour-binding specificity") and the more stable is the bond between astatine and the tumour specific carrier compound the better are the chances of therapeutic efficacy. The last decade has seen major efforts to achieve this aim by cooperation of chemists, biologists and physicians.

## A. Inorganic $^{211}At$ Species

After a great deal of theoretical speculation the first convincing experimental results on curing tumour in a living organism by means of radiation of $^{211}At$ published in 1981 were obtained by a Harvard Medical School team[90]. Bloomer and his colleagues[90,91] used $^{211}At^-$ bound to tellurium colloid to treat experimental malignant ascites in the ovaries of C3H mice. Single graded doses of radiocolloid were administered by intraperitoneal injections. As can be seen from Figure 8, doses of ~0.9-1.8 MBq (25-50 μCi) were curative in all animals. Doses of ~2.8 MBq (75 μCi) and higher turned out to be toxic causing death in 7-10 days whereas administration of lower activities resulted in considerable increasing median survival of treated animals. Comparison of the effect of $^{211}At$-Te colloid with that of the most often used β emitting radiocolloids has shown that none of the latter could result in a complete cure despite the more or less substantial increase of median survival for treated animals. Animals cured by $^{211}At$-Te colloid — observed for at least 200 days — appeared sleek, agile and alert. Long term histological changes established in the thyroid can be prevented by preliminary blocking of this

tissue using an excess of KI or $KClO_4$. Apart from this, short term acute alterations were detected in the gastrointestinal tract which disappeared after 36 hours[91].

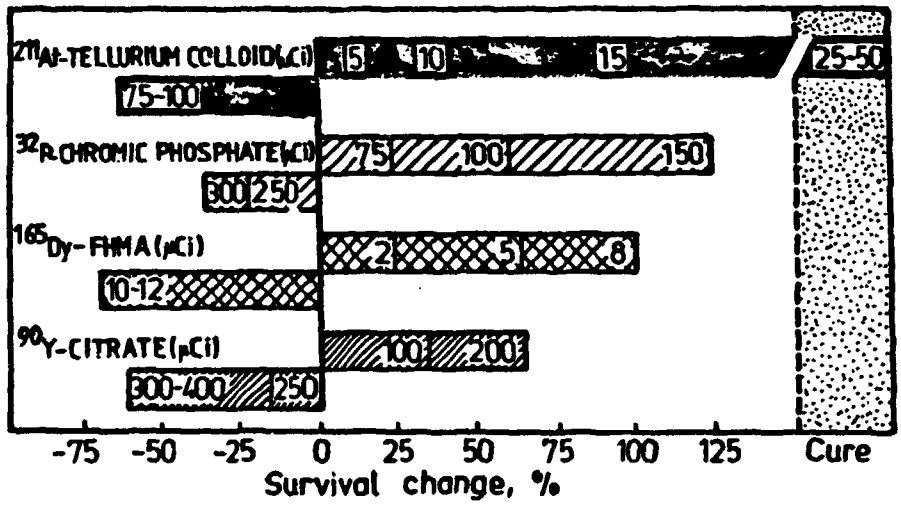



FIG. 8. Median survival change of mice treated with α and β emitting radiocolloids. (Reproduced from W. D. Bloomer, W. H. McLaughlin, R. M. Lambrecht, R. W. Atcher, S. Mirzadeh, J. L. Madara, R. A. Milius, M. R. Zalutsky, S. J. Adelstein and A. P. Wolf, *Int. J. Radiat. Oncol., Biol., Phys.*, **10**, 341 (1984) by permission of Pergamon Press)
(Negative results indicate that experimental mice died of treatment-related toxicity before control animals died of tumour.)
FHMA — ferrihydroxide macroaggregate;
1 μCi = 37 kBq; 1 mCi = 37 MBq

The significance of this first *in vivo* tumour killing study by means of α radiation of $^{211}$At isotope should not be underrated even if some other teams trying to repeat the experiment under slightly different conditions have found smaller curative effect[92] and more expressed histopathological changes in most organs of the experimental animals[93].

More recently $^{211}$At adsorbed on silver coated human serum albumin (HSA) microspheres has been used by Wunderlich, Doberenz and their colleagues[46, 94-99] to study the accumulation of the isotope in some tumour bearing tissues of mice and pigs, and in one special case for human therapy[100].

Microspheres produced from denaturated HSA are first treated with $AgNO_3$ to produce a silver coating and then labelled within 30 minutes at ~50°C

with yields of ~90%. Binding of $^{211}$At to microspheres has proved to be stable for more than 30 hours *in vivo*.

As an intermediate step between experiments with mice and application for human therapy of tongue carcinoma, pigs were used since they react similarly to human beings in the ENT region. It has been established that labelled microspheres accumulate in the tongue and larynx after intraarterial injection and remain for about 12 hours in the target organs with negligible loss of $^{211}$At. Figure 9 shows the accumulation and decay of activity in tongue and in the abdomen of a pig after injection in relation to the radioactivity of the original suspension.

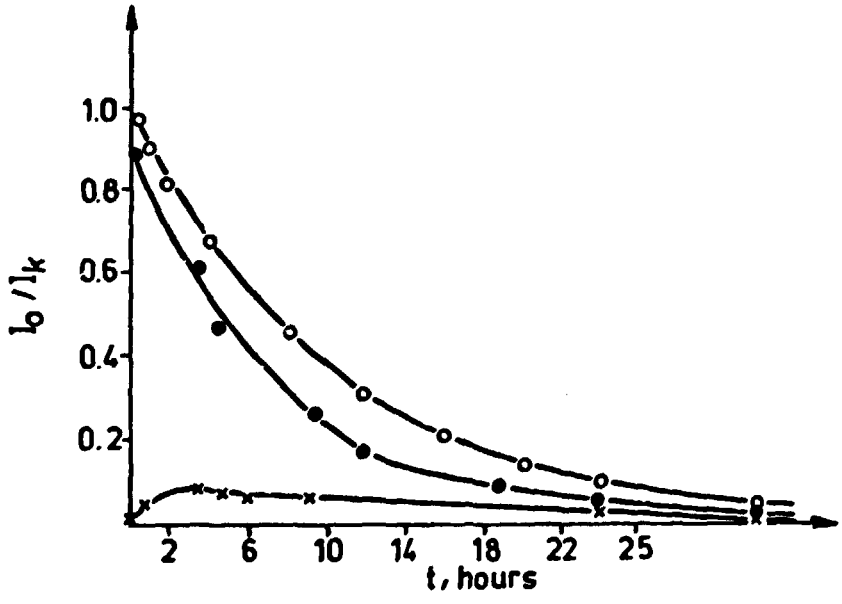



FIG. 9. Activity ($I_k$) in the region of the tongue (•) and the abdomen (x) related to the activity ($I_o$) of the original suspension of $^{211}$At-microspheres (o) depending on the time elapsed after administration to a pig. (Reproduced from G. Wunderlich, W.-G. Franke, R. Hlics and W. Doberenz, *Med. Nucl.*, **1**, 25 (1989) by permission of the authors.)

The accumulation ratio of activity in lingual tumour to that of the background has been determined as 20 to 1 and a mean equivalence dose has been calculated to be 4.0 Sv in the abdomen in contrast to 1000 Sv in the tongue[96]. These results have encouraged therapeutic application and

$^{211}$At-HSA microspheres were used for human radioendotherapy (at a given patient's request) first in November 1988. Administration of 200 MBq (~5.4 mCi) $^{211}$At activity caused complete tumour remission as a result of tumour cell and capillary system destruction in the treated region. Only a slight depression of thyroid function was observed as a side effect[46,100].

Other solid particles (S- or Au-colloids, gelatin, targesin, etc.) have similarly been examined for their ability to transfer the $^{211}$At isotope to tumour bearing tissues[46,97,99]. Application of solid carriers is, however, limited by their lack of biological specificity. Therefore radiocolloids or labelled microspheres are primarily used for local administration into the tumourous tissue itself.

## B. $^{211}$At-Labelled Proteins

A more general application for alpha endoradiotherapy was expected from labelling proteins which — participating in the metabolic processes — could serve as selective carriers of $^{211}$At to tumour bearing tissues. Especially promising possibilities seemed to be offered by labelling antibodies seeking tumour associated antigens. In searching for optimal conditions the following aspects have to be considered:

a) Specificity and affinity of antibody towards the tumour associated antigen. New techniques producing monoclonal antibodies have significantly contributed to this aspect[101,102].

b) Rapid antibody uptake and a relatively long half-life in the tumour compared to the speed of clearance from the healthy tissues. In some cases light chain fragments of antibodies are applied owing to their faster clearance from the blood and other radiation sensitive organs (*vide infra*).

c) Stability of $^{211}$At binding to the antibody *in vivo* and decay characteristics determining the radiation dose absorbed in different types of tumours. According to absorbed dose calculations made by Humm[103] the short range high LET $\alpha$ radiation of $^{211}$At provides maximal destruction in solid tumours of moderate size (ø = 100-500 μm).

In the last 10-15 years a variety of proteins, antibodies and their light chain fragments have been labelled with $^{211}$At without loss of target specificity by means of one or the other technique described in Section III.

The first results on synthesizing astatinated antibodies, studying their biodistribution and tumour killing effect *in vitro* and *in vivo* were published by Vaughan and his coworkers[42,104-108]. One of the labelled biomolecules, the IgG class monoclonal antibody [$^{211}$At]astato-BK19.9, shows specific reactivity for the human transferrin receptor appearing on the surface of rapidly dividing human cells. Its extreme cytotoxicity *in vitro* was able to be demonstrated by the $D_{37}$ value (i.e. 37% cell population survival) which was obtained for 7-12 $^{211}$At atoms/cell concentration[42,106]. The effect *in vivo* is, however, less dramatic because, according to the biodistribution results, the concentration of $^{211}$At in tumour was always found to be equal to or lower than in the blood of experimental animals (mice), as can be seen from the data of Table 12. Loss of the astatine label — most probably in $At^-$ form — from the blood pool causes its accumulation in normal tissues, mainly in the spleen, lungs, and reproductive organs. This represents the most important limitation concerning therapeutic applications.

Harrison and Royle's experiments[109,110] with $^{211}$At-labelled anti-Thy 1.1 IgG,OX7 monoclonal antibody appeared more promising. When used for treating Thy 1.1 T cell lymphoma in mice, the astatinated antibody was stable *in vivo* and considerably increased the median survival time. Injection of 80-90 kBq (~2.2-2.4 μCi) [$^{211}$At]astato-OX7 resulted in a complete cure — i.e. survival over 200 days — in 78% of the treated mice.

Later experiments by Harrison[111], however, have led to the conclusion that in most cases conjugates of $^{211}$At with whole antibodies (e.g. IgG) cannot be successfully used for tumour therapy because of their slow clearance from the blood, thereby inducing damage to the bone marrow and the circulating white cells. Furthermore, due to their size the labelled antibodies may fail to penetrate the tumour mass thus leaving malignant cells beyond the ~65 μm range of α particles emitted by $^{211}$At. Therefore, Harrison suggested that labelling light chain fragments should be explored since these should be able to penetrate the tumour and be eliminated from the blood more rapidly.

TABLE 12. Specific activity index (SAI) of tumour and of normal tissues of mice 12 hours after injection of $^{211}$At-BK 19.9[108]

| Tissue | S.A.I.[a] |
|---|---|
| Blood | 1.00 |
| Thyroid | 0.46 |
| Lung | 0.83 |
| Liver | 0.42 |
| Spleen | 0.62 |
| Gut | 0.35 |
| Kidney | 0.44 |
| Muscle | 0.32 |
| Tumour | 0.91 |

[a]S.A.I = $^{211}$At cpm g$^{-1}$ (tissue) : $^{211}$At cpm g$^{-1}$ (blood)

Zalutsky and colleagues have been continuing their investigations in this direction. Two antibody fragments have been labelled with $^{211}$At: one of them, monoclonal Me1-14 F(ab')2, is reactive with antigens present in some tumours, e.g. human gliomas; the other a non specific antibody fragment, RPC 5 F(ab')2, not binding to any known antigens[66]. Administration of these $^{211}$At-conjugates to mice bearing D-54 MG subcutaneous human glioma xenografts (human tumour model) offered a possibility for establishing their biodistribution and radiation dose absorbed in the tumour compared to normal tissues. According to the results shown in Fig. 10, the specific binding and affinity of Me1-14 F(ab')2 fragment to tumour cells is retained also after labelling with astatine. The radiation dose absorbed by the tumour is 4-5 times higher for this labelled fragment than that for the non-specific [$^{211}$At]astato-RPC 5. In a further study,

however, a disturbingly rapid loss of $^{211}$At label from the antibody fragments was observed by the same authors[68].

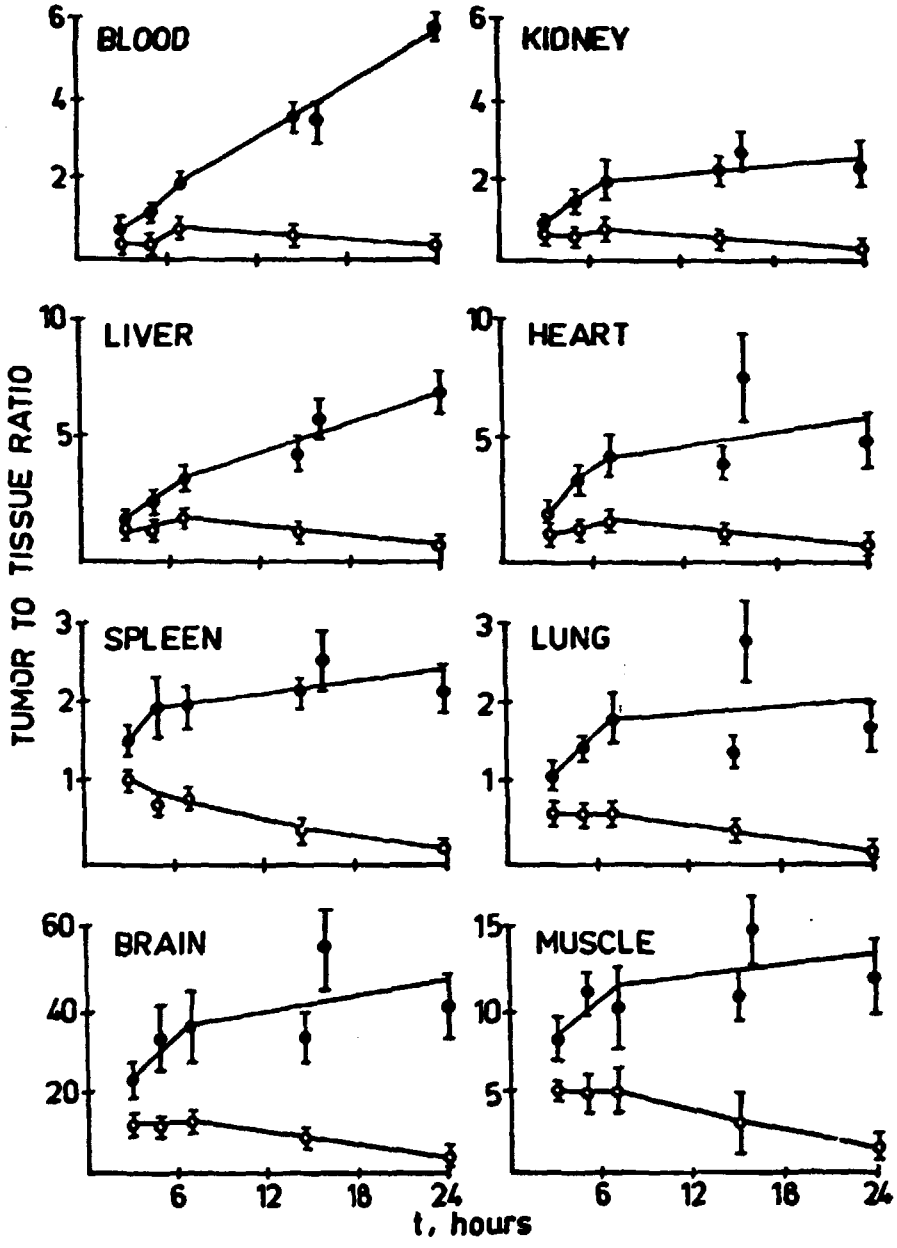



FIG. 10. Tumour to normal tissue uptake ratios in mice for tumour-specific Me1-14 F(ab')2 (•) and non-specific RPC 5 F(ab')2 (o), both labelled with $^{211}$At. (Reproduced from M. R. Zalutsky, P. K. Garg, A. M. Friedman and D. D. Bigner, *Proc. Natl. Acad. Sci. U.S.A.*, **86**, 7149 (1989) by permission of the authors.)

So far it seems that the attractive idea of specifically binding antibodies carrying radioisotopes for destroying target cells ("magic bullets"), is not easy

to realize because of the complicated nature of metabolic processes. Moreover, Vaughan and several other researchers[112] have expressed a general scepticism about the possibility of tumour killing by means of radiolabelled antibodies without causing inadmissible radiation damage to healthy tissues.

### C. $^{211}$At-Labelled Drugs

Currently, it would seem that the most promising approach is to use metabolically directed anti-cancer drugs (or biomolecules selectively accumulating in the tumour bearing tissue) as carriers of astatine.

Brown and coworkers[33,34,57] have developed and investigated 6-[$^{211}$At]astato-2-methyl-1,4-naphthoquinol bis (dihydrogenphosphate salt) or 6-$^{211}$At-MNDP (see (2) in Section III.A.2) from this aspect. The mechanism of intracellular localization is based on the presence of oncogenically expressed tumour-membrane phosphatase isoenzymes which perform the dephosphorilation of the MNDP molecule followed by transport of quinone metabolite to the cell nucleus where it is covalently bound to chromatin. The presence of $^{211}$At could also be used to determine the intracellular site of the therapeutic action of the drug by means of α particle track autoradiography. Such investigations have confirmed the heterogeneous distribution of $^{211}$At-labelled MNDP metabolite in the tumour with specific localization in malignant cell nuclei[113-115].

Biodistribution studies[34,116-119] in a murine CMT-93 rectal tumour model have indicated a significant tumour uptake over the first 12 hour period after administration of 6-$^{211}$At-MNDP, as can be seen from the data in Table 13. However, after the first 6 hours $^{211}$At also accumulated in the lung, spleen and stomach all of which are specifically At$^-$ localizing organs. This phenomenon is interpreted as a consequence of gradual metabolic cleavage of the C—At bond of naphthoquinol[117]. Despite side-effects due to deastatination, a 50-60% survival rate after 12 months has been determined for the animals treated with 92.5-222 kBq (2.5-6 μCi) 6-$^{211}$At-MNDP. A lower survival rate has been

obtained for animals treated with higher than ~260 kBq (7 μCi) doses of the astatinated drug; this finding can be explained by late manifestation of radiation induced histopathological changes in the pulmonary tissue[84,119].

TABLE 13. Tumour to blood ratio for 6-$^{211}$At-MNDP

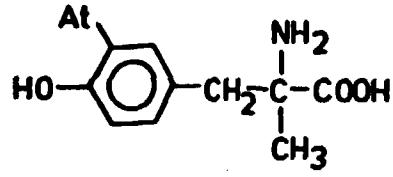
| Time, hours | Tumour to blood ratio |
|---|---|
| 0.5 | 3.32 ± 0.56 |
| 1.0 | 3.62 ± 0.61 |
| 3.0 | 4.05 ± 1.12 |
| 6.0 | 1.71 ± 0.37 |
| 12.0 | 2.29 ± 0.24 |
| 24.0 | 3.08 ± 0.59 |
| 48.0 | 1.32 ± 0.22 |

Reproduced from I. Brown, R. N. Carpenter and J. S. Mitchell, *Int. J. Appl. Radiat. Isot.*, **35**, 843 (1984) by permission of Pergamon Press.

Advantage has been taken of the high binding affinity of phenothiazine and its derivatives to melanine — an abundant component of melanoma tumours — by Link and colleagues[120-122] to prepare 4-[$^{211}$At]astato-methylene blue or $^{211}$At-MTB (see (*5*) in Section III.A.2) to study its accumulation in pigmented and non-pigmented melanoma cells *in vitro* and *in vivo*. The results demonstrated a cell-killing effect for pigmented melanoma: only 4% of $10^5$ melanotic cells survived *in vitro* after addition of 11 kBq (~0.3 μCi) of $^{211}$At-MTB. Autoradiographic studies have proved selective accumulation of the radiolabelled dye by pigmented melanoma cells[122].

For *in vivo* investigation 4MBq (~108 μCi) of $^{211}$At-MTB was injected into the tail vein of mice 24 hours after injecting the tumour cells. The fraction of

surviving melanotic cells 17 days later was estimated as 2.1%. Moreover, the animals showed no symptoms indicating toxic effects even 1 year after treatment. On the other hand, amelanotic melanoma cells were not affected significantly by $^{211}$At-MTB[122].




(31)

$^{211}$At may also be selectively accumulated by melanoma cells if administered as L-3-[$^{211}$At]astato-alpha-methyltyrosine or $^{211}$At-AMT (31) since tyrosine is the principal precursor in melanin synthesis. Uptake and efflux of $^{211}$At-AMT by B 16 murine melanoma cells as well as its cytotoxic effect have been studied by McLaughlin and colleagues[123,124]. From Figure 11

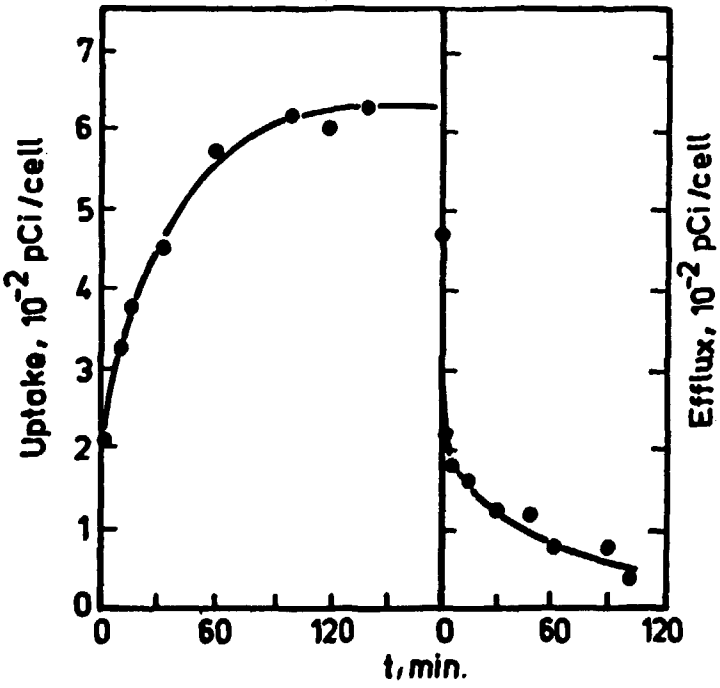



FIG. 11. Uptake and efflux $^{211}$At-labelled alpha-methyltyrosine in B16 melanoma cells as a function of time (values corrected for $^{211}$At-decay). (Reproduced from W. H. McLaughlin, W. M. Thramann, Jr., R. M. Lambrecht, R. A. Milius and W. D. Bloomer, *J. Surg. Oncol.*, 37, 192 (1988) by permission of Academic Press.) 1pCi = 37 Bq

a sharp initial rise in the uptake can be seen reaching a saturation level in ~90 minutes. After exposing the melanoma cells to ~315 kBq (8,5 μCi) $ml^{-1}$ for 3 hours by incubation, the efflux shows a drastic 65% loss of radioactivity in the first 10 minutes with, subsequently, a much more gradual decrease. The residual 35% of α activity forms the basis for using $^{211}$At-AMT as a potential diagnostic or therapeutic agent.

Clonogenic survival of B16 melanoma cells measured after exposure to graded doses of $^{211}$At-AMT for 45 minutes is represented in Figure 12, showing that this radiolabelled modified melanin precursor is exquisitely cytotoxic. The linear cell survival curve, without a "shoulder" in the low dose region, is characteristic for high LET α radiation[125].

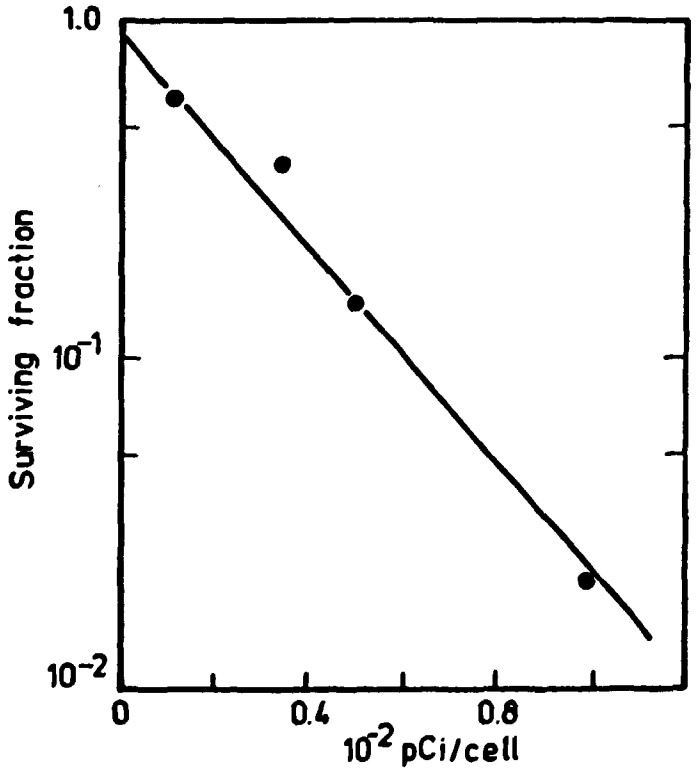



FIG. 12. Clonogenic survival of B16 melanoma cells exposed to $^{211}$At-labelled alpha-methyltyrosine for 45 min incubation period. (Reproduced from W. H. McLaughlin, W. M. Thramann, Jr., R. M.Lambrecht, R. A. Milius and W. D. Bloomer, *J. Surg. Oncol.*, **37**, 192 (1988) by permission of Academic Press.)
1pCi = 37 Bq

The same team [63,124] has pointed out that accumulation of astatosteroids in estrogen receptor positive cells may have a significant destructive (radiotoxic) effect on these and also on the neighbouring receptor negative tumour cells. In practice so far only one $^{211}$At-labelled steroid: 6-[$^{211}$At]astato-methyl-19-norcholest-5(10)-en-3β-ol or NCL-6-$^{211}$At (see (3) in Section III.A.2) has been used in animal biodistribution studies. Liu and his coworkers[37] have demonstrated a remarkable accumulation of NCL-6-$^{211}$At in the adrenal glands, as can be seen from Table 14. This may enable its application in adrenal tumour therapy although 12 hours after the administration ~3-5% of radioactivity was found in the thyroid, thereby indicating slow deastatination of the steroid *in vivo*.

TABLE 14. Distribution of NCL-6-$^{211}$At in mice tissue 12 hours after administration[37]

| Tissue | Relative values[a] |
|---|---|
| Adrenal | 139.95 |
| Liver | 9.99 |
| Kidney | 6.48 |
| Lung | 12.98 |
| Spleen | 17.48 |
| Stomach | 8.62 |
| Heart | 5.17 |
| Blood | 4.65 |

[a]Values corrected for $^{211}$At decay and expressed as percentage of administered dose per gram of tissue

• • •

Despite the considerable advances made in methods of synthesis and biomedical investigations of $^{211}$At-labelled compounds, application of this isotope for human therapy is still not considered realistic, mainly because of the risks of simultaneous irradiation of healthy tissues[112,126-129].

For all practical purposes the chemical toxicity can be ignored due to the negligible amounts of astatine applied: Only $5x10^{-10}$ g $^{211}$At is needed to induce 37 MBq (1 mCi) α activity. However, as has recently been established by Cobb and colleagues[129], pathological changes in mice following injection of 61 kBq (~1.6 μCi) $^{211}$At per gram body weight are clearly noticeable. The most obvious toxicological effect is exhibited by the thyroid, the spleen, the gonads, the lymph nodes, bone marrow and salivary glands. Lesser changes are observed in the stomach and nasal passages. From these and earlier studies it can be concluded that the thyroid gland, the gonads and lymphocytes are most likely to be affected if human beings were to be treated with higher doses of $^{211}$At. Moreover, it should of course be mentioned that not all health hazards that may be encountered in human therapy can necessarily be determined from animal experimentation.

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## VI. References


1. K. Berei and L. Vasáros, in *The Chemistry of Functional Groups*, Suppl. D (Eds. S. Patai and Z. Rappoport), John Wiley and Sons, New York, 1983, pp. 405-440.

2. Astatine, *Gmelin Handbook of Inorganic Chemistry*, 8th Edition, (Eds. H.K. Kugler and C. Keller), Springer, Berlin, 1985.

3. *Congress Edition of Nucl. Med.*, **26**, No. 4. (1987).

4. *Radiochim. Acta* (Special Issue on Astatine Chemistry), **47**, Nos. 2/3 (1989).

5. T. S. Perova and V. S. Libov, *Spektrokhim. Vnutri-Mezhmol. Vzaimodeistvii*, No. 2, 126 (1978).

6. W. J. Stevens and M. Krauss, *J. Phys.*, B **16**, 2921 (1983).

7. V. P. Glushko, V. A. Medvedev, G. A. Bergman, L. V. Gurvich, V. S. Yungman, A. F. Vorobev, V. P. Kolesov, L. A. Reznitskii, B. V. Mikhailov, G. L. Galchenko and M. Kh. Karapetyants, *Termicheskie Konstanty Veshchestv*, Pt. I, Nauka, Moscow, 1966.

8. G. A. Krestov, *Radiokhimiya*, **4**, 690 (1962); *Soviet Radiochem.*, **4**, 612 (1962).

9. G. A. Krestov, *Izv. Vysshikh Uchebn. Zavedenii Khim. Khim. Tekhnol.*, **6**, 754 (1963).

10. G. A Krestov, *Teor. Eksperim. Khim.*, **1**, 479 (1965).

11. D. S. Kaganyuk, *Radiokhimiya*, **25**, 814 (1983).

12. G. A. Krestov, *Izv. Vysshikh Uchebn. Zavedenii Khim. Khim. Tekhnol.*, **11**, 762 (1968).

13. G. A. Krestov, *Izv. Vysshikh Uchebn. Zavedenii Khim. Khim. Tekhnol.*, **6**, 228 (1963).

14. E. H. Appelman, E. N. Sloth and M. H. Studier, *Inorg. Chem.*, **5**, 766 (1966).

15. J. Merinis, Y. Legoux and G. Bouissieres, *Radiochem. Radioanal. Lett.*, **11**, 59 (1972).

16. K. Otozai and N. Takahashi, *Radiochim Acta*, **31**, 201 (1982).

17. E. H. Appelman, *UCRL-9025*, 1-113 (1960).

18. A. H. W. Aten Jr., in: *Beitrage zur Physik und Chemie des 20. Jahrhunderts*, (Eds. O. R. Frisch, F. A. Paneth, F. Laves and P. Rosbaud), Vieweg, Braunschweig, 1959, pp. 121-123.

19. A. H. W. Aten Jr., *Advan. Inorg. Chem. Radiochem.*, **6**, 207 (1964).

20. E. H. Appelman, *MTP Intern. Rev. Sci. Inorg. Chem. Ser. One*, **3**, 181 (1972).

21. L. Vasáros and K. Berei, in *Gmelin Handbook of Inorganic Chemistry, 8th Edition, Astatine* (Eds. H. K. Kugler and C. Keller), Springer, Berlin, 1985, pp. 107-128.

22. K. Berei and L. Vasáros, in *Gmelin Handbook of Inorganic Chemistry, 8th Edition, Astatine* (Eds. H. K. Kugler and C. Keller), Springer, Berlin, 1985, pp. 210-259.

23. G. W. M. Visser, *Radiochim. Acta*, **49**, 97 (1989).

24. L. Vasáros, Yu. V. Norseyev, D. D. Nhan and V. A. Khalkin, *Radiochem. Radioanal. Lett.*, **47**, 313 (1981).

25. L. Vasáros, Yu. V. Norseyev, D. D. Nhan and V. A. Khalkin, *Radiochem. Radioanal. Lett.*, **47**, 403 (1981).

26. L. Vasáros, Yu. V. Norseyev, D. D. Nhan and V. A. Khalkin, *Radiochem. Radioanal. Lett.*, **50**, 275 (1982).

27. L. Vasáros, K. Berei and Yu. V. Norseyev, *Radiochim. Acta*, **49**, 119 (1989).

28. L. Vasáros, Yu. V. Norseyev, D. D. Nhan and V. A. Khalkin, *Radiochem. Radioanal. Lett.*, **59**, 347 (1983).

29. Yu. V. Norseyev, L. Vasáros and Z. Szűcs, *Radiokhimiya*, **30**, 182 (1988).

30. H. Elias, Ch. Arnold and G. Kloss, *Int. J. Appl. Radiat. Isot.*, **24**, 463 (1973).

31. C.-Y. Shiue, G.-J. Meyer, T. J. Ruth and A. P. Wolf, *J. Labelled Compd. Radiopharm.*, **18**, 1039 (1981).

32. Z. Szűcs, Yu. V. Norseyev, D. D. Cuong and L. Vasáros, *Radiokhimiya*, **33**, 64 (1991).

33. I. Brown, *Radiochem. Radioanal. Lett.*, **53**, 343 (1982).

34. I. Brown, *J. Labelled Compd. Radiopharm.*, **19**, 1389 (1982).

35. L. Vasáros, Yu. V. Norseyev, V. I. Fominikh and V. A. Khalkin, *Radiokhimiya*, **24**, 95 (1982); *Soviet Radiochem*, **24**, 84 (1982).

36. G. W. Gokel and H. D. Durst, *Aldrichim. Acta*, **9**, 3 (1976).

37. Boli Liu, Yutai Jin, Zhenghao Liu, Chen Luo, M. Kojima and M. Maeda, *Int. J. Appl. Radiat. Isot.*, **36**, 561 (1985).

38. I. Brown, R. N. Carpenter, E. Link and J. S. Mitchell, *J. Radioanal. Nucl. Chem., Lett.*, **107**, 337 (1986).

39. I. Brown, R. N. Carpenter, J. S. Mitchell, and E. Link, *J. Labelled Compd. Radiopharm.*, **23**, 1245 (1986).

40. G. W. M. Visser and E. L. Diemer, *Radiochem. Radioanal. Lett.*, **51**, 135 (1982).

41. H. Zollinger, *Acc. Chem. Res.*, **6**, 335 (1973).

42. A. T. M. Vaughan, W. J. Bateman and J. Cowan, *J. Radioanal. Chem.*, **64**, 33 (1981).

43. A. Harrison and L. Royle, *Int. J. Appl. Radiat. Isot.*, **35**, 1005 (1984).

44. Changhou Yi, Jannan Jin, Shuyuan Zhang, Ketai Wang, Dayuan Zhang and Maolun Zhou, *J. Radioanal. Nucl. Chem., Artic.*, **129**, 377 (1989).

45. G. Wunderlich, S. Fischer, R. Dreyer and W.-G. Franke, *J. Radioanal. Nucl. Chem., Lett.*, **117**, 197 (1987).

46. G. Wunderlich, *Dr. Sci. Diss. Techn. Univ. Dresden*, Dresden, 1989.

47. T. Reimann, in *Sem. Frschgr. Radiochem. Techn. Univ. Dresden*, Dresden, 1990, pp. 100-102.

48. T. Reimann, *Ph.D. Thesis. Techn. Univ. Dresden*, Dresden, 1992.

49. L. Vasáros, Yu. V. Norseyev and V. A. Khalkin, *Dokl. Akad. Nauk SSSR*, **266**, 120 (1982); *Proc. Acad. Sci. USSR*, **262/267**, 297 (1982).

50. Yu. V. Norseyev, D. D. Nhan, V. A. Khalkin, N. Q. Huan and L. Vasáros, *J. Radioanal. Nucl. Chem., Lett.* **95**, 137 (1985).

51. R. W. Taft, *J. Phys. Chem.*, **64**, 1805 (1960).

52. L. Vasáros, Yu. V. Norseyev, D. D. Nhan and V. A. Khalkin, *Radiochem. Radioanal. Lett.*, **54**, 239 (1982).

53. Yu. V. Norseyev, D. D. Nhan, V. A. Khalkin, N. Q. Huan and L. Vasáros, *J. Radioanal. Nucl. Chem., Lett.*, **94**, 185 (1985).

54. Yu. V. Norseyev, L. Vasáros, D. D. Nhan, and N. Q. Huan, *Radiokhimiya*, **29**, 474 (1987); *Soviet Radiochem.*, **29**, 457 (1987).

55. G. W. M. Visser, E. L. Diemer and F. M. Kaspersen, *Int. J. Appl. Radiat. Isot.*, **32**, 905 (1981).

56. H. H. Coenen, S. M. Moerlein and G. Stöcklin, *Radiochim. Acta*, **34**, 47 (1983).

57. I. Brown, *Int. J. Appl. Radiat. Isot.*, **33**, 75 (1982).

58. G. W. M. Visser, E. L. Diemer and F. M. Kaspersen, *J. Labelled Compd. Radiopharm.*, **18**, 799 (1981).

59. Boli Liu, Yutai Jin, and Taihua Li, *Beijing Shifan Daxue Xuebao, Ziran Kexueban*, **3**, 57 (1985); Chem. Abstr. **106**, No. 5321 (1987).

60. G. W. M. Visser and E. L. Diemer, *Int. J. Appl. Radiat. Isot.*, **33**, 389 (1982).

61. R. A. Milius, W. H. McLaughlin, R. M. Lambrecht, A. P. Wolf, J. J. Carroll, S. J. Adelstein and W. D. Bloomer, *Appl. Radiat. Isot., Artic.*, **37**, 799 (1986).

62. D. E. Seltz, G. L. Tonnessen, R. N. Hanson, S. Hellman and S. J. Adelstein, *J. Organomet. Chem.*, **186**, C33 (1980).

63. K. M. R. Pillai, W. H. McLaughlin, R. M. Lambrecht and W. D. Bloomer, *J. Labelled Compd. Radiopharm.*, **24**, 1117 (1987).

64. M. R. Zalutsky and A. S. Narula, *Appl. Radiat. Isot., Artic.*, **39**, 227 (1988).

65. A. S. Narula and M. R. Zalutsky, *Radiochim. Acta*, **49**, 131 (1989).

66. M. R. Zalutsky, P. K. Garg, A. M. Friedman and D. D. Bigner, *Proc. Natl. Acad. Sci. U.S.A.*, **86**, 7149 (1989)

67. P. K. Garg, C. L. Harrison, D. D. Bigner and M. R. Zalutsky, *J. Labelled Compd. Radiopharm.*, **26**, 293 (1989)

68. P. K. Garg, C. L. Harrison and M. R. Zalutsky, *Cancer Res.*, **50**, 3514 (1990)

69. D. S. Wilbur, M. D. Hylarides, S. Hadley, J. Schroeder and A. R. Fritzberg, *J. Labelled Compd. Radiopharm.*, **26**, 316 (1989).

70. D. S. Wilbur, M. D. Hylarides and A. R. Fritzberg, *Radiochim. Acta*, **47**, 137 (1989).

71. K. Berei and L. Vasáros, in *Handbook of Hot Atom Chemistry* (Eds. J.-P. Adloff, P. P. Gaspar, M. Imamura, A. G. Maddock, T. Matsuura, H. Sano and K. Yoshihara), Kodansha, Tokyo, 1992, pp. 80-84.

72. K. Berei and L. Vasáros, *Radiochim. Acta*, **43**, 61 (1988).

73. K. Berei, L. Vasáros, Yu. V. Norseyev and J. Sutyor, *Radiochim. Acta*, **42**, 17 (1987).

74. L. M. Stock and H. C. Brown, in *Adv. Phys. Org. Chem.*, Vol. 1, (Ed. V. Gold), Academic Press, New York, 1963, pp. 35-154.

75. Yu. V. Norseyev and V. D. Nefedov, in *Issled. Khim. Tekhnol. Primen. Radioakt. Veshchestv*, Leningrad, 1977, pp. 3-8; Chem. Abstr. **94**, No. 3539 (1981).

76. L. Vasáros, Yu. V. Norseyev and V. A. Khalkin, *Report JINR-R-12 188*, (1979); Chem. Abstr. **96**, No 198792 (1982).

77. L. Vasáros, Yu. V. Norseyev and V. A. Khalkin, *Report JINR-R-6-80-158*, (1980).

78. L. Vasáros, Yu. V. Norseyev and V. A. Khalkin, *Report JINR-R-12-81-511*, (1981).

79. L. Vasáros and K. Berei, *Kém. Közl.*, **63**, 64 (1985).

80. L. Vasáros, Yu. V. Norseyev, D. D. Nhan, V. A. Khalkin and N. Q. Huan, *J. Radioanal. Nucl. Chem., Lett.*, **87**, 31 (1984).

81. G. W. M. Visser, E. L. Diemer and F. M. Kaspersen, *Recl. Trav. Chim.* **99**, 93 (1980).

82. G. W. M. Visser, E. L. Diemer and F. M. Kaspersen, *Int. J. Appl. Radiat. Isot.*, **31**, 275 (1980).

83. W. D. Bloomer and S. J. Adelstein, *Radioakt. Isot. Klin. Forsch.*, **15**, 227 (1982); Chem. Abstr. **99**, No. 18894 (1983).

84. I. Brown, *Appl. Radiat. Isot.*, **37**, 789 (1986).

85. I. Brown, in *Adv. Inorg. Radiochem.*, Vol. 31 (Eds. H. J. Emeléus and A. G. Sharpe), Academic Press, New York, 1987, pp.789-798.

86. K. Berei, *Strahlenschutz-Kerntechn., Suppl. D*, (Ed. E. W. Giebeler), GIT-Verlag, Darmstadt, 1988, pp.25-32.

87. L. I. Jardine, *Report LBL-246*, (1972); Chem. Abstr. **77**, No. 107876, (1972).

88. M. A. Ritter, J. W. Cleaver and C. A. Tobias, *Nature (London)*, **266**, 653 (1977).

89. W. J. Bateman, *Ph.D. Thesis, Univ. of Birmingham*, Birmingham, 1983.

90. W. D. Bloomer, W. H. McLaughlin, R. D. Neirinckx, S. J. Adelstein, T. J. Ruth and A. P. Wolf, *Science*, **212**, 340 (1981).

91. W. D. Bloomer, W. H. McLaughlin, R. M. Lambrecht, R. W. Atcher, S. Mirzadeh, J. L. Madara, R. A. Milius, M. R. Zalutsky, S. J. Adelstein and A. P. Wolf, *Int. J. Radiat. Oncol., Biol., Phys.*, **10**, 341 (1984).

92. N. L. Shmakova, Yu. V. Norseyev, A. A. Vaynson, Z. Szücs, T. A. Fadeyeva, T. E. Fomenkova, V. A. Khalkin and A. P. Cherevatyenko, *Report JINR-R-19-89-172*, (1989); INIS Atomindex **22**, No. 043506 (1991).

93. Shuyuan Zhang, Xizhong Wang, Zhisu Wang, Wenyuan Cheng, Jannan Jin, Jiazao Zhang, Daoquan Xu, Youesheng Shao, Changrong Luo, Juan Wang, Kejia Wu and Maolun Zhou, *J. Radioanal. Nucl. Chem., Artic.*, **131**, 73 (1989).

94. G. Wunderlich, E. Henke, F. Rösch, W.-G. Franke and R. Dreyer, *Radiobiol., Radiother.*, **27**, 387 (1986).

95. I. Doberenz, G. Wunderlich, W. Doberenz, J.-G. Heidelbach, W.-G. Franke, R. Dreyer, S. Fischer, P. Nelz and L. Kessler, *HNO-Prax.*, **13**, 53 (1988).

96. G. Wunderlich, W.-G. Franke, R. Hlics and W. Doberenz, *Med. Nucl.*, **1**, 25 (1989).

97. G. Wunderlich, W.-G. Franke, S. Fischer and R. Dreyer, *Radiobiol., Radiother.*, **29**, 212 (1988).

98. I. Doberenz, W. Doberenz, G. Wunderlich, W.-G. Franke, J.-G. Heidelbach, H. Nitzsche, S. Fischer, P. Nelz, R. Dreyer and L. Kessler, *NUC Compact*, **19**, 105 (1988); INIS Atomindex **20**, No. 005686 (1989).

99. G. Wunderlich, S. Fischer, R. Dreyer, W. Doberenz and I. Doberenz, *Radiochim Acta*, **47**, 153 (1989).

100. I. Doberenz, W. Doberenz, G. Wunderlich, W.-G. Franke, J.-G. Heidelbach, S. Fischer, R. Dreyer and L. Kessler, *NUC Compact*, **21**, 124 (1990);

101. W. D. Bloomer, R. Lipsztein and J. F. Dalton, *Cancer*, **55, Suppl., 9**, 2229 (1985).

102. R. W. Kozak, T. A. Waldmann, R. W. Atcher and O. A. Gansow, *Trends Biotechnol.*, **13**, 165 (1986).

103. J. L. Humm, *J. Nucl. Med.*, **27**, 1490 (1986).

104. A. T. M. Vaughan, *Int. J. Appl. Radiat. Isot.*, **30**, 576 (1979).

105. G. Brown, W. Bateman, J. Cowan, A. G. Fisher, D. Toksoz and A. T. M. Vaughan, *Protides. Biol. Fluids*, **29**, 937 (1981).

106. A. T. M. Vaughan, W. Bateman, and A. G. Fisher, *Int. J. Radiat. Oncol., Biol., Phys.*, **8**, 1943 (1982).

107. A. T. M. Vaughan, W. Bateman, G. Brown, and J. Cowan, *Int. J. Nucl. Med. Biol.*, **9**, 167 (1982).

108. W. Bateman, A. T. M. Vaughan and G. Brown, *Int. J. Nucl. Med. Biol.*, **10**, 241 (1983).

109. A. Harrison and L. Royle, *NCI Monogr.*, **3**, 157 (1987).

110. A. Harrison and L. Royle, *NATO-ASI-Ser., Ser. A.*, **152**, 445 (1988).

111. A. Harrison, *Radiochim. Acta*, **47**, 157 (1989).

112. A. T. M. Vaughan, A. R. Bradwell, P. W. Dykes and P. Anderson, *Lancet*, **1986**, 1492.

113. J. S. Mitchell, I. Brown and R. N. Carpenter, *Experientia*, **39**, 337 (1983).

114. J. S. Mitchell, I. Brown and R. N. Carpenter, *Prog. Clin. Biol. Res.*, **166**, 327 (1984).

115. J. S. Mitchell, I. Brown and R. N. Carpenter, *Experientia*, **41**, 925 (1985).

116. I. Brown, R. N. Carpenter, J. S. Mitchell and S. F. Russell, in *Radiation Research* (Eds. J. J. Broerse and G. W. Barendsen), Martinus Nijhoff, Amsterdam, 1983, Lect. D4-05.

117. I. Brown, R. N. Carpenter and J. S. Mitchell, *Int. J. Appl. Radiat. Isot.*, **35**, 843 (1984).

118. J. S. Mitchell, I. Brown and R. N. Carpenter, in *Strahlenschutz in Forschung*, Vol. 26, (Eds. H. A. Ladner, C. Reiners, W. Boerner and J. Schutz), Thieme, Stuttgart, 1985, pp. 51-57.

119. I. Brown and R. N. Carpenter, *Radiochim. Acta*, **47**, 143 (1989).

120. E. Link, I. Brown, J. S. Mitchell, and R. N. Carpenter, in *Proc. 6th European Wrksh. Melanin Pigment.* Murcia, 1985, p. 33.

121. E. Link, I. Brown, R. N. Carpenter and J. S. Mitchell, *J. Invest. Dermatol.*, **87**, 385 (1986).

122. E. Link, I. Brown, R. N. Carpenter and J. S. Mitchell, *Cancer Res.*, **49**, 4332 (1989).

123. W. H. McLaughlin, W. M. Thramann, Jr., R. M. Lambrecht, R. A. Milius and W. D. Bloomer, *J. Surg. Oncol.*, **37**, 192 (1988).

124. W. D. Bloomer and W. H. McLaughlin, *Radiochim. Acta*, **47**, 149 (1989).

125. A. I. Kassis, C. R. Harris, S. J. Adelstein, T. J. Ruth, R. M. Lambrecht and A. P. Wolf, *Radiat. Res.*, **105**, 27 (1986).

126. K. Yokoro, A. Kunii, J. Furth and P. W. Durbin, *Cancer Res.*, **24**, 683 (1964).

127. G. Ueda and T. Mori, *Amer. J. Path.*, **51**, 601 (1967).

128. A. Harrison and L. Royle, *Health Phys.*, **46**, 377 (1984).

129. L. M. Cobb, A. Harrison and S. A. Butler, *Hum. Exp. Toxicol.*, **7**, 529 (1988).

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