Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXVIII, No. 2

The Synthesis and Biodistribution of 3-(4'-[^{125}I]-Iodophenyl)-4-aminobutyric acid, a Radioiodinated Analogue of Baclofen

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SUMMARY

Baclofen has been found to bind to receptors in the central nervous system that are specific for γ-aminobutyric acid (GABA), a well known inhibitory neurotransmitter. This paper describes the synthesis of a radioiodinated analog of baclofen as part of an effort to develop receptor probes useful in single photon emission computed tomography. Preliminary biodistribution studies showed the radioiodinationed analog to be essentially stable to in vivo deiodination and have a distribution profile similar to that of baclofen.

Keywords: GABA, Baclofen, Radioiodination, Radioiodinated baclofen analog

INTRODUCTION

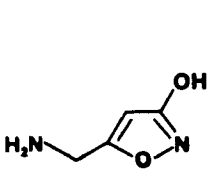
γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system (1). Two types of GABA receptors have been characterized and designated as $GABA_A$ and $GABA_B$ (2,3). These receptors are

0362–4803/90/020123–14$07.00

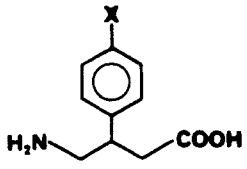
Received January 6, 1989
Revised August 10, 1989

selectively activated by muscimol (1) and baclofen, respectively.

In contrast to GABA, muscimol (1) and baclofen can penetrate the blood brain barrier, although to a limited



Muscimol (1)



X=Cl=Baclofen

X=I= (8)

extent. This property was an important consideration in our goal to design a radiodiagnostic that could delineate the binding sites for GABA agonists in the central nervous system by single photon emission computed tomography (SPECT). Moreover, since iodine-123 is an ideal nuclide for SPECT studies, the replacement of the chlorine in baclofen with an iodine (8) seemed an attractive starting point. The greater lipophilicity of an iodine over chlorine substituent was also an advantage. Thus, the purpose of this study was to devise a route for the synthesis of 8, label it with radioiodine and conduct a preliminary study to determine its biodistribution pattern similarity with baclofen and to ascertain its in vivo stability towards deiodination.

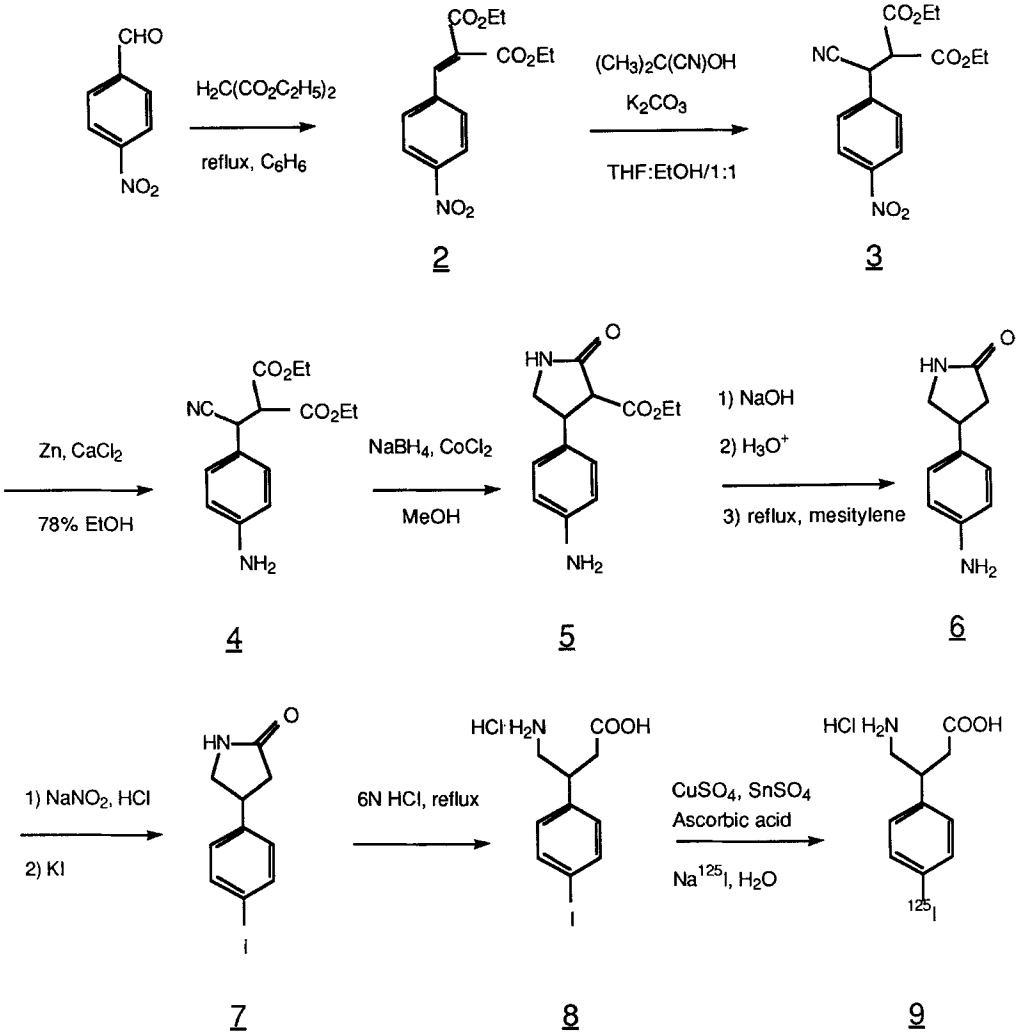
CHEMISTRY

Synthesis of the 4-iodo analog of baclofen, 8 was accomplished via a synthetic route starting from p-nitrobenzaldehyde as outlined in Scheme I. A Knoevenagel condensation between p-nitrobenzaldehyde and diethyl

malonate (4) in the presence of catalytic amounts of p-toluenesulfonic acid and piperidine (5) gave p-nitrobenzalmalonic acid diethyl ester 2 in an excellent yield. Introduction of a cyano group at the benzylic position of 2 via a Michael addition of cyanide was achieved by employing acetone cyanohydrin as the cyanide source under basic conditions, bringing about high yield of diethyl cyano(p-nitrophenyl)methyl-malonate 3. Our first attempt to introduce a cyano group into 2 using potassium cyanide (6) was unsuccessful, the reaction gave a tarry mixture probably due to polymeric decomposition of the initial product 3 under strongly alkaline conditions. Reduction of the aromatic nitro group in 3 with zinc powder and $CaCl_2$ in 78% EtOH (7) afforded the corresponding amino compound, diethyl p-aminophenyl(cyano)methyl malonate 4 in essentially quantitative yield, with the cyano group at the benzylic position remaining unaffected. Reduction of the benzylic cyano group was achieved by employing the $NaBH_4$-$CoCl_2$ reagent system (8), whereby successive reduction and cyclization took place to give ethyl 2-oxo-4-(4-aminophenyl)-3-pyrrolidinecarboyxlate 5. We also examined the reduction of cyano and/or nitro groups on 3 or 4 utilizing catalytic hydrogenation with PtO_2, Pd/C or Raney nickel under atmospheric pressure of H_2. In each case, the reaction was sluggish and gave a complex mixture of products and starting material. Conversion of 5 to 4-(4-aminophenyl)-2-pyrrolidinone 6 was achieved by alkaline hydrolysis of the ester group followed by decarboxylation in mesitylene. The conversion of 6 to 4-(4-iodophenyl)-2-pyrrolidinone 7 was done by first converting 6 to the corresponding diazonium salt and the diazonium group subsequently displaced by iodide. The synthesis of iodo-

baclofen, 3-(4-iodophenyl)-4-aminobutyric acid hydrochloride 8, was achieved by heating the lactam 7 to reflux in 6N HCl for 3.5 hours. The synthesis of the [^{125}I] iodinated baclofen analog, 9 was completed by exchange radiolabeling of 8 in an aqueous solution containing $Na^{125}I$ in the presence of catalytic $CuSO_4$, and the reductants $SnSO_4$ and ascorbic acid according to the procedure of Mertens et al. (9).

Scheme 1



BIODISTRIBUTION RESULTS

Biodistribution studies of 9 and [^{3}H] baclofen are shown on Table I. Similar patterns of tissue uptake and renal clearance were observed for both compounds. At no time point was the brain/blood ratio greater than one in either case. Moreover, the iododinated baclofen analog showed little in vivo deiodination which may be the result of its inherent chemical stability along with its rapid clearance.

Interestingly, racemic 9 showed a slightly greater accumulation in brain tissue at early time points when compared to (-) baclofen. Since GABA agonist action has been shown to be highly stereoselective for (-) baclofen (2), future studies require a) enantiomeric resolution of 9, b) assessment of each enantiomers affinity for the $GABA_B$ receptors and c) eventual preparation of radioiodinated (-) 9 in high specific activity.

TABLE I

Radioactivity of various organs expressed as percent of administered dose per gram of tissue

	Minutes after administration						
TISSUE	1	3	5	10	15	30	60
			COMPOUND 9				
BLOOD	2.33	1.51	1.11	0.65	0.52	0.44	0.23
BRAIN	0.11	0.06	0.07	0.05	0.05	0.05	0.03
HEART	0.63	0.52	0.39	0.26	0.24	0.29	0.20
KIDNEY	6.26	11.06	10.22	11.65	7.09	9.83	2.84
LIVER	1.62	2.06	1.52	1.02	0.82	0.71	0.32
THYROID	0.87	0.55	0.54	0.35	0.38	0.53	1.21
			[^{3}H] BACLOFEN				
BLOOD	1.67	1.35	1.11	0.73	0.46	0.45	0.16
BRAIN	0.03	0.04	0.04	0.03	0.03	0.03	0.02
HEART	0.50	0.39	0.33	0.26	0.20	0.24	0.16
KIDNEY	6.50	13.84	14.92	6.26	3.58	3.98	1.47
LIVER	1.05	1.17	1.12	1.11	0.80	0.88	0.19
THYROID	0.40	0.40	0.48	0.24	0.20	0.36	0.06

EXPERIMENTAL

MATERIALS AND METHODS:

Melting points are uncorrected and were measured with either a Yanagimoto micro melting point apparatus or a Thomas-Hoover apparatus. Proton magnetic resonance (^{1}H NMR) spectra were obtained on either a JEOL PS-100 or Bruker WP270SY instrument using Me_4Si as an internal standard. Infared (IR) spectra were obtained on either a JASCO IRA-1 or a Perkin-Elmer 281 spectrometer. Radiochromatograms of thin layer chromatography plates were obtained on a Vangard 930 autoscanner. The HPLC system consisted of an Altex 110A pump, Excalibur 5 μm (4.6 x 250 mm) C_{18} reverse phase column, Gilson 111 UV monitor (254 nm) and an in-line Bicron Analyst (Bicron Electronic Products, Newbury, Ohio) survey meter with a model G1LE low energy gamma scintillation probe and strip chart output. Elemental analysis was performed by either the Service Center of the Elementary Analysis of Organic Compounds, Kyushu University, or Midwest Microlabs Ltd., Indianapolis, IN. Column chromatography was performed on silica gel (70-230 mesh, Merck). The ^{125}I used was a no-carrier-added solution of $Na^{125}I$ (100 mCi/ml) in reductant-free dilute NaOH (pH 7-11) obtained from The Amersham Corporation, Arlington Hts., IL. [^{3}H]-(-)-Baclofen (sp. act. = 39.8 Ci/mmol) was purchased from Dupont NEN Research Products, Boston, MA. Tetrahydrofuran (THF) was distilled from $LiAlH_4$ under argon just prior to use. All other solvents were of commercial grade and were used without further purification. All organic or inorganic reagents were purchased from commercial sources and used directly.

Diethyl 4-nitrobenzalmalonate (2): A solution of p-nitro-benzaldehyde (50g, 0.33 mole), diethyl malonate (53 g.

0.33 mole), piperidine (5.64 g. 0.066 mole) and p-toluenesulfonic acid monohydrate (6.3 g. 0.033 mole) in benzene (700 ml) was heated to reflux and the generated water removed with the aid of a Dean-Stark trap. When the reaction was complete (ca. 20h), the reaction mixture was allowed to cool to room temperature and benzene (200 ml) was added. The benzene solution was transferred to a separatory funnel, washed successively with water (twice), saturated sodium bicarbonate, and brine. The organic phase was dried over sodium sulfate and concentrated to dryness under reduced pressure. The resulting solid was recrystallized from ethanol to give 84.3 g (87%) of 2 as slightly greenish-yellow needles, m.p. 91-92°C. IR (nujol mull) 1725 cm^{-1} (C=O ester); ^{1}H-NMR (100 MHz, $CDCl_3$) δ : 8.24 (d, J = 9 Hz, 2H, Aryl 3,5-H), 7.76 (s, 1H, PhCH=), 7.60 (d, J = 9 Hz, 2H, Aryl 2,6-H), 4.35 (q, J = 7 Hz, 4H, $COOCH_2$ x 2), 1.35 (t, J = 7 Hz, 3H, CH_3), 1.28 (t, J = 7 Hz, 3H, CH_3). Analysis calculated for $C_{14}H_{15}No_6$: C, 57.34; H, 5.16. Found: C, 57.21; H, 4.97.

Diethyl cyano(p-nitrophenyl)methyl-malonate (3): To a stirred mixture of compound 2 (43.75 g. 0.149 mole) and acetone cyanohydrin (38.1 g, 0.448 mole) in THF/EtOH (1:1, 300 ml) was added dropwise a solution of potassium carbonate (3.94 g, 0.0067 mole) in 10 ml of water. The color of the reaction changed from pale yellow to purple and then turned to reddish orange at the end of the reaction. After the starting material had disappeared as indicated by TLC (30-45 min.), the solution was acidified with 3N HCl and the solvents evaporated under reduced pressure. The residual orange oil was dissolved in ether and the ether solution washed three times with water and dried over sodium sulfate.

Removal of the solvent under reduced pressure left an orange oil, which gradually solidified upon standing at room temperature. The solid obtained was filtered with the aid of a cold n-hexane/EtOH (1:1), washed with the same solvent, and dried under vacuum to give 39.1 g (81.8 %) of almost pure cyanated product 3 as a pale yellow solid. Recrystallization from ethanol gave an analytically pure sample as pale yellow needles, m.p. 75-75°C. IR (nujol mull) 2250 (CN), 1760 and 1730 cm^{-1} (C=O ester); ^{1}H-NMR (100 MHz, $CDCL_3$) δ : 8.26 (d, J = 9 Hz, 1H, Aryl 3,5-H), 7.62 (d, J = 9 Hz, 2H, Aryl 2,6-H), 4.65 (d, J = 9 Hz, 1H ArCH), 4.25 (q, J = 7 Hz, 2H, $COOCH_2$), 4.10 (q, J = 7 Hz, 2H, $COOCH_2$), 3.90 (d, J = 9 Hz, 1H, $CH(CO_2Et)_2$), 1.30 (t, J = 7 Hz, 3H, CH_3), 1.16 (t, J = 7 Hz, 3H, CH_3). Analysis calculated for $C_{15}H_{16}N_2O_6$: C, 56.25; H, 5.03. Found: C, 56,22; H, 4.89.

Diethyl p-aminophenyl(cyano)methylmalonate (4): To a stirred mixture of compound 3 (5.0 g, 15.6 mmole) and zinc powder (33.5 g) in 78% EtOH (110 ml) was added $CaCl_2$ (1.1 g) dissolved in water (1.5 ml). The brownish reaction mixture was immersed in a preheated oil bath and refluxed for 10 min (the color changed to almost colorless at the end of the reaction). The mixture was filtered through a Celite-pad and the filtered zinc was washed several times with hot ethanol. The filtrates were combined and evaporated under reduced pressure. The residual oil was dissolved in ether and the ether solution was washed (water and then brine), dried (Na_2SO_4) and then evaporated to dryness to afford compound 4 (4.35 g, 96%) as a yellow oil. IR (neat) 3440, 3340, and 3200 (NH_2), 2240 (CN), 1750 and 1730 cm^{-1} (C=O ester). This compound was subjected to next reaction without further purification.

Ethyl 2-oxo-4-(4-aminophenyl)-3-pyrrolidinecarboxylate (5): To a stirred solution of compound 4 (4.35 g, 15.0 mmole) in methanol (80 ml) was added $CoCl_2 \Delta 6H_2O$ (6.75 g, 28.4 mmole). The resulting dark purple solution was cooled in an ice bath and solid sodium borohydride (5.5 g, 145.5 mmole) was added over a period of 30 minutes (hydrogen gas evolved violently upon the addition of $NaBH_4$ and a black solid precipitated). The ice bath was then removed and the reaction mixture stirred for 1 hour at room temperature. 3N HCl (50 ml) was added to the reaction mixture and the mixture stirred until dissolution of the black precipitate occurred. Methanol was removed by distillation and the remaining aqueous mixture was transferred to a continuous extraction apparatus. After alkalinization with aqueous ammonia (insoluble cobalt complex precipitated), the mixture was extracted with ether (200 ml) for 24 hours. The solid obtained upon evaporation of the ether extract was washed with cold ether/ethanol (10:1) and dried under vaccuum to give 1.7 g (46%) of 5 as a pale yellow powder. Recystallization from ethanol gave an analytically pure sample, m.p. 149-150°C. IR (nujol mull) 3440, 3320 and 3190 ($ArNH_2$ and lactam NH), 1735 (C=O ester), and 1705 cm^{-1} (C=O lactam); ^{1}H-NMR (100 MHz, $CDCl_3$) δ: 7.05 (d, J = 8 Hz, 2H, Aryl 2,6-H), 6.95 (br s, 1H, lactam NH) 6.60 (d, J = 8 Hz, 2H, Aryl 3.5-H), 4.05-3.25 (m, 4H, lactam H's), 3.68 (s, 2H, Aryl NH_2), 1.25 (t, J = 7 Hz, 3H, CH_3). Analysis calculated for $C_{13}H_{16}N_2O_3$: C, 62.89; H, 6.50. Found: C, 63.10; H, 6.78.

4-(4-Aminophenyl)-2-pyrrolidinone (6): Compound 5 (4.35 g, 17.5 mmole) was dissolved in (10 ml) hot ethanol. To this solution, aqueous 10M NaOH (2 ml) was added and the white solid (sodium salt) which formed was collected by filtration. The residue was washed with a small amount of

absolute ethanol, subsequently dissolved in water (15 ml) and treated with a calculated amount of hydrochloric acid (5.8 ml of 3N HCl). The resulting white solid (free acid) was collected by filtration, washed successively with water, ethanol and ether and then dried under vacuum. The product was suspended in mesitylene (15 ml) and the mixture was heated at 165-179 °C in an oil bath under a gentle stream of argon until decarboxylation was complete (the suspension changes to two-phase solution at the end of the reaction). After cooling, the solidified material was separated from the mixture, ground to a powder, washed with benzene, and dried under vacuum to give 2.5 g (81%) of 6. Recystallization from ethanol gave an analytically pure sample, m.p. 154-155°C. IR (nujol mull) 3340, 3280, and 3200 (Aryl NH_2 and lactam NH), 1680 cm^{-1} (C=O lactam); ^1H-NMR (100 MHz, DMSO-d_6 + D_2O) δ : 6.96 (d, J = 9 Hz, 2H, Aryl 2,6-H), 6.36 (d, J = 9 Hz, 2H, Aryl 3.5-H), 3.64-3.26 (m, 2H, CH_2NHCO), 3.24-2.96 (m, 1H, ArCH), 2.60-2.05 (AB of ABX system, δ_A = 2.46, δ_B = 2.16, J_{AB} = 16 Hz, J_{AX} = 9 Hz, J_{BX} = 9 Hz, 2H, CH_2CONH). Analysis calculated for $C_{10}H_{12}N_2O$: C, 68.16; H, 6.86. Found: C, 68,39: H.6.71.

4-(4-Iodophenyl)-2-pyrrolidinone (7): To an ice bath-cooled solution of compound 6 (0.5 g, 2.84 mmole) in 3N HCl (5 ml) was added sodium nitrite (0.216 g, 3.13 mmole) dissolved in water (1.0 ml). After stirring for 20 minutes, potassium iodide (0.566 g, 3.41 mmole) dissolved in water (5 ml) was added and the reaction mixture stirred for ten minutes. The ice bath was then removed and the mixture was heated in an oil bath at 70-80°C until evolution of N_2 gas ceased. After cooling, the reaction mixture was extracted three times with chloroform. The chloroform extracts were combined and washed successively with saturated sodium

bicarbonate, aqueous sodium thiosulfate, water and brine. The washed extracts were dried over sodium sulfate and evaporated to dryness under reduced pressure. The crude product was purified by column chromatography on silica gel. Elution with $CHCl_3$/MeOH (50:1) gave 0.37 g (45.4%) of 7 as a pale yellow solid. Analytically pure material was obtained by recrystallization from benzene, m.p. 143-145° C. IR (nujol mull) 3160 and 3080 (NH), 1720 cm^{-1} (C=O lactam); ^{1}H-NMR (100 MHz, $CDCl_3$) δ : 7.68 (d, J = 8 Hz, 2H, Aryl 3,5-H), 7.02 (d, J = 8 Hz, 2H, Aryl 2,6-H), 6.53 (br, 1H, NH), 3.90-3.50 (m, 2H, CH_2CONH), 3.50-3.25 (m, 1H, ArCH), 2.90-2.28 (AB of ABX system, δ_A = 2.72, δ_B = 2.42, J_{AB} = 16 Hz, J_{Ax} = 8 Hz, 2H, CH_2CONH). Analysis calculated for $C_{10}H_{10}INO$: C, 41.84; H, 3.51. Found: C, 42.21: H, 3.72.

3-(4-Iodophenyl)-4-aminobutyric Acid Hydrochloride (8): Compound 7 (0.1 g, 0.348 mmole) suspended in 6N HCl (10 ml), was heated at reflux under a stream of nitrogen for 3.5 hr. After cooling to room temperature, the solvent was completely removed by lyophilization to afford 0.118 g (100%) of almost pure 8 as a white powder. Recrystallization from EtOH/Et_2O (2:1) gave analytically pure material (0.092 g, 77%). m.p. 190-195°C (dec). ^{1}H-NMR (270 MHz, DMSO-d_6 + D_2O) δ: 7.69 (d, J = 8.1 Hz, 2H, Aryl 3,5-H), 7.15 (d, J = 8.2 Hz, 2H, Aryl 2,6-H), 3.33 (m, 1H, ArCH), 3.13-2.91 (m, 2H, CH_2NH_2), 2.89-2.51 (AB of ABX system, δ_A = 2.85, δ_B = 2.56, J_{AB} = 16.3 Hz, J_{AX} = 5.0 Hz, J_{BX} = 9.5 Hz, 2H, CH_2COOH). Analysis Calculated for $C_{10}H_{13}ClINO_2$: C, 35.16; H, 3.84; N, 4.10; I, 37.15. Found: C, 35.13; H, 3.66; N, 4.22; I, 36.92.

3-(4-[^{125}I] Iodophenyl)-4-aminobutyric Acid Hydrochloride (9): To a 2.0 ml crimp capped vial was added

8 (1.3 mg), $SnSO_4$ (0.5 mg), $CuSO_4 \cdot 5H_2O$ (150 µg), ascorbic acid (6.0 mg) and oxygen free water (50 µl). Following addition of an aqueous sodium iodide solution (5 µl, 2.57 mCi), the vial was sealed, purged with nitrogen and heated to 100° C. After 30 minutes, the vial was cooled and the water removed under a gentle stream of N_2. The residue was purified by silica gel column chromatography, eluted with $CHCl_3$:MeOH/1:1 followed by $CHCl_3$:MeOH:H_2O/4:4:1. Radiochromatograms of the appropriate collected fractions indicated a pure product which co-migrated with 8. The product was isolated in a radiochemical yield of 1.74 mCi (67.7%). This corresponds to a specific activity of approximately 458 mCi/mmol. Rf = 0.51 in $CHCl_3$:MeOH:H_2O/4:4:1.

TISSUE DISTRIBUTION

Tissue distribution studies were performed with female Sprague-Dawley rats from Charles River, Portage, MI. weighing 200-260 grams. One animal at each time point was used in these preliminary studies. All animals received a tracer dose of 7.2-11.1 µCi of the radiolabeled compounds in water by intravenous injection into the tail vein while under light ether anesthesia. The animals were then placed under general ether anesthesia and sacrificed at the indicated time points by exsanguination. Major organs were excised, blotted dry and disected free of connective tissue. Large organs were minced with scissors. Weighed samples containing radioiodine were placed in gelatin capsules and counted on a Searle 1185 well counter (efficiency = 84-86%). Weighed samples containing tritium were dissolved as previously described (10), 10 ml of Safety-solv cocktail (RPI, M. Prospect, IL) added and counted on a Tracor 6881

liquid Scintillation counter. Radioactivity concentration in each organ was expressed as percent of administered dose per gram of tissue.

ACKNOWLEDGEMENTS

This research was supported by USPHS grant CA-08349 and a Fogarty Senior International Fellowship (IF06 TW01103-1 BI-5) to REC.

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