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DEVELOPMENT OF AN EXCHANGE-LABELLING PROCEDURE AND INVESTIGATION OF THE RADIOCHEMICAL STABILITY AND BIODISTRIBUTION CHARACTERISTICS FOR (^{131}I) *META*-IODOBENZYLGUANIDINE SULPHATE (^{131}I) MIBG

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ABSTRACT

A simple exchange-labelling procedure for the radioiodination of *meta*-Iodobenzylguanidine (MIBG) has been developed which facilitates the production of (^{131}I) MIBG both quickly and efficiently. Stability studies indicate that labelled material if stored at reduced temperature undergoes little breakdown. Comparative biodistribution studies with a commercial source have been used to authenticate both the identity and purity of the product.

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BIOLOGICAL LOCALIZATION; CHROMATOGRAPHY; ELECTROPHORESIS; GUANIDINES; IMPURITIES; IODINE 131; ISOTOPIC EXCHANGE; LABELLED COMPOUNDS; LABELLING; RADIONUCLIDE KINETICS; RATS; TEMPORAL DOSE DISTRIBUTIONS; TISSUE DISTRIBUTION.

EDITORIAL NOTE

The Australian Nuclear Science and Technology Organisation (ANSTO) replaced the Australian Atomic Energy Commission (AAEC) on 27 April 1987. Reports issued after April 1987 have the prefix ANSTO with no change of the symbol (E, M, S or C) or numbering sequence.

CONTENTS

1. INTRODUCTION

Since its introduction by Wieland *et al.* [1979] (^{131}I) MIBG has been advocated as the radiopharmaceutical of choice for imaging of neuroblastoma and pheochromocytoma based on some excellent clinical results including:

Shapiro *et al.* [1982], Nakajo *et al.* [1983], Shapiro *et al.* [1984] and Hoefnagel *et al.* [1985]

and on extensive animal biodistribution studies including:

Mice - Heggli *et al.* [1983], Guilloteau *et al.* [1984] and Deckart *et al.* [1986];

Rats - Swanson *et al.* [1981], Letiec *et al.* [1986], Nakajo *et al.* [1986] and Troncone *et al.* [1986];

Dogs - Wieland *et al.* [1980], Swanson *et al.* [1981] Heggli *et al.* [1983], Wieland *et al.* [1984], Guilloteau *et al.* [1984], Tobes *et al.* [1985] and Troncone *et al.* [1986];

Rabbits - Ma *et al.* [1984];

Monkeys - Wieland *et al.* [1981], Swanson *et al.* [1981] and Ma *et al.* [1984];

Bovines - Jaques *et al.* [1984], Tobes *et al.* [1985] and Gasnier *et al.* [1986].

Several modifications to the exchange labelling procedure [Angelberger *et al.* [1982], Mangner *et al.* [1982], Wieland *et al.* [1982], Eisenhunt *et al.* [1984], Mertens *et al.* [1984], Doremalen *et al.* [1985]] and numerous chromatography systems [Angelberger *et al.* [1979], Weiland *et al.* [1980], Mangner *et al.* [1982] and Doremalen *et al.* [1985]] have been proposed for estimates of radiochemical purity.

In this study a method for the exchange labelling of *meta*-iodobenzylguanidine sulphate (MIBG) with iodine-131 has been developed which is both simple and efficient. The chemical stability of the labelled material was assayed at various intervals post manufacture using either paper chromatography or electrophoresis to estimate the radiochemical purity; we have found that both procedures yield similar results. Biodistribution studies in rats were performed together with a comparison of chemical and biological data with a commercial source.

2. EXPERIMENTAL

2.1 Exchange Labelling

Inactive 'cold' MIBG (Emka-Chemie, West Germany) is exchange labelled with iodine-131 according to the method described by Doremalen and Janssen [1985] with a slight modification. To a 10 mL Wheaton vial is added 0.5 mg of MIBG, 100 mμL of 0.157 M $Cu(NO_3)_2 . 3H_2O$ and 0.1 to 0.3 mL of 0.02 N NaOH containing 200 to 500 MBq of iodine-131. The vial is sealed with a teflon coated stopper [Wheaton, USA] and capped with an aluminium closure. It is then placed in a pre-heated aluminium block and incubated at 150°C for 45 minutes. The vial is allowed to cool for 10 minutes (important to achieve uniform labelling) and then 1 mL of isotonic phosphate buffer containing 23 mg/mL of sodium dihydrogen phosphate dihydrate, 2.8 mg/mL of disodium hydrogen phosphate (anhydrous) and 1% v/v benzyl alcohol/water at pH 4.0 to 7.0) is added. After mixing, the solution and 2 x 1 mL washes of isotonic phosphate buffer are transferred to an anion-exchange column containing 0.25 cm^3 of DEAE Sephadex A25 Anion-exchange resin (Pharmacia,

Sweden). The eluent from the column is passed through a 0.22 micron filter into a sterile 10mL vial then sterilised by autoclaving at 132°C for 6 minutes. Autoclaving under these conditions did not significantly increase the free iodide-131 content.

Assessments of radiochemical purity were determined by paper chromatography (stationery phase - Whatman No.1 paper; mobile phase - 60:15:25 v/v nBuOH/CH_3COOH/H_2O with R_f(MIBG(^{131}I)) = 0.9 and R_f(free ^{131}I) = 0.2) or electrophoresis (support - cellulose acetate paper (Oxoid, London); electrolyte - 403 mL of 0.025 M barbitone, 100 mL of 0.5 M sodium barbitone and 100 mL of 0.5 M sodium chloride; conditions - 400 V, 15mA, 8 min.).

In order to confirm the identity and purity of the ANSTO produced (^{131}I) MIBG four paper chromatography strips were developed. The first was spotted with iodide-131 in 0.02 NaOH, the second with ANSTO manufactured (^{131}I) MIBG, the third with Amersham (UK) manufactured (^{131}I) MIBG and the fourth with a mixture of ANSTO and Amersham (UK) manufactured (^{131}I) MIBG of approximately equal activity. In figure 1 a composite chromatograph is presented which illustrates the data from the mixture of ANSTO and Amersham (^{131}I) MIBG superimposed on the iodine-131 control. A sharp, single, peak for the mixture, clearly differentiated from the peak due to free iodine-131, provides an unambiguous test for the purity and homogenicity of the two (^{131}I) MIBG solutions.

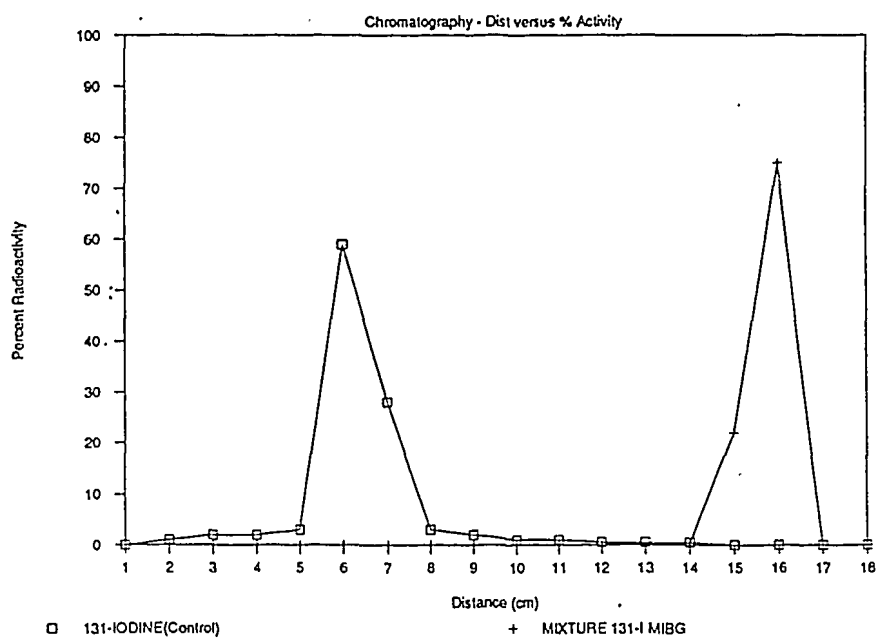

FIGURE 1 MIXTURE OF ANSTO MIBG AND AMERSHAM MIBG

2.2 Stability Trials

Two stability trials were conducted. In the first trial 5 batches of (^{131}I) MIBG were prepared, each batch being divided into two portions with one portion being stored at ambient temperature (10 to 19°C) and the other stored frozen (-12 to -15°C). Each portion was studied over a 13-day period for *in vitro* radiolysis. In the second trial 3 batches of (^{131}I) MIBG were prepared, stored at -12 to -15°C, and studied over a 47-day period. Radiochemical purity was determined by paper chromotography.

2.3 Biodistribution Studies

Biodistribution studies were conducted in male specific pathogen free AAW rats aged 8 to 10 weeks. Groups of 3 to 4 lightly anaesthetised animals were injected intravenously via the tail veins with 90 microlitres of (^{131}I) MIBG with activity 10 to 20 MBq. After injection the animals are placed in metabolic cages for collection of urine and faeces. To examine the biodistribution of the compound, groups were sacrificed at time intervals between 5 minutes and 24 hours for tissue samples. For inter-comparison at different batches of (^{131}I) MIBG, animals were sacrificed at 1 hour post injection. For comparison with a commercial source, parallel studies were conducted with batches of (^{131}I) MIBG manufactured by Amersham (UK).

3. RESULTS

3.1 Exchange Labelling

Twelve batches of (^{131}I) MIBG were prepared and assessed for overall yield and labelling efficiency. The radiochemical purity was determined using either electrophoresis or paper chromotography, results are given in Table 1.

TABLE 1

YIELDS AND LABELLING EFFICIENCIES FOR THE PRODUCTION OF (^{131}I)

Batch No.	Activity (MBq)[1]	Overall Yield (%)[2]	Labelling Efficiency (%)
1	192	76	99
2	201	78	99
3	267	81	96
4	276	79	99
5	288	82	98
6	293	81	97
7	296	81	99
8	300	84	98
9	312	74	98
10	336	82	98
11	351	86	99
12	479	77	97
		Mean = 80	Mean = 98

[1] Activities measured by ion-chamber; accuracy = ± 10%

[2] Chemical yield based on recovery of labelled material; accuracy = ± 20%

3.2 Stability Trials

Results of the first trial are shown in figures 2 and 3. The results for the second trial which was conducted over a longer time interval are shown in figure 4.

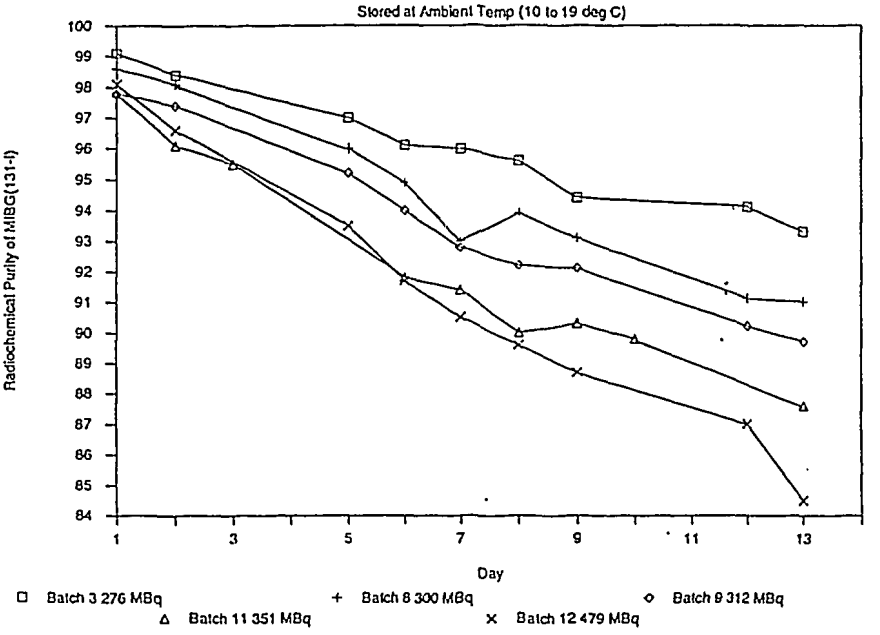

FIGURE 2 Radiochemical Purity Of (^{131}I) MIBG

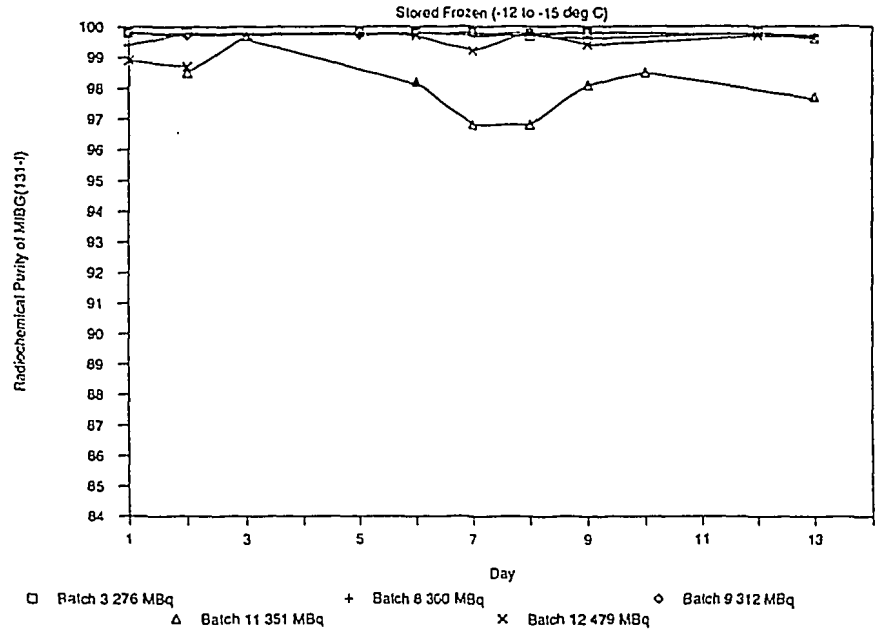

FIGURE 3 Radiochemical Purity of (^{131}I) MIBG

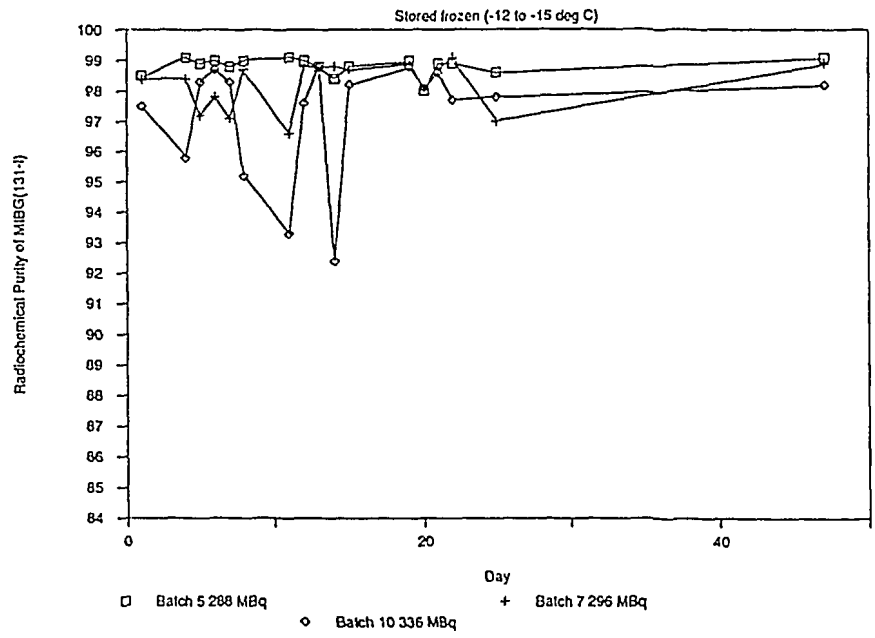

FIGURE 4 Radiochemical Purity of (^{131}I) MIBG

3.2.1 Biodistribution Studies

The variation of biodistribution versus time for the compound is presented in table 2. In table 3 the biodistribution of batches of ANSTO and Amersham (^{131}I) MIBG are compared with literature values at a standardized time of one hour post injection. In figures 5 and 6 data for the uptake of (^{131}I) MIBG in units of percent injected dose versus time is given, and in figure 7 the tissue/blood ratio versus time is presented for selected tissues.

TABLE 2

BIODISTRIBUTION OF (^{131}I) MIBG IN RATS VERSUS TIME *

Time (h)	0.1	0.5	1	3	24
Liver	8.29	9.85	6.53	2.08	0.44
Kidney	7.43	2.76	1.96	1.21	0.22
Muscle	47.84	34.98	31.72	30.48	8.58
Lung	7.59	3.49	2.86	1.23	0.19
Heart	3.16	2.28	2.22	1.35	0.17
Blood	6.89	2.00	2.21	1.84	0.68
Urine	0.03	6.96	9.83	21.38	59.61
GIT **	7.13	9.59	12.03	15.06	11.03
Thyroid	0.03	0.05	0.08	0.29	1.79
Adrenal	0.21	0.15	0.15	0.11	0.04
Pancreas	0.86	0.68	0.52	0.33	0.03

* % injected dose g^{-1}.

** GIT = gastrointestinal tract; per cent injected dose.

TABLE 3

BIODISTRIBUTION OF (^{131}I) MIBG IN RATS AT 1 HOUR *

	ANSTO 20/7/87	ANSTO 29/7	ANSTO 17/9	Amersham 25/2/88	Amersham 9/6	Amersham 1/7	Amersham 14/7	Amersham 15/8	Nakajo et al	Swanson et al**
Liver	0.697	0.673	0.67	0.48	0.52	0.42	0.60	0.78	0.36	-
Spleen	0.626	0.758	0.81	0.68	0.67	0.65	0.65	0.75	0.64	-
Kidney	0.575	0.809	0.68	0.55	0.55	0.59	0.52	0.62	-	-
Muscle	0.244	0.222	0.21	0.18	0.19	0.19	0.15	0.25	-	-
Skin	0.164	0.151	0.11	0.15	0.14	0.16	0.12	0.19	-	-
Bone	0.150	0.144	0.11	0.08	0.15	0.14	0.10	0.19	-	-
Lungs	1.229	1.773	1.32	1.10	1.17	1.49	1.00	1.47	1.00	-
Heart	1.595	2.468	1.99	1.56	1.43	1.63	1.69	1.94	1.92	4.40
Blood	0.097	0.099	0.08	0.08	0.08	0.09	0.07	0.09	0.12	-
Thyroid	7.099	5.699	15.48	2.84	5.67	5.25	0.52	5.95	-	-
Adrenal	1.636	2.119	-	1.49	1.74	1.77	2.43	2.39	-	-
Brain	0.015	0.018	0.02	0.01	-	-	-	-	-	-
Pancreas	0.608	0.672	0.71	0.46	-	-	-	3.73	-	-

* % Injected dose .g^{-1} (means of 3-4 rats)

** Figures standardised on a 250 gram rat

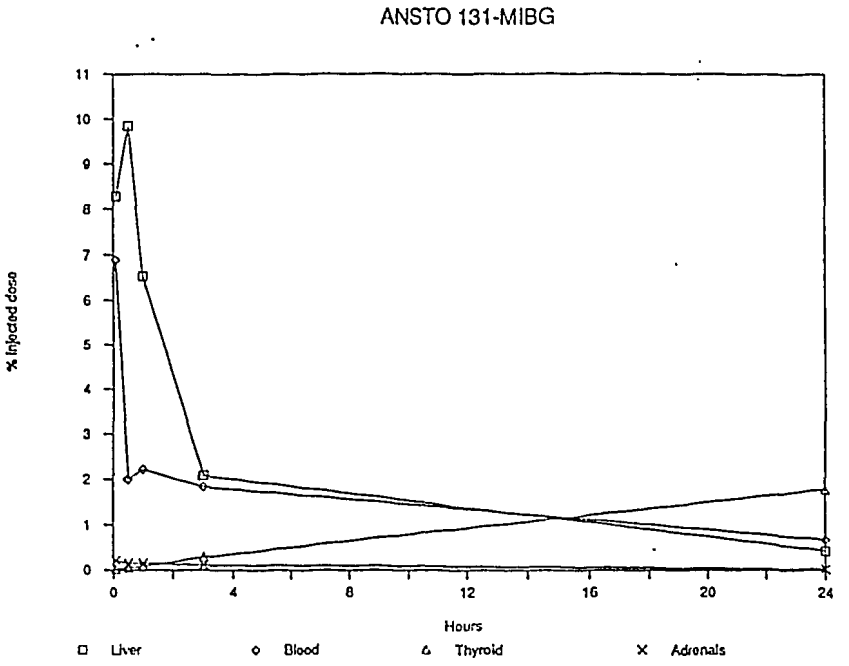

FIGURE 5 ORGAN UPTAKE VERSUS TIME

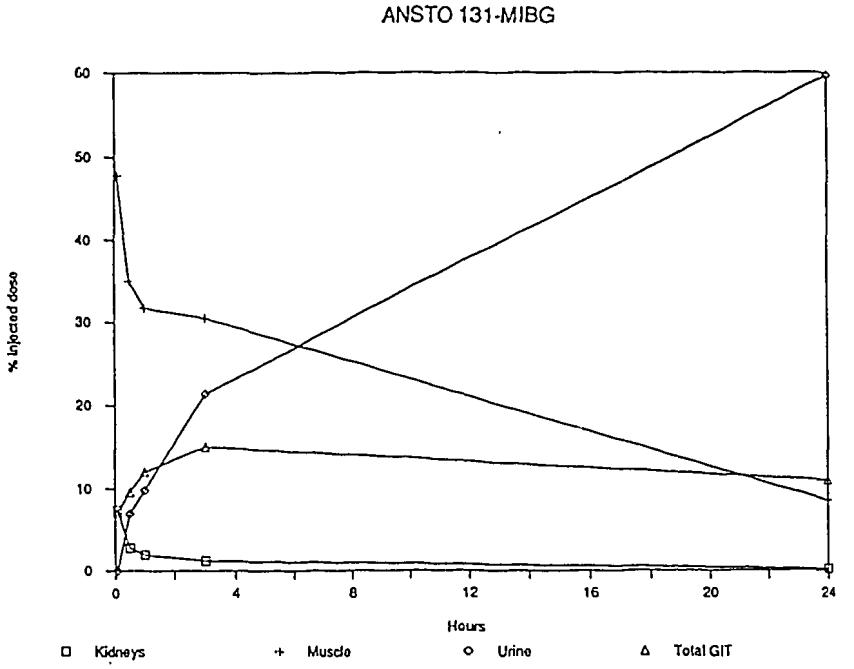

FIGURE 6 ORGAN UPTAKE VERSUS TIME

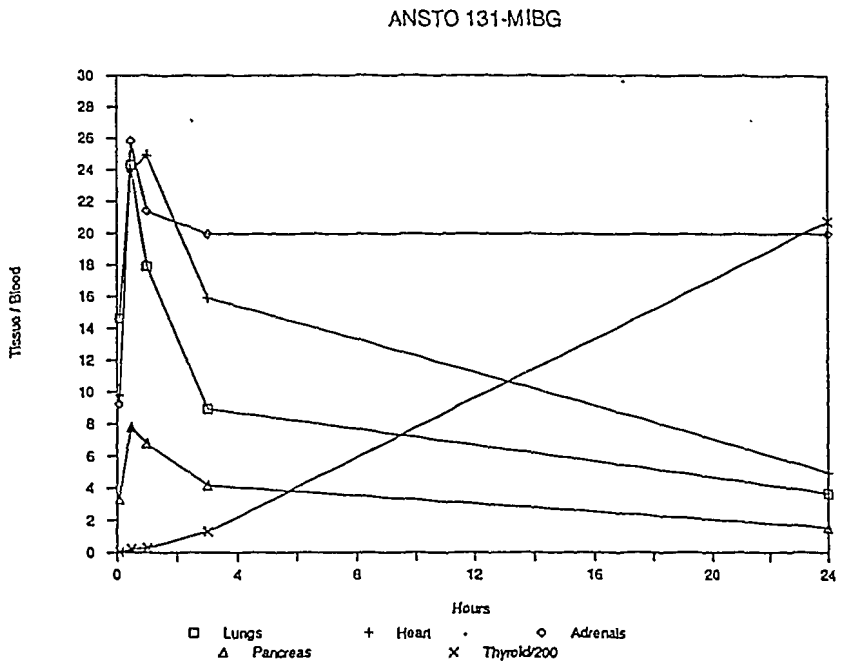

FIGURE 7 TISSUE/BLOOD RATIOS

4. DISCUSSION

The exchange labelling procedure used in this study, which is based on the procedure described by Dormalen and Janssen [1985] has been found to give consistently high and reliable yields with few problems. Initial experience with this procedure indicated that it was necessary to use unstabilised iodide-131 in order to achieve high yields, and that a teflon faced stopper should be used to minimise iodine adsorption in the reaction vessel. In addition, it is found preferable to use a 2 x 1 mL rather than 1 x 1 mL wash of isotonic phosphate buffer in order to maximise the elution of labelled material from the anion-exchange column.

In general the published chromatography methods for the determination of radiochemical purity have been found to be unreliable and time consuming. The analytical procedures adopted in this study include one based on electrophoresis and one on paper chromatography. The electrophoresis method is fast and gives good separation however it does appear that some cellulose-acetate media perform better than others. The paper chromatography system is quite slow (15 cm, 1 to 2 hours) but gives excellent separation. In this study the two procedures were deemed equivalent and were used interchangeably.

The stability studies while indicating a marked thermal instability of the labelled material at room temperature show excellent stability at temperatures below -12°C illustrating the need for low temperature storage conditions during shipment. The results also indicate a radiolytic breakdown effect which will pose some logistical problems if therapeutic levels of (^{131}I) MIBG are to be manufactured.

The biodistribution studies indicate the compound localises predominantly in the muscular tissue and is excreted via the gut.

5. CONCLUSION

The procedure outlined by Doremalen and Janssen [1985] has, with slight modification, been found to give excellent results in terms of high overall yields (typically 80%) and a high labelling efficiency (typically 98%). Comparative chromatographic analysis and biodistribution studies with a commercial source have been used to authenticate both the identity and purity of the labelled material.

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