Radiolabeling with Organomercury Compounds: Part 2. High Specific Activity Synthesis of Iodine-125 and Iodine-131-6-Iodocholest-5-en-3β-ol and Its Tissue Distribution in Rats

Richard J. Flanagan, F. Peter Charleson, Eyvind I. Synnes, and Leonard I. Wiebe

Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta; and Merck Frosst Canada Inc., Pointe Claire—Dorval, Québec, Canada

Iodine-125 (^{125}I) and iodine-131- (^{131}I) 6-iodocholest-5-en-3β-ol has been prepared directly from 6-chloromercuricholest-5-en-3β-ol and [^{125}I] or [^{131}I]sodium iodide. This method produces material of "no-carrier-added" specific activity and excellent radiochemical purity. The entire procedure is complete in 10 min and can be carried out in 95% ethanol. The biodistribution of this new high specific activity form of [^{131}I]-6-iodocholest-5-en-3β-ol has been measured in rats and found to be very similar to that found for low specific activity [^{131}I]-6-iodocholest-5-en-3β-ol produced by exchange labeling. The whole-body elimination curve over a 4-day period was measured and a dependence between the rate of elimination and specific activity was detected. Products of three different specific activities in addition to "no-carrier-added" material were studied.

J Nucl Med 27:1165–1171, 1986

As part of a current study* to evaluate the use of organomercury compounds as substrates for halogen radiolabeling, we have examined the use of 6-chloromercuricholest-5-en-3β-ol **1** in the preparation of iodine-131- (^{131}I) 6-iodocholest-5-en-3β-ol **2** (Fig. 1). We feel mercury is the most promising of the many heteroatoms being investigated as organometallic intermediates for halogen radiolabeling. Other heteroatoms of interest include boron (*1,2*), thallium (*3*), and tin (*4*). Although all of these methods show promise, organomercury compounds show important advantages over other heteroatoms from the point of view of preparation and stability. There are several synthetic methods available for aliphatic, vinylic, aromatic, and heterocyclic organomercury compounds (*5,6*), whereas organoboron compounds are generally only available from the addition of boranes to olefins and acetylenes (*7*).

Iodine-131 6-iodocholest-5-en-3β-ol **2** is a compound which shows great promise as an adrenal imaging agent (*8*). It has been shown to concentrate in adrenal tissue over a period of 4 days. The current methods of preparing this compound are based on nucleophilic exchange labeling using 6-iodocholest-5-en-3β-ol **3** and [^{131}I]NaI in n-propanol (*9*) or in a melt at 160° (*10*). These methods have the disadvantage of requiring forcing conditions since it has been shown that **3** is resistant to nucleophilic exchange (*11*). Furthermore, the product of this reaction has a very low specific activity that causes corresponding problems in dose preparation due to the low solubility of 6-iodocholest-5-en-3β-ol in saline. Attempts to improve the speed of the labeling reaction through the use of copper(I) and copper(II) salts has led to contamination of the reaction product with water soluble copper.

We have improved the speed of the reaction and increased the specific activity of the product by synthesizing **2** directly from 6-chloromercuricholest-5-en-3β-ol **1**. We have also demonstrated the general applicability of the method by preparing [^{125}I]-6-iodocholest-5-en-3β-ol **4** and [^{123}I]-6-iodocholest-5-en-3β-ol **5** in a similar fashion. The attractiveness of this method is

Received Apr. 23, 1985; revision accepted Dec. 11, 1985.

For reprints contact: Richard J. Flanagan, PhD, Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2N8.

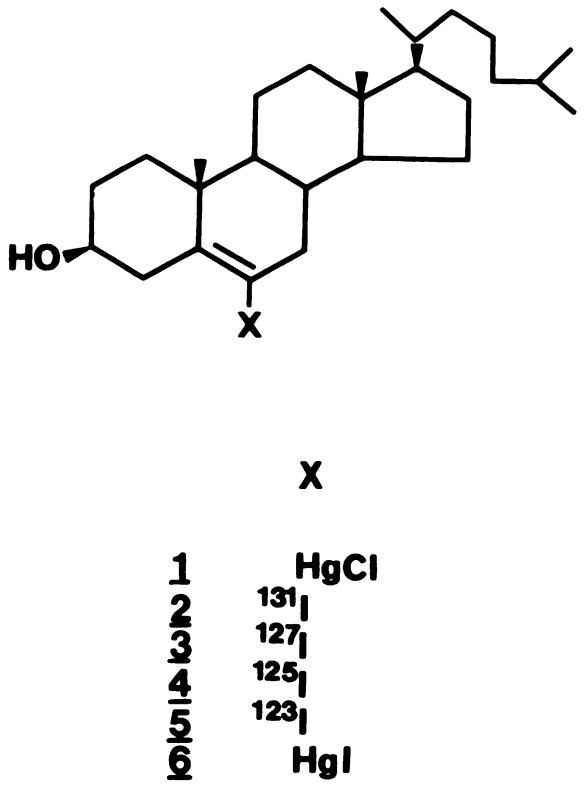

FIGURE 1
Chemical structures of 6-substituted cholesterol analogs

that it can be carried out very simply and quickly at room temperature. 6-Chloromercuricholest-5-en-3β-ol undergoes a very fast reaction with iodine in the zero-valent state in a large variety of solvents. The conditions chosen for this study involve iodine monochloride as the substrate and ethanol as solvent. The resulting ethanolic solution is easily purified by prep. high performance liquid chromatography (HPLC) to remove unreacted organomercury compound. After being diluted with saline and sterilized by filtration it is then suitable for injection.

MATERIALS AND METHODS

All reagents and solvents were reagent grade and were used without further purification. The [^{131}I] and [^{125}I] sodium iodide used were "radiochemical" grades that did not contain any sodium thiosulfate or sodium hydroxide and usually had a concentration of 1 mCi (37 MBq/μl). Before labeling, this material was diluted to a concentration of 1 mCi (37 MBq/ml) with 95% ethanol. No effort was made to remove the original water. The reported specific activity of this material is 3.16 KCi (117 TBq/mmol).[‡]

6-Chloromercuricholest-5-en-3β-ol was prepared, the purity was determined, and the structure confirmed as previously described (*11*) and (*12*). A typical labeling experiment was carried out as follows; A solution of [^{131}I]iodine monochloride was prepared by adding two Iodobeads[†] to a solution of 3.7 MBq of [^{131}I]sodium iodide in 1 ml of 95% ethanol. To this solution, a solution of 1 mg of **1** in 1 ml of 95% ethanol was quickly added. The reaction was allowed to proceed for 3 min at which point analysis by thin layer chromatography (*vide infra*) indicated the formation of [^{131}I]-6-iodocholest-5-en-3β-ol. There was always some residual radioactivity at the origin—usually 10–15%. This material was assumed to be radioiodine species that were resistant to conversion to iodine monochloride. The ethanol was flash evaporated using a vacuum system with a pressure of <0.5 Torr and the residue partitioned between 1 ml of water and 1 ml of ether. The ether layer was drawn off and evaporated in a stream of nitrogen and the residue taken up in the HPLC solvent and chromatographed. The radioactive peak corresponding in retention time to that for 6-iodocholest-5-en-3β-ol was collected and the solvent removed in a stream of nitrogen. The residue at this point consisted of pure [^{131}I]-6-iodocholest-5-en-3β-ol **2**. The radiochemical purity was >99%. Free radioiodine was <0.03%. Typical radiochemical yields were 80 to 85% of starting radioactivity.

Whatman MK6F[§] silica gel microplates were used throughout the study. They were developed to 6.0 cm using methanol/chloroform (7:93) as solvent. In order to obtain reproducible Rf values for "no-carrier-added" materials, it was necessary to add a small amount of carrier. For this purpose, a standard carrier solution of cholesterol, 6-iodocholest-5-en-3β-ol **3** and 6-chloromercuricholest-5-en-3β-ol **1** in methylene chloride was applied to each plate prior to spotting with the radioactive solution. After developing, the positions of these compounds were visualized using uv light at 254 nm. The radioactive content of these plates was determined using either an in-house plate scanner based on a NaI(Th) crystal or a Berthold LB 2853 Linear Analyzer. While the NaI(Th) system gave better resolution, the Berthold system was more sensitive.

HPLC analysis was carried out using a Waters CN[¶] radial compression column and methanol/chloroform/hexane 10:20:70 as solvent with a flow-rate of 2 ml/min. Preparative HPLC separations were performed with a Whatman M9 10/50 PAC Partisil column and methanol/chloroform/hexane 5:20:75 as solvent using a flow-rate of 4 ml/min. The column effluent was passed through a narrow diameter nylon tube through a detector consisting of a NaI(Th) crystal in a lead castle. The sensitivity of this system could be changed by varying the number of coils of the plastic tube adjacent to the crystal. Since the retention times observed during HPLC analysis were quite short (<10 min), fraction collection was done by hand.

Peak detection was by monitoring the uv absorbance of the eluate at 245 nm. Since neither **1** or **2** contain

significant chromophores the level of sensitivity is lower than that normally found for most compounds. Examination of the uv spectrum of **1** and **2** showed a maximum at 245 nm. This figure is close to the uv cutoff for the solvent mixtures described above. It was found that reagent grade hexane was unsatisfactory, in that the uv cutoff was invariably above 245 nm. The use of HPLC grade hexane solved this problem.

Carrier 6-iodocholest-5-en-3β-ol was not required to obtain reproducible retention times, in contrast to the behavior observed on TLC analysis. During preparative HPLC analysis of the reaction product, [^{131}I]-6-iodocholest-5-en-3β-ol was easily detected by the radioactivity detector but no corresponding peak was observed by the uv detector.

The radiolabeled [^{131}I]-6-iodocholest-5-en-3β-ol was dissolved in 100 μl of 95% ethanol and diluted to 1 ml with 1% Tween 80 in saline. The Tween 80 was considered unnecessary in the case of the no-carrier-added studies, but because some of the distribution studies involved added carrier 6-iodocholest-5-en-3β-ol it was felt necessary to include it in all the studies. Carrier 6-iodocholest-5-en-3β-ol was added in three concentrations. 1 μg/ml, 10 μg/ml, and 100 μg/ml where these figures represent the concentration of carrier in the final dose preparation. This carrier was added to the dose in the 100 μl of 95% ethanol. The final dose was filtered through a 0.22-μ Millipore filter to ensure sterility.

Tissue distribution studies were carried out using male Sprague Dawley rats supplied by the University of Alberta Small Animal Program. The average weight was 200 g. A total of 16 animals were used in each study involving differing carrier concentrations. Typically, 16 animals were injected on a Monday and four animals were killed and dissected on each subsequent day. Prior to injection each animal was given mild anesthesia by placing it in bell jar containing diethyl ether vapor for about 1 min. Dose administration was by i.v. tail vein injection. Upon dissection, the tissues were rinsed in saline, dried by dabbing on paper tissue, briefly air dried and weighed. These tissues were then measured in a gamma counter.

The same animals were also used to measure the whole-body elimination curves for [^{131}I]-6-iodocholest-5-en-3β-ol. After injection each animal was counted using a gamma camera** with a Pho/Gamma HP crystal to obtain an overall value for the dose injected. Each animal was counted in conjunction with a standard which consisted fo a single dose in 250 ml of water. By comparing the counts of each animal with that for the standard, a measure of dose variation was obtained and a correction factor was generated. The true value of the injected dose was then calculated. On each of the subsequent days of the study each animal was counted using the same geometry to determine the amount of dose excretion in the previous 24 hr. As a result, data for 24 hr excretion are based on 16 animals and 48 hr excretion data are based on 12 animals.

Samples for neutron activation analysis were irradiated at a flux of 10^{12} neutrons/sec/cm^2 for a period fo 10 min. After a 1-min cool time they were counted using a Aptec Associates Win-15 GeLi detector coupled to a Nuclear Data ND-660 computer. The gamma line studied was the 285 keV line of ^{199m}Hg. All samples were measured in conjunction with internal standards of mercuric nitrate.

RESULTS

6-Chloromercuricholest-5-en-3β-ol **1** or indeed any chloromercury compound can undergo two distinct reactions with iodine depending on the valence of the latter. Thus iodide reacts at the mercury atom of **1** to give 6-iodomercuri-cholest-5en-3-βol **6** while iodine or iodine monochloride react to give **3.** Since the formation of **6** was undesirable the [^{131}I]NaI was converted to iodine monochloride via the use of chloramine-T before adding the 6-chloromercuricholest-5-en-3β-ol. Iodobeads (an immibolized form of chloramine-T) was also found to be a good means of accomplishing the same conversion without the added problem of removing or separating the excess chloramine-T. No loss of radioiodine due to volatility of the iodine monochloride in the short time of the reaction was observed.

The solvent of choice for labeling experiments was 95% ethanol. This was chosen because earlier studies (*11*) had identified it as an excellent medium for the reaction of halogens with organomercury compounds. It also was an excellent choice for the preparation of the final dose for injection since it needed only to be diluted with saline. In practice because of the need for HPLC chromatography this latter advantage was lost. Reverse phase absorption chromatograpny was not used, but rather normal phase absorption chromatography which involved the removal of the ethanol from the reaction product, was required. It was found that the Iodobeads were quite stable to solvents such as 95% ethanol over the short time scale used for reactions.

The general labeling procedure used was to react the [^{131}I]NaI with Iodobeads in ethanol followed by the addition of 6-chloromercuricholest-5-en-3β-ol **1**. It was also discovered that the reaction of iodine with **1** to form **6** under these conditons, either did not occur, which seems unlikely, or else was sufficiently reversible to play no role in the reaction sequence. The continuous presence of the oxidizing agent (i.e., Iodobeads) throughout the course of the reaction was found to be important. If the Iodobeads were present only at the beginning then the amount of inorganic radioiodide present in the reaction solution on completion was much higher.

Using these methods, labeling was rapid and complete in 1 min. It is quite possible that reaction is indeed quicker than this but this was the shortest interval measured. The radiochemical yield often showed slight variations due to factors presumed to be associated with the age and specific activity of the radioiodide but it always fell between 85 and 100%. The radiochemical purity of the product was also excellent, with [^{131}I]-6-iodocholest-5-en-3β-ol being the only identifiable product other than inorganic radioiodide. In practice, the analysis of the reaction product was carried out in two ways. The quantity of inorganic radioiodide was determined from TLC analysis of the crude reaction product and the purity of the radiolabeled organic compound(s) was measured by HPLC after extraction of these compounds into ether.

Preparative HPLC was used to purify the reaction product and separate the unreacted chloromercury compound from the product. Towards this end it was originally planned to use reverse phase absorption chromatography but this presented difficulty detecting the presence of 6-iodocholest-5-en-3β-ol during elution due to its poor absorption in the uv. The limiting factor was the solubility of the 6-iodocholest-5-en-3β-ol in the aqueous solvents used in reverse phase chromatography. By changing to normal phase chromatography using polar amino cyano (PAC) columns and a hexane/chloroform mixture as solvent the concentration of the 6-iodocholest-5-en-3β-ol was increased to the point where it was detectable by uv absorption.

The [^{131}]-6-iodocholest-5-en-3β-ol prepared in this fashion showed excellent stability in ethanol but not in the hexane chloroform solvents used for chromatography. In the latter, after 24 hr at room temperature, decomposition products would start to appear. It was apparent that this process was hastened by nonpolar solvents but did not occur in polar solvents such as ethanol or ethanol/water mixtures. This decomposition process is known to involve oxidation and is well known for many 5-cholestenes (*13*). This oxidation reaction was not a problem in the short time period (10 min) during which the [^{131}I]-6-iodocholest-5-en-3β-ol was dissolved in these solvents. The pure fractions of [^{131}I]-2 collected from the HPLC were quickly concentrated *in vacuo* and redissolved in ethanol. In this form there was no detectable deterioration over a number of days.

The [^{131}I]-2 was prepared for injection by diluting the ethanol solution to 10% in saline. Because the effect of carrier on the tissue distribution of 6-iodocholest-5-en-3β-ol was to be studied a number of doses were also prepared in which varying amounts of carrier 6-iodocholest-5-en-3β-ol was added to the dose. To ensure that all of this carrier material stayed in solution 1% Tween-80 was added. The Tween-80 was also added to the no-carrier-added dose to ensure the formulation equivalence of all the doses.

The biologic behavior of [^{131}I]-6-iodocholest-5-en-3β-ol was studied in male Sprague Dawley rats. A tissue distribution study and a whole-body elimination study were carried out simultaneously on the same animals. Thus, the excretion was correlated with the uptake in specific tissues. This took the form of injecting 16 animals on Day 0 and on each subsequent day removing four animals for whole-body counting followed by dissection for the tissue distribution study. Thus the excretion data for Day 1 is based on 16 animals and for Day 4 is based on four animals. No attempt was made to distinguish between urinary and fecal excretion.

The results of the biodistribution studies are contained in Table 1 and Fig. 2. While Table 1 shows the tissue distribution for no carrier added material Fig. 2 shows the adrenal/blood ratio for all the specific activites prepared. Table 1 contains the tissue/blood ratios for all organs and the %dose/tissue for every organ except muscle and skin. A value of 6.5% of body weight was used to determine the total blood weight. The adrenal glands, as expected, showed an excellent uptake of the labeled material with tissue/blood ratios of 200 or greater after 4 days. The slow rate of buildup for activity in the thyroid and stomach was evidence for the great stability of 6-iodocholest-5-en-3β-ol towards deiodination. Of note was the increased activity in the lungs and spleen. After 24 hr the lungs contained nearly 1% of the injected dose. It was not ascertained whether this uptake was related to physical trapping of radioactivity associated with Tween 80 miceles. However, gradual increase in concentration with time was supportive of a biochemical mechanism.‡

Figure 3 shows the whole-body excretion curve for the injected dose and demonstrates clearly the effect of specific activity on the rate of clearance of the dose from the body. The material of higher specific activity shows a slower overall rate of elimination. While the specific activities of the various doses varied by a factor of 1,000 the adrenal/blood ratios differed by less than an order of magnitude and the rate of elimination by even less. It is clear that there is an advantage to be gained from using as high a specific activity as possible, with a correspondingly small chemical dose.

The possibility of mercury contamination of the [^{131}I]-6-iodocholest-5-en-3β-ol was also examined since this material was to be used for i.v. injection. Accordingly a 3.7 MBq sample of "no-carrier-added" [^{131}I]-6-iodocholest-5-en-3β-ol was allowed to decay over a period of 4 wk and then examined by Neutron Activation Analysis using the University of Alberta Slowpoke Reactor Facility. The lower limit of detection for [^{199m}Hg] using this method was 5 μg of mercury. No mercury levels above this limit of detection were found.

DISCUSSION

There are few accounts in the literature of radiolabeling experiments involving organomercury com-

TABLE 1
Tissue Distribution of i.v. Doses of No-Carrier-Added [^{131}I]-6-Iodocholest-5-en-3β-ol in Male Sprague Dawley Rats

Tissue	Time after injection (hr)			
	24	48	72	96
Blood	1.00(0.0)*	1.00(0.0)	1.00(0.0)	1.00(0.0)
	4.58(1.2)†	2.97(0.7)	1.96(0.4)	1.39(0.2)
Liver	1.92(0.2)	2.21(0.3)	2.31(0.4)	2.45(0.4)
	8.06(1.3)	5.54(0.5)	3.71(0.3)	2.76(0.3)
Spleen	2.40(0.2)	2.60(0.3)	2.67(0.4)	3.19(0.33)
	0.52(0.1)	0.38(0.03)	0.24(0.04)	0.23(0.04)
Stomach	0.36(0.1)	0.60(0.2)	0.54(0.2)	0.90(0.2)
	0.46(0.2)	0.42(0.06)	0.35(0.02)	0.37(0.01)
Testicle	0.20(0.01)	0.31(0.05)	0.37(0.08)	0.37(0.1)
	0.14(0.04)	0.15(0.05)	0.11(0.02)	0.13(0.02)
Kidney	0.65(0.03)	0.93(0.20)	1.13(0.26)	1.92(0.2)
	0.46(0.12)	0.40(0.14)	0.32(0.08)	0.38(0.03)
Adrenal	36.56(3.5)	80.49(13.3)	134.74(53.2)	208.54(22.5)
	0.61(0.16)	0.85(0.23)	0.84(0.35)	1.00(0.14)
Thymus	0.31(0.05)	0.58(0.19)	0.80(0.29)	1.28(0.16)
	0.06(0.02)	0.07(0.03)	0.06(0.02)	0.08(0.02)
Lungs	2.23(0.16)	3.08(0.48)	3.32(0.61)	4.40(0.25)
	0.90(0.23)	0.80(0.23)	0.53(0.10)	0.50(0.04)
Heart	0.71(0.08)	1.02(0.28)	1.12(0.25)	1.64(0.12)
	0.19(0.05)	0.17(0.04)	0.13(0.03)	0.12(0.02)
Brain	0.06(0.01)	0.10(0.01)	0.12(0.03)	0.23(0.03)
	0.04(0.01)	0.04(0.01)	0.03(0.007)	0.04(0.002)
Prostate	0.23(0.03)	0.38(0.08)	0.47(0.16)	0.78(0.15)
	0.01(0.003)	0.01(0.003)	0.009(0.004)	0.009(0.001)
Thyroid	10.26(2.8)	14.51(2.4)	19.91(5.3)	29.60(13.8)
	0.33(0.06)	0.26(0.05)	0.20(0.04)	0.20(0.03)
Seminal vesicles	0.30(0.03)	0.50(0.01)	0.65(0.14)	1.10(0.14)
	0.01(0.005)	0.01(0.005)	0.01(0.004)	0.01(0.004)
Skin	0.44(0.06)*	0.77(0.15)	0.96(0.30)	1.52(0.14)
Muscle	0.19(0.03)*	0.31(0.06)	0.40(0.11)	0.69(0.08)

*Tissue/blood ratio (±1 s.d.).
† %Dose/tissue (±1 s.d.).

pounds and iodine (*14,15*). Earlier studies (*11,16,17*) had shown the general applicability of vinylic organomercury compounds for this type of radiolabeling. However, it was not clear what modifications in method or reagents would be required to carry out this reaction on the exceedingly small scale required for no-carrier-added experiments. Thus, it was pleasing to discover that the same ease of use, found in the macro scale application of these compounds, was also apparent in their use in the submicro scale. Since 6-iodocholest-5-

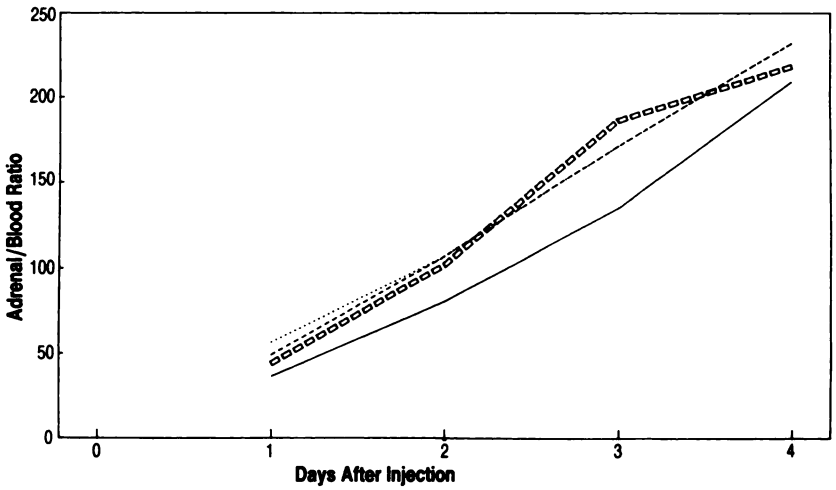

FIGURE 2
Adrenal uptake of [^{131}I]-6-iodocholest-5-en-3β-ol by i.v. injection in male Sprague Dawley rats. Plot showing adrenal/blood up to 4 days postinjection of [^{131}I]-6-iodocholest-5-en-3β-ol at four different specific activities. Each point for Day 1 represents 16 animals, for Day 2, 12 animals, Day 3, 8 animals, and for Day 4, 4 animals

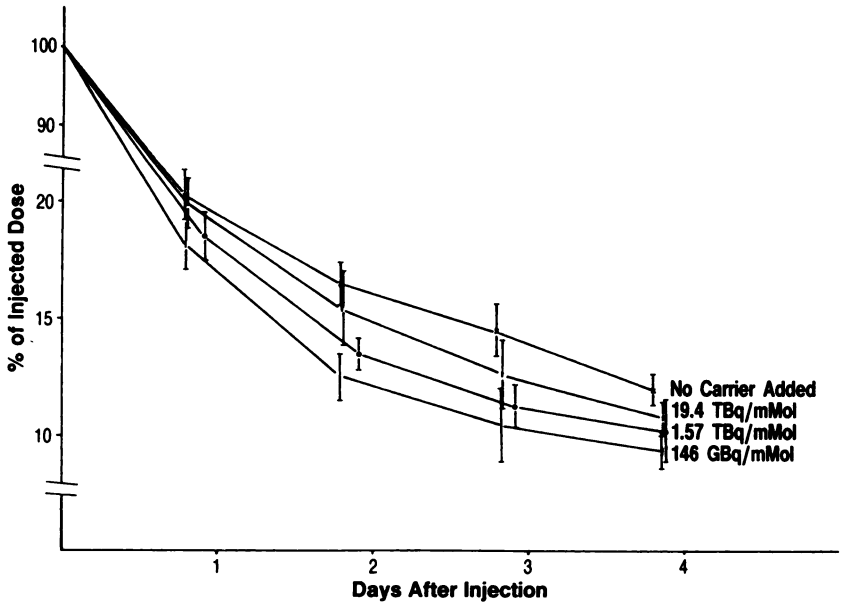

FIGURE 3
Whole body elimination of radioactivity after injection of [^{131}I]-6-iodocholest-5-en-3β-ol in male Sprague Dawley rats. Plot showing rate of elimination of doses of [^{131}I]-6-iodocholest-5-en-3β-ol with time at four different specific activities. Each point for Day 1 represents 16 animals, for Day 2, 12 animals, Day 3, 8 animals and for Day 4, 4 animals

en-3β-ol is known to slowly build up in adrenal tissue over a period of 4 days it was of interest to see if this time period could be reduced by increasing the specific activity.

The results from Table 1 and Fig. 2 show that the biodistribution of the "no-carrier-added" material is indeed very similar to that found for material of lower specific activity in terms of tissue/blood ratios (*8*). The %dose/adrenal however, displayed a trend of increasing values as a function of time after injection for the no-carrier-added material. This effect was not observed for the lower specific activity products. Development of a mathematical model to describe this slow accumulation of radioactivity in the adrenal while blood levels and total body retention levels decline is currently underway. Preliminary data do not support simple models in which slow uptake from the blood pool is related to the % of cardiac output reaching the adrenal gland.[‡‡] The picture is complicated by the slower clearing of blood radioactivity and higher (but declining) residual radioactivity in kidney and liver after administering "no-carrier-added" [^{131}I]-6-iodocholest-5-en-3β-ol.

The high adrenal/blood ratios observed for all specific activities were excellent for imaging purposes. In addition, the high percentage of injected radioactivity in the adrenal underline the potential of this agent for clinical scintigraphic studies. The advantage of no-carrier-added 6-iodocholest-5-en-3β-ol over lower specific activity preparations of that material is particularly evident when an uptake index, such as the mathematical product of tissue/blood ratio and %dose/organ is calculated (Table 2). Although the "tumor index" is

TABLE 2
Selective Concentration Indices for Adrenal Tissue Using [^{131}I]-6-Iodocholest-5-en-3β-ol in Male Sprague Dawley Rats

Specific activity	Index parameter	Time after injection (hr) 24	48	72	96
No-carrier-added	Tumor index*	515	1,595	2,833	4,618
	Mod. tumor index†	22.5	68.4	114.13	209.4
524 Ci/mmol	Concentration index‡	25.5	36.9	39.7	44.6
(19.4 TBq/mmol)	Tumor index	530	1,871	3,384	3,922
	Mod. tumor index	19.1	63.9	107.1	104.7
42.4 Ci/mmol)	Concentration index	19.0	28.6	28.6	22.7
(1.57TBq/mmol)	Tumor index	742	1,432	3,474	4,165
	Mod. tumor index	28.1	51.6	128.7	119.0
3.95 Ci/mmol	Concentration index	28.5	24.2	38.6	34.7
(146 GBq/mmol)	Tumor index	1,448	2,995	4,953	5,702
	Mod. tumor index	51.7	98.6	175.8	213
	Concentration index	45.5	53.1	58.3	44.5

* Tumor index = (adrenal/blood) × (% dose/g tissue).
† Modified tumor index = (adrenal/blood) × (% dose/organ).
‡ Concentration index = (counts/g tissue)/(counts/g body weight).

frequently presented as the product of tissue/blood and %dose/g tissue (*18*), the use of %dose/organ is considered to be more meaningful when dealing with small organs as the adrenal.

CONCLUSIONS

In summary, 6-iodocholest-5-en-3β-ol has been easily prepared at specific activities ranging from no-carrier-added to 3.95 Ci (146 GBq/mmol). Tissue studies in male rats indicated a complex distribution of radiaoctivity as a function of specific activity when a constant formulation of Tween 80/EtOH/H_2O was used. The best combination of tissue radioactivity and tissue/blood ratios were found using no-carrier-added 6-iodocholest-5-en-3β-ol or the lowest specific activity (146 GBq/mmol) 6-iodocholest-5-en-3β-ol after 48 hr.

The synthesis of no-carrier-added [^{131}I]-6-iodocholest-5-en-3β-ol from 6-chloromercuricholest-5-en-3β-ol has been achieved using a practical and simple method. The resultant high specific material allows easier dose formulation since the quantity of compound present is extremely small. The biodistribution of this high specific activity material was very similar to that of the lower specific activity material prepared by exchange labeling. The whole-body elimination curve on the other hand showed an inverse relationship between specific activity and rate of clearance.

FOOTNOTES

* The preceding paper in this series is Part 1 "The Syntheses and Structure of 6-Halo-cholest-5-en-3-β-ols," *Can J Chem* 63:2853, 1985.

† The Pierce Chemical Company, Rockford, IL.

‡ Atomic Energy of Canada Limited.

§ Whatman Inc., Clifton, NJ.

¶ Waters Associates, Milford, MA.

**Nuclear Chicago, Des Plaines, IL.

†† Recent experiments to determine the effect, if any, of Tween-80 on the biodistribution of ^{131}I-6-iodocholest-5-en-3β-ol have shown that in the case of the "no carrier added" material there is no observable difference.

‡‡ Butler GJ, Wiebe LI, Flanagan RJ: work in progress.

ACKNOWLEDGMENTS

We wish to acknowledge an NSERC/Frost Fellowship and an Alberta Heritage Foundation for Medical Research-Research Allowance for one of us (RJF). The animal studies were carried out by Mr Thomas Woo under a Medical Research Council Summer Studentship. Neutron Activation Analysis studies were undertaken at the University of Alberta SLOWPOKE reactor, with the assistance of Mr. John Duke and Mr. Peter Ford.

REFERENCES

1. Kabalka GW, Gooch EE: A mild and convenient procedure for the conversion of alkenes into alkyl iodides via the reaction of iodine monochloride with organoboranes. *J Org Chem* 45:3578, 1980
2. Kalbalka GW, Gooch EE, Sastry KAR: Rapid and mild synthesis of radioiodine labelled radiopharmaceuticals. *J Nucl Med* 22:908, 1981
3. Gilliland DL, Basmadjian GP, Marchand AP, et al: Iodine labelled radiopharmaceuticals from arylthallium bis(trifluoroacetate) intermediates. *J Radioanal Chem* 65:107 1981
4. Adam MJ, Pate BD, Ruth TJ, et al: Cleveage of aryltin bonds with elemental fluorine. A rapid synthesis of ^{18}F-Fluorobenzene. *J Chem Soc Chem Comm* 733: 1981
5. Larcock RC: Organomercurials in organic synthesis. *Tetrahedron* 38:1713, 1982
6. Houben-Weyl A: *Methoden der Organischen Chemie, XIII, 2b*, Stuttgart, Georg Thieme Verlag, 1974
7. Brown HC: *Organic Synthesis via Boranes*, New York, John Wiley and Sons, 1975
8. Bo-Li L, Yu-Tai J, Zhenghao L: ^{131}-I-6-Iodocholesterol, an agent for imaging the adrenal gland. *J Nucl Radiochem (China)* 2:107, 1980
9. Bo-Li L, Yu-Tai J, Zhengao L: Labelling of 6-iodocholesterol with iodine-131. *J Lab Comp Radiopharm* 18:112, 1980
10. Bo-Li L, Yu-Tai J, Maeda M, et al: Labelling of 6-Iodocholesterol in melt. *Radioisotopes* 30: 275, 1981
11. Flanagan RJ, Charleson FP, Synnes EI: Radiolabelling with organomercury compounds. Part 1. The synthesis and structure of 6-halocholest-5en-3β-ols. *Can J Chem:* in press
12. Levin RH, Spielman MA: The mercuration of cholesterol. *J Am Chem Soc* 62:920, 1940
13. Kirk DV, Hartshorn MP: *Steroid Reaction Mechanisms*, Amsterdam, Elsevier Publishing, 1968
14. Jones RL: 4-Chloro-2-iodophenoxyacetic acid labelled with ^{131}I. *J Chem Soc* 4080, 1952
15. Agrawal KC, Ray FE: Tumour localization agents. Radioactive iodofluoroenaminesulphonic acids. *J Med Chem* 9:729, 1966
16. Bo-Li L, Yu-Tai J, Zhong-Yun P, et al: Radiobromine cholesterol analogues. *J Lab Comp Radiopharm* 19:1089, 1982
17. Visser GWM, Diemer EL, Kasparen FM: The preparation of aromatic astatine compounds through aromatic mercury compounds. Part 2. *J Lab Comp Radiopharm* 18:789, 1981
18. Emrich D, von zur Muhlen A, Willgeroth F, et al: Comparison of tumour uptake and kinetics of different radiopharmaceuticals under experimental conditions. *Acta Radiol (Ther) (Stockholm)* 11: 566, 1972