TUMOR LOCALIZING AGENTS. IX.[1] RADIOIODINATED CHOLESTEROL

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19-Iodocholesterol-^{125}I was synthesized for study as a possible agent for photoscanning the adrenal gland and associated tumors. In contrast to previous radioiodinated steroids, it was found to be much less prone to rapid *in vivo* deiodination. Preliminary tissue distribution studies and biochemical analyses have revealed a marked similarity in the behavior of the radioiodinated derivative with the natural steroid. The concentration of radioactivity in the adrenal cortex of dogs at 48 hours was found to greatly exceed that found in other organs.

Over the past several years an effort has been made in our laboratories to develop a radiopharmaceutical suitable for photoscanning the adrenal gland and associated tumors. Despite the rapid advances in Nuclear Medicine, the adrenal glands remain one of the few organs to be visualized by radioisotope scanning techniques. Similar to the use of radioiodine in thyroid disease, a radiolabeled drug that selectively concentrates in the adrenal gland

would not only be of diagnostic value, but also have potential therapeutic applications.

Our synthetic approaches to this problem have proceeded along the following lines:

1. To radiolabel drugs reported or suspected to have a predilection for adrenal tissue, and
2. To radiolabel naturally occurring compounds known to be biosynthesized and/or stored in the adrenals.

We have previously reported the synthesis of various radioiodinated analogs of 1-(o-chlorophenyl)-1-(p-chlorophenyl)-2,2-dichloroethane (o,p'-DDD) based on the reported predilection of o,p'-DDD for adrenal tissue.[2] While several of the radioiodinated analogs did concentrate in the dog adrenal cortex more than any other tissue shortly after administration, the target to non-target ratio was insufficient to achieve successful adrenal photoscans.[3]

Our alternate approach to adrenal localizing agents has been to investigate the feasibility of radioiodinated steroids. There are numerous reports in the literature citing the ability of various radiolabeled steroid hormones to concentrate in the adrenal cortex. For example, Hanngren *et al.*[4] observed accumulation of ^{14}C-cortisone in the adrenal cortex of mice five minutes after administration. The radioactivity soon subsided from the cortex, however, and very little remained 20 minutes after injection. Similar studies with ^{14}C-estrone or ^{14}C-diethylstilbestrol showed that the adrenal cortex had the highest level of activity of any tissue examined for as long as 60 minutes following injection.[5]

We previously reported the synthesis of 21-iodoprogesterone-^{125}I and 21-iodopregnenolone acetate-^{125}I as possible adrenal

scanning agents.[6] This work was prompted by the reports by Bengtsson et al.[7] and Laumas and Farooq[8] that uptake of radiolabeled progesterone by the adrenal cortex far exceeded that of any other tissue. In our study, however, the radioiodinated steroid analogs rapidly underwent in vivo deiodination, and it was quite apparent that other sites on the steroid nucleus less prone to enzymatic dehalogenation would have to be found.

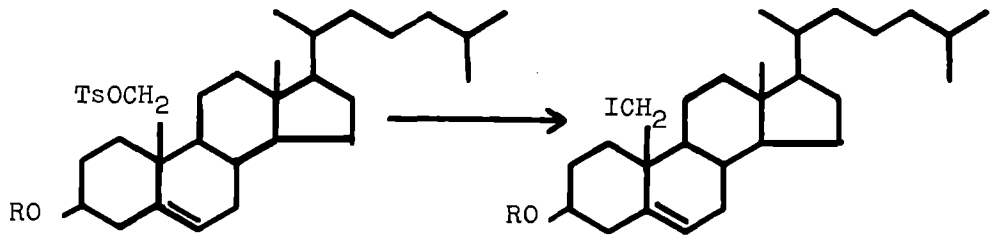
It has been known for a number of years that the adrenals are particularly rich in cholesterol. In most animals, the adrenals are the richest source of cholesterol where it is present mainly in the esterified form.[9] This information suggested that an appropriately radioiodinated cholesterol might have the features required for an adrenal scanning agent.

Several papers have reported the ability of ^{14}C-cholesterol to concentrate in adrenal tissue,[10] but it was Appelgren's[11] findings which prompted us to take a more serious look at utilizing cholesterol as a platform for radioiodine. Appelgren[11] gave ^{14}C-cholesterol by I.V. injection to mice and studied the distribution at various times by whole body and detailed autoradiography. The strongest accumulation appeared in the adrenal cortex and at 24 hours the level of radioactivity was about 20 times that of blood. A strong accumulation was also seen in the ovary and corpora lutea. More recent electron microscopic autoradiographs have demonstrated that most of the adrenal cholesterol is stored in the lipid droplets of the adrenal cortical cells.[12]

Shortly after we initiated our study, Nagai, Solis and Koh[13] reported on ^{131}I-cholesterol as a possible adrenal scanning agent. These workers labeled cholesterol by iodination with sodium iodide-

^{131}I and chloramine-T. Although they did not characterize their radioiodinated product or products, one would expect the iodine to add across the 5, 6-double bond to give a diiodo derivative Subsequent tissue distribution studies in mice with this agent revealed, however, that the adrenal-to-liver concentration at 24 hours was only 2.70 and that significant *in vivo* deiodination had occured. These workers later found that radioiodinated stigmasterol prepared in a similar manner was superior to the cholesterol derivative in its ability to concentrate in the adrenals, but the target to non-target ratio was still insufficient for radiographic delineation of these organs.

In our own studies, we attempted to find a position on the steroid molecule that could be readily functionalized with iodine but which at the same time would not markedly alter the basic cholesterol structure. In addition, the position would have to be somewhat resistant to *in vivo* dehalogenation. On this basis, substitution at the neopentyl, C-19 position of cholesterol seemed a logical choice for our initial studies. Cholest-5-ene-3,19-diol 3-acetate was prepared in a manner similar to that described by Kalvoda *et al.*[14] Treatment of this alcohol with p-toluenesulfonyl chloride in pyridine afforded the desired tosylate, I. Selective hydrolysis of the acetate ester of I gave II which was subsequently treated with sodium iodide in isopropanol to yield 19-iodocholesterol (III). Radioiodination was readily achieved by isotope exchange of III with sodium iodide-125 in refluxing acetone. 19-Iodocholesterol acetate-^{125}I (IV) was prepared from I in a similar manner.



I R = Ac

II R = H

III R = H

IV R = Ac

Tissue distribution studies in dogs with 19-iodocholesterol-^{125}I have been completed and the details are reported elsewhere.[15] In brief, the radioiodinated derivative was found to behave remarkably similar to the natural steroid. Table I compares the tissue distribution of radioiodinated cholesterol with that found for cholesterol-4-^{14}C in dogs at 48 hours. In all instances the concentration of radioactivity in the adrenal cortex was found to greatly exceed that in other organs. The high radioactivity found in the thyroids of the dogs receiving the radioiodinated derivative, particularly the dog not previously dosed with potassium iodide solution, reflects the expected in vivo metabolism and deiodination of the compound.

For purposes of photoscanning, it was important that the concentration of radioactivity in the adrenal gland be considerably greater than the nearest major organs, liver and kidney. In all three dogs receiving 19-iodocholesterol-^{125}I, the adrenal cortex-to-liver and adrenal cortex-to-kidney ratios were approximately 30, and this value has been found to exceed 100 at 8 days after administration.[15] This selective concentration of 19-iodocholesterol-^{125}I in the adrenal cortex has made it possible to visualize the adrenal glands in a living dog with a 5-inch photoscanner.[15]

TABLE I

Concentration of Cholesterol-4-^{14}C and 19-Iodocholesterol-^{125}I in Various Dog Tissues 48 Hours Following IV Administration (expressed as % administered dose/gm of tissue)

Radiolabel	Adrenal	Liver	Kidney	Thyroid	Spleen	Bile	Testes	Ovaries	Blood
^{14}C	0.152	0.008	0.008	0.014	0.017	-	-	0.020	0.020
^{14}C	0.298	0.015	0.012	0.005	0.028	0.192	0.006	-	0.008
^{125}I*	0.498	0.017	0.017	0.088	0.020	0.156	-	0.075	0.016
^{125}I*	0.379	0.013	0.012	0.038	0.020	0.118	0.007	-	0.017
^{125}I	0.243	0.007	0.009	2.323	0.015	0.205	0.007	-	0.008

* Dogs predosed orally with saturated solution of potassium iodide.

Extraction of the adrenal cortices by the Folch procedure[16] revealed that all of the radioactivity was contained in the chloroform phase and none in the methanol-water phase. There appeared, therefore, to be no inorganic iodide-125 in the adrenals after the injection of 19-iodocholesterol-^{125}I.

The analysis of the chloroform phase from 19-iodocholesterol-^{125}I treated dogs showed much greater variation from results obtained with a similar extract of the cholesterol-4-^{14}C adrenals. In the former, a significant amount of radioactivity always was associated with the cholesterol-ester fraction (25-35%). Only small amounts were found in the cholesterol fraction (4-6%) and the remainder stayed at the origin. In the case of the cholesterol-4-^{14}C adrenal extract, 80-85% of the radioactivity was located in the cholesterol ester fraction and the rest migrated as free cholesterol.

In several cases, the radioiodinated cholesterol-ester fraction was extracted from the tlc plate and rechromatographed in the same solvent system. The lipid staining material again moved with the ester fraction, but only 24% of the radioactivity could be recovered from this fraction. The variability in the chromatographic distribution of ^{125}I radioactivity and the failure of the radioactivity to migrate with the lipid when rechromatographed is probably the result of the instability of 19-iodocholesterol under the conditions of analysis. This instability also has hampered attempts to rigorously characterize the chemical structure of the radioiodinated ester fraction.

Since 19-iodocholesterol was apparently capable of *in vivo* esterfication in a manner similar to cholesterol, it seemed possible

that certain radioiodinated adrenocorticosteroids and their excretory metabolites might also be formed. However, analysis of urine samples obtained from the bladder of one of the dogs treated with 19-iodocholesterol-^{125}I revealed no radioiodinated steroid metabolites and all of the urinary radioactivity could be accounted for as inorganic iodide. Additional studies are now in progress to evaluate 19-iodocholesterol-^{125}I as a biochemical tool.

EXPERIMENTAL[17]

Cholest-5-ene-3β,19-diol 19-toluene-p-sulfonate (II). -- A solution of cholest-5-ene-3β,19-diol 3-acetate 19-toluene-p-sulfonate[18] (I, 200 mg) in dioxane (7 ml) was added dropwise to a solution of NaOH (100 mg) in aqueous methanol (10 ml). The solution was stirred at room temperature for 2 hr. and then poured into ice-water. The resulting mixture was extracted with ether and the extract washed with water and dried over anhydrous sodium sulfate. Removal of the solvent and crystallization of the residue from acetone-water afforded pure II (120 mg, 52%): mp 121-123°; nmr δ 0.58 (s,3,C_{18}-protons), 2.53(s,3,$CH_3C_6H_4$-), 3.95 and 4.09 (d,2,Jgem=10cps,C_{19}-protons), 5.50(s,1,vinylic proton), 7.31 and 7.73 (dd,4,J_o=8cps, aromatic protons).

Anal. Calcd for $C_{34}H_{52}SO_4$: C,73.33; H,9.41. Found: C,73.77; H,9.55.

19-Iodocholest-5-en-3β-ol (III). -- A solution of II (200 mg) and sodium iodide (100 mg) in isopropanol (15 ml) was gently refluxed under nitrogen for 4 hr. The solution was concentrated to about 5 ml *in vacuo* and poured into ice-water. Extraction with ether and work up as above furnished an oily residue which solidified upon trituration with petroleum ether (bp 30-40°). Recrystallization from methanol gave pure III(115 mg. 62%): mp 106-109°; nmr δ 0.69 (s,3,C_{18}-protons), 3.24 and 3.51 (dd,2,Jgem=11cps, C_{19}-protons), and 5.53 (s,1,vinylic proton).

Anal. Calcd for $C_{27}H_{45}IO$: C,63.27; H,8.85. Found: C,63.45; H,8.91.

19-Iodocholest-5-en-3β-ol acetate (IV). -- A solution of I (300 mg) and sodium iodide (200 mg) in isopropanol (20 ml) was treated as above. Recyrstallization of the crude product from acetone-methanol afforded pure IV (110 mg, 42%): mp 91-93° (reported[19] mp 97°); nmr δ 0.76 (s,3,C_{18} protons), 2.0 (s,3, CH_3COO-), 3.30 and 3.54 (dd,2,Jgem=11 cps,C_{19}-protons), and 5.61 (s,1,vinylic proton).

Anal. Calcd for $C_{29}H_{47}IO_2$: C,62.79; H,8.54. Found: C,62.78; H,8.57.

Radioiodinated Steroids - Isotope Exchange:

A. A solution of $Na^{125}I$ (5 mc) was placed in a 25 ml round bottom flask and the water removed by azeotropic distillation with benzene. A solution of III (100 mg) in acetone (7 ml) was added and the mixture refluxed under an atmosphere of nitrogen for 4 hr. The solution was allowed to cool and poured into water. The resulting mixture was extracted with ether and the ether extract washed with water and dried over anhydrous sodium sulfate. The ether was evaporated and the residue chromatographed over alumina (activity III). Elution with petroleum ether (bp 30-40°)-ether (1:1) gave 19-iodocholesterol-^{125}I (80 mg) with a specific activity of 28.25 μCi/mg (52% exchange). Thin layer chromatography using chloroform-ethanol (1:1) gave a single spot (R_f=0.66) coincident with the single radioactive peak appearing on the radiochromatogram.

B. Water from $Na^{125}I$ solution (3 mc) was removed as described above. A solution of IV (100 mg) in acetone (5 ml) was added and the clear solution gently refluxed with stirring for 4 hr. under nitrogen. The solution was concentrated to about 1/2 the original volume and poured into ice water. The precipitate was collected, washed well with water, and recrystallized from methanol-acetone. This gave 19-iodocholesterol acetate-^{125}I with a specific activity of 8.87 μCi/mg (68% exchange). Tlc using benzene: hexane (1:2) showed a single spot (R_f=0.74) coincident with the radioactive area shown by a radiochromatogram.

Tissue Distribution Studies: Cholesterol-4-^{14}C in benzene solution was obtained from New England Nuclear Corporation, Boston, Mass. and the benzene was removed by distillation under reduced pressure. The cholesterol-4-^{14}C thus obtained and 19-iodocholesterol-^{125}I were both dissolved in 90% ethanol such that the resulting solutions contained 100-200 μCi/ml.

Each dog was given 50-150 μCi of radiolabeled steroid by injection into the foreleg vein and the syringe was flushed twice with blood. The dogs were sacrificed at 48 hours by injection of rapid-acting lethal solution (Haver-Lockhart Laboratories, Kansas City, Missouri).

In all dogs, 17 to 20 tissues were routinely dissected free, cleaned of fat and foreign material, and weighed. All tissues were done in duplicate and additional samples were obtained of tissue of special interest. The adrenals were the first tissues to be removed, weighed, and then frozen for separation of cortex from medulla. ^{14}C-labeled tissues were placed in counting vials after weighing, digested in 70% NaOH (0.3 ml) overnight, and heated for at least 30 seconds in near boiling water the next day to complete digestion. The digests were allowed to cool and 30% H_2O_2 (3 drops) and thixotropic liquid counting system[26] added. The radioactive

contents were then assayed in a liquid scintillation system. The radioiodine-containing tissues were counted directly in disposable plastic test tubes in an automated well counter. The concentration of radioactivity in each tissue was expressed in terms of the % administered dose/gm of tissue which was calculated as follows:

$$\frac{\text{CPM/mg} \times 1000 \times 100}{2.2 \times 10^6 \times \mu\text{Ci admin. dose}}$$

Lipid Analysis of Adrenal Cortex: The total lipid was extracted from the adrenal cortex by the Folch extraction procedure.[16] The chloroform extract was evaporated with a stream of nitrogen and the residue lipids fractionated by tlc on silica gel H. The solvent system hexane:ether:acetic acid(90:10:1 v/v) was used to separate the lipids according to their general class; phospholipids, sterols, fatty acids, triglycerides and sterol esters. In several initial experiments inorganic iodide-^{125}I was added to adrenal cortex tissue prior to the extraction. The inorganic iodide-^{125}I was completely recovered in the methanol-water phase of the extraction and no radioactivity from inorganic iodide-^{125}I was found in the chloroform extract.

Urine Analysis: Urine samples (2-3 ml) obtained from a dog dosed with 19-iodocholesterol-^{125}I were chromatographed on ion exchange resin (Dowex-1) according to the procedure of Galton and Pitt-Rivers.[21] Inorganic iodide binds strongly to the resin and can be removed only with elution by 3N KBr. This was verified for urinary inorganic iodide by adding inorganic iodide-^{125}I to 3 ml of normal urine and subjecting this to the fractionation procedure.

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$CDCl_3$ at a concentration of 10%, with tetramethylsilane as internal reference. Thin layer chromatograms (t.l.c.) were run with 1-in. wide Eastman Chromagrams, Type K301R, with fluorescence indicator and detected with uv light and iodine vapor. Chromagrams of radioiodinated compounds were scanned with an Atomic Associates RCS-363 radiochromatogram scanner. The specific activities were determined with a Picker Isotope Calibrator No. 632-500. The sodium iodide-125 was obtained from Mallinckrodt Nuclear, St. Louis, Missouri.

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