Visualization of Nonfunctioning Adrenal Adenomas with Iodocholesterol: Possible Relationship to Subcellular Distribution of Tracer

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[^{131}I] 19-iodocholesterol (I-131 C) correctly located adrenocortical adenomas in four patients who had no clinical or biochemical evidence of excessive steroid production. Three of the four "nonfunctioning" adenomas showed significant quantities of lipid histologically. To clarify the discordance between uptake and adrenal-steroid excretion, the subcellular location of I-131 C was studied. Normal rats and rats treated with ACTH or aminoglutethimide (AG) were injected intravenously with I-131 C or [^{3}H] cholesterol (H-3 C) and killed after three days. The homogenized adrenals were subjected to subcellular fractionation.

Treatment with AG increased both the amount and the percentage of both I-131 C and H-3 C contained in the lipid fraction. ACTH treatment decreased H-3 C content but did not change I-131 C content in the lipid layer, suggesting an impairment of I-131 C mobilization from lipid droplets. The data demonstrate that excess steroid production is not necessary for I-131 C uptake and provide an explanation why certain biochemically nonfunctioning adrenocortical adenomas are visualized with I-131 C.

J Nucl Med 19: 458–463, 1978

Uptake of [^{131}I] 19-iodocholesterol (I-131 C) by adrenal glands has been demonstrated in normal subjects and individuals with a variety of adrenal disorders. In each of these instances, steroid production was either normal or excessive (*1–3*). In addition, iodocholesterol uptake is increased by ACTH (*4*) and is suppressed after administration of dexamethasone (*5*). This has been interpreted to mean that iodocholesterol uptake parallels adrenal steroid production. Thus in practice, an increased uptake by an individual adrenal gland is generally interpreted to represent hyperfunction of that gland, or an autonomously functioning tumor.

On the other hand, little or no uptake of I-131 C by functioning adrenal carcinomas (*1,2,6*), by functioning adenomas (*1,6*), or by normal adrenal glands (*6*), indicates the existence of a more complex relationship between adrenal steroid biosynthesis and I-131 C uptake.

We have observed four patients who had no evidence of excess steroid production and yet I-131 C imaging correctly lateralized the "nonfunctioning" adrenal tumor. This suggests a dissociation between adrenal I-131 C uptake and adrenal function as defined by the routine biochemical and clinical testing of steroid secretion.

As a possible explanation for this discordance between I-131 C uptake and adrenal function, we postulated that I-131 C concentration parallels native

Received Aug. 10, 1977; revision accepted Dec. 5, 1977.

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cholesterol uptake rather than steroid biosynthesis. To test this hypothesis we have examined the subcellular distribution of I-131 C in rat adrenals, as well as the influence of ACTH and aminoglutethimide on its location.

METHODS

Clinical evaluation. From 1972 to 1976, 71 adrenal scans were performed in our institution. A retrospective review revealed four adrenal-tumor patients who had no clinical or biochemical evidence of excess steroid production, as measured by routine laboratory testing, and yet the tumor was correctly located by I-131 C imaging. The scans in these individuals were performed by injection of 2 mCi [^{131}I] 19-iodocholesterol intravenously, along with administration of SSKI or Lugol's solution. Serial adrenal imaging was performed on days 2–7 until optimal visualization was obtained. Renal outlines were defined by imaging after administration of either [^{197}Hg] chlormerodrin or later Tc-99m DMSA.

In vitro experiments. Male Sprague-Dawley rats (250–300 g) were injected intravenously with a 0.5-cc solution containing either 50 μCi of I-131 C (specific activity 1.8 μCi/μg) or [^{3}H] cholesterol (1.3 μCi/μg). The injectate was prepared by adding 3.0 cc of rat serum to the reconstituted cholesterol solution and diluting to the appropriate volume with normal saline. When [^{3}H] cholesterol was used, the benzene carrier was first removed by lyophilization. Each experiment involved a group of six rats with all determinations being performed upon individual animals. The animals were injected intravenously with either I-131 C or [^{3}H] cholesterol and then for the next 3 days they were given subcutaneously either 20 mg of aminoglutethimide twice a day, 4 units of ACTH get twice a day, or no treatment. On the fourth day, under ether anesthesia, the animals were exsanguinated by intracardiac puncture. Each pair of adrenals was promptly removed and placed in iced buffer (0.05 *M* sodium phosphate, 5 m*M* magnesium chloride, and 0.154 *M* sodium chloride at pH 7.5) (*7*). After weighing, the adrenals were homogenized using a hand-held Teflon homogenizer and were then subjected to differential centrifugation by a modification of the methods of Moses (*8*) and Garren (*7*) (Fig. 1). After diluting the adrenal homogenates to 5 cc with iced buffer in plastic ultracentrifuge tubes, duplicate aliquots were removed and counted in a NaI well counter for [^{131}I] idocholesterol, and in a liquid-scintillation counter for [^{3}H] cholesterol. This count multiplied by the dilution factor was used to represent total counts per homogenate. The remainder of the homogenate was centrifuged twice at 30,000 rpm for 30 min in an ultracentrifuge. The floating lipid layer was removed by a Pasteur pipette after each spin. The tube was punctured near its base and the supernatant aspirated. It was then cut in half and the residual lipid adhering to the sides was washed into counting vials containing the remainder of the lipid fraction. The pellet was resuspended and combined with supernatant. (As a check for loss of radioactivity due to adsorption on glass, the homogenizers were washed with either acetone or biosolve depending on the tracer used. This radioactivity represented less than 1% of the total counts initially present in the homogenate.) The "lipid-free" homogenate was centrifuged twice at 500 g for 10 min with an intervening resuspension of the pellet with buffer. The pellet obtained after this low-speed spin was quantitatively transferred to a counting vial and will be referred to as the crude cell fraction. The pellet obtained after centrifuging the supernatant twice at a higher speed (10,000 g for 10 min) consisted primarily of mitochondria. The counts remaining in the supernatant were considered to represent label associated with microsomes and soluble proteins. The above fractions were examined under an election microscope and the predominance of the expected organelles was confirmed. Enzymatic study of the microsomal-soluble protein supernatant (courtesy of Dr. Tony Bramley) demonstrated the absence of mitochondrial cytochrome oxidase, whereas microsomal NADPH cytochrome C reductase was readily identified. All glassware, pipettes, and centrifuge tubes were siliconized before each experiment. All stages of the above fractionation were either refrigerated or performed on ice.

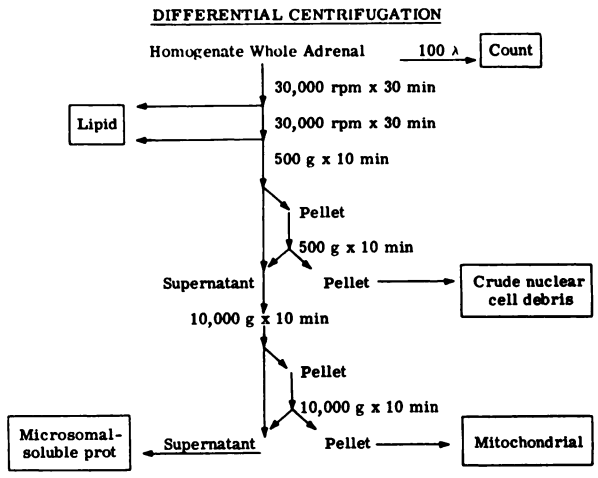

FIG. 1. Subcellular fractionation. All experiments were performed on pairs of adrenal glands. Boxes represent individual subcellular fraction that were counted.

Extraction with benzene (3:1 benzene:buffer) re-

TABLE 1. CLINICAL AND LABORATORY DATA

Patient No.	Age/sex	Side of tumor	Adrenal uptake	Lipid content of adenoma	Plasma corticoids AM/PM	2 mg dexamethasone	17 ketosteroids	17 ketogenic	Aldosterone
1	45/F	R	R	3+	22/28	1/4	13.7	3.6	2
2	56/F	L	L	4+	14/11	4/5	12.6	11.0	4
3	62/M	L	L	4+	12/6		7.5	9.2	3
4	58/F	L	L	4+	24/17		5.0	5.1	4
					Normals AM (7–28 μg/dl) PM (2–18 μg/dl)		Normals (4–17 mg/ 24 hr)	Normals (2–12 mg/ 24 hr)	Normals (2–16 μg/ 24 hr)

* Graded 0–4; 0 = no lipid, 4 = essentially complete replacement by lipid.

moved over 99% of the radioactivity present in the total homogenate (two rats) and in the lipid fraction (three rats) of animals previously injected with I-131 C. This confirmed that [^{131}I] iodide had not been removed from the cholesterol carrier during the preparative procedures.

To ensure that I-131 C did not distribute nonspecifically during processing of the fractions, 0.4 μCi of the iodocholesterol injectate was added to the homogenate of a previously unlabeled pair of adrenals. This homogenate was then processed as described above.

Designating cpm in the total homogenate as 100%, recoveries obtained by adding the cpm of the individual fractions varied from 86% to 120%. Distribution of each fraction was calculated both as percentage of the sum of the individual fractions and as cpm per milligram of adrenal wet weight. Comparisons were made by using the single tailed nonpaired student's t test. Results are expressed as mean ± s.d.

RESULTS

Clinical material. 1. *Nonfunctioning adenomas—clinical presentation.* Ten of 71 patients who had I-131 C adrenal scans performed at this institution were found to have both normal adrenal function and surgically proven adrenal cortical adenomas. Four of these 10 adenomas showed uptake of [^{131}I] cholesterol and were correctly located by adrenal scanning. Three of the four patients were women with ages ranging from 45 to 62 yr. Two of the four had mild hypertension that was unchanged by the operation. A suprarenal mass was initially discovered in three of the patients on a routine flat film. In the other, a mass was noted during a urogram performed as part of a workup for hypertension. As shown in Table 1, all four of these patients had normal plasma adenocortical steroids, and urinary 17-ketosteroids, 17-ketogenic steroids, and aldosterone. Normal diurnal variation in plasma corticoids was present in three of the four patients. In the fourth patient, dexamethasone suppression (2 mg daily) was normal. Renin (2.1 peripheral upright, right renal vein 3.6, left renal vein 4.6, IVC 3.5) available only in Patient #8, showed no evidence of suppression. Plasma sodium and potassium were normal in all patients. The adrenal scans are shown in Figure 2.

2. *Histologic evaluation.* The adenomas varied in size from 4 × 3.5 × 2.5 cm to 5.5 × 4.7 × 2.5 cm. In two of the patients the noninvolved adrenal was palpated at surgery and considered normal. Histologic section from three of the four adenomas dem-

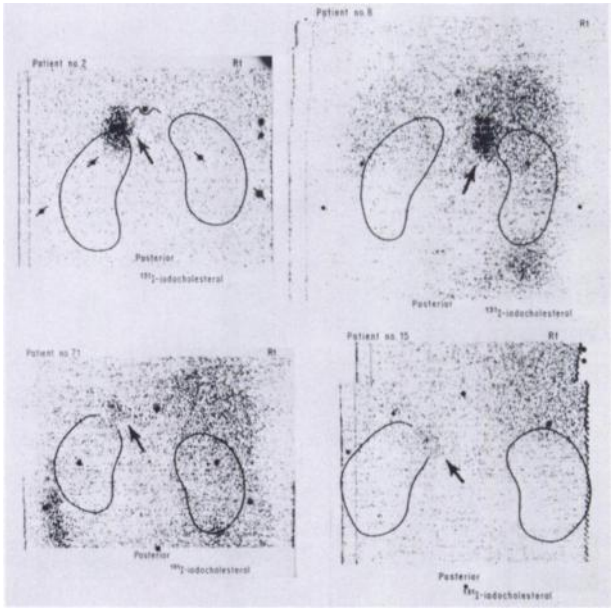

FIG. 2. Scans in four patients, none of whom had biochemical evidence of excess steroid production, yet adrenal imaging demonstrated enhanced uptake of [^{131}I] 19-iodocholesterol (arrows) on the side of the surgically verified tumor.

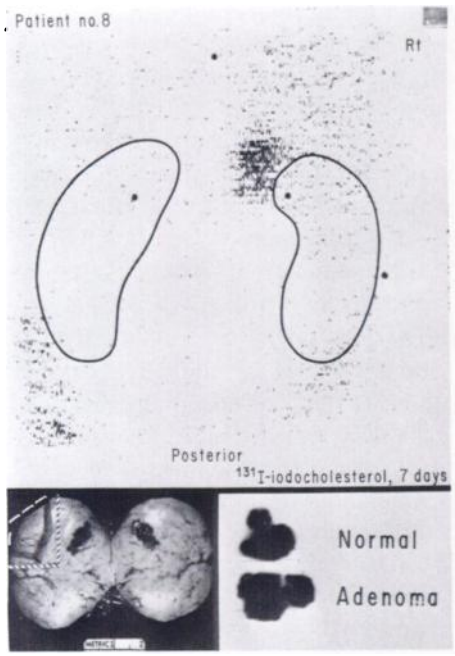

FIG. 3. Top: Adrenal scan of Patient #8. Bottom left: removed tumor. Right: autoradiogram of adenoma area (see box at left) for comparison with that of neighboring normal area.

onstrated increased lipid content, whereas the fourth showed complete absence of lipid. Autoradiography of the tumor and the normal adrenal of Patient #8 showed grossly equal radioactivity in each (Fig. 3), thereby confirming the presence of the I-131 C within the tumor.

Experimental animals. 1. *Subcellular sites.* The percentage distribution of I-131 C in the various cell fractions, and that of [^{3}H] cholesterol, is summarized in Table 2. The percentage distribution of I-131 C in the lipid fraction was slightly greater than that of [^{3}H] cholesterol (90 ± 2.3% against 86 ± 2.5%, $p < 0.005$). In contrast, the percentage distribution of I-131 C in the microsomal soluble protein fraction was less than that of [^{3}H] cholesterol (1.2 ± 0.5% against 3.4 ± 0.48%, $p < 0.001$). The percentage distribution of the mitochondrial and crude nuclear fractions was the same for both agents.

As shown in Table 2, nonspecific redistribution of I-131 C during preparation was minimal. Iodocholesterol added to the homogenate of a previously unlabeled adrenal fraction resulted in a distribution that differed markedly from that observed with the in vivo labeling.

2. *Aminoglutethimide.* This agent, known to block steroidogenesis and to cause an increase of adrenocortical lipid (*9*), resulted in a grossly lipoid appearance and in the expected increase in adrenal weight relative to that in the untreated animals. With either tracer (I-131 C or [^{3}H] C), this drug caused a significant increase in the absolute amount (cpm per mg of wet adrenal) present in the lipid fraction (Fig. 4). In addition, it resulted in a significant, but not dramatic, increase in the percentage of both I-131 C and [^{3}H] cholesterol found in the lipid fraction compared with that of untreated controls (I-131 C: 93 ± 1.5% against 90 ± 2.3%, $p < 0.05$; ^{3}H: 92 ± 1.3% against 86 ± 2.5%, $p < 0.001$). Thus, an agent that blocks steroidogenesis, thereby causing a compensatory increase in cholesterol uptake, also results in a cocomitant increase in iodocholesterol uptake.

3. *ACTH.* ACTH administration caused a small increase in adrenal weight relative to that of the untreated control. The percentage distribution in the lipid layer of either iodocholesterol of [^{3}H] cholesterol, relative to that of controls and to one another, was unchanged by treatment with ACTH. However, as noted in Fig. 5, treatment with ACTH significantly decreased the lipid content of [^{3}H] cholesterol, though it did not do this for [^{131}I] iodocholesterol. This would be consistent with an impaired mobilization of iodocholesterol from the lipid droplets.

DISCUSSION

Uptake of [^{131}I] 19-iodocholesterol by the adrenal cortex has been observed in both normal individuals and in patients suffering from a variety of conditions all of which have been characterized by excessive hormone production (*1–3*). Concentration of iodocholesterol by presumably "nonfunctioning" adrenal tumors has been reported by Beierwaltes et al. in two cases (*10*), although they could not obtain patho-

TABLE 2. RELATIVE DISTRIBUTION OF [^{131}I] 19-IODOCHOLESTEROL AND [7-^{3}H] CHOLESTEROL IN RAT ADRENAL GLANDS

Cell fraction	[^{131}I] 19-iodocholesterol (%)		[^{3}H] cholesterol (%)	Added* tracer
Lipid Droplet	90 ± 2.3	P < 0.005	86 ± 2.5	17
Mitochondrial	4.8 ± 1.3	N.S.	5.8 ± 0.6	1
Crude Nuclei & Cell Debris	3.5 ± 1.4	N.S.	4.7 ± 1.5	30
Microsomal & Soluble Protein	1.2 ± 0.52	P < 0.001	3.4 ± 0.48	52

* 0.4 μCi [^{131}I] 19-iodocholesterol added to homogenate of unlabeled adrenal gland.

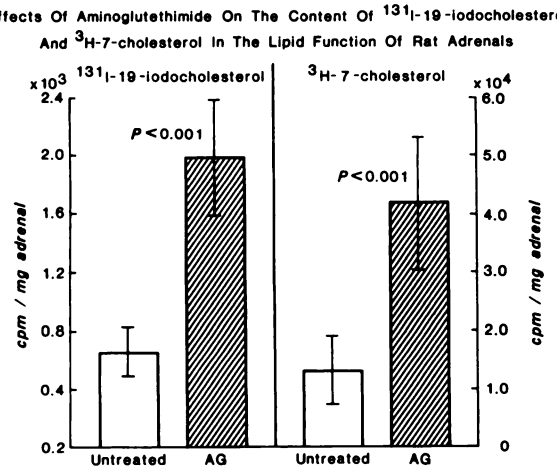

FIG. 4. Aminoglutethimide resulted in increase in content of either [^{131}I] 19-iodocholesterol [7-^{3}H] cholesterol present in lipid fraction of rat adrenal when compared with untreated controls. Data expressed as mean ± s.d.; n = six rats for I-131 C; n = five rats [7-^{3}H] C.

logic confirmation of the actual presence of tumors. In all four of our patients, plasma corticoids were normal, as were values for urinary 17-ketosteroids, 17-ketogenic steroids, and aldosterone. Peripheral and renal-vein renins, available in only one patient, showed no evidence of suppression. Iodocholesterol imaging was performed in an attempt to evaluate a suprarenal mass discovered fortuitously during other radiologic procedures. In all four, iodocholesterol correctly lateralized the tumor. In one, autoradiography confirmed that the tracer was present in the tumor. The scans in the other three strongly suggest that enhanced uptake by the tumor occurred.

It is possible that these "nonfunctioning" adenomas were producing corticosteroid metabolites not detected by the assays employed. The normal diurnal variation in plasma corticoids in three of the four

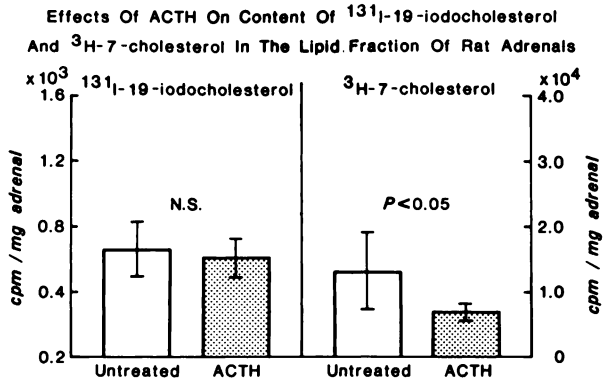

FIG. 5. ACTH decreased content of [7-^{3}H] cholesterol in lipid fraction of rat adrenals but did not affect the [^{131}I] 19-iodocholesterol when compared with untreated controls. Data expressed as mean ± s.d.; n = rats for each group.

patients, and the normal suppression with low doses of dexamethasone, argue against this possibility, since both findings imply dependence on ACTH secretion and would not be expected to occur if endogenous ACTH were already suppressed. Furthermore, peripheral and renal-vein renins, in the one patient from which they were obtained, showed no evidence of suppression. When additional similar patients are encountered, determination of serum ACTH concentration will be necessary to answer this question definitely.

The observation of a marked accumulation of lipid in three of the four adenomas suggested a possible mechanism for visualization of these tumors. Moses et al., using i.v. administration of [^{3}H] cholesterol combined with autoradiography and differential centrifugation, have shown that 75–80% of adrenal cholesterol is contained within the lipid droplets of rat adrenocortical cells (*8*). These lipid droplets presumably subserve a storage function, since ACTH decreases droplet cholesterol content (*7,9*), whereas inhibitors of steroid biosynthesis such as aminoglutethimide and cycloheximide cause an increase in accumulation of cholesterol in the lipid fraction (*9,11*). Thus, if the intracellular distribution of iodocholesterol is similar to that of cholesterol, adequate accumulation of the tracer in these lipid-rich tumors may be anticipated.

No data exist in the literature describing the intracellular location of iodocholesterol. In order to define this location we used in vivo labeling of rat adrenals with either iodocholesterol or [^{3}H] cholesterol, followed by differential centrifugation to separate the various subcellular components. These experiments demonstrated that the distribution of iodocholesterol closely mimics that of [^{3}H] cholesterol with the exception that the percentage distribution of iodocholesterol is slightly greater than that of [^{3}H] cholesterol but slightly less in the microsomal soluble-protein fraction. The results obtained for the [^{3}H] cholesterol distribution are similar to those reported by Moses et al. (*8*). That the radioactivity found in the lipid fraction represents iodocholesterol rather than free iodide is strongly suggested by its essentially complete extraction from the lipid fraction by benzene.

In order to examine whether the ability of the rat adrenal to concentrate iodocholesterol would persist when steroidgenesis is absent, treatment with aminoglutethimide was employed as a model. This drug blocks steroid biosynthesis, presumably by inhibiting the enzyme system involved in the conversion of cholesterol to pregnenolone (*12*). The glucocorticoid deficiency caused by the blockade results in a compensatory increase in endogenous ACTH, which in

turn increases cholesterol uptake. The blockade prevents further metabolism of cholesterol and results in increased adrenal cholesterol content (*9*). Treatment of rats with this agent, therefore, resulted in an increase in both the content and percentage distribution of iodocholesterol found in the lipid fraction. Changes of similar magnitude were also observed when [^{3}H] cholesterol was administered. This supports the tenet that it is cholesterol uptake, and not steroid production, that is necessary for concentration of iodocholesterol by the adrenal. The findings by others of partial enzymatic defects in adrenocortical neoplasms (*13*) support the concept that a discordance between cholesterol uptake and steroid biosynthesis may exist clinically.

Neither radioiodinated adrenal corticoids nor labeled excretory metabolites have been described after administration of iodocholesterol (*14*). Treatment with ACTH, as reported here and previously (*7*), caused a significant decrease in [^{3}H] cholesterol content in lipid droplets did not change the content or iodocholesterol in the lipid layer. The apparent inability of iodocholesterol to reach the mitochondrial and microsomal enzymatic sites necessary for steroid biosynthesis may offer an explanation why I-131-labeled adrenal corticoids have not been found.

The data presented here demonstrate that excess steroid hormone production is not necessary for iodocholesterol uptake, and they provide an explanation why certain biochemically "nonfunctioning" adrenocortical adenomas are visualized with iodocholesterol imaging. These data suggest that [^{131}I] iodocholesterol scanning is an appropriate technique for the location and identification of adrenocorticoid tissue, regardless of its functional state.

ACKNOWLEDGMENT

We wish to acknowledge the help of M. K. Dewanjee in the quality control of the radiopharmaceuticals used.

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