^{131}I-LABELED 19-IODINATED AND 6β-IODOMETHYL-19-NOR STEROIDS: EFFECT OF STRUCTURAL MODIFICATION ON THE ADRENAL ACCUMULATION

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ABSTRACT

19-Iodinated and 6β-iodomethyl-19-nor derivatives of cholesterol and 17-ketosteroid labeled with ^{131}I were tested in rats to determine the critical structural features required for maximal adrenal uptake. The introduction of the 17-keto group in place of the 17β-side chain of cholesterol caused most of the radioactivity to be taken up by the thyroids. Fluorination at the C-3 position had deleterious effects on the adrenal concentration and led to the loss of adrenal specificity. A β-hydroxy group at the C-3 position is substantially required for adrenal uptake.

INTRODUCTION

A variety of radioiodinated steroids have been investigated as potential adrenal imaging agents (1). The first successful clinical use of radioiodinated steroids in the detection and diagnosis of human adrenal disorders was with ^{131}I-labeled 19-iodocholesterol (CL-19-^{131}I) (2,3). The original idea for CL-19-^{131}I as a radiodiagnostic agent was based on the knowledge that the adrenal cortex stores cholesterol for ultimate use in corticosteroid hormone biosynthesis, and concentrates intravenously administered ^{14}C-labeled cholesterol to a greater extent than other steroid analogs (4).

Recently considerable interest in 6β-substituted 19-norcholest-5(10)-ene steroids has been stimulated by the discovery that ^{131}I-labeled 6β-iodomethyl-19-norcholest-5(10)-en-3β-ol (NCL-6-^{131}I), an isomer of 19-iodocholesterol, provides better human adrenal images than CL-19-^{131}I because of its strikingly increased adrenal uptake and

greater adrenal to non-target tissue ratios. NCL-6-^{131}I is now the most promising agent for clinical use (5-8).

Continued interest in preparing steroids labeled with other gamma emitters has resulted in the synthesis and clinical testing of ^{123}I-labeled NCL-6-I, which has provided high-quality images of adrenal glands in short intervals and reduced radiation doses to patients (9). ^{75}Se-labeled 6-methylselenomethyl-19-norcholest-5(10)-en-3β-ol has been also developed as a ^{75}Se-labeled agent for adrenal scintigraphy (10). More recently, the synthesis and tissue distribution studies of ^{123m}Te-labeled 23-(isopropyl telluro)-24-nor-5α-cholan-3β-ol have also been reported, and good adrenal uptake of this agent has been demonstrated in rats (11).

Our previous structure distribution studies with 6β-substituted 19-norcholesterols have shown that the 6β-methyl analog, the parent steroid of NCL-6-I, has a higher adrenal affinity than cholesterol itself (12). Furthermore, the replacement of iodine with bromine in NCL-6-I resulted in a decrease of the selective adrenal uptake (13). We have also reported that the addition of an ethyl group at the C-24 position in NCL-6-^{131}I did not decrease adrenal selectivity or concentration, but slightly raised its uptake by the liver (14). In a continuing effort to obtain further information regarding the structural requirements necessary for adrenal localization, the present paper studies the tissue distribution of some of structurally modified 19-iodinated and 6β-iodomethyl-19-nor steroids labeled with ^{131}I in rats (15), and compares them to the distribution pattern of NCL-6-^{131}I.

MATERIALS AND METHODS

Steroid Synthesis

All melting points are uncorrected. ^{1}H-NMR spectra were obtained with a JNM PS-100 spectrometer with tetramethylsilane as internal reference and ir spectra were taken on a JASCO IRA-1 spectrometer. Optical rotations were determined for solutions in chloroform at ambient temperature with a JASCO DIP-SL automatic polarimeter.

The radioiodinated steroids were purified by thin-layer chromatography (TLC)(silica gel 60F254, 0.25 mm, Merck), after removal of the reaction solvent, and analyzed by TLC using a radiochromatogram scanner (Aloka 101) and the same silica gel plates. In all cases, more than 95% of the radioactivity coincident with the spot corresponding to non-radioactive steroids was found in a single spot. The structural formulae of the radioiodinated steroids are shown in Fig. 1.

3β-Hydroxy-19-iodo-5-androsten-17-one acetate

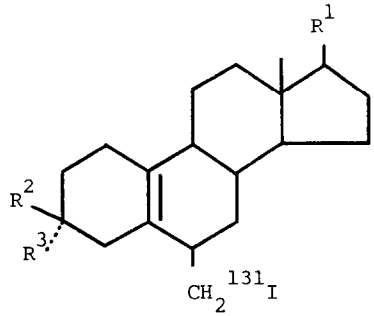
A solution of 3β,19-dihydroxy-5-androsten-17-one 3-acetate 19-*p*-toluenesulfonate (2.6 g)(16) in 2-propanol (200 ml) was heated under reflux for 3 hr with sodium iodide (2 g) under nitrogen. The solution was concentrated to a small volume *in vacuo* and extracted with a mixture of chloroform and water. The chloroform phase was successively washed with water, 1% sodium thiosulfate, and water, and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was recrystallized from acetone to give 3β-hydroxy-19-iodo-5-androsten-17-one acetate (1.1 g) as needles: m.p. 161°C; $[\alpha]_D^{20}$ = -14° (c 0.95); IR (Nujol) 1740 and 1260 cm^{-1}; ^{1}H-NMR($CDCl_3$) δ 1.00(3H, s, C-18 Me), 2.05 (3H, s, OCOMe), 3.27 and 3.65(2H, dd, C-19 CH_2I, J 11 Hz), 4.60(1H, m, C-3 H), and 5.75 ppm(1H, m, olefinic); Anal. Calcd. for $C_{21}H_{29}O_3I$: C, 55.27; H, 6.40. Found: C, 55.16; H, 6.42.

3β-Hydroxy-19-iodo-5-androsten-17-one (AO-19-I)

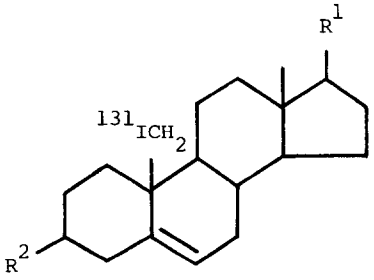
A solution of sodium hydroxide (100 mg) in 20% aqueous methanol (60 ml) was added dropwise to a solution of 3β-hydroxy-19-iodo-5-androsten-17-one acetate (1.48 g) in dioxane (50 ml). The reaction mixture was then stirred for 3.5 hr at room temperature and poured into ice-water and extracted with chloroform. The chloroform was then washed with water and dried (Na_2SO_4). Removal of the solvent gave AO-19-I (1 g) as colorless needles, m.p. 167°C, after recrystallization from chloroform: $[\alpha]_D^{20}$ = -18° (c 0.98); IR(Nujol) 1740 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 0.88(3H, s, C-18 Me), 3.30 and 3.65(2H, dd, C-19 CH_2I, J 11 Hz), 3.95 (1H, s, C-3 H), and 5.80 ppm(1H, m, olefinic); Anal. Calcd. for $C_{19}H_{27}O_2I$: C, 55.08; H, 6.57. Found: C, 55.27; H, 6.70. TLC Rf value: 0.19 [chloroform-acetone (95:5)], 0.42 [benzene-ethyl acetate (3:5)].

3β-Hydroxy-6β-iodomethyl-5(10)-estren-17-one (NAO-6-I)

A solution of AO-19-I (900 mg) in acetonitrile (80 ml) was refluxed for 10 hr. After complete removal of the solvent, the residue was repeatedly chromatographed on silica gel (100 mesh, Mallinckrodt) using chloroform to give NAO-6-I (400 mg) in the glassy state: $[\alpha]_D^{20}$ = +47.5° (c 1.23); IR(Nujol) 3400 and 1740 cm^{-1}; ^{1}H-NMR($CDCl_3$) δ 0.90(3H, s, C-18 Me), 1.78(1H, OH, D_2O exchangeable), 3.08(1H, t, J 10 Hz), 3.50 (1H, dd, J 10, 2.5 Hz), and 4.00 ppm(1H, m, C-3 H); Anal. Calcd. for $C_{19}H_{27}O_2I$: C, 55.08; H, 6.57. Found: C, 55.01; H, 6.69. TLC Rf value: 0.45 [benzene-ethyl acetate (3:5)], 0.22 [chloroform-acetone (95:5)].



NCL-6-^{131}I	: $R^1 = C_8H_{17}$	R^2 = OH	R^3 = H
NAO-6-^{131}I	: R^1 = =O	R^2 = OH	R^3 = H
αNCL-6-^{131}I	: $R^1 = C_8H_{17}$	R^2 = H	R^3 = OH
FNCL-6-^{131}I	: $R^1 = C_8H_{17}$	R^2 = F	R^3 = H
MNCL-6-^{131}I	: $R^1 = C_8H_{17}$	$R^2 = OCH_3$	R^3 = H



CL-19-^{131}I	: $R^1 = C_8H_{17}$	R^2 = OH
AO-19-^{131}I	: R^1 = =O	R^2 = OH
FCL-19-^{131}I	: $R^1 = C_8H_{17}$	R^2 = F

Fig. 1. Structural representation of ^{131}I-labeled steroids

^{131}I-3β-Hydroxy-19-iodo-5-androsten-17-one (AO-19-^{131}I)

A solution of AO-19-I (1.9 mg) and dry Na^{131}I (2.5 mCi) in dry acetone (2 ml) was refluxed for 4 hr under nitrogen. The crude product was subjected to TLC using chloroform-methanol (95:5) in the usual manner to give 645 μCi of AO-19-^{131}I, with specific activity of 500 mCi/mmol.

^{131}I-3β-Hydroxy-6β-iodomethyl-5(10)-estren-17-one (NAO-6-^{131}I)

NAO-6-I (2 mg) was reacted with dry Na^{131}I (2.3 mCi) in acetonitrile (2 ml) for 4.5 hr at reflux temperature and the crude product chromatographed on silica gel using chloroform-methanol (95:5) to give 943 μCi of NAO-6-^{131}I, with specific activity of 416 mCi/mmol.

^{131}I-3β-Fluoro-19-iodocholest-5-ene (FCL-19-^{131}I)

A solution of 3β-fluoro-19-iodocholest-5-ene (1.5 mg)(17) and dry Na^{131}I (3.2 mCi) in dry acetone (2 ml) was refluxed for 4 hr under nitrogen. TLC using chloroform-acetone (95:5) gave 190 μCi of FCL-19-^{131}I, with specific activity of 360 mCi/mmol.

^{131}I-6β-Iodomethyl-19-norcholest-5(10)-en-3α-ol (αNCL-6-^{131}I)

This labeled steroid was prepared as described earlier (18), and had specific activity of 460 mCi/mmol.

^{131}I-3β-Fluoro-6β-iodomethyl-19-norcholest-5(10)-ene (FNCL-6-^{131}I)

A solution of 3β-fluoro-6β-iodomethyl-19-norcholest-5(10)-ene (1.1 mg)(17) and dry Na^{131}I (2.6 mCi) in acetonitrile (2 ml) was refluxed for 12 hr and the crude product chromatographed on silica gel using n-hexane-benzene (4:1) to afford 1.05 mCi of FNCL-6-^{131}I, with specific activity of 824 mCi/mmol.

^{131}I-3β-Methoxy-6β-iodomethyl-19-norcholest-5(10)-ene (MNCL-6-^{131}I)

3β-Methoxy-6β-iodomethyl-19-norcholest-5(10)-ene (2 mg)(17) and dry Na^{131}I (2.4 mCi) was heated at 90°C for 1 hr in a sealed tube. The crude product was chromatographed on silica gel using chloroform-acetone (95:5) to give 210 μCi of MNCL-6-^{131}I, with specific activity of 530 mCi/mmol.

Stability of ^{131}I-Labeled Steroids

The radioiodinated steroids obtained were dissolved in ethanol, and polysorbate 80 (3-5%) and sufficient 0.9% NaCl were added to give 3-5% ethanol solutions (αNCL-6-^{131}I was formulated as a 26% ethanol solution) having a radioactive concentration of about 0.1 mCi/ml. The radiochemical purity of the ^{131}I-labeled steroids solutions through the course of storage at 5° and 20°C in the dark was checked by TLC using a radiochromatogram scanner.

Tissue Distributions in Rats

The preparations were administered by intraveneous route to male Wistar rats weighing 120-220 g. Rats received through the tail vein a dose of 10-20 μCi/animal. Groups of two or three rats were killed at various time intervals after the injection by cutting off the carotid artery under ether anesthesia after the administration of heparin. Immediately after death, samples of blood were taken and major organs

were minced with scissors. Samples of tissues were counted in an automatic gamma well counter (Aloka JDG-752), and were corrected for radioactive decay and counting efficiencies. The concentration in each organ was expressed as percentage of injected dose per gram. Each voiding of urine and feces was also collected separately and assayed for radioactivity.

RESULTS AND DISCUSSION

Table 1 shows the change of purity of the formulated ^{131}I-labeled steroids during the storage at 5° and 20°C in the dark. ^{131}I-3β-Fluoro-19-iodocholest-5-ene (FCL-19-^{131}I) and ^{131}I-3β-methoxy-6β-iodomethyl-19-norcholest-5(10)-ene (MNCL-6-^{131}I) were relatively stable and showed only 10% decomposition after 6 days at 20°C. On the other hand, a marked loss of radioactivity of ^{131}I-3β-hydroxy-19-iodo-5-androsten-17-one (AO-19-^{131}I) was observed with the elevation of temperature; even after storage at 5°C, 50% of deiodination occurred in 3 days. ^{131}I-3β-Hydroxy-6β-iodomethyl-5(10)-estren-17-one (NAO-6-^{131}I) maintained its radiochemical purity more than 95% even after 12 days at 5°C, though 72% of its radioactivity was lost in 6 days at 20°C. When stored at 5°C, ^{131}I-6β-iodomethyl-19-norcholest-5(10)-en-3α-ol (αNCL-6-^{131}I) and ^{131}I-3β-fluoro-6β-iodomethyl-19-norcholest-5(10)-ene (FNCL-6-^{131}I) retained more than 95% radiochemical purity for 12 days.

The distribution of radioactivity in the tissues of male rats was determined at time intervals varying 0.5 hr to 7 days following i.v. administration of the ^{131}I-labeled steroids, and the results are summarized in Table 2. The adrenal to non-target ratios for several selected tissues are presented in Table 3. In Fig. 2, the percentage injected dose values for the labeled steroids in the adrenal glands of rats are compared with our previous data reported for NCL-6-^{131}I and

Table 1. Radiochemical purity (%) of the radioiodinated steroids at various time intervals*

Steroids	Storage temperature(°C)	Days after preparation 0	2	3	4	6	12
AO-19-^{131}I	5	>95		50			
	20	>95		0			
NAO-6-^{131}I	5	>95			>95	>95	>95
	20	>95			41	28	8
FCL-19-^{131}I	5	>95			>95	90	90
	20	>95				89	
αNCL-6-^{131}I	5	>95	>95		>95		>95
	20	>95	>95		>95		80
FNCL-6-^{131}I	5	>95	>95		>95	>95	>95
	20	>95	>95		>95	>95	>95
MNCL-6-^{131}I	5	>95	90		90	90	90
	20	>95	90		90	90	90

* The radiochemical purity (%) was determined by thin-layer chromatography on silica gel 60F 254 (Merck). As developing solvent system, chloroform-methanol (95:5) for AO-19-^{131}I and NAO-6-^{131}I, and chloroform-acetone (95:5) for the others were used.

CL-19-^{131}I (5).

AO-19-^{131}I and NAO-6-^{131}I showed essentially no adrenal uptake and most of the radioactivity was found in the thyroids.

Previous studies have shown that the ring A in 5(10)-unsaturated steroids is an inverted half-chair and NCL-6-^{131}I has the β-hydroxy group at the C-3 position (18,19). The 3-hydroxy group in CL-19-^{131}I is also β-oriented. Thus, it became necessary to ascertain the configurational requirement of the hydroxy group at the C-3 position in 19-norcholesterol for adrenal affinity. Comparison of the tissue distribution data for αNCL-6-^{131}I and NCL-6-^{131}I demonstrates that the β-hydroxy group at the C-3 position is required for maximal adrenal uptake. αNCL-6-^{131}I showed a considerable selective localization of radioactivity in adrenals, though level of uptake was highest in the thyroid. However, the adrenal concentration of radioactivity is less

Table 2. Distribution of radioactivity in rat tissues at time intervals after i.v. administration of the ^{131}I-labeled steroids[a]

Tissue	^{131}I-steroids	Time after injection								
		0.5 hr	1 hr	3 hr	6 hr	12 hr	1 day	3 day	5 day	7 day
Adrenal	AO-19-^{131}I		0.13	0.40	0.11	0.03	0.02	0.01		
	NAO-6-^{131}I		1.17	0.38	0.30	0.13	0.21	0.06		
	FCL-19-^{131}I		9.51	11.68	16.66	23.89	22.88	14.74	3.61	1.30
	αNCL-6-^{131}I						33±3	69±14	68±19	99±17
	FNCL-6-^{131}I	6.56±0.44	7.83±0.49	8.08±3.16	10.57±0.69	13.77±0.56	20.64±4.03	12.71±1.67	5.96±0.97	3.45±0.29
	MNCL-6-^{131}I						26.78±2.08	41.85±8.42	21.27±5.41	12.72±3.62
Liver	AO-19-^{131}I		0.42	0.18	0.13	0.06	0.02	0.01		
	NAO-6-^{131}I		1.73	0.44	0.26	0.08	0.05	0.03		
	FCL-19-^{131}I		7.54	8.61	8.25	5.27	6.31	0.94	0.48	0.18
	αNCL-6-^{131}I						2.84±0.57	1.29±0.13	0.71±0.15	0.42±0.09
	FNCL-6-^{131}I	4.94±0.42	5.55±0.59	7.97±1.81	9.32±0.95	8.86±0.26	7.45±0.89	3.01±0.74	0.88±0.11	0.49±0.11
	MNCL-6-^{131}I						7.59±0.21	2.45±0.32	1.23±0.39	0.33±0.07
Kidney	AO-19-^{131}I		0.24	0.51	0.17	0.08	0.03	0.01		
	NAO-6-^{131}I		1.40	0.51	0.32	0.14	0.05	0.02		
	FCL-19-^{131}I		0.82	0.66	0.69	0.57	0.56	0.24	0.18	0.09
	αNCL-6-^{131}I						1.64±0.24	1.29±0.15	0.96±0.08	0.73±0.08
	FNCL-6-^{131}I	1.06±0.08	1.04±0.05	0.64±0.22	0.46±0.05	0.40±0.05	0.58±0.06	0.50±0.02	0.32±0.01	0.27±0.04
	MNCL-6-^{131}I						0.80±0.01	0.71±0.09	0.61±0.11	0.27±0.04
Spleen	AO-19-^{131}I		0.16	0.35	0.12	0.06	0.02	0.01		
	NAO-6-^{131}I		0.72	0.47	0.27	0.08	0.04	0.01		
	FCL-19-^{131}I		40.28	30.49	33.79	16.03	7.15	0.75	0.26	0.15
	αNCL-6-^{131}I						9.08±1.30	2.18±0.25	1.08±0.16	0.68±0.19
	FNCL-6-^{131}I	4.29±1.12	26.42±1.12	44.96±18.9	25.78±1.84	46.02±2.04	18.88±4.86	2.91±1.67	0.62±0.09	0.24±0.06
	MNCL-6-^{131}I						10.62±2.22	1.91±0.19	0.86±0.16	0.33±0.02
Testicle	AO-19-^{131}I		0.16	0.27	0.14	0.06	0.02	0.01		
	NAO-6-^{131}I		0.53	0.32	0.24	0.07	0.02	0.01		
	FCL-19-^{131}I		0.11	0.16	0.23	0.22	0.28	0.12	0.08	0.04
	αNCL-6-^{131}I						0.37±0.33	0.27±0	0.22±0.04	0.19±0.01
	FNCL-6-^{131}I	0.08±0	0.08±0.01	0.08±0.01	0.11±0.01	0.12±0.02	0.26±0.03	0.25±0.01	0.15±0.01	0.11±0.02
	MNCL-6-^{131}I						0.41±0.04	0.35±0.04	0.27±0	0.11±0.02

Table 2. Distribution of radioactivity in rat tissues at time intervals after i.v. administration of the ^{131}I-labeled steroids[a] (continued)

Tissue	^{131}I-steroids	Time after injection								
		0.5 hr	1 hr	3 hr	6 hr	12 hr	1 day	3 day	5 day	7 day
Blood	AO-19-^{131}I		0.87	0.36	0.28	0.12	0.04	0.01		
	NAO-6-^{131}I		1.32	0.80	0.54	0.15	0.06	0.02		
	FCL-19-^{131}I		6.27	3.22	2.28	1.14	0.78	0.13	0.07	0.03
	αNCL-6-^{131}I						2.41±0.29	0.80±0.06	0.39±0.07	0.22±0
	FNCL-6-^{131}I	6.89±0.10	7.20±0.96	3.08±1.10	1.74±0.16	0.93±0.07	0.96±0.10	0.29±0.06	0.09±0	0.05±0.01
	MNCL-6-^{131}I						1.08±0.10	0.41±0.08	0.20±0.06	0.05±0.01
Thyroid	AO-19-^{131}I		74	36	118	166	204	81		
	NAO-6-^{131}I		32	182	303	339	384	303		
	FCL-19-^{131}I		5	9	27	54	138	193	242	148
	αNCL-6-^{131}I						45±7	98±8	69±7	91±6
	FNCL-6-^{131}I	5±0	2±0	6±1	7±1	29±4	96±3	284±57	243±57	235±44
	MNCL-6-^{131}I						84±8	158±15	132±26	86±34
Urine[b]	AO-19-^{131}I						69	81	88	
	NAO-6-^{131}I						68	73	68	
	FCL-19-^{131}I						10	29	31	41
	αNCL-6-^{131}I						7	13	18	19
	FNCL-6-^{131}I						8	20	27	31
	MNCL-6-^{131}I						8	15	17	22
Feces[b]	AO-19-^{131}I						2	3	9	
	NAO-6-^{131}I						9	9	8	
	FCL-19-^{131}I						7	30	24	30
	αNCL-6-^{131}I						7	21	28	35
	FNCL-6-^{131}I						6	22	26	26
	MNCL-6-^{131}I						8	25	33	51

a Values represent mean % dose/g of tissue for three rats with SD of mean with αNCL-6-^{131}I, FNCL-6-^{131}I and MNCL-6-^{131}I. With AO-19-^{131}I, NAO-6-^{131}I and FCL-19-^{131}I, values represent mean % dose/g of tissue for two rats.

b The excretion of radioactivity in urine and feces was expressed as a percentage of injected dose.

Table 3. Adrenal-to-tissue ratios after i.v. administration of the ^{131}I-labeled steroids*

^{131}I-Steroids	Time after injection	Adrenal-to-tissue concentration ratios			
		Liver	Kidney	Spleen	Blood
AO-19-^{131}I	1 hr	0.3	0.5	0.8	0.1
	3 hr	2.2	0.8	1.1	1.1
	6 hr	0.8	0.6	0.9	0.4
	12 hr	0.5	0.4	0.5	0.3
	1 day	1.0	0.7	1.0	0.5
	3 days	1.0	1.0	1.0	1.0
NAO-6-^{131}I	1 hr	0.7	0.8	1.6	0.9
	3 hr	0.9	0.7	0.8	0.5
	6 hr	1.2	0.9	1.1	0.6
	12 hr	1.6	0.9	1.6	0.9
	1 day	4.2	4.2	5.2	3.5
	3 days	2	3	6	3
FCL-19-^{131}I	1 hr	1.2	11.5	0.2	1.5
	3 hr	1.3	17.6	0.4	3.6
	6 hr	2.0	24.1	0.5	7.3
	12 hr	4.5	41.9	1.4	20.9
	1 day	3.6	40.8	3.2	29.3
	3 days	15.6	61.4	19.6	113
	5 days	7.5	20.0	13.8	51.5
	7 days	7.2	14.4	8.6	43.3
αNCL-6-^{131}I	1 day	11.6	20.1	3.6	13.7
	3 days	53.4	53.4	31.6	86.2
	5 days	95.7	70.8	62.9	86.2
	7 days	235	135	145	450
FNCL-6-^{131}I	0.5 hr	1.3	6.1	1.5	1.0
	1 hr	1.4	7.5	0.3	1.0
	3 hr	1.0	12.6	0.2	0.4
	6 hr	1.1	22.9	0.4	6.0
	12 hr	1.5	34.4	0.3	14.8
	1 day	2.7	35.5	1.1	21.5
	3 days	4.2	25.4	4.3	43.8
	5 days	6.7	18.6	9.6	66.2
	7 days	7.0	12.7	14.3	69.0
MNCL-6-^{131}I	1 day	3.5	33.4	2.5	24.7
	3 days	17.0	58.9	21.9	102
	5 days	17.2	34.8	24.7	106
	7 days	53.6	65.6	53.6	354

*Values are calculated from %dose/g data summarized in Table 2.

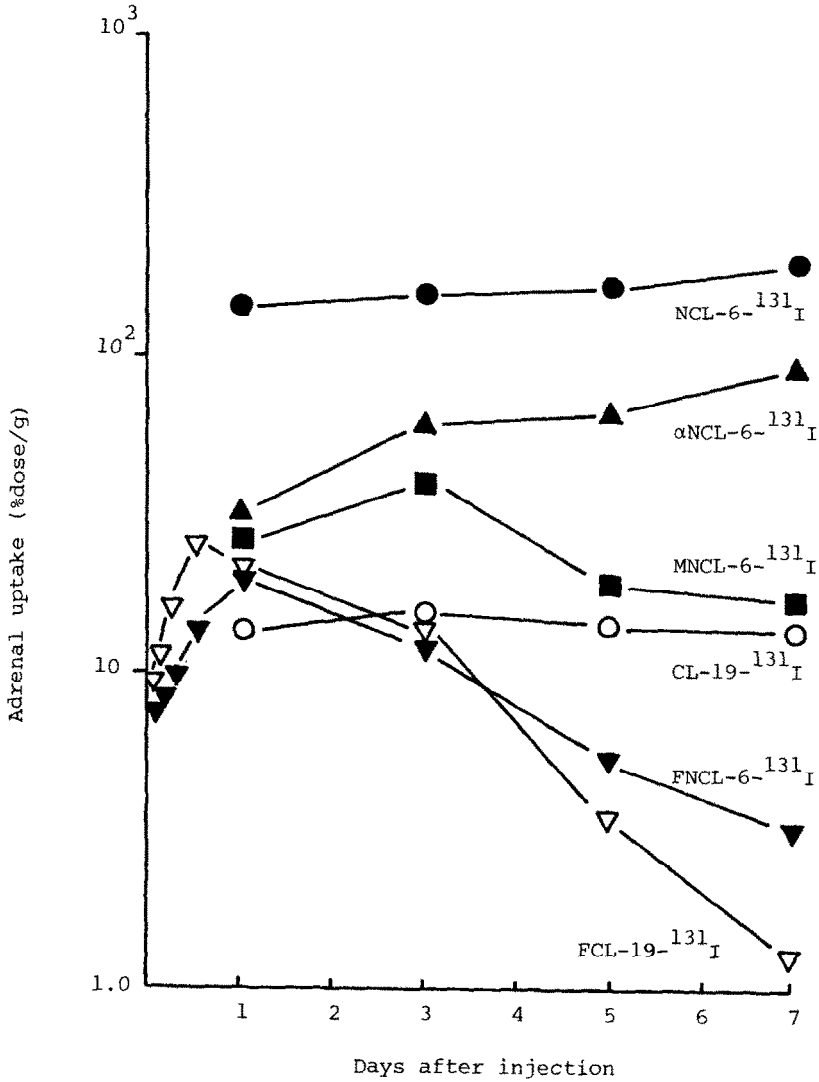


Fig. 2. Comparison of adrenal uptake after i.v. administration of the ^{131}I-labeled steroids. Detailed description of the tissue distribution data for NCL-6-^{131}I and CL-19-^{131}I is included in our previous paper (ref. 5)

than that achieved with NCL-6-^{131}I over the 7-day period (5). Also the concentration of αNCL-6-^{131}I in the liver, spleen, and blood was few times higher than that observed with NCL-6-^{131}I.

The importance of hydroxy group at the C-3 position for adrenal affinity was further supported by the result of tissue distribution study with MNCL-6-^{131}I. MNCL-6-^{131}I showed only moderate adrenal accumulation at 3 days after administration, indicating that the introduction of methoxy group in place of hydroxy group leads to the partial loss of adrenal specificity, although the adrenal concentration of MNCL-6-^{131}I was comparable to that of CL-19-^{131}I (5). Furthermore, in contrast with NCL-6-^{131}I, the adrenal uptake of MNCL-6-^{131}I started to decrease significantly at 3 days after injection. Fukushi, Umeda, and Ito et al (20) have noted that nonesterified NCL-6-^{131}I is incorporated by adrenal tissue. Once in the adrenals, however, NCL-6-^{131}I is rapidly esterified and stored. Thus, the substantially decreased adrenal specificity of MNCL-6-^{131}I can be attributed to the lack of the hydroxy group at the C-3 position.

While it is known that fluorination at a specific site in a steroid molecule enhances the biological activity, little has been reported about the effects of fluorination on tissue distribution. Furthermore, the availability of ^{18}F (β^+ decay, $T_{1/2}$= 110 min) for radiopharmaceutical synthesis meant that cholesterol analogs suitably labeled with this nuclide would have potential diagnostic value in the adrenals. Therefore, we investigated the effects of fluorination at the C-3 position in NCL-6-^{131}I and CL-19-^{131}I on adrenal affinity. Unfortunately, the disposition patterns of FCL-19-^{131}I and FNCL-6-^{131}I were considerably different from those of the other ^{131}I-labeled

steroids: the adrenal uptake of FCL-19-^{131}I and FNCL-6-^{131}I reached a maximum of about 20% dose/g within 12-24 hr and started to diminish after 1 day. Over a 7-day period, at no time intervals was the adrenal-to-liver ratio greater than 15. Thus, the lack of localization and selectivity in the adrenals of both FCL-19-^{131}I and FNCL-6-^{131}I indicates that the introduction of fluorine in place of hydroxy group at the C-3 position results in the loss of adrenal specificity.

The data obtained in this investigation and previous structure-distribution studies shows that the structural features required for good adrenal uptake in rats of 19-norcholesterol analogs include a 3β-hydroxy group, a 17β-side chain of the cholesterol type, and 6β-iodomethyl or selenomethyl group. However, the limitations of diagnostic imaging of the adrenal glands with radioiodinated NCL-6-I are the time required (2-7 days) to complete the study and the prolonged biological half-life in adrenals, all which are undesirable.

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