SELENIUM-75-19-SELENOCHOLESTEROL— A NEW ADRENAL SCANNING AGENT WITH HIGH CONCENTRATION IN THE ADRENAL MEDULLA

Salil D. Sarkar, Rodney D. Ice, William H. Beierwaltes, Satinder P. Gill, Suppiah Balachandran, and Garabed P. Basmadjian

University of Michigan Medical Center, Ann Arbor, Michigan

A tissue distribution study with ^{75}Se-19-selenocholesterol in rats, rabbits, and dogs showed high adrenal concentrations and good adrenal images. In the dog, higher concentrations were obtained in the adrenal medulla than in the cortex at Days 1 and 7 after dosing. Extraction and thin-layer chromatography of the adrenal lipid in dogs given this compound showed that ^{75}Se in the adrenal is still attached to the steroid moiety. A reduction in production costs is expected from its longer shelf life. Selenium-75-19-selenocholesterol is being evaluated in humans not only for routine use as a adrenal cortex scanning agent, but also for the detection of pheochromocytomas and other sympathetic tissue tumors, especially neuroblastomas.

The value of ^{131}I-19-iodocholesterol as an adrenal scanning agent has been shown in the assessment of patients suspected of having Cushing's syndrome (*1–3*), aldosteronism (*4–6*), pheochromocytomas (*7*), and adrenal remnants following "total" adrenalectomy (*8*). The physical properties of ^{131}I, however, make it less than ideal as a label. A more suitable label might be ^{75}Se, which offers several advantages over ^{131}I (*9*): a lower beta absorbed dose (7% that of ^{131}I) and a higher usable photon yield (1.74 photons per disintegration at energies between 100 and 400 keV, compared to 0.92 for ^{131}I). This may permit a decrease of the administered dose. The longer physical half-life will affect the absorbed dose but will also increase shelf life and reduce patient costs.

The present tissue distribution study was undertaken to evaluate the efficacy of ^{75}Se-19-selenocholesterol (NP-65) as a potential adrenal scanning agent. That the adrenal medulla in dogs took up higher concentrations of NP-65 than the adrenal cortex was an unexpected finding.

MATERIALS AND METHODS

Radiopharmaceutical. Selenium-75-19-selenocholesterol in ethanol was obtained from Amersham/Searle Corp. (Arlington Heights, Ill.). Ten millicuries of the ethanolic solution were evaporated to dryness under vacuum. To the dry NP-65 was added 0.4 ml of 20% polysorbate-80 in ethanol; the vial was swirled until the NP-65 dissolved. Then 0.2 ml of ascorbic acid injection, U.S.P., was added as an antioxidant, followed by 3.9 ml of bacteriostatic sodium chloride, U.S.P. Radiochemical purity was established with thin-layer chromatography using: a) silica gel G, chloroform:ethanol, 1:1, $R_f = 0.64$; and b) silica gel G, 100% chloroform, $R_f = 0.32$. These are the same solvent systems used to separate 6-iodomethylcholesterol from 19-iodocholesterol (*10*). The method of preparation, as well as analytic measurements using infrared and nuclear magnetic resonance, ensured that the label was at the 19-position of the compound. The only observed threat to purity, 19-selenoxycholesterol, was prevented from forming by adding ascorbic acid to the formulation. Figure 1 shows the chemical structure of ^{75}Se-19-selenocholesterol (NP-65), together with that of ^{131}I-19-iodocholesterol (NM-145) for comparison.

Rats. *Intravenous dose.* Fifty-five mature female Sprague–Dawley rats, weighing 200–260 gm, were each given 25 μCi of ^{75}Se-19-selenocholesterol (sp. act. 4.2–5.5 mCi/mg) through a femoral vein after intraperitoneal sodium pentobarbital anesthesia (0.05 mg/gm body wt). The rats fasted for 12 hr before and 6 hr after administration of the dose. Six additional female rats were similarly given 25 μCi of ^{131}I-19-iodocholesterol (sp. act. 1.3 mCi/mg) to

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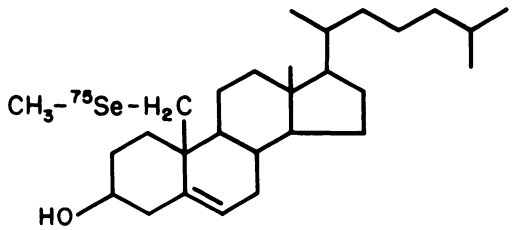
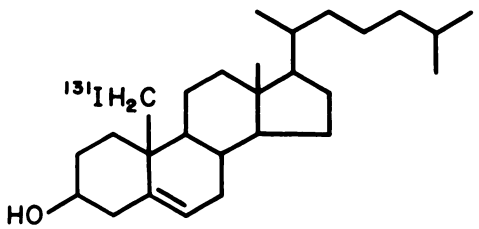
For reprints contact: William H. Beierwaltes, Nuclear Medicine Sect., Univ. of Michigan Medical Center, Ann Arbor, Mich. 48104.



^{75}Se-19-Selenocholesterol
(^{75}Se-19-Methylselenocholest-5(6)-en-3β-ol)



^{131}I-19-Iodocholesterol
(^{131}I-19-Iodocholest-5(6)-en-3β-ol)

FIG. 1. Structural formulas of NP-65 and NM-145 (for comparison).

compare relative product uptakes using the same techniques.

Oral dose. Fifty mature female Sprague–Dawley rats were given 25 μCi of ^{75}Se-19-selenocholesterol by oral gavage, other conditions remaining the same as for the intravenous doses (except for the lack of sodium pentobarbital anesthesia before dosing).

Rabbits. Each of 22 mature female white rabbits, weighing 3.3–4.5 kg, was given 400 μCi of ^{75}Se-19-selenocholesterol (sp. act. 11.2 mCi/mg) through an ear vein.

Dogs. Each of 27 mature female mongrel dogs, weighing 7–14 kg, received 100 μCi/kg body wt of ^{75}Se-19-selenocholesterol (sp. act. 4.2–5.5 mCi/mg) through a cephalic vein. All of the animals in this study were fed regular commercial food and were not treated with any drug other than the sodium pentobarbital.

Adrenal scans. Adrenal scintiscans were performed on the dogs at various time intervals after dosing and just before death. The scans were obtained with the dogs in the prone position, under intravenous sodium pentobarbital anesthesia, using a Picker rectilinear scanner (Mentor, Ohio) equipped with a 5-in. crystal and a medium-energy 3-in. fine-focus collimator.

Animal sacrifice. The animals were killed at 2, 6, and 12 hr after dosing, and at Days 1, 2, 4, 5, 7, 10, 15, and 20. The 2-hr interval was omitted in the rats dosed orally, whereas the rats receiving ^{131}I-19-iodocholesterol were killed at Days 1 and 5 only. The rats were killed by excising the heart under ether anesthesia, and the rabbits and dogs were killed with an intravenous injection of sodium pentobarbital. Five rats (three each for rats receiving ^{131}I-19-iodocholesterol), two rabbits, and two dogs were killed at each of the stated time intervals. Additional dogs were killed at Days 1, 2, 4, 5, and 7.

Tissue samples. Routinely, 18 tissues were obtained from the rats including the adrenals, thyroid, liver, spleen, kidney, ovaries, stomach, small intestines, large intestines, pancreas, lungs, heart, brain, parotids, fat, muscle, blood, and urine. Gallbladder and bile were also obtained in the rabbit and dog. Duplicate samples of all tissues were obtained in the dog, and the adrenal cortex and medulla were separated by placing the adrenal gland on dry ice immediately after removal, then sectioning it sagittally and scooping out the medulla.

Measurement of radioactivity. Tissue samples from rats, rabbits, and dogs were cleaned of adipose and connective tissue and weighed. They were then placed in tubes to which 2.5 ml of distilled water was added and counted in an automatic gamma well counter for 10 min. Correction was made for radioactive decay and counter efficiency. Additionally, tissue radioactivity in a rat was measured similarly in samples of whole blood, red blood cells, plasma, and plasma proteins (obtained by their precipitation with 20% trichloroacetic acid) at Day 3 after dosing.

The tissue concentrations, expressed as percent kilogram dose per gram (*11*), were calculated as follows:

$$\frac{\mu\text{Ci in organ}}{\mu\text{Ci in dose}} \times \frac{\text{body weight (kg)}}{\text{organ weight (gm)}} \times 100 = \%\ \text{kg dose/gm.}$$

The peak adrenal concentrations were also expressed as percent dose per total organ, which was obtained as follows:

$$\frac{(\%\ \text{kg dose/gm}) \times \text{organ wt (gm)}}{\text{body wt (kg)}} = \%\ \text{dose/total organ.}$$

Standard adrenal weights were used: 0.05 gm/100 gm body wt in rats and 0.01 gm/100 gm body wt in rabbits and dogs.

Extraction and separation of radioactive products from adrenals. Total lipid extraction, using the method of Folch et al (*12*), was carried out on several adrenal samples from dogs. Tissue samples of adrenal cortex and medulla were weighed and homogenized in 7.0 ml of absolute methanol per gram of tissue for 3 min. Chloroform (14 ml/gm tissue) was added to the homogenates and the sample was allowed to extract for 1 hr under constant agitation.

A lipid and nonlipid biphasic system was then obtained by adding 0.2 ml of water per gram of tissue sample and centrifuging at 500 rpm for 10 min. Aliquots of the chloroform (lipid) and methanol–water (nonlipid) fractions were counted in an automatic gamma well counter. The proportion of radioactivity in the lipid fraction was calculated. Thin-layer chromatography (silica gel G, 100% $CHCl_3$) was also performed on the lipid fraction.

RESULTS

Rats. *Intravenous dose.* Table 1 presents the relative tissue distribution of ^{75}Se-19-selenocholesterol and ^{131}I-19-iodocholesterol in rats. The highest con-

TABLE 1. DISTRIBUTIONS OF ^{75}Se-19-SELENOCHOLESTEROL AND ^{131}I-19-IODOCHOLESTEROL IN FEMALE RATS* (% kg dose/gm ± 1 s.e.m.)

	^{75}Se-selenocholesterol								^{131}I-19-iodocholesterol*	
Tissues	Day 1	Day 2	Day 4	Day 5	Day 7	Day 10	Day 15	Day 20	Day 1	Day 5
Adrenals	4.302	5.490	6.696	4.763	5.472	5.596	5.879	4.041	2.347	2.508
	±0.396	±0.273	±0.263	±0.316	±0.522	±0.207	±0.340	±0.357	±0.131	±0.187
Liver	0.319	0.192	0.116	0.102	0.050	0.052	0.040	0.036	0.120	0.128
	±0.015	±0.010	±0.003	±0.009	±0.012	±0.002	±0.002	±0.007	±0.028	±0.001
Spleen	0.167	0.255	0.146	0.094	0.077	0.055	0.038	0.030	0.141	0.011
	±0.120	±0.012	±0.004	±0.017	±0.004	±0.002	±0.001	±0.002	±0.027	±0.001
Blood	0.151	0.117	0.050	0.039	0.001	0.020	0.014	0.011	0.080	0.003
	±0.015	±0.009	±0.003	±0.003	±0.000	±0.001	±0.002	±0.001	±0.024	±0.001
Kidneys	0.108	0.153	0.150	0.122	0.115	0.112	0.091	0.066	0.050	0.008
	±0.012	±0.012	±0.021	±0.016	±0.013	±0.005	±0.002	±0.004	±0.011	±0.001
Lungs	0.703	0.397	0.313	0.226	0.150	0.089	0.063	0.042	0.137	0.012
	±0.048	±0.026	±0.032	±0.015	±0.014	±0.004	±0.003	±0.004	±0.023	±0.001
Thyroid	0.103	0.111	0.077	0.061	0.045	0.028	0.024	0.018	43.604	17.983
	±0.011	±0.007	±0.003	±0.005	±0.004	±0.007	±0.006	±0.001	±4.174	±2.003
Ovaries	4.286	3.485	4.096	2.573	2.248	2.045	1.302	1.462	1.024	0.642
	±0.472	±0.150	±0.327	±0.141	±0.264	±0.054	±0.045	±0.102	±0.073	±0.058
Heart	0.207	0.136	0.122	0.083	0.060	0.036	0.022	0.016	0.041	0.005
	±0.001	±0.042	±0.003	±0.010	±0.005	±0.010	±0.001	±0.001	±0.007	±0.001
Small intestine	0.202	0.185	0.124	0.076	0.059	0.043	0.029	0.024	0.053	0.006
	±0.022	±0.015	±0.006	±0.005	±0.002	±0.002	±0.002	±0.004	±0.005	±0.001

* Intravenous injection.

TABLE 2. DISTRIBUTION OF ^{75}Se-19-SELENOCHOLESTEROL IN FEMALE RABBITS* (% kg dose/gm ± 1 s.e.m.)

	Days after dose							
Tissue	1	2	4	5	7	10	15	20
Adrenal	2.278	3.573	3.847	2.983	3.638	2.295	2.204	1.711
	±0.184	±1.670	±0.080	±0.302	±0.174	±0.205	±0.205	±0.123
Liver	1.041	0.420	0.292	0.209	0.195	0.085	0.048	0.038
	±0.079	±0.021	±0.045	±0.036	±0.062	±0.012	±0.017	±0.001
Bile	0.492	0.676	0.838	0.218	0.797	0.128	0.135	0.048
	±0.480	±0.089	±0.017	±0.024	±0.321	±0.060	±0.074	±0.011
Spleen	0.766	0.467	0.221	0.158	0.122	0.062	0.036	0.030
	±0.093	±0.257	±0.031	±0.025	±0.028	±0.001	±0.010	±0.003
Blood	0.703	0.135	0.079	0.046	0.039	0.014	0.013	0.010
	±0.481	±0.069	±0.030	±0.012	±0.002	±0.003	±0.001	±0.001
Kidneys	0.347	0.278	0.254	0.197	0.160	0.144	0.076	0.045
	±0.026	±0.018	±0.003	±0.058	±0.017	±0.003	±0.012	±0.010
Lungs	0.378	0.715	0.438	0.295	0.253	0.115	0.060	0.048
	±0.364	±0.367	±0.024	±0.064	±0.006	±0.016	±0.023	±0.004
Ovaries	0.470	0.471	0.377	0.288	0.341	0.197	0.190	0.098
	±0.419	±0.066	±0.014	±0.055	±0.037	±0.026	±0.052	±0.019
Heart	0.200	0.180	0.149	0.096	0.090	0.044	0.019	0.015
	±0.012	±0.005	±0.008	±0.026	±0.016	±0.003	±0.005	±0.001
Small intestine	0.223	0.166	0.136	0.089	0.084	0.052	0.029	0.017
	±0.004	±0.003	±0.015	±0.025	±0.027	±0.010	±0.003	±0.003

* Intravenous injection.

centration of ^{75}Se-19-selenocholesterol in the adrenals was 6.7% kg dose/gm (2.9% dose per total adrenal in a rat weighing 230 gm) and occurred at Day 4. High uptakes persisted through Day 20. At Days 1 and 5, the uptakes were twice those of ^{131}I-19-iodocholesterol.

Concentrations in the liver, spleen, blood, kidney, lungs, thyroid, ovaries, heart, and small intestines are shown in Table 1. Peak concentrations of ^{75}Se-19-selenocholesterol in the large intestines, pancreas, stomach, brain, parotids, adipose tissue, muscle, and urine were 0.1% kg dose/gm or less. The highest adrenal-to-liver concentration ratio was 148 (at Day 15). The adrenal-to-liver ratios of 14 and 48 at Days 1 and 5 were, respectively, ½ and twice those of ^{131}I-19-iodocholesterol. At Day 3 there was a concentration of 0.07% kg dose/gm (0.22% dose/gm) in whole blood and red blood cells, 0.6% kg dose/gm (0.20% dose/gm) in the plasma, and 0.15% kg dose/gm (0.5% dose/gm) in plasma proteins.

Oral dose. The highest adrenal uptake [0.9% kg dose/gm (0.4% dose per total organ for a rat weighing 230 gm)] occurred at Day 20. The highest adrenal-to-liver concentration ratio (91) also occurred at Day 20.

Rabbits. Table 2 shows the distribution of ^{75}Se-19-selenocholesterol in the adrenals, liver, bile, spleen, blood, kidneys, lungs, ovaries, heart, and small intestines of rabbits. The highest uptake in adrenals was 3.9% kg dose/gm (0.4% dose to total organ in a 4-kg rabbit) and occurred at Day 4. The highest adrenal-to-liver concentration ratio was 46 (at Day 15). Peak concentrations in the thyroid, large intestines, pancreas, stomach, brain, adipose tissue, muscle, and urine were 0.1% kg dose/gm or less. Two male rabbits were also studied and showed a testicular concentration of 0.61% kg dose/gm ± 0.002 (s.e.m.) at Day 2.

Dogs. *Concentration in adrenals.* The adrenal cortex showed a selenocholesterol uptake of 1.7% kg dose/gm at Day 1, increasing to a peak concentration of 4.5% at Day 15 (approximately 0.4% dose/total organ for a 10-kg dog—see Table 3). The adrenal medulla showed higher mean uptakes than the adrenal cortex (Table 3) at Day 1 (2.2% kg dose/gm) and at Day 7 (5.6% kg dose/gm).

Concentration in other tissues. The distribution of ^{75}Se-19-selenocholesterol in bile, liver, spleen, lungs, ovaries, small intestines, heart, kidneys, thyroid, and blood is shown in Table 3. The ovarian uptake was high (2.6%) in one of three dogs at Day 4 and in

TABLE 3. DISTRIBUTION OF ^{75}Se-19-SELENOCHOLESTEROL IN FEMALE DOGS*
(% kg dose/gm ± 1 s.e.m.)

Tissue	Days after dose							
	1	2	4	5	7	10	15	20
Adrenal cortex	1.667	2.074	2.917	2.448	3.062	3.096	4.494	2.630
	±0.051	±0.250	±0.375	±0.132	±0.638	±0.113	±0.299	±0.238
Adrenal medulla	2.188	1.375	2.680	1.547	5.605	2.561	3.142	1.792
	±0.728	±0.478	±0.814	±0.178	±1.392	±0.327	±0.862	±0.093
Bile	4.746	1.318	3.302	0.763	0.152	0.419	0.418	0.155
	±0.318	±1.293	±2.050	±0.254	±0.064	±0.154	—	±0.051
Liver	0.561	0.372	0.271	0.148	0.337	0.122	0.112	0.084
	±0.063	±0.054	±0.050	±0.006	±0.145	±0.017	±0.022	±0.011
Spleen	0.771	0.397	0.247	0.138	0.154	0.095	0.083	0.029
	±0.058	±0.038	±0.039	±0.007	±0.034	±0.006	±0.014	±0.002
Lungs	0.434	0.444	0.315	0.216	0.164	0.120	0.049	0.039
	±0.072	±0.071	±0.051	±0.017	±0.040	±0.011	±0.005	±0.011
Ovaries	0.287	0.340†	1.034	0.177	1.189‡	0.496	0.044	0.122
	±0.028	±0.039	±0.511	±0.036	±0.486	±0.343	±0.034	±0.040
Small intestine	0.269	0.271	0.203	0.122	0.080	0.100	0.065	0.026
	±0.041	±0.023	±0.025	±0.006	±0.014	±0.011	±0.009	±0.002
Heart	0.149	0.204	0.126	0.083	0.078	0.059	0.053	0.017
	±0.011	±0.029	±0.021	±0.005	±0.013	±0.004	±0.010	±0.003
Kidneys	0.356	0.347	0.183	0.128	0.063	0.131	0.045	0.030
	±0.098	±0.076	±0.059	±0.011	±0.009	±0.016	±0.013	±0.003
Thyroid	0.128	0.187	0.118	0.080	0.227	0.073	0.167	0.029
	±0.021	±0.027	±0.021	±0.003	±0.077	±0.002	±0.073	±0.006
Blood	0.317	0.583	0.154	0.088	0.028	0.064	0.025	0.013
	±0.027	±0.295	±0.040	±0.014	±0.013	±0.019	±0.007	±0.001

* Intravenous doses.
† 2.6% kg dose/gm in 1 of 3 dogs.
‡ 1.6% and 1.9% kg dose/gm, respectively, in 2 of 3 dogs.

TABLE 4. ADRENAL-TO-TISSUE CONCENTRATION (% kg dose/gm) RATIOS OF ^{75}Se-19-SELENOCHOLESTEROL IN FEMALE DOGS

Tissue	Days after dose							
	1	2	4	5	7	10	15	20
Ratios of adrenal cortex to tissue								
Liver	3.0	6.0	11.0	17.0	9.0	25.0	40.0	31.0
Blood	5.0	4.0	19.0	28.0	109.0	48.0	182.0	195.0
Spleen	2.0	5.0	12.0	18.0	20.0	33.0	54.0	92.0
Small intestine	6.0	8.0	14.0	20.0	38.0	31.0	69.0	101.0
Large intestine	16.0	11.0	17.0	31.0	24.0	43.0	82.0	124.0
Ratios of adrenal medulla to tissue								
Liver	4.0	4.0	10.0	11.0	17.0	21.0	28.0	21.0
Blood	7.0	2.0	17.0	18.0	200.0	40.0	128.0	133.0
Spleen	3.0	4.0	11.0	11.0	36.0	27.0	38.0	63.0
Small intestine	8.0	5.0	13.0	13.0	70.0	26.0	48.0	69.0
Large intestine	21.0	7.0	16.0	20.0	45.0	35.0	57.0	84.0

two of three dogs at Day 7 (1.6% and 1.9% kg dose/gm, respectively). In the gallbladder, peak concentration was 0.5% kg dose/gm; and that in the stomach and large intestines, 0.2% kg dose/gm. In the pancreas, brain, muscle, adipose tissue, and urine, the peak uptakes were 0.1% kg dose/gm or less.

Three male dogs showed a testicular concentration at Days 1, 2, and 5 of 0.05, 0.09, and 0.08% kg dose/gm, respectively. A pregnant dog of 3–4 weeks' gestation (not included in Table 3) showed ovarian and fetal uptakes of 1.7% and 0.04% kg dose/gm, respectively, at Day 2.

Adrenal-to-tissue ratios. As shown in Table 4, the highest concentration ratios of adrenal cortex to liver and adrenal medulla to liver were 40 and 28, respectively, at 15 days; the peak ratios of adrenal cortex to blood and adrenal medulla to blood were 195 (Day 20) and 200 (Day 7), respectively. The highest concentration ratios of adrenal cortex and adrenal medulla to spleen, small intestines, and large intestines occurred at Day 20.

Radioactive products in adrenals. The adrenal cortical and medullary radioactivities were completely recovered in the chloroform phase (lipid fraction); none appeared in the methanol (nonlipid fraction). Thin-layer chromatography of the lipid fraction showed a peak having the same R_f value as that of ^{75}Se-19-selenocholesterol.

Scintillation scans. Adrenal images of good quality (Fig. 2) were obtained in the dogs. These images compare favorably with those obtained with ^{131}I-19-iodocholesterol.

DISCUSSION

Selenium-75-19-selenocholesterol showed high adrenal concentration in rats, rabbits, and dogs and yielded good-quality adrenal images (Fig. 2). Radioactivity measurement in adrenal lipid extracts and thin-layer chromatography showed that ^{75}Se in the adrenal gland is still attached to the steroid moiety. In view of these properties and the expected reduction in the costs of production, this agent could conceivably be developed for routine clinical use in various adrenal disorders (*1–8*).

The markedly high concentrations in the adrenal medulla (Table 3) are of particular interest to us. A similar finding, though not as marked, has been noted with another new adrenal imaging agent (*13*); these effects may be related to lipid involvement in adrenal medullary function (*14,15*). We are thus evaluating the potential use of this agent in detecting structural abnormalities of the adrenal medulla, such as pheochromocytomas less than 3 cm in diameter (*7*) and neuroblastomas (*16*).

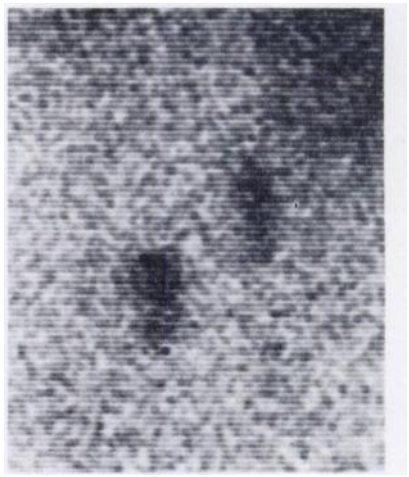


FIG. 2. Posterior image of adrenals in dog, 10 days after administration of ^{75}Se-19-selenocholesterol.

TABLE 5. HUMAN DOSIMETRY: COMPARISON OF ^{75}Se-19-SELENOCHOLESTEROL* WITH ^{131}I-19-IODOCHOLESTEROL

	Selenocholesterol (rads/mCi)	Iodocholesterol (rads/mCi)
Adrenals	32.7	30.0
Ovaries	4.7	2.9
Thyroid	1.7	28.0
Whole body	1.5	0.9

* Extrapolated from tissue distribution data in dogs.

The high ovarian uptake in some dogs (Table 3) may be related to increased ovarian function, e.g., undetected pregnancy or the luteal phase of an estrus cycle. We have noted similar uptakes in pregnant dogs. It would be interesting to study the concentration of this agent in functional ovarian tumors in humans.

Although an orally administered scanning agent would be preferable, this possibility seems unlikely with ^{75}Se-19-selenocholesterol since adrenal concentration in rats given oral doses was relatively low up to Day 20.

The estimated absorbed dose to humans (Table 5) is approximately equal to that of ^{131}I-19-iodocholesterol for the adrenals, ovaries, and whole body, and $\frac{1}{16}$ that of ^{131}I-19-iodocholesterol for the thyroid.

A relative drawback of this agent, necessitating scanning at longer intervals after the dose, is the high background activity in the blood and liver at the earlier time intervals. The former probably represents binding of this compound to plasma proteins, while the latter, together with increased concentrations in the spleen and lungs, is probably related to mechanical phagocytosis or metabolism of cholesterol in the reticuloendothelial system (*17,18*).

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