Iodine-125 Cholesteryl Iopanoate for Measuring Extent of Atherosclerosis in Rabbits

Mark R. DeGalan, Susan W. Schwendner, R. W. Scott Skinner, Marc A. Longino, Milton Gross, and Raymond E. Counsell

Departments of Pharmacology and Internal Medicine, Division of Nuclear Medicine, University of Michigan Medical School and Veteran's Administration Medical Center, Ann Arbor, Michigan

Rabbits rendered atherosclerotic by mechanical aortic de-endothelialization and 6 wk of cholesterol feeding were administered estradiol-17β-cypionate, an anti-atherogenic agent in rabbits. These animals were compared to a similar, untreated group and control animals fed a regular non-atherogenic diet. Iodine-125 cholesteryl iopanoate ([^{125}I]CI), a nonhydrolyzable cholesteryl ester derivative, was administered intravenously at regular intervals throughout the study. Six days after the last dose of [^{125}I]CI, the animals were scanned with a gamma camera. After animals were killed, tissue distribution of the ^{125}I radioactivity showed a significant decrease of [^{125}I]CI accumulation in the aorta of estrogen-treated as compared to untreated, cholesterol-fed animals. However, the accumulation of [^{125}I]CI in the aortas was insufficient to accurately define the presence of atheroma by gamma camera scintigraphy.

J Nucl Med 29:503–508, 1988

Atherosclerosis is the underlying factor leading to such cardiovascular diseases as stroke, aneurysm, and myocardial infarction, and represents the major cause of death in the United States (*1*). Over the past decade, the causative factors associated with atherosclerosis have become more apparent and have created interest in developing new anti-atherogenic agents. However, many therapeutic approaches have been hampered by the inability of the available noninvasive methods to directly quantify the anti-atherogenic efficacy of drug therapy. Standard approaches such as monitoring changes in the levels of plasma cholesterol or lipoprotein concentrations have been utilized to monitor the effectiveness of drug therapy, but such analyses may not correlate with intimal plaque formation (*2*). Current methods for measuring drug-induced attenuation of atherosclerosis involve excising the aortas of drug-treated and untreated atherosclerotic animals and comparing the involved surface areas and/or the total cholesterol or cholesteryl ester content of the aorta. Despite describing the involved intimal area, surface mapping does not assess lesional depth. Furthermore, measuring aortic cholesterol or cholesteryl ester content is a time-consuming process. A third approach attempts to quantify the atherosclerotic process by employing newer noninvasive modalities (i.e., magnetic resonance imaging, ultrasound and computed tomography) (*3–5*). These techniques are presently more qualitative than quantitative in nature and at this time are not well suited for small animal investigations.

The purpose of this study was to develop an animal model whereby the efficacy of drugs to influence the progress of atherosclerosis could be evaluated by radioscintigraphy. While current methods requiring postmortem analysis are practical for drug evaluation in small laboratory animals, a noninvasive method would be particularly valuable for studies in expensive non-human primates such as the rhesus monkey where lesion development is more analogous to that observed in humans.

Since atherosclerosis may be considered a dynamic cholesteryl ester storage disorder in which excess esters of cholesterol accumulate within tissues such as the media of arteries, it was logical to assume that a radiolabeled cholesteryl ester-like probe may also accumulate within afflicted vessels. Such a probe could gain entry into atheroma presumably as a component of serum lipoproteins. If such a cholesteryl ester analog were resistant to the action of intracellular cholesteryl ester hydrolases, its presence in lesions would closely reflect the process of cholesteryl ester accumulation during the

Received Aug. 15, 1986; revision accepted Nov. 30, 1987.

For reprints contact: Raymond E. Counsell, PhD, Dept. of Pharmacology, M6322 Medical Science Building I, University of Michigan Medical School, Ann Arbor, MI 48109-0626.

development of atherosclerosis. One such probe, [^{3}H] cholesteryl linoleyl ether, developed by Stein et al. (*6*) has proven useful in measuring influx rates of natural cholesteryl esters into aortas of cholesterol-fed rabbits.

Previously we have described a radioiodinated cholesteryl ester iodine-125 cholesteryl iopanoate ([^{125}I]CI), as a probe for the in vitro measurement of atherosclerosis in animals (*7,8*). These reports showed [^{125}I]CI to be resistant to hydrolysis in vivo and selective for atheroma over normal aortic tissue. Moreover, the amount of [^{125}I]CI accumulating in the atherosclerotic lesions was found to be directly related to the content of natural cholesteryl esters within these lesions (*8*).

Thus, a study was undertaken to assess the value of [^{125}I]CI as an in vivo tracer for measuring the attenuation of atherosclerosis in cholesterol-fed rabbits treated with estradiol-17β-cypionate, an anti-atherogenic compound in rabbits (*9*). A de-endothelialized cholesterol-fed rabbit model was used, primarily because the vascular lesion is rapidly induced and well-characterized (*10*). A secondary objective was to determine if the accumulation of [^{125}I]CI in atheroma of atherosclerotic rabbits could be distinguished noninvasively using gamma-camera scintigraphy.

MATERIALS AND METHODS

Fourteen female New Zealand White rabbits (Langshaw Farms, Augusta, MI) (2.34–3.20 kg) were randomly divided into three groups at the beginning of the study. One group was maintained on normal rabbit chow (n = 4) and the other two groups on rabbit chow supplemented with 2% cholesterol (Purina Special Diet Plant, Richmond, IN) (n = 10); food and water were given *ad libitum*. Rabbits receiving cholesterol-enriched chow were subjected to aortic balloon catheter (3F Fogarty) de-endothelialization under xylazine (9 mg/kg) and ketamine (Butler, Brighton, MI) (50 mg/kg) anesthesia as previously described (*7*). Immediately after surgery, one cholesterol-fed group received 1.5 mg of estradiol-17β-cypionate i.m. in a corn oil vehicle, once every week until animals were killed. The other cholesterol-fed group did not receive estrogen and served as the atherosclerotic control group.

Cholesteryl iopanoate was synthesized, radiolabeled, and formulated in Tween-20/saline as described previously (*11*). Radiopurity was determined by silica gel thin-layer chromatography (TLC) (Merck silica gel 60 f^{2} plates, Darmstadt, West Germany) with a solvent system of hexane:ethyl acetate 5:2 (v/v). Plates were visualized under uv light and scanned on a radiochromatogram scanner (Berthold 6000 Radiochromatogram Scanner; Berthold Instruments, Pittsburgh, PA) before being cut into 1 cm sections and counted in a gamma counter (Searle 1185 Gamma Counter, TM Analytic, Inc., Elk Grove Village, IL) (^{125}I counting efficiency 82%). Ten or 11 days after the start of diet, all three groups of animals were injected with 7–10 μCi of [^{125}I]CI in the marginal ear vein. Subsequent doses were given in a similar manner twice weekly for 5 wk; the cumulative dose ranged from 83.1–90.0 μCi/animal.

Following the last injection of [^{125}I]CI, rabbits were maintained on their respective diets for 6 days to allow for adequate blood clearance of [^{125}I]CI prior to imaging. Rabbits were anesthetized as described above and posterior abdominal images were collected for 30-min periods (~100,000 counts/image) using a gamma-camera (Sigma 410 Mobile Gamma Camera, Ohio Nuclear, Solon, OH) equipped with a high sensitivity collimator interfaced to a digital minicomputer (Medtronix Medical Data Systems, Ann Arbor, MI). The location of the aorta was confirmed by simultaneous administration of technetium-99m-(^{99m}Tc) labeled red blood cells (*12*). Pulse height discrimination of the photopeaks of ^{99m}Tc and ^{125}I demonstrated <1% of the ^{99m}Tc activity in the ^{125}I window. The region of ^{125}I activity corresponding to the position of the aorta was analyzed for radioactivity and compared to an adjacent, nontarget region for ten rabbits. All values were determined by computerized analysis and were normalized and corrected for sample size in the digitalized images.

In order to determine the minimum detectable concentration of radioactivity necessary for imaging the atherosclerotic plaque of rabbit aorta, the following procedure was undertaken. A series of small plastic microcentrifuge tubes were filled with 20 μl of various concentrations of aqueous Na^{125}I. The amount of radioactivity contained within each tube was determined by counting in a gamma counter. These tubes were then placed in order of increasing radioactivity in the aorta of a female rabbit that had been killed by exsanguination as before and eviscerated. The abdominal cavity was packed with absorbant towels to replace tissues removed during evisceration. The edges of the imaging field were determined using a radioactive source and marked on the animal's back with needles to serve as a reference for subsequent imaging. Image acquisition was performed as described earlier. After acquisition of counts, discrete areas of radioactivity along the axis of the aorta were matched with their sources. In this manner, the lowest quantity of radioactivity necessary for aortic visualization could be ascertained.

While under anesthesia, rabbits were killed and various tissues were removed in order to assess the tissue distribution of [^{125}I]CI. Representative tissue samples were taken, weighed, and counted for ^{125}I content as previously described (*7*). Body fluids, such as bile were removed using a syringe and analyzed as above.

Aortas were removed, trimmed of adventitial fat, rinsed with saline, and opened longitudinally. Selected aortas were stained with Oil Red O, photographed, wrapped with plastic wrap, and exposed to Kodak XAR-5 x-ray film for 40 hr at −70°C for autoradiography. Aortas were cut into 1 cm sections, weighed, and counted as other tissues to determine uptake of probe.

The in vivo stability of [^{125}I]CI was determined by extracting samples of liver, adrenal, and plasma using chloroform:methanol 2:1 (v/v) according to the protocol of Folch et al. (*13*). The percent of radioactivity in the organic phase remaining as intact compound was determined by thin layer chromatography (TLC) using the solvent system and counting protocol outlined previously (*7*). Unlabeled cholesteryl iopanoate was used as a standard.

A sample of plasma taken at time of death was also analyzed by polyacrylamide gel electrophoresis (PAGE) as described elsewhere to determine the distribution of [^{125}I]CI within the plasma compartment (*7*). Also, plasma collected at time of

death was analyzed for total cholesterol content using a commercially-available enzymatic assay kit (Worthington Diagnostics, Freehold, NJ).

RESULTS

The uptake of [^{125}I]CI into arteries of cholesterol-fed rabbits was an order of magnitude higher than that found in the aortas of the control group (Table 1). In contrast, simultaneous treatment of hypercholesterolemic rabbits with estradiol-17β-cypionate resulted in a 64% decrease in aortic radioactivity. These differences correlated with the severity of atheroma observed by gross inspection. For example, Figure 1 compares an autoradiogram of aortas taken from estrogen-treated and untreated cholesterol-fed rabbits with photographs of the aortas after Oil Red O staining. The autoradiograms clearly illustrate the reduction in atheroma formation associated with estrogen treatment.

At the time of death, the total cholesterol levels in the estrogen-treated group were found to be 2,484 ± 209 mg/dl compared to 1,940 ± 248 mg/dl for the untreated cholesterol-fed group. This value for the control group was 278 ± 161 mg/dl.

As shown by the tissue distribution of [^{125}I]CI listed in Table 1, high concentrations of [^{125}I]CI were found in the adrenals and liver of chow-fed rabbits. When compared to the chow-fed, normolipidemic rabbits, these levels were reduced 70% and 26%, respectively, in the cholesterol-fed, hyperlipidemic rabbits. Estrogen treatment did not alter this pattern of accumulation in the hypercholesterolemic rabbit; however, a reduction in the concentration of [^{125}I]CI was seen in the ovary. Other tissues containing high concentrations of radioactivity included bone marrow, spleen, and thyroid. As is usual for radioiodine-containing diagnostics, thyroid levels were presumed to reflect the sequestration of radioiodide produced by low rates of metabolic deiodination of tracer.

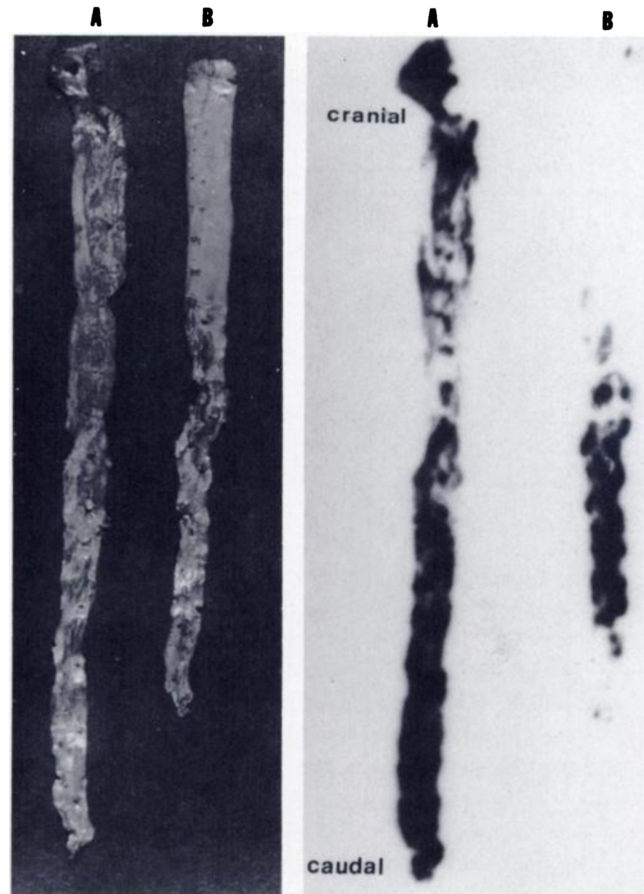

FIGURE 1
Aortas of either untreated (A) or estradiol-17β-cypionate-treated (B) cholesterol-fed rabbits. Left: Oil Red O stained. Right: en face autoradiogram. Aortas were removed from animals 6 days after the last administration of [^{125}I]CI.

Table 2 demonstrates the relative distribution of [^{125}I]CI within the various components of the plasma compartment as determined by PAGE. Hypercholesterolemia shifted the majority of the probe in the plasma

TABLE 1
Tissue Distribution of [^{125}I]Cholesteryl Iopanoate
% Administered (kg) Dose/g (Average ± s.e.m.)

Tissue	Chow-fed (n = 4)	Cholesterol-fed (n = 5)	Estrogen-treated cholesterol-fed (n = 5)
Adrenal	6.077 ± 0.318	1.833 ± 0.155†	1.908 ± 0.179†
Aorta	0.013 ± 0.004	0.187 ± 0.021†	0.067 ± 0.015†,‡
Blood	0.034 ± 0.006	0.048 ± 0.012	0.084 ± 0.015
Bone marrow§	0.194 ± 0.023	0.302 ± 0.051	0.323 ± 0.036*
Liver	1.504 ± 0.201	1.111 ± 0.067*	1.013 ± 0.090*
Ovary	0.214 ± 0.031	0.230 ± 0.017	0.106 ± 0.010†,‡
Spleen	0.840 ± 0.129	0.916 ± 0.137	0.819 ± 0.096
Thyroid	3.898 ± 0.966	3.287 ± 0.454	3.260 ± 0.413

* $p < 0.05$ versus control group.
† $p < 0.01$ versus control group.
‡ $p < 0.01$ versus untreated atherosclerotic group.
§ Taken from femur.

TABLE 2
Percent Radioactivity Associated with Plasma Fractions Separated by 3% Polyacrylamide Gel Electrophoresis (% Radioactivity on Gel (average ± s.e.m.))

Fraction	Chow-fed	Cholesterol-fed	Estrogen-treated Cholesterol-fed
	(n = 4)	(n = 5)	(n = 5)
CM-VLDL	7.0 ± 3.6	36.0 ± 6.1[†]	43.1 ± 8.2[†]
VLDL-IDL	9.4 ± 5.4	38.8 ± 9.8[*]	30.2 ± 4.0[†]
LDL	26.3 ± 8.1	11.7 ± 3.4	17.6 ± 6.2
HDL	25.8 ± 11.5	6.5 ± 1.6	3.9 ± 1.1[*]
Albumin	13.4 ± 6.0	2.3 ± 0.8[*]	1.1 ± 0.4[*]
Below Albumin	17.9 ± 7.5	4.6 ± 2.3	4.1 ± 1.8[*]

[*] $p < 0.05$ versus control group.
[†] $p < 0.01$ versus control group.

from those fractions having greater density (d > 1.019 g/ml) to those of lesser density (d < 1.019 g/ml), especially beta very low density lipoprotein (β-VLDL).

Lipid extraction and TLC analysis demonstrated that between 89–91% of the radioactivity extracted from the adrenals of the three groups of animals partitioned into the organic phase and between 83–87% of this radioactivity co-migrated with a cholesteryl iopanoate standard on TLC. Likewise, the values for liver were comparable (87–92% partitioned into the lower, chloroform phase, with 80–84% co-migrating with cholesteryl iopanoate). The majority of the radioactivity found in plasma at sacrifice was also intact probe, although less so than the other tissues studied (72–87% in organic phase, 61–65% of this co-migrating with [^{125}I]CI). We believe that these other forms of radioactivity present in the plasma account for the levels associated with the albumin and below albumin band on PAGE, which is especially pronounced in the chow-fed control group.

Scintigraphic Studies

Scintigraphic images of representative animals from the chow-fed control (A), the untreated cholesterol-fed (B), and the estrogen-treated cholesterol-fed (C) groups are shown in Figure 2. These are shown along with the image obtained following administration of ^{99m}Tc-labeled red blood cells to a normal chow-fed rabbit (D). The estrogen-treated animals exhibited a reduction in radioactivity in the abdominal region over that of the untreated cholesterol-fed animals, particularly in the region of the aorta. Such observations correlated with the reduction in aortic radioactivity shown in Figure 1 for the estrogen-treated group. However, phantom studies involving implantation of calibrated amounts of Na^{125}I into the aorta of a normal rabbit revealed that ~0.2 μCi/cm was the minimal amount of aortic radioactivity needed for imaging. Since the highest levels of radioactivity found in the aortas of untreated atherosclerotic rabbits were at least 30-fold lower (0.002–0.006 μCi/cm) than this minimal level, the differences observed in Figure 2 cannot be due to differences in aortic activity but rather to differences in the accumulation of radioactivity at other abdominal sites. This was confirmed when exvisceration of the aorta in one rabbit failed to significantly alter the scintigraphic image.

DISCUSSION

The reduction of atheroma noted by gross inspection and the resultant reduction of [^{125}I]CI uptake into aortas of estradiol-treated cholesterol-fed rabbits was achieved without a decrease in plasma total cholesterol. This suggests that estrogens attenuate atherosclerosis through a nonhypolipidemic mechanism. Recently, Hough and Zilversmit (*9*) reported that estrogen reduced the extent of atherosclerosis during cholesterol feeding without a concomitant reduction in plasma cholesterol. Although the mechanism for this anti-atherogenic action of estrogen is unknown, it has been reported that estrogens decrease collagen synthesis in the walls of arteries during hypercholesterolemia and that this effect may account for their anti-atherosclerotic action (*14*).

While this study was able to show abdominal scintigraphic differences between estrogen-treated and untreated atherosclerotic rabbits, these observed differences could not be attributed solely to differences in aortic radioactivity. Studies are now in progress to identify those abdominal tissues other than aorta which display significant retention of cholesteryl ester in the atherosclerotic animal.

Several tissues in normal animals, such as liver and adrenal, contained high concentrations of [^{125}I]CI and these organs are readily observed in the scintiscan (Fig. 2A). Moreover, the levels of radioactivity in these tissues were observed to decline in the hypercholesterolemic state. This observation was not surprising since tissues such as liver and adrenal are known to contain high concentrations of lipoprotein receptors that down-regulate during periods of hypercholesterolemia (*15,16*). Moreover, PAGE results indicated that [^{125}I]CI rapidly becomes associated with plasma lipoproteins after administration, and thus could easily be internalized in the above tissues by a lipoprotein receptor-mediated uptake process. Plasma lipoproteins are also known to provide cholesterol as precursor for hormone synthesis in the ovary (*17,18*), and this is a plausible explanation for the high concentration of radioactivity in this tissue. A reduction of ovarian radioactivity was noted in rabbits treated with estrogen and this may be related to normal hormonal feedback inhibition of ovarian steroidogenesis resulting in decreased exogenous cholesterol uptake.

In agreement with previous studies (*19,20*), cholesterol feeding led to pronounced hypercholesterolemia and a vast elevation in the d < 1.019 g/ml lipoproteins

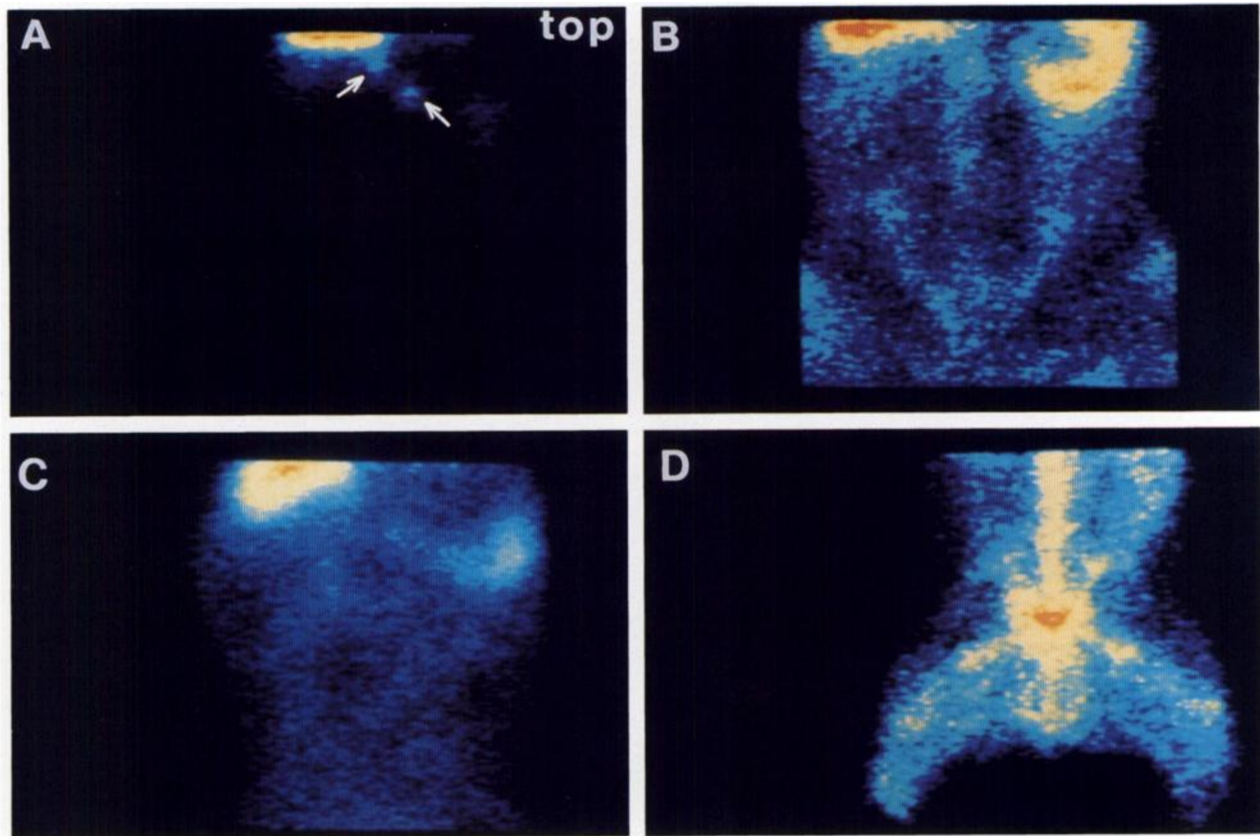

FIGURE 2
Posterior abdominal images obtained after administration of [^{125}I]CI to (A) a control rabbit (note two adrenals below the liver indicated by arrows), (B) a cholesterol-fed rabbit, and (C) an estrogen-treated cholesterol-fed rabbit. (D) is an image obtained following administration of [^{99m}Tc]RBCs to a normal rabbit.

(especially β-VLDL). The higher levels of radioactivity in the blood of cholesterol-fed animals over that of control animals could thus be ascribed to "metabolic dilution" of the tracer as well as diminished clearance of the d < 1.019 g/ml lipoproteins, which is known to occur in cholesterol-fed rabbits as a result of saturation and reduction in the number of hepatic receptors (*21, 22*).

Bone marrow also contained an elevated concentration of radioactivity in the hypercholesterolemic rabbits and is perhaps a result of the sequestration of lipoproteins containing [^{125}I]CI through the scavenger-mediated mechanisms intrinsic to the cells of the reticuloendothelial system (*23,24*). Several studies have shown β-VLDL to be taken up by macrophages via a specific cell-surface receptor different from that of the LDL and modified-LDL receptors (*25,26*). Thus, the high bone marrow [^{125}I]CI accumulation found in both of the cholesterol-fed groups is compatible with these previous findings.

The extraction and separation of the radioactivity contained in liver, adrenal, and plasma demonstrated the stability of [^{125}I]CI in vivo. The apparent plasma degradation of probe may be due to both metabolism within the plasma as well as the presence in the plasma of metabolites of [^{125}I]CI formed within tissues (e.g., liver and adrenal). The apparent resistance of [^{125}I]CI to hydrolysis was also supported by the lack of activity found in the bile (Table 1), since iopanoic acid, an expected product of hydrolysis, is known to quickly concentrate within bile. Although the stability of [^{125}I]CI in the aortas was not examined in this study, extraction of an atherosclerotic aorta in a previous study found [^{125}I]CI to be present as the intact compound (*7*).

In summary, [^{125}I]CI may serve as a useful tracer for the measurement of atherosclerosis in laboratory animals and for the assessment of the anti-atherogenic effect of hypolipidemic therapy. In order for such a tracer to be useful for scintigraphic purposes, however, a considerable improvement in the aortic uptake vis-a-vis surrounding tissues will be needed. Since lipoproteins are known to be involved in the transport of cholesteryl esters to atheroma, studies are now in progress to incorporate [^{125}I]CI into various lipoproteins and chemically-modified lipoproteins. Hopefully such studies will generate the type of information ultimately required to achieve selective localization of radiodiagnostics in atheroma.

ACKNOWLEDGMENTS

The authors thank Wendelyn Schmidt, Tammy Granroth, Darrell Looney, and Lisa Santay for technical assistance, and Linda Busha for typing the manuscript. This research was supported by grants from the American Heart Association of Michigan and the National Institutes of Health (CA-08349).

REFERENCES

1. Solberg LA, Strong JP. Risk factors and atherosclerotic lesions. A review of autopsy studies. *Arteriosclerosis* 1983; 3:187–198.
2. Day CE, Philips WA, Schurr PE. Animal models for an integrated approach to the pharmacologic control of atherosclerosis. *Artery* 1979; 5:90–109.
3. Strandness Jr. DE. Noninvasive evaluation of arteriosclerosis. Comparison of methods. *Arteriosclerosis* 1983; 3:103–116.
4. Blakenhorn DH. Noninvasive methods for evaluation of atherosclerosis in man. *Metabolism* 1985; 34:78–81.
5. Budinger TF. Overview of imaging technologies: Present and future trends. *Atherosclerosis Reviews.* New York: Raven Press, 1983:7–12.
6. Stein Y, Stein O, Halperin G. Use of [^{3}H]cholesteryl linoleyl ether for the quantitation of plasma cholesteryl ester influx into the aortic wall in hypercholesterolemic rabbits. *Arteriosclerosis* 1982; 2:281–289.
7. DeGalan MR, Schwendner SW, Weichert JP, et al: Radioiodinated cholesteryl iopanoate as a probe for the *in vivo* visualization of atherosclerotic lesions in animals. *Pharm Res* 1986; 3:52–55.
8. DeGalan MR, Schwendner SW, Gross MD, et al. Assessment of the anti-atherogenic efficacy of a hypolipidemic drug by scintigraphy. In: *Current applications in radiopharmacology.* New York: Pergamon Press, 1986:109–117.
9. Hough JL, Zilversmit DG. Effect of 17-beta-estradiol on aortic cholesterol content and metabolism in cholesterol-fed rabbits. *Arteriosclerosis* 1986; 6:57–63.
10. Jokinen MP, Clarkson TB, Prichard RW. Recent advances in molecular pathology. Animal models in atherosclerosis research. *Exp Mol Pathol* 1985; 41:1–28.
11. Seevers RH, Groziak MP, Weichert JP et al: Potential Tumor-or organ-imaging agents. 23. Sterol esters of iopanoic acid. *J Med Chem* 1982; 25:1500–1503.
12. Eckelman WC, Richard P, Hauser W et al: Technetium-labeled red blood cells. *J Nucl Med* 1971; 12:22–24.
13. Folch J, Lees M, Sloane-Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 1957; 226:497–509.
14. Fischer GM, Swain ML. Effects of estradiol and progesterone on the increased synthesis of collagen in atherosclerotic rabbit aortas. *Atherosclerosis* 1985; 54:177–185.
15. Slater HR, Shepherd J, Packard CJ. Receptor-mediated catabolism and tissue uptake of human low density lipoprotein in the cholesterol-fed, atherosclerotic rabbit. *Biochim Biophys Acta* 1982; 713:435–446.
16. Pittman RC, Carew TE, Attie AD, et al. Receptor-dependent and receptor-independent degradation of low density lipoprotein in normal rabbits and in receptor-deficient mutant rabbits. *J Biol Chem* 1982; 257:7994–8000.
17. Azhar S, Menon M, Menon KMJ. Receptor-mediated gonadotropin action in the ovary. Demonstration of acute dependence of rat luteal cells on exogenously supplied sterol precursor (sterols) for gonadotropin-induced steroidogenesis. *Biochim Biophys Acta* 1981; 665:362–375.
18. Tureck RW, Strauss JF. Progesterone synthesis by luteinized human granulosa cells in culture: The role of *de novo* sterol synthesis and lipoprotein-carried sterol. *J Clin Endocrin Metab* 1982; 54:367–373.
19. Shore VG, Shore B, Hart RG. Changes in apolipoproteins and properties of rabbit very low density lipoproteins on induction of cholesteremia. *Biochemistry* 1974; 13:1579–1585.
20. Roth RI, Gaubatz JW, Gotto AM, et al. Effect of cholesterol feeding on the distribution of plasma lipoproteins and on the metabolism of apolipoprotein E in the rabbit. *J Lip Res* 1983; 24:1–11.
21. Kovanen PT, Brown MS, Basu SK, et al. Saturation and suppression of hepatic lipoprotein receptors: a mechanism for the hypercholesterolemia of cholesterol-fed rabbits. *Proc Natl Acad Sci USA* 1981; 78:1396–1400.
22. Kovanen PT. Regulation of plasma cholesterol by hepatic low-density lipoprotein receptors. *Am Heart J* 1987; 113:464–469.
23. Slater HR, Packard CJ, Bicker S, et al. Effects of cholestyramine on receptor-mediated plasma clearance and tissue uptake of human low density lipoprotein in the rabbit. *J Biol Chem* 1980; 225:10210–10231.
24. Slater HR, Packard CJ, Shepherd J. Receptor-independent catabolism of low density lipoprotein. Involvement of the reticuloendothelial system. *J Biol Chem* 1982; 257:307–310.
25. Goldstein JL, Ho YK, Brown MS, et al. Cholesteryl ester accumulation in macrophages resulting from receptor-mediated uptake and degradation of hypercholesterolemic canine β-very low density lipoproteins. *J Biol Chem* 1980; 255:1839–1848.
26. Bates SR, Coughlin BA, Mazzone T, et al. Apoprotein E mediates the interaction of β-VLDL with macrophages. *J Lip Res* 1987; 28:787–797.