LOCALIZATION AND RETENTION OF I^{131} FROM FED TRIOLEIN IN THE ATHEROSCLEROTIC INFILTRATION OF RABBIT AORTAS *

By MEYER FRIEDMAN, SANFORD O. BYERS, LELAND FELTON AND PAXTON CADY

(From the Harold Brunn Institute, and the Department of Radiology, Mount Zion Hospital and Medical Center, San Francisco, Cal.)

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Until recently, many clinical and the laboratory investigators considered an atherosclerotic artery to be a relatively inert tissue and its excess lipid and cholesterol concentration the passive, chronic result of intramural deposit. The demonstration by Siperstein, Chaikoff and Chernick (1), by Eisley and Pritham (2) and Werthessen, Nyman, Holman and Strong (3) of the ability of the aorta of some animals to synthesize cholesterol and the discovery by Biggs and associates (4) of the presence of recently fed cholesterol in human atherosclerotic artery suggested the participation of such an artery in the general day to day cholesterol metabolism of the mammal. Azarnoff has found (5) that aortae of humans, dogs, rats and cats synthesize not cholesterol itself, but another unidentified digitonin-precipitable sterol. Zilversmit, Shore and Ackerman (6) showed not only the existence of amazingly active phospholipid synthesis on the part of the aorta of an atherosclerotic rabbit but also demonstrated a differential activity in such synthesis between an atherosclerotic plaque and neighboring normal areas.

However, the triglyceride dynamics of the atherosclerotic artery are not well known. The present report describes some features of the participation of an atherosclerotic vessel in the disposition of an orally administered triglyceride tagged with I^{131}.

METHODS

Seven rabbits were fed a commercial pellet diet enriched with cholesterol (2 per cent) and cotton seed oil (2 per cent) for three months, after which time they were placed again on ordinary Purina rabbit chow for another three months. Then each of these previously hypercholesteremic rabbits was given by stomach tube 2.0 ml. of iodinated triolein containing 0.73 mc. of I^{131} (Raolein®, Abbott [1]) for three successive days. For control purposes, two normal rabbits were given the same dosage of I^{131} triolein. For additional control purposes, one previously hypercholesteremic rabbit and two normal rabbits were given 2.0 ml. of NaI solution containing 0.73 mc. of NaI^{131} for three successive days. All rabbits were given KI (2 mg. of KI per Gm. of Purina rabbit chow) for two days prior to the feeding of radioactive iodine in order to prevent significant absorption of I^{131} by the thyroid gland.

Seven days after the initial administration of the radioactive triolein or sodium iodide all of the animals were sacrificed. The aorta of each of the previously hypercholesteremic animals was examined and its degree of atherosclerosis was grossly estimated. The atheromata consisted of smooth white glistening thickened areas without fibrosis or lesions either grossly or histologically. Microscopic sections stained with Sudan IV showed the presence of both intra- and extracellular lipid. Then a section of the aorta beginning from the attachment of the semilunar valves was so cut from each rabbit that the total area of intima always equaled approximately 550 sq. mm. This first section was used for determination of total radioactivity. A second section of aorta also was obtained for radioautography. The adventitial fat of these aortic sections was removed with meticulous care. The segments then were weighed. Then, in addition, a section of adrenal, adventitial fat of the aorta, perirenal fat, liver, kidney and thyroid were obtained, weighed, cut up into small fragments and thoroughly washed.

The aorta of each of the normal rabbits given either triolein I^{131} or NaI^{131} was similarly sectioned, cleaned of adventitial fat and weighed.

The I^{131} content of the organ and samples was determined as follows: Organ fragments were placed in uni-

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[1] This material is made by iodinating commercial triolein with iodine monochloride in the proportion of one atom of iodine to 1,000 molecules of triolein. The resulting iodinated fat is then mixed with peanut oil to increase volume in order to facilitate handling. While more than 90 per cent of the I^{131} is lipid bound, in our hands chromatographic analysis has shown the presence of at least five I^{131} containing lipids.

TABLE I

Radioactivity of tissues after I^{131} administration

Rabbit	Aorta (550 sq. mm.)			Adrenal			Aortic fat			Renal fat			Liver			Kidney			Thyroid			Athero-sclerosis
	*cps**	*wt.*†	*cps/mg.*	*cps*	*wt.*	*cps/mg.*	*cps*	*wt.*	*cps/mg.*	*cps*	*wt.*	*cps/mg.*	*cps*	*wt.*	*cps/mg.*	*cps*	*wt.*	*cps/mg.*	*cps*	*wt.*	*cps/mg.*	*grade*
										Normal rabbit + triolein I^{131}												
C–11	0.080	145	0.001																			0
C–12	0.084	159	0.001																			0
Average	0.082	152	0.001																			
										Normal rabbit + NaI^{131}												
R–4	366	585	0.63	39	160	0.24	189	520	0.36				159	795	0.20	107	495	0.22	106	730	0.15	0
R–5	291	350	0.83	39	150	0.26							194	715	0.27	254	695	0.37	140	750	0.19	0
Average	328	468	0.73	39	155	0.25	189	520	0.36				177	755	0.24	181	595	0.29	123	740	0.17	
										Atherosclerotic rabbits + triolein I^{131}												
										A. Rabbits with marked atherosclerosis												
2031	3,555	1,205	2.95	4,510	660	6.83	752	640	1.18	264	230	1.15	1,215	2,055	0.59	1,425	1,720	0.83	257	950	0.27	5
2053	2,300	800	2.88	8,520	770	11.06				1,257	425	2.95	1,467	2,030	0.72	550	995	0.55	471	1,055	0.45	6
2023	2,910	995	2.92	2,400	475	5.05	918	300	3.06	785	205	3.83	639	975	0.66	1,020	1,140	0.89	502	730	0.69	6
2026	2,060	590	3.49	2,076	330	6.29	767	365	2.10	852	235	3.63	790	1,030	0.77	981	1,185	0.83	179	610	0.29	4
Average	2,706	898	3.06	4,376	559	7.3	812	435	2.11	790	274	2.89	1,028	1,523	0.69	994	1,260	0.78	352	836	0.43	5
										B. Rabbits with minimal atherosclerosis												
2057	1,125	535	2.10	4,560	410	11.1	2,473	525	4.7	3,830	215	17.8	266	285	0.93	226	425	0.53	223	755	0.30	2
2057‡	442	255	1.73																			0
2054	443	425	1.02	3,685	535	6.9	337	455	0.74	154	135	1.14	402	1,125	0.36	264	1,030	0.26	485	795	0.61	1
Average	666	404	1.62	4,123	473	9.0	1,405	490	2.72	1,992	175	9.47	334	705	0.65	245	728	0.40	354	775	0.45	1
										Atherosclerotic rabbits with NaI^{131}												
2028	146	290	0.5	99	620	0.16	40	205	0.1				139	620	0.22	135	535	0.25	101	835	0.12	

* Counts per second.

† Sample weight in milligrams.

‡ An additional segment of aorta showing less atherosclerosis was obtained and measured in this animal.

form glass test tubes, 0.9 per cent saline added to a constant volume of 2 ml., and all samples counted in a well-type scintillation counter with a spectrometer scaler long enough to ensure counting errors of less than 3 per cent. Counts obtained were corrected for decay to the date of injection.

Dental (no screen) film packs were exposed for two half-lives to the second aorta segments of all rabbits. Tissues were maintained hydrated by wrapping with Saran Wrap® and preserved by refrigeration. In the atherosclerotic rabbits given either triolein I^{131} or NaI^{131}, films also were exposed to a section of adrenal and of aortic adventitial fat.

RESULTS

The data indicated convincingly that I^{131} from orally ingested triolein was capable of entering and remaining in an atherosclerotic plaque. Thus in the four rabbits exhibiting thick and confluent aortic atherosclerosis, the radioactivity of the first section of atherosclerotic aorta on a unit weight basis (see Table I) was as intense as that found either in the aortic adventitial fat or in the perirenal fat but not as great as that observed in the adrenal. It is of interest too that the radioactivity of the aortic sections obtained from the severely atherosclerotic rabbits (see Table I) was four times as great as sections of similar area obtained from less severely atherosclerotic rabbits. On a weight basis, however, the intensity appears to be only about twice as great.

On the other hand, the aortic segments of normal rabbits given I^{131} triolein failed to exhibit significant radioactivity (see Table I). As compared with the radioactivity of aortic segments from atherosclerotic rabbits given triolein I^{131}, relatively little radioactivity was found in the aortic segments of either the normal or the atherosclerotic rabbits given NaI^{131}. Additionally, only minimal excess concentration of I^{131} was found in the fatty tissues of these animals given NaI^{131} in contrast to the marked preferential concentration in such tissues of I^{131} from triolein. Apparently any fatty tissue, including the adrenal gland, appears capable of preferentially receiving and retaining, for a time at least, I^{131} from triolein. This capability is much less manifest in the case of inorganic iodine. Such organs, however, as the liver, the kidney and the previously iodide "blocked" thyroid gland either receive or retain relatively little either of triolein I^{131} or NaI^{131} when assayed four days after the last of three daily doses.

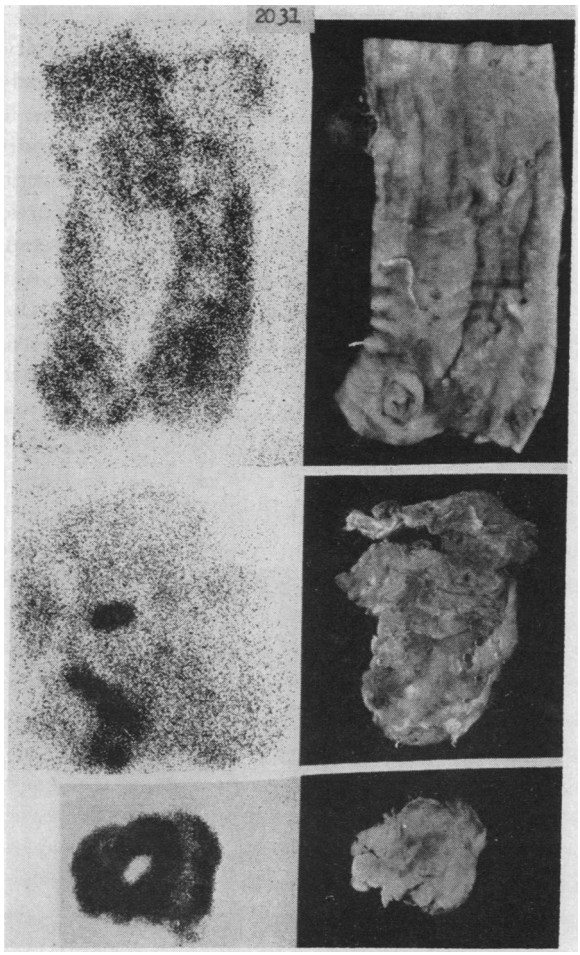

FIG. 1. PHOTOGRAPH OF A LONG SEGMENT OF ATHEROSCLEROTIC AORTIC FRAGMENT (TOP), AORTIC ADVENTITIAL FAT (MIDDLE) AND MEDIAN CROSS-SECTION OF ADRENAL (BOTTOM) AND THEIR RESPECTIVE RADIOAUTOGRAPHS OF RABBIT 2031 GIVEN I^{131} TRIOLEIN

Note the greater density of the aortic radioautograph in its parts corresponding to the atherosclerotic areas of the aorta itself. Also note the intense density of the adrenal radioautograph as well as the relatively clear zone corresponding to the medullary area of the adrenal.

The radioautographs revealed results quite consonant with those obtained by the well scintillation counter. Thus (see Figure 1) films exposed to sections of the atherosclerotic aorta, aortic fat and adrenal gland from animals receiving triolein I^{131} all revealed radioactivity present therein. Also, as suggested by the results obtained with the counter, the films indicated considerably more

activity in the adrenal gland than in the other fat containing tissues. Moreover, this activity clearly was most intense in the cortex of the adrenal gland. It will be observed (see Figures 1 and 2) that the film of the aortic segments indicates activity primarily from the atherosclerotic plaques and almost none from the normal aortic intima lying between these plaques. As expected, the films (see Figures 3 and 4) exposed to the aortic fragments of the normal rabbit given triolein I^{131} and to the aortic fragments of the atherosclerotic rabbit given NaI^{131}, respectively, revealed no significant radioactivity. It is of interest too that the aortic adventitial fat and adrenal gland of the rabbit given NaI^{131} also failed (see Figure 4) to exhibit significant radioactivity on film.

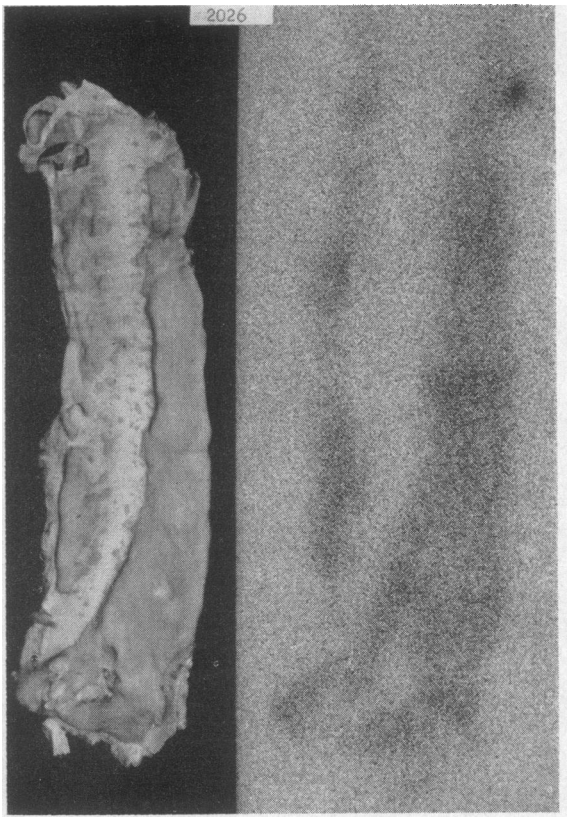

FIG. 2. PHOTOGRAPH OF A LONG SEGMENT OF ATHEROSCLEROTIC AORTIC FRAGMENT OF RABBIT 2026 GIVEN I^{131} TRIOLEIN (SUDAN STAIN) AND CORRESPONDING RADIOAUTOGRAPH

Note again the correspondence between the darker portions of the radioautograph and the atherosclerotic areas of the aorta stained here with Sudan. The emulsion grain is responsible for the background.

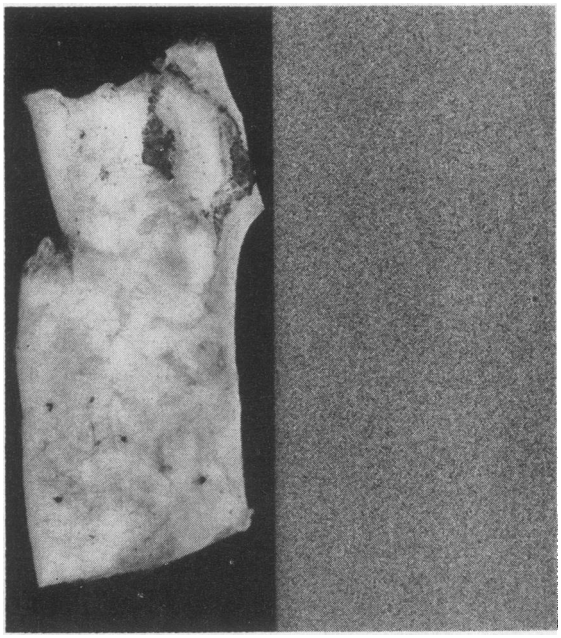

FIG. 3. PHOTOGRAPH OF A LONG SEGMENT OF AORTIC FRAGMENT AND AUTOGRAPH OF NORMAL RABBIT C-11 GIVEN I^{131} TRIOLEIN

Note the background imparted by the emulsion grain and the complete absence of radioactivity as indicated by the autograph.

DISCUSSION

The foregoing results demonstrate decisively that the atherosclerotic aorta of the rabbit is both permeable to and retentive of I^{131} when the latter is administered in lipid form, primarily as triolein I^{131}. Furthermore, the greater radioactivity exhibited by those aortas having the greatest amount of lipid infiltration, the negligible radioactivity of the normal aorta, and finally the localization of the activity afforded by the radioautographs all lead inescapably to the conclusion that it is the atherosclerotic infiltration itself which is responsible for the activity detected.

The ability of the I^{131} to penetrate and to remain in an atherosclerotic process is probably due to its combination with lipid and the probable solubility of this lipid in the lipid of the plaque. This seems true for a number of reasons. It should be recalled that although fed fat is absorbed into the intestinal wall as a mixture of tri-di- and monoglycerides and fatty acids, nevertheless the fat particles in the intestinal lymph contain triglycerides only. Partial glycerides and long chain

fatty acids have disappeared by synthesis into triglyceride during passage across the intestinal wall (7). The triglycerides are stabilized as chylomicrons with phospholipid, cholesterol and protein (8) and it is possible for some of the phospholipid to be formed in the intestinal mucosa from dietary constituents (7). However, Zilversmit and co-workers have shown (6, 9) that plasma phospholipid is entirely excluded from the normal or atheromatous artery of the rabbit. Hence it is fair to conclude that the I^{131} whose preferential localization in the atheromatous plaque was observed in the present study entered the blood stream in the form of chylomicron triglyceride. Additional reasons for this conclusion are: *1*) relatively little I^{131} was detected in an atherosclerotic infiltration when administered as NaI^{131}, and *2*) other tissues rich in fat, such as adrenal cortex, aortic adventitial and perirenal fat, were also found to localize I^{131} after its administration as triolein I^{131}.

Because seven days were permitted to elapse between feeding I^{131} and killing the animals, it is not possible from these experiments to state whether the atherosclerotic plaque is more or less prone to localize lipid bound I^{131} than the other organs and fatty tissues assayed. Sufficient time has been allowed for considerable metabolic alteration. This was allowed by design, in the hope of obtaining just such radioautographs as were in fact obtained. Only serial studies will determine to what extent the differential localization observed at the end of seven days reflects differences in intrinsic permeability, retention or lipid turnover rate in the tissues studied. Such studies are now under way. Nevertheless, the present experiments indicate that a significant differential localization of I^{131} from triglyceride may take place in a supposedly passive atherosclerotic process.

SUMMARY

Four days after the last of three daily doses of triolein I^{131} was fed to atherosclerotic rabbits, radioactivity was found in the atherosclerotic aorta. Moreover this activity was concentrated preferentially in the atherosclerotic infiltration itself. Radioactivity also was found in other fat containing tissues such as the adrenal cortex, aortic and perirenal fat. In contrast, little activity was found in "non-fatty tissues" such as adrenal medulla, liver, kidney and thyroid. In normal rabbits no radioactivity was found in the aorta after triolein I^{131}.

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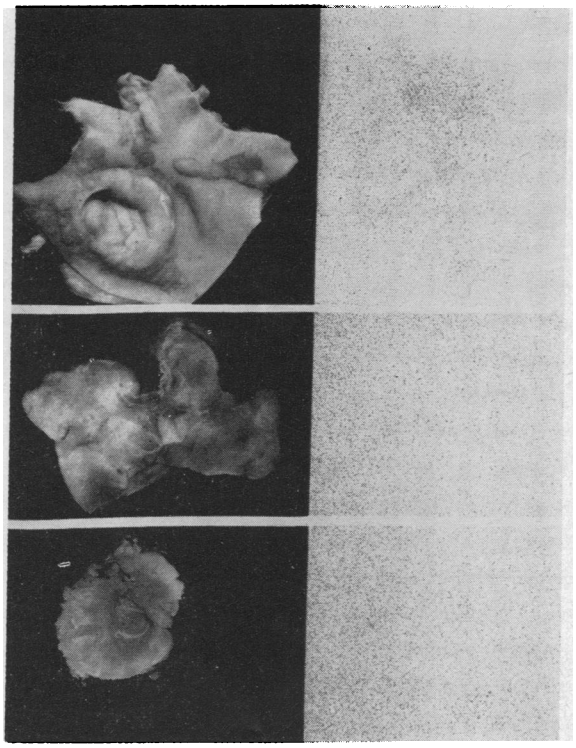

FIG. 4. PHOTOGRAPH OF A LONG SEGMENT OF ATHEROSCLEROTIC AORTIC FRAGMENT (TOP), AORTIC ADVENTITIAL FAT (MIDDLE) AND MEDIAN CROSS-SECTION OF ADRENAL (BOTTOM) AND THEIR RESPECTIVE AUTOGRAPHS OF RABBIT 2028 GIVEN RADIOACTIVE IODIDE

Note absence of radioactivity in all tissues including the atherosclerotic aorta.

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