Selective accumulation of low density lipoproteins in damaged arterial wall

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Abstract To determine whether damaged arterial wall selectively accumulates lipoproteins, normocholesterolemic rabbits were injected with human radiolabeled low density lipoproteins, high density lipoproteins, and/or albumin 24 hr to 12 weeks after balloon-catheter de-endothelialization of the abdominal aorta. When ^{125}I-labeled low density lipoproteins and ^{99m}Tc-labeled albumin were injected simultaneously, the amount of ^{125}I-low density lipoprotein present 24 hr later in abdominal aortas increased steadily, for several weeks, above the amount present at 24 hr in control animals. The increase correlated closely with the degree of re-endothelialization and reached an average maximum for the whole abdominal aorta of three times control when re-endothelialization was between 75 and 85% complete. By contrast, the amounts of ^{99m}Tc-albumin or ^{125}I-labeled high density lipoprotein in balloon-damaged abdominal aortas, and the amounts of ^{125}I-low density lipoprotein, ^{125}I-high density lipoprotein, or ^{99m}Tc-albumin in undamaged thoracic aortas of injured animals showed no such increase. As early as 2 weeks after de-endothelialization, en face radioautographs made following injection of ^{125}I-labeled low density lipoproteins revealed localized areas of greatest radioactivity around the leading edges of regenerating endothelial islands, broad areas of intermediate radioactivity corresponding to the de-endothelialized areas, and very little radioactivity in the re-endothelialized areas. This pattern occurred rarely with ^{125}I-labeled high density lipoproteins and not at all with ^{125}I-labeled albumin. The results suggest that low density lipoproteins are selectively accumulated by the healing rabbit aorta and that the accumulation is greatest in regions where the endothelium is actively regenerating.—**Roberts, A. B., A. M. Lees, R. S. Lees, H. W. Strauss, J. T. Fallon, J. Taveras, and S. Kopiwoda.** Selective accumulation of low density lipoproteins in damaged arterial wall. *J. Lipid Res.* 1983. **24:** 1160–1167.

Supplementary key words balloon-catheter • de-endothelialization • normocholesterolemic rabbits • endothelial regeneration • radioautography

We have shown previously (1, 2) that both ^{125}I-labeled low density lipoproteins (^{125}I-LDL) and ^{125}I-labeled albumin rapidly enter the intact aorta of the normolipidemic rabbit in vivo. Within 10 min after injection of as little as 4–5 mg of LDL or albumin, the radiolabeled protein began to accumulate in the aorta and the amount present did not begin to decrease until 4 to 24 hr after injection (1). The half-lives of LDL and albumin in plasma were 21 and 31 hr, respectively (1, 2). Although the absolute amount of labeled protein accumulated at any time point was small and was greater for albumin than LDL, the profiles of protein accumulation across the arterial wall were qualitatively similar for both LDL and albumin (1).

Since these findings were made in the normal aorta, the question remained unanswered whether albumin and lipoproteins would behave differently in the damaged aorta. Of particular interest was whether there was specific accumulation of LDL by the healing aorta. Other investigators have found that lipid-filled foam cells were concentrated under the leading edge of regenerating endothelium in experimentally injured rabbit (3, 4) and atherosclerotic human (5) arteries. Since plasma LDL is thought to be an important source of such lipid, we measured the in vivo 24-hr accumulation by abdominal and thoracic aortas of ^{125}I-LDL and ^{99m}Tc-albumin, ^{125}I-HDL, or ^{125}I-albumin administered from 24 hr to 3 months after balloon-catheter de-endothelialization of the abdominal aorta. We also made radioautographs of the aortas at several times after de-endothelialization.

METHODS

Lipoproteins and albumin

LDL was prepared by heparin and manganous chloride precipitation (200 units and 0.05 mmol per ml of

Abbreviations: LDL, low density lipoproteins; HDL, high density lipoproteins; EDTA, disodium ethylenediaminetetraacetic acid; ^{125}I-LDL, ^{125}I-labeled LDL.

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plasma, respectively) of fresh, normal, human plasma, and resolubilization in 2 M NaCl with 1 mM EDTA, pH 8.6. This was followed by flotation in a discontinuous density gradient (1.006 and 1.084 g/ml) in a Beckman L2-65B ultracentrifuge at 100,000 *g* for 22 hr at 12°C. The yellow LDL band in the middle of the centrifuge tube was removed by puncture of the tube with a syringe and a 25-gauge needle. No residual heparin was detectable when resolubilized LDL was tested with anti-heparin antiserum. HDL was prepared by ultracentrifugation of the heparin-manganous chloride supernate at a density of 1.21 g/ml. Following 24 to 36 hr of dialysis against buffer containing 0.2 M NaCl and 1.0 mM disodium EDTA, pH 8.6, each lipoprotein preparation was filtered through a 0.22-micron filter (Millipore Corp., Bedford, MA). Lipoprotein purity was determined by double immunodiffusion in agarose (6) against the following rabbit antisera to human proteins: anti-LDL, anti-VLDL, anti-HDL, anti-albumin, anti-immunoglobulins, and anti-whole serum. Protein concentration was measured by the method of Lowry et al. (7).

Human albumin was obtained from Pentex Division, Miles Laboratories, Elkhart, IN for ^{125}I labeling; ^{99m}Tc-labeled albumin was prepared from the "Cardiolyte" kit (New England Nuclear, Boston, MA) which contains human albumin. Labeling was accomplished by stannous chloride reduction of pertechnetate in the presence of albumin.

Protein iodination

Iodination of LDL, HDL, and albumin was carried out by a previously described modification of the McFarlane iodine monochloride technique (1). After 24 to 36 hr of dialysis against buffer containing 0.2 M NaCl and 1.0 mM EDTA, pH 8.6, more than 97% of the radioactivity was precipitable by 10% trichloroacetic acid. Final specific activities were between 100 and 400 cpm/ng protein. From 2.5 to 4.5% of the radioactivity was bound to lipid.

Animals and surgery

Male New Zealand white rabbits (2–3 kg) were obtained from ARI Breeding Labs, West Bridgewater MA, and maintained on Wayne rabbit ration (Allied Mills, Inc., Special Feed Division, Chicago, IL). Their distal aortas were stripped of endothelium by the Baumgartner technique (8). After an animal was anesthetized with ketamine and ether, the left femoral artery was isolated, and a short midline lower abdominal incision was made to allow exposure of the distal aorta. A 4F Fogarty embolectomy catheter was introduced through an arterotomy in the femoral artery and advanced under direct vision into the abdominal aorta. It was inflated to a pressure 3 PSI above the balloon inflation pressure. Three passes were made through the distal aorta with the inflated catheter to remove the aortic endothelium before removing the catheter, ligating the femoral artery, and closing the wounds.

The uninjured control animals had their aortas and femoral arteries exposed and isolated, but no catheter was placed into the arterial tree.

The animals were returned to their cages for recovery intervals ranging from 1 day to 12 weeks prior to injection with labeled proteins; 24 hr after injection they were killed.

Labeled protein injections

Most rabbits were injected intravenously with both ^{125}I-LDL and ^{99m}Tc-albumin. Twenty-five rabbits were injected with ^{125}I-HDL. The rabbits received 130–300 μCi of ^{125}I bound to 1.0–4.5 mg of lipoprotein protein. The dose of ^{99m}Tc was 16 times the activity of the ^{125}I, so that 24 hr after injection when the animals were killed, the activity levels of ^{125}I and ^{99m}Tc were approximately equal. Three animals were given ^{125}I-albumin (165 μCi and 2.2 mg albumin per rabbit) so that en-face radioautography could be performed with albumin.

Fixation and quantitation of aortic specimens

Twenty-four hours after injection of radiolabel, each animal was injected intravenously with 4 ml of a 0.5% solution of Evans Blue dye (Allied Chemical Co., National Aniline Division, New York, NY), which stains de-endothelialized aorta (9). Thirty minutes later, the animal was deeply anesthetized, the right femoral artery was exposed and cannulated with a 1-mm (internal diameter) plastic cannula for aortic perfusion, and a long midline laparotomy incision was made. The inferior vena cava was isolated below the right renal vein, cannulated with a 2-mm (internal diameter) plastic cannula, and the animal was killed with an overdose of intravenous barbiturate. The aorta was perfused with 10% formalin under a pressure of approximately 100 cm H_2O in a retrograde manner from the femoral catheter, with drainage through the vena cava. After 30 min of perfusion, the aortas were excised. The adventitia was removed completely, and the remaining aorta was washed in formalin, divided into abdominal (injured), and thoracic (uninjured) regions, and counted immediately for ^{99m}Tc in a Packard Auto Gamma Scintillation Spectrometer. To eliminate spillover of ^{99m}Tc counts into the ^{125}I window, specimens were counted for ^{125}I 5 days later. An aliquot of the injected dose was counted simultaneously with the aortas in order to correct the results for isotope decay.

After counting, the aortas were pinned out, immersed in 10% trichloroacetic acid, and photographed. The photos of the vessels were enlarged to 8 × 10 prints. The area of the luminal surfaces and the area of the portions that had been re-endothelialized (i.e., were not stained with Evans Blue) were measured with a "Graf-Pen" (Science Accessories Corp., Southport, CT).

En-face radioautography

The fixed, opened vessels were covered with one layer of plastic (Saran) wrap, placed on high-speed X-ray film (Kodak Orthofilm OH-1), and stored for 2 to 3 weeks in a Kodak "X-Omatic Cassette" (24 × 30 cm) prior to development. The fixed aortas were stored for 1 week before radioautography was begun (28 half-lives of ^{99m}Tc). Thus, since the activity levels of ^{99m}Tc and ^{125}I were approximately equal at the time of excision, only the ^{125}I was imaged by the autoradiograph.

Statistics

Statistical analysis was performed using the least-significant-difference procedure for multiple comparisons with a single control group, described by Dunnett (10).

RESULTS

Almost 2% of the injected ^{99m}Tc-albumin and 0.6% of the injected ^{125}I-LDL were present in the intact abdominal aorta 24 hr after injection (**Table 1**). For ^{125}I-HDL, the amount was 1.5% (**Table 2**). Two days after injury, when re-endothelialization had not yet begun (**Table 3**), the percent of the injected dose present in ballooned abdominal aorta was not significantly above the levels in abdominal aortas of uninjured control animals for albumin, but was 150% above control ($P < 0.002$) for LDL, and 280% above control ($P < 0.005$) for HDL (Tables 1 and 2, **Fig. 1**). At 4 days, when mean re-endothelialization was only 13% (Table 3), the relative amounts of both ^{125}I-LDL and ^{99m}Tc-albumin fell sharply to slightly below control values (Table 1, Fig. 1). Thereafter, striking differences in accumulation of LDL and albumin by the abdominal aorta developed. While the percent of injected ^{125}I-LDL in the ballooned abdominal aorta rose steadily as re-endothelialization progressed, the percent of ^{99m}Tc-albumin did not. Four to 8 weeks after injury (29–57 days), LDL accumulation in ballooned abdominal aorta was three times that in unballooned control abdominal aorta ($P < 0.02$ and <0.01, respectively; Table 1, Fig. 1). In contrast, albumin accumulation by the abdominal aorta was slightly below control between 4 and 29 days, and rose just above control at 57 days; none of the differences from control were statistically significant. Between 8 weeks and 12 weeks (57–85 days), accumulation of both LDL and albumin decreased to control levels (Table 1, Fig. 1). The pattern for ^{125}I-HDL uptake by abdominal aorta between 29 and 57 days was similar to that of albumin and unlike that of LDL (Table 2).

The patterns of radiolabeled protein accumulation by uninjured thoracic aorta also varied with time, but much less than they did in abdominal aorta. In contrast to the abdominal aorta, where the pattern of LDL accumulation was distinct from the patterns of HDL and

TABLE 1. Percent of injected ^{125}I-LDL and ^{99m}Tc-albumin present per gram of aorta correlated with time after de-endothelialization

	Radiolabeled Protein Present (percent of injected dose)					
	^{125}I-LDL			^{99m}Tc-Albumin		
	n	Abdominal Aorta Mean ± SEM (*P*)	Thoracic Aorta Mean ± SEM (*P*)	n	Abdominal Aorta Mean ± SEM	Thoracic Aorta Mean ± SEM
Control	8	0.6 ± 0.1	0.6 ± 0.1	8	1.9 ± 0.4	1.7 ± 0.6
Time after injury (days)						
2	6	1.4 ± 0.2 (<0.002)	0.9 ± 0.2	6	2.7 ± 0.3	1.1 ± 0.2
4	6	0.5 ± 0.1	0.3 ± 0.1 (<0.05)	6	1.5 ± 0.2	0.8 ± 0.1
8	6	1.2 ± 0.2 (<0.02)	0.4 ± 0.1	6	1.8 ± 0.2	0.6 ± 0.1
15	7	1.5 ± 0.3 (<0.02)	0.5 ± 0.1	6	1.4 ± 0.3	0.9 ± 0.1
29	7	1.7 ± 0.4 (<0.02)	0.4 ± 0.1	5	1.6 ± 0.3	0.6 ± 0.1
57	5	1.7 ± 0.3 (<0.01)	0.7 ± 0.2	5	2.8 ± 0.7	1.7 ± 0.3
85	8	0.7 ± 0.1	0.2 ± 0.1 (<0.01)	8	2.0 ± 0.2	0.9 ± 0.1

The abdominal aortic endothelium was removed with a balloon catheter on day 0. The thoracic aorta was left intact. Twenty-four hours after radiolabeled protein injections, aortas were excised, washed, divided, and counted as described in Methods. The counts per minute per gram of abdominal or thoracic aorta were divided by the total counts injected to obtain the percent of radiolabel present. The *P* values in each column are for the comparison of experimental with control data in the same column. Where no *P* values are given, *P* was >0.05.

TABLE 2. Percent of injected ^{125}I-HDL present per gram of aorta correlated with time after de-endothelialization

	n	Abdominal Aorta Mean ± SEM (*P*)	Thoracic Aorta Mean ± SEM (*P*)
Control	6	1.5 ± 0.3	1.3 ± 0.2
Time after injury (days)			
2	6	5.7 ± 1.0 (<0.005)	3.0 ± 0.3 (<0.001)
29	3	1.7 ± 0.6	0.8 ± 0.1 (<0.05)
36	5	1.8 ± 0.2	1.0 ± 0.2
57	5	1.9 ± 0.2	0.9 ± 0.1

The abdominal aortic endothelium was removed with a balloon catheter on day 0. The thoracic aorta was left intact. Twenty-four hours after injection of ^{125}I-HDL, aortas were excised, washed, divided, and counted as described in Methods. The cpm per gram of abdominal or thoracic aorta were divided by the total counts injected to obtain the percent of radiolabel present. The *P* values are reported as in Table 1.

albumin accumulation, in thoracic aorta the patterns of accumulation of all three proteins were quite similar. Of particular interest were decreases in accumulation between 4 and 29 days and at 85 days. While abdominal aortic LDL accumulation was rising steadily, thoracic LDL accumulation was below control at 4 days ($P < 0.05$), and rose gradually back to control by 57 days, when levels of LDL in abdominal aorta were peaking, and then, at 85 days, decreased ($P < 0.01$) below control again (Table 1). After the initial rise at 2 days ($P < 0.001$), thoracic HDL accumulation (Table 2) fell below control at 29 days ($P < 0.05$). Thoracic aortic accumulation of ^{99m}Tc-albumin (Table 1) did not rise at 2 days; like thoracic LDL accumulation, it was consistently below control from 4 through 29 days; it rose to control at 57 days, when LDL radioactivity in thoracic aorta was also rising to control, with a suggestion of a decrease again at 85 days.

Re-endothelialization of abdominal aortas progressed steadily with time (Fig. 1), but at each time beyond 2 days the range of re-endothelialization was broad (Table 3), as much as 75% between highest and lowest at 15 days, and never falling below about 30%. Since, when expressed as a function of time, the profiles of both LDL accumulation by abdominal aorta and re-endothelialization correlated quite closely with each other (Fig. 1) in spite of the variability of the latter with time, we next analyzed percent of injected dose present as a function of percent re-endothelialization. Initially, after all the endothelium was removed from the abdominal aortas, they accumulated 2.5 times more LDL than the abdominal aortas of uninjured animals ($P < 0.002$). With only 5% re-endothelialization, the amount of ^{125}I-LDL present in abdominal aortas fell to control levels; this was followed by a gradual steady rise to a peak just over three times control when re-endothelialization was between 75 and 85% complete ($P < 0.005$), followed by a decrease back to control as re-endothelialization approached completion (**Table 4, Fig. 2**). Although the amount of ^{125}I-LDL present in the uninjured thoracic aorta rose slightly when the abdominal aortic endothelium was absent, it fell to one-half of control when re-endothelialization was 40–49% complete ($P < 0.05$). At between 50 and 85% endothelial regeneration, the percent of the injected ^{125}I-LDL present in thoracic aorta rose from one-half of control to control and then fell back to less than half of control as regeneration neared completion ($P < 0.01$). The late small rise in LDL accumulation in thoracic aorta coincided with the maximum levels of LDL accumulated by abdominal aorta.

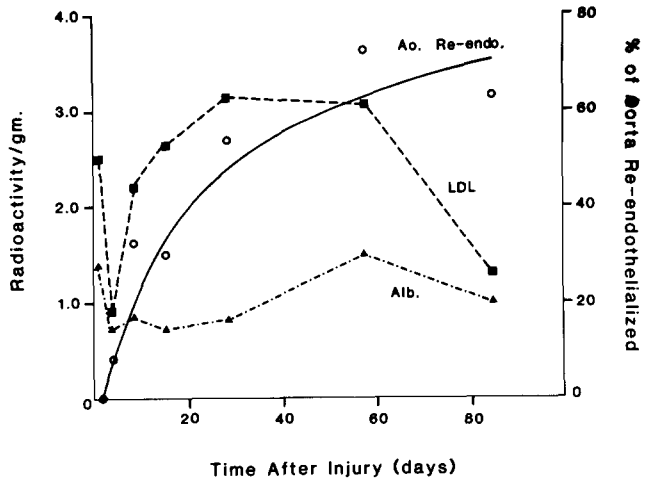

Fig. 1. Endothelial regeneration and fraction of radiolabeled protein present in rabbit abdominal aorta as a function of time after de-endothelialization. The abdominal aortic endothelium was removed with a balloon catheter on day 0. The percent of the aorta that had re-endothelialized, O—O, and the amount of radiolabeled LDL ■ – – – ■ and albumin ▲ – • – • – ▲ that were present at different times following injury, graphed on the ordinate as a fraction of control, were calculated as described in Methods. The values from which the fractional amounts of protein present were derived are given in Table 1. Ao., aortic; Re-endo., re-endothelialization; Alb., albumin.

TABLE 3. Percent of luminal surface of abdominal aorta re-endothelialized as a function of time after injury

Time after injury	n	Mean ± SEM	Range
days			
2	6	0	
4	6	13 ± 4	4–32
8	7	33 ± 5	18–51
15	20	30 ± 3	10–85
29	16	54 ± 4	29–82
57	9	72 ± 4	57–95
85	8	63 ± 7	46–94

The total luminal surface area and the area re-endothelialized were measured as described in Methods.

TABLE 4. Percent of injected radioactivity present per gram of aorta correlated with percent endothelial regeneration

	Radiolabeled Protein Present (percent of injected dose)					
	^{125}I-LDL			^{99m}Tc-Albumin		
	n	Abdominal Aorta Mean ± SEM (*P*)	Thoracic Aorta Mean ± SEM (*P*)	n	Abdominal Aorta Mean ± SEM	Thoracic Aorta Mean ± SEM
Control	8	0.6 ± 0.1	0.6 ± 0.1	8	1.9 ± 0.4	1.7 ± 0.6
Percent endothelial regeneration						
0	6	1.4 ± 0.2 (<0.002)	0.9 ± 0.2	6	2.7 ± 0.3	1.1 ± 0.2
1–9	2	0.6	0.3	2	1.6	0.8
10–19	5	0.9 ± 0.3	0.4 ± 0.1	5	1.3 ± 0.2	0.7 ± 0.1
20–29	2	0.8	0.3	2	1.6	1.0
30–39	9	1.3 ± 0.3 (<0.02)	0.4 ± 0.1	9	1.6 ± 0.2	0.7 ± 0.1
40–49	5	1.2 ± 0.1 (<0.005)	0.2 ± 0.1 (<0.05)	5	2.5 ± 0.2	1.0 ± 0.1
50–59	5	1.7 ± 0.6	0.6 ± 0.3	4	1.7 ± 0.1	0.8 ± 0.1
60–69	2	1.2	0.4	2	1.5	1.1
70–79	7	1.7 ± 0.3 (<0.005)	0.7 ± 0.2	4	2.4 ± 0.6	1.2 ± 0.3
80–89	2	1.7	0.7	2	3.0	2.1
90–99	2	0.4	0.2	2	1.6	0.8

The percent of re-endothelialization (obtained as described in Table 3) by decile was used to group the measurements of percent radiolabel present (calculated as described in Table 1). The *P* values are reported as in Table 1.

Albumin accumulation remained distinctly different from abdominal aortic LDL accumulation when the data were analyzed as a function of percent regeneration (Table 4, **Fig. 3**). During most of the regenerative process, the amount of albumin present in abdominal aorta varied slightly above and below control; and in thoracic aorta it was consistently, but not statistically significantly, less than control ($P < 0.2$ at 10–19%, 30–39%, and 50–59% regeneration). Between 65 and 85% regeneration, accumulation rose above the depressed levels of the preceding period in both thoracic and abdominal aorta, before falling back below control as regeneration approached completion.

Additional evidence for preferential sequestration of LDL in regions of regenerating endothelium came from en-face radioautographs of the entire rabbit aorta. In the thoracic regions at any time, and in completely de-endothelialized abdominal aorta, the radioautographic

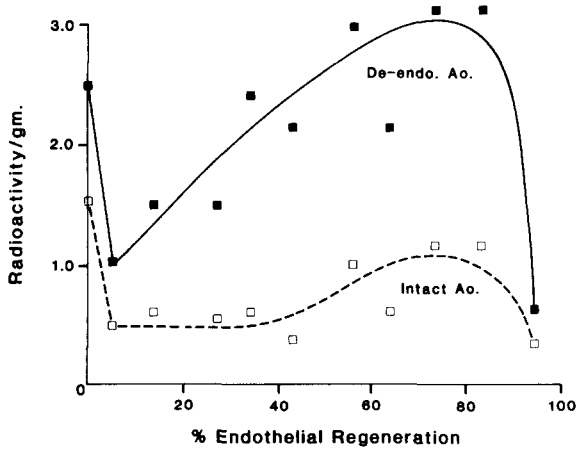

Fig. 2. The fraction of ^{125}I-LDL present in rabbit aorta as a function of endothelial regeneration. The fractions of LDL present in de-endothelialized abdominal aorta ■——■ and intact thoracic aorta □– – –□, graphed on the ordinate as a fraction of control, were related to the percent of endothelial regeneration present 24 hr after ^{125}I-LDL injection. The values from which the fractional amounts of ^{125}I-LDL present were derived are given in Table 4. De-endo., de-endothelialized; Ao., aorta.

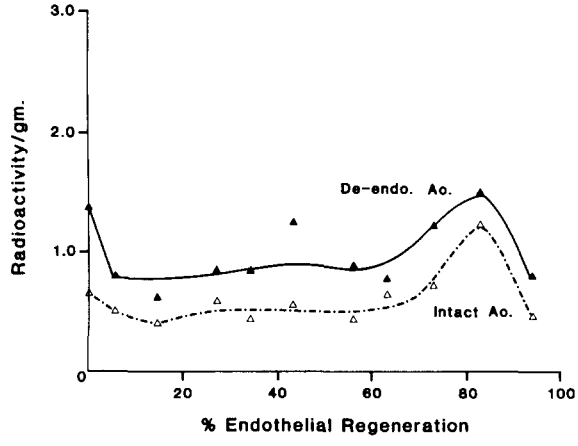

Fig. 3. The fraction of ^{99m}Tc-albumin present in rabbit aorta as a function of endothelial regeneration. The fractions of albumin present in de-endothelized abdominal aorta ▲——▲ and intact thoracic aorta △–·–·–△, graphed on the ordinate as a fraction of control, were related to the percent of endothelial regeneration present 24 hr after ^{99m}Tc-albumin injection. The values from which the fractional amounts of ^{99m}Tc-albumin present were derived are given in Table 4. See Fig. 2 for abbreviations.

images from rabbits injected with ^{125}I-LDL were faint, diffuse, and relatively uniform in intensity. As re-endothelialization progressed, three levels of radioactive intensity became apparent and each activity level, when compared to the Evans-blue staining, corresponded to an anatomic area in the abdominal aortas (**Fig. 4**). The centers of regenerated endothelial islands had very little activity. The blue, still de-endothelialized pseudo-intima corresponded with areas of moderate radioactivity. The actively regenerating edges of endothelial islands showed pronounced radioactivity, although there was some variation in the intensity of activity among different animals or different islands in the same vessel.

The ^{125}I-albumin radioautographs had a relatively uniform distribution of radioactivity, with no evidence of increased uptake at the endothelial edge. The ^{125}I-HDL studies showed some concentration of activity at the endothelial edge in an occasional vessel, but this was a rare event compared with the consistent, marked localization of LDL around the regenerating endothelial edges.

DISCUSSION

Many investigators have pursued the etiology of atherosclerosis through the use of experimental models. Minick, Stemerman, and Insull (3, 4) found that after balloon-catheter injury, hypercholesterolemic rabbits' aortas developed their most marked fatty proliferative response under the edge of the regenerating endothelium, not in the de-endothelialized areas, as had been expected. Similar results were found by Falcone, Hajjar, and Minick (11), when, using the same rabbit balloon injury model, they characterized and quantitated the lipid concentration of the different areas of the healing aorta and found three times more free and ester cholesterol in the re-endothelialized vessel than in the pseudo-intima. Smith et al. (5) found a similar pattern of lipoprotein localization in human arteries, with up to twelve times more LDL in lesions covered with endothelium than in those that had no endothelium.

These studies all indicated an endothelial role in the deposition of lipids and lipoproteins in healing arterial wall; however, the form in which these moieties arrived at their place of deposition and the pattern of deposition in relation to healing both remained unclear. In this study we briefly exposed healing rabbit aortas to labeled LDL, HDL, and albumin in order to examine the specificity of lipoprotein sequestration and to correlate the course of accumulation of radioactivity with vessel healing. We found that LDL was preferentially sequestered by the healing aorta, in high concentration at the edges

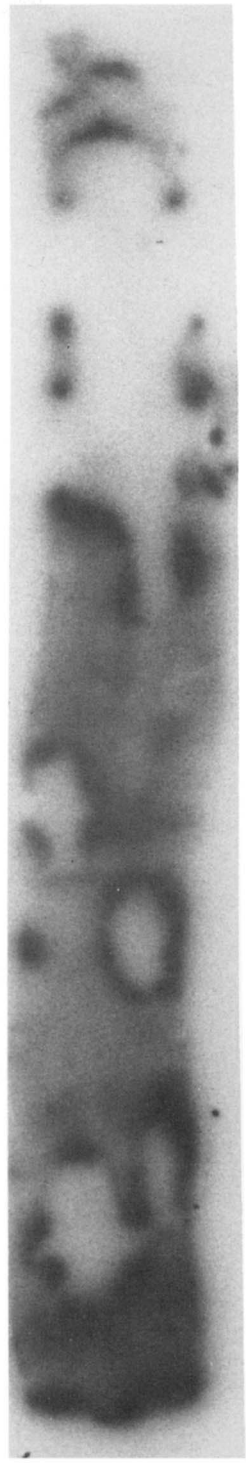

Fig. 4. En-face radioautograph of the abdominal aorta of a rabbit injected with ^{125}I-LDL. ^{125}I-LDL was injected 8 weeks after de-endothelialization of the abdominal aorta and allowed to circulate for 24 hr. One hour before removal of the aorta, Evans blue dye was injected. The areas of least activity in the radioautograph correspond to the centers of endothelial islands, which did not stain with Evans blue. The middle level of radioactivity corresponds with areas that lacked endothelial coverage and thus, did stain with the dye. The most pronounced areas of radioactivity occurred around the leading edges of the endothelial islands where active regeneration was in progress.

of regenerating endothelial islands, and to a lesser extent in still de-endothelialized areas.

Of particular interest was the finding that the preferential uptake of LDL did not develop immediately. In the first 2 days after de-endothelialization, there appeared to be an initial stage of nonspecific uptake of all three proteins by the abdominal aorta in response to the acute trauma. By 4 days after injury, a different response began. The onset of this second stage was marked by a sharp fall in LDL accumulation by the abdominal aorta from 2.5 times more than control at 2 days, to control at 4 days, although mean re-endothelialization had progressed only from zero to 13%. Thereafter, the abdominal aorta began a selective accumulation of LDL. LDL content increased gradually and the increase correlated closely with the gradual increase in endothelial regeneration. No such increase occurred with either HDL or albumin.

The quantitation of LDL accumulation presented in Tables 1 and 4, and in Fig. 2 considerably underestimates the amount of LDL accumulated focally in regions of regenerating endothelium. The focal nature of LDL uptake was apparent only in the radioautographs. For technical reasons, it was necessary to count the whole abdominal aorta. If one assumes that most of the measured increase in LDL accumulation actually took place in less than 10% of the area counted, it is apparent that the focal uptake was far greater than three times control. Although the de-endothelialized areas showed some LDL accumulation on the radioautographs, this was relatively small in comparison with the intense accumulation in regions of regenerating endothelium.

Although the half-life of human ^{125}I-LDL in the rabbit is less than that of ^{125}I-albumin (21 and 31 hr, respectively) (1, 2), this difference obviously does not pertain to the greater accumulation of ^{125}I-LDL in ballooned abdominal aorta as compared either with ^{125}I-LDL accumulation in intact thoracic aorta in the same animals, or in abdominal aorta of control animals. However, the shorter half-life of LDL may contribute to the observed lower accumulation of ^{125}I-LDL as a percent of the injected dose in both the abdominal and thoracic aorta than occurred with ^{99m}Tc-albumin. When the accumulation of both radiolabels in experimental animals is expressed as a fraction of that in control animals, the effect of the difference in half-lives is eliminated.

In addition to the LDL accumulation related to regenerating endothelium, there may be another process that modifies protein accumulation. During the course of healing, when endothelial regeneration in the *abdominal* aorta was between 5 and 50%, the amounts of both ^{125}I-LDL and ^{99m}Tc-albumin in the *thoracic* aorta were consistently below control, although this was statistically significant only for LDL, at one point. The reason for the apparent below-normal accumulation, as well as the late return to normal levels is unclear. Since the phenomenon occurred in the uninjured thoracic aorta, it suggests that a humoral factor may be involved, but the source and nature of such a factor, if present, is unknown.

The focal accumulation of LDL in areas of regenerating endothelium may occur not only because LDL can provide cholesterol to the dividing endothelial cells, but also because there are more elements under the regenerating endothelium that bind LDL than there are in the pseudo-intima. Minick et al. (4) recognized that after endothelial injury the rabbit intima had more layers of smooth muscle below the endothelial edge than it did below the centers of the endothelial islands, or in the pseudo-intima. Also using rabbits, Richardson, Ihnatowycz, and Moore (12) found significantly more extracellular chondroitin sulfate and dermatan sulfate in the intima under the healing endothelium than in the pseudo-intima. These glycosaminoglycans, which can bind LDL to form insoluble complexes (13), are produced by modified smooth muscle cells, which also synthesize extracellular collagen (14, 15). Again using the rabbit balloon injury model, Chidi, Klein, and DePalma (16) demonstrated that for the first 30 days after intimal injury, there was a steady increase in the amount of collagen under the regenerating endothelium, which was not found in the pseudo-intima. There is much evidence that intact LDL crosses normal endothelium into the subendothelial area (1, 17–19). If this occurred, then the subendothelial glycosaminoglycans and collagen could bind the intact LDL. The location of the majority of radioactivity was most likely extracellular because all analyses were made 24 hr after injection and almost all intracellular ^{125}I-LDL would have been degraded to iodotyrosine by that time (20) and have diffused into the circulation. However, radioactive LDL bound to proliferating endothelial cells and smooth muscle cells, as well as LDL internalized by these cells but not yet excreted as iodotyrosine, would be measured also.

If a similar selective accumulation of LDL occurs in human arteries following injury, a subject at risk for atherosclerosis might have arterial regions that had peak levels of LDL accumulation all the time as a result of repeated injury and healing. If LDL removal could not keep up with accumulation, this could lead to the formation of atherosclerotic plaques.

This research was supported in part by grants NS 10828 and HL21460 from the National Institutes of Health, and in part by a grant from the Ambrose Monell Foundation.

Manuscript received 17 November 1982, in revised form 4 March 1983, and in re-revised form 6 June 1983.

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