PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 173, 427–435 (1983)

Long-Term Effects of Deendothelialization of Rabbit Aorta: *In Vitro* Synthesis of DNA, Protein, and Lipid (41666)

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Abstract. To study the long-term local effects of a single balloon catheter deendothelialization of the aorta in the rabbit, the incorporation of [^{3}H]leucine and [^{3}H]thymidine into protein and DNA, respectively, and [^{14}C]acetate and [^{14}C]mevalonate into sterols was measured in incubations of intima–media sections prepared from vessels taken 1 year following the procedure. The uptake of [^{3}H]thymidine by the tissue was essentially the same as in the nonballooned controls, but the incorporation of [^{3}H]leucine and [^{14}C]acetate into tissue residue (proteins and glycoproteins) was approximately nine times and four times control values, respectively. At the same time, sections from the ballooned animals incorporated over six times the amount of radioactive acetate into nonsaponifiable lipids and cholesterol than did controls. In animals ballooned 3 months before sacrifice, when about half of the aortic luminal surface was covered with endothelium, intima–media tissue from both covered and uncovered areas showed increased uptake of labeled precursors into protein, nonsaponifiables, and cholesterol but there was no significant difference in incorporation between reendothelialized and nonendothelialized areas. The persistence of increased metabolic activity in the vessel following the loss of endothelium could be a contributing factor in the atherogenic process.

The sequence of events at the luminal surface of the aorta of experimental animals following *in vivo* deendothelialization is well known. It involves adherence of platelets to the injured area, followed by release of mitogenic factor(s) by the platelets (1) which stimulate(s) proliferation and migration of the underlying smooth muscle cells (SMC) through and above the internal elastic lamina to form a thickened neo-intima. Finally, a single layer of endothelial cells, growing out from endothelium at branch orifices of the vessel, covers the thickened, injured area (2–6). In the rabbit, this procedure has been used to study the atherogenic process by several groups of investigators (7–11). They found that maximum proliferative response to deendothelialization occurs by 1 week after injury, and by about 2 months, this injury-related activity seems to subside. It takes approximately 1 year for endothelial cells to completely re-cover the apparently passivated neo-intimal SMC (4, 9, 12).

We have recently shown that segments of the intima–media prepared from rabbit aorta which had been deendothelialized with a balloon catheter 6 days to 4 months prior to sacrifice, incorporated increased amounts of radioactivity from [^{3}H]thymidine and [^{3}H]leucine into DNA and protein, respectively, and from [^{14}C]acetate (Ac) and [^{14}C]mevalonate (MVA) into lipids as compared with control tissue in short-term incubation studies (13). At 6 days, all of these processes associated with the proliferative response of the SMC of the vessel to deendothelialization were accelerated in the tissue incubations. By 60 days after balloon injury, the samples prepared from the aorta showed no incorporation of [^{3}H]thymidine above that of nonballooned control tissue, but the uptake of [^{3}H]leucine and [^{14}C]Ac and [^{14}C]MVA into protein and lipids continued at an elevated rate; indeed, 120 days after catheterization of the aorta, this higher rate of metabolic activity persisted in the tissue prepared from the vessel. Whether this increased incorporation of radioactivity is preferentially associated with tissue from areas lacking endothelial cover ("blue areas" which take up Evan's blue dye) or with reendothelialized areas ("white areas" where the dye is excluded) is not known. Differences in lipid content and metabolic activity between blue and white areas of aortic tissue from rabbits up to 5 months after deendothelialization have been observed (14–17).

0037-9727/83 $1.50

We now report measurements on the metabolic activity of intima–media tissue from aortae of animals maintained on a normal diet for 1 year after deendothelialization of the vessel, and compare the activities of deendothelialized with reendothelialized aorta in rabbits subjected to balloon injury. To these ends, the incorporation of [^{14}C]Ac and [^{14}C]MVA into sterols and the uptake of [^{3}H]leucine and [^{3}H]thymidine into protein and DNA, respectively, were measured in aortic tissue from rabbits ballooned 1 year prior to sacrifice. In addition, nonballooned controls from the above study permitted a comparison of these parameters with those of a younger group of control rabbits so as to ascertain any effects of age. In a second study, the incorporation of the labeled precursors into intima–media tissue was compared in nonendothelialized (blue) and reendothelialized (white) areas of aorta taken from rabbits sacrificed 3 months after balloon injury.

TABLE I. SPECIFIC ACTIVITY AND AMOUNT OF RADIOACTIVITY IN EACH INCUBATION

Pairs	Specific activity (Ci/mmole)	Study[a] (cpm × 10^{-6}) I	Ia	II
[^{14}C]Ac*	0.057	17	17	12
+				
[^{3}H]Leu	5.0	10	10	14
[^{14}C]MVA	0.022	6	6	14
+				
[^{3}H]Thym	2.0	13	13	7

[a] I: Rabbits ballooned 12 months prior to sacrifice and age-matched controls; age 15 months at sacrifice. Ia: Rabbits, no treatment; age 6 months at sacrifice. II: Rabbits ballooned 3 months prior to sacrifice and age-matched controls; age 6 months at sacrifice.

* Ac, acetate; Leu, leucine; MVA, mevalonate; Thym, thymidine.

Materials and Methods. *Animals.* New Zealand male rabbits were used in all studies and were maintained on cholesterol-free rabbit chow. In the investigations of the long-term effects of ballooning (Study I, Table I), both the experimental and control animals were housed in individual cages at the Pocono Animal Farms, Canadensis, Pennsylvania; balloon catheterization of the aorta was carried out by us at this facility. The rabbits were brought to the Montefiore laboratories for acclimatization 1 week before sacrifice. At the beginning of this study, the rabbits were 12 weeks old and weighed about 2.5 kg. In the other studies reported here (Studies Ia and II, Table I), the rabbits were kept in our animal quarters throughout the period of investigation.

Deendothelialization of the aorta. Rabbits were balloon deendothelialized under pentobarbital and ether anesthesia via the iliac artery up to the level of the aortic arch by a modification (13) of the Baumgartner procedure (18).

Preparation of tissue. Injection of Evan's blue dye 1 hr prior to sacrifice, removal of the aorta, and stripping of the adventitia in ice-cold Dulbecco's medium was carried out as described earlier to afford essentially intima–media tissue (13). In the 1 year post-ballooning study, this preparation was sectioned perpendicularly to its axis to give rings about 1 mm long (13). In the experiment where we compared the metabolic activity of the white with that of the blue areas of the ballooned aorta, we carefully sectioned the intima–media into the appropriate areas and cut the pieces into approximately 2-mm squares; the control tissue was manipulated identically. Completeness of removal of adventitia was evaluated by randomly picking two segments from each aorta and slides were prepared and examined by light microscopy; no adhering adventitia was observed in any sample.

Incubations. The procedure was identical to that previously used (13). To minimize possible differences between the metabolic activity of thoracic and abdominal aortic tissue (20, 21), approximately equal portions of the respective segments were combined for each incubation. Material sufficient for eight incubations was obtained from each rabbit, about 12 fragments per reaction vessel. Elapsed time for readying the tissue for incubation amounted to about 45 min; during this interval, all procedures were carried out in ice-cold Dulbecco's medium. Tissue fragments were incubated in 2 ml Krebs–Ringer bicarbonate buffer containing the labeled substrates, under a 95% air–5% CO_2 atmosphere at 37°C. The tracers were added to the buffer

in pairs so that each incubation contained two labeled substrates, paired as [1−^{14}C]acetate plus L-[4,5-^{3}H(*n*)]leucine or [2-^{14}C]mevalonate plus [methyl-^{3}H]thymidine. The specific activity of the substrates and amount of radioactivity incubated in each vial are listed in Table I. After incubations for the times indicated in the tables, the radioactive buffer solution was removed from the segments, which were then rinsed with cold Dulbecco's medium, 0.9% NaCl, and finally with 20% trichloroacetic acid (TCA); this procedure took about 10 min.

Extraction, isolation, and counting procedures. After removing the 20% TCA solution, the tissue fragments were washed with 5-ml portions of 5% TCA, then 0.9% NaCl; these washes were discarded. Lipids were extracted from the segments by sequential treatment with 5 ml each of hot acetone:ethanol (1:1), then at room temperature with acetone:ether (1:1), and ether (three times); these washings were combined and concentrated to yield the total lipid extract. The delipidated residue was finally washed with 5% TCA, 0.9% NaCl, methanol (twice), and ether; these washings were discarded and the residue was air dried and weighed.

[^{3}H]cholesterol (500 cpm) was added to the lipid extract to measure completeness of recovery during the subsequent steps. This was permissible since it has been shown that ^{3}H in sterols derived from [^{3}H]leucine under the conditions of the study is negligible (22) and it was found in exploratory studies that ^{3}H from labeled thymidine did not enter the lipid fraction. The mixture was saponified, then extracted to afford the nonsaponifiable fraction. Thin-layer chromatography and extraction of the cholesterol from the plate, as well as estimation of its recovery were carried out identically as described (13). Radioactivity was measured by liquid scintillation spectrometry by the double-labeled mode (23) in the nonsaponifiable fraction, in the cholesterol fraction and in the tissue residue; the latter was solubilized (24) prior to addition of the scintillant. Data are expressed as percentage dose per milligram dry weight of tissue, or as a ratio of radioactivities of comparable fractions in experimental vs control rabbits (13). DNA content of the dry tissue residue was measured by a modification (25) of the Burton method (26).

Results. *Study I. Single balloon catheterization 12 months before sacrifice.* Five rabbits were subjected to balloon injury and segments of aortic intima–media were obtained at sacrifice 1 year later; these were incubated for 4 or 8 hr as described. Five animals from the same initial group, maintained under exactly the same conditions for the same time as the experimental rabbits served as controls; they were not ballooned but were injected with Evan's blue before sacrifice and their aortic tissue was identically processed. In contrast to rabbits deendothelialized 2 or 4 months prior to sacrifice, where about 50% of the denuded area had been re-covered by regenerated endothelium (14), the 12-month ballooned rabbits showed almost complete regeneration (>90%) of the aortic luminal endothelium, as judged by Evan's blue staining in the areas still free of endothelial cover (14, 15, 27). The total weight of the dried aortic tissue, free of adventitia, in the ballooned animals, 32.7 ± 10.8 (SD) mg/kg body weight was significantly greater than that in the age-matched controls, 18.4 ± 3.92 mg/kg body weight ($P = 0.02$) indicating increased neo-intimal mass in response to injury. No significant difference was observed in the DNA concentration of the tissue from the ballooned rabbits as compared with the controls, 4.32 ± 0.57 μg DNA/mg tissue residue vs 4.17 ± 0.55 μg DNA/mg tissue residue, respectively.

The aortic tissue from both the control and deendothelialized rabbits remained metabolically active during the 8 hr of incubation as evidenced by continued incorporation of labeled leucine, thymidine, acetate, and mevalonate into tissue constituents during this time (Fig. 1).

Incorporation of [^{3}H]thymidine, [^{3}H]leucine, and [^{14}C]acetate into aortic tissue segments. Table II shows that incorporation of [^{3}H]thymidine by aortic segments into the nonextractable portion of the tissue after 4- and 8-hr incubations, a measure of DNA synthesis, did not differ statistically between the experimental group and the control rabbits. This is in accord with our findings that the proliferative activity of the ballooned vessel returns to baseline 2 months after injury (28)

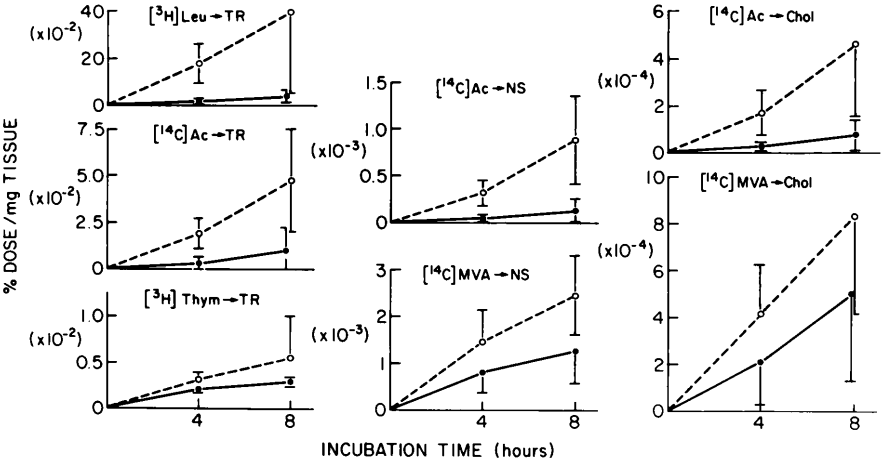

FIG. 1. Incorporation of labeled precursors into tissue components by segments of aortic intima–media from rabbits ballooned 1 year before sacrifice. Segments were incubated in Krebs–Ringer bicarbonate buffer for 4 or 8 hr: ●, control rabbits; ○, deendothelialized rabbits. Each rabbit furnished material for eight incubations; four containing [^{14}C]Ac + [^{3}H]Leu and four containing [^{14}C]MVA + [^{3}H]Thym. Two from each set were incubated for 4 and 8 hr, respectively; thus, every rabbit provided a duplicate incubation for each condition. Each point on the figure represents data from five duplicate determinations from the control and ballooned animals ($N = 5$). The standard deviations are indicated by the lines through the points. Leu, leucine; Ac, acetate; Thym, thymidine; MVA, mevalonic acid; Chol, cholesterol; NS, nonsaponifiable fraction; TR, dried, extracted tissue residue.

and remains approximately at that level for up to 1 year. The uptake of [^{3}H]leucine into this same fraction, marking protein synthesis, 1 year after balloon catheterization, remained 10-fold higher in the experimental animals (Fig. 1 and Table II).

Incorporation of [^{14}C]acetate (Ac) and [^{14}C]mevalonate (MVA) into tissue nonsaponifiable lipids and cholesterol (Fig. 1 and Table II). One year after ballooning, an increased uptake of [^{14}C]Ac into the nonsaponifiable fraction and cholesterol, averaging 6-fold over the aortic tissue segments from the control rabbits, was observed ($P < 0.01$). [^{14}C]MVA incorporation into the nonsaponifiable fraction and cholesterol of the intima–media ex-

TABLE II. INCORPORATION OF RADIOACTIVITY INTO SEGMENTS OF RABBIT AORTA 1 YEAR AFTER BALLOON DEENDOTHELIALIZATION

	Ratio of ballooned/control*†						
	Tissue residue			Nonsaponifiable		Cholesterol	
Tissue incub. time (hr)	[^{3}H]Leu	[^{14}C]Ac	[^{3}H]Thym	[^{14}C]Ac	[^{14}C]MVA	[^{14}C]Ac	[^{14}C]MVA
4	10.2 ± 4.2	5.7 ± 2.9	1.4 ± 0.20§	6.0 ± 2.8	1.8 ± 0.64§	6.9 ± 3.1	1.9 ± 0.93‖
8	9.4 ± 4.9‡	4.9 ± 2.8	1.9 ± 0.58‖	6.7 ± 3.2	1.9 ± 0.54	6.7 ± 3.6‡	1.6 ± 0.64‖

* Ratio of radioactivity in aortic segments of ballooned rabbits (% dose/mg tissue) to that in nonballooned control animals run at the same time. Abbreviations: See Table I.

† Data represent ratio of incorporation of radioactivity (mean ± SEM) into aortic segments of five ballooned and five control rabbits. The ratios were obtained by dividing the average % dose/mg tissue (ballooned) by the corresponding average for the controls which were taken from data in Fig. 1. The error represents the standard error of the ratio of two means (Kendall MG, Stuart A, The Advanced Theory of Statistics. New York, Hafner, 3rd ed, Vol 1:p232, 1969).

‡ $P < 0.02$; § $P < 0.05$; ‖ $P > 0.05$, not significant; all other values, $P < 0.01$.

ceeded that of the labeled acetate (13), since the six-carbon precursor is closer to sterols in the biosynthetic sequence; however, utilization of labeled MVA appeared less affected by the ballooning procedure, showing only about a 2-fold increased uptake into the nonsaponifiable fraction ($P < 0.01$). The incorporation into cholesterol was also increased but was below significance ($P > 0.05$).

Study Ia. Comparison of incorporation of labeled precursors into tissue constituents by aortic segments from 15- and 6-month-old rabbits. The availability of the control animals in Study I (15 months of age at sacrifice) permitted a comparison of the metabolic activity of the aortic tissue from these rabbits with that of a younger group. Accordingly, five rabbits, 6 months old, raised under the same conditions, were sacrificed at the same time as the animals in Study I. The aortae were removed, processed, and incubated concurrently and extracted identically to the controls of Study I. The weight of the aortic tissue, essentially intima–media, after stripping the adventitia, was the same in the 15- and 6-month-old rabbits, 18.4 ± 3.9 (SD) mg/kg body weight and 18.6 ± 4.1 mg/kg body weight, respectively. Data from the incubations with labeled precursors are presented in Table III. Of the seven parameters investigated, six showed no significant difference between the aortic segments from the young and the older rabbits. Only the incorporation of labeled thymidine into DNA appeared related to age; the 6-month-old animals showed a slightly higher uptake ($P < 0.05$). This was in accord with the concentration of DNA in the aortic intima–media which was greater in the younger rabbits (5.43 ± 0.63 μg DNA/mg of tissue) than in the older ones (4.17 ± 0.55), $P < 0.02$.

Study II. Comparison of incorporation of labeled precursors into intima–media of reendothelialized (white) vs deendothelialized (blue) aorta. Five rabbits, 3 months old, were subjected to balloon catheterization and were sacrificed 3 months later. The aorta, approximately 50% reendothelialized at this time, was sectioned into white and blue areas and portions of each were incubated for 8 hr with the labeled precursors as described above. Six nonballooned rabbits served as controls, with their aortic intima–media processed in the same manner. Thus, the uptake of radioactivity into the blue and white areas in the aorta from the same animal could be compared and these could also be compared with the uptake of tracers by tissue from controls, as shown in Fig. 2.

With the exception of the incorporation of [^{3}H]thymidine into DNA, where no significant difference between tissues from control and ballooned aorta could be seen, all experimental animals showed about a 5–7-fold increase in uptake of [^{3}H]leucine and [^{14}C]Ac into the nonlipid portion of the tissue, a 2.5–4-fold increase in uptake of [^{14}C]MVA into sterols and cholesterol, and about a 2-fold increase in the incorporation of labeled acetate into lipids; the latter parameter was not statistically significant by the F test (29). These results are

TABLE III. INCORPORATION OF RADIOACTIVITY INTO SEGMENTS OF AORTA FROM 6- AND 15-MONTH-OLD RABBITS

Tissue incub. time (hr)	Ratio of young/old animals*†						
	Tissue residue			Nonsaponifiable		Cholesterol	
	[^{3}H]Leu	[^{14}C]Ac	[^{3}H]Thym	[^{14}C]Ac	[^{14}C]MVA	[^{14}C]Ac	[^{14}C]MVA
4	1.6 ± 0.77	0.71 ± 0.43	1.4 ± 0.22‡	2.3 ± 1.3	0.84 ± 0.38	2.0 ± 1.1	0.79 ± 0.52
8	2.4 ± 1.4	0.93 ± 0.76	1.5 ± 0.21‡	3.1 ± 2.31	0.76 ± 0.36	2.6 ± 2.0	0.64 ± 0.36

* Ratio of radioactivity in aortic segments of 6-month-old rabbits (% dose/mg tissue) to that in 15-month-old rabbits sacrificed at the same time. These animals were *not* ballooned. Abbreviations: See Table I.

† Data represent ratio of incorporation of radioactivity (mean ± SEM) into aortic segments of five young and five older rabbits. Ratios obtained by dividing % dose/mg tissue of the younger animals by the corresponding values of the older rabbits. See Table II.

‡ $P < 0.01$, all other values are not significant.

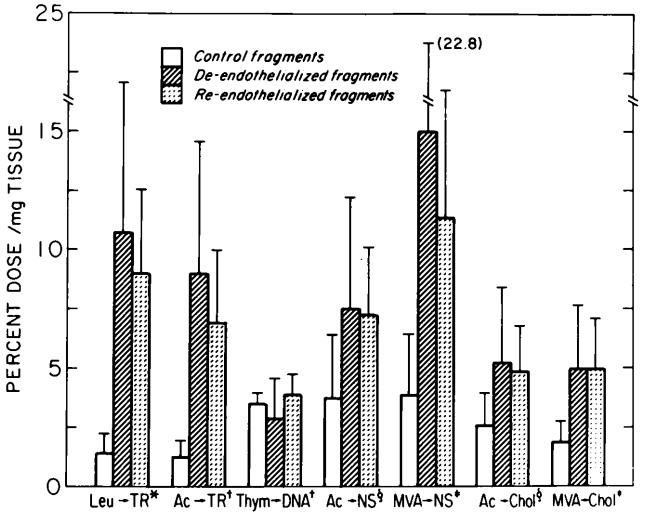

FIG. 2. Incorporation of labeled precursors into tissue components by fragments of aortic intima–media from rabbits ballooned 3 months before sacrifice: a comparison of deendothelialized, reendothelialized, and control tissue. The standard deviations are indicated by the lines through the bars. For abbreviations, see Fig. 1. *, ordinate $\times 10^2$; †, ordinate $\times 10^3$; §, ordinate $\times 10^5$; ‡, ordinate $\times 10^4$.

similar to, and confirm the data reported earlier (13) in rabbits which were balloon catheterized 2 and 4 months before sacrifice. Comparing the deendothelialized (blue) and reendothelialized (white) aortic neo-intima–media tissues with respect to uptake of labeled precursor from the incubation medium, no significant differences in incorporation could be seen ($\alpha > 0.25$). The incorporation of [^{3}H]leucine and [^{3}H]thymidine into protein and DNA, respectively, and the uptake of [^{14}C]Ac or [^{14}C]MVA into nonsaponifiable lipids by blue areas appeared essentially identical to that of the white areas.

Discussion. The increased uptake of labeled leucine and, to a lesser extent, of acetate and MVA by aortic segments from rabbits on a cholesterol-free diet and subjected to a single balloon catheter deendothelialization has been shown to persist at least 1 year after the procedure. This is long after increased SMC proliferation as judged by uptake of [^{3}H]-thymidine for DNA synthesis had subsided to control levels. One can only speculate about the implications of the prolonged effects of the catheterization. Clearly, the data indicate that protein synthesis by the SMC remains elevated under the conditions of our study; this may possibly include increased turnover of glycoproteins associated with glycosaminoglycans and other connective tissue elements (30, 31). Increased utilization of lipid precursors may indicate stimulated membrane formation (31). Whether the accelerated uptake of labeled precursors by the aortic intima–media represents biosynthesis of extracellular matrix or intracellular constituents cannot be ascertained in this study.

In an earlier study where rabbits were ballooned 2 months prior to sacrifice, the mass of the aortic tissue after lipid extraction and dehydration was 38% greater than the mass of the control tissue (13). In the present study where the rabbits were deendothelialized *1 year* before sacrifice, identically treated aortic tissue from the experimental animals still showed approximately the same difference, about 43% greater mass than corresponding tissue from the control rabbits. Since the DNA concentration was approximately the same in the ballooned and nonballooned tissue, this suggests a greater rate of cellular proliferation, a process known to occur during the first month after deendothelialization of the aorta as a response to injury in the rabbit (4, 9, 12). If the rate of incorporation of [^{3}H]thymidine into the tissue fragments has any relevance to the *in vivo* situation, then the observations

that there are no significant differences in [^{3}H]thymidine uptake and DNA concentration between the experimental and control tissue imply that 1 year after balloon injury, the cells from both groups might have comparable proliferative activities. Then how can the increased incorporation of labeled leucine into protein and/or peptide moieties of glycoproteins and increased [^{14}C]Ac uptake into lipids in the aortic segments from the ballooned rabbits be explained? One would think that such metabolic activity might result in increased quantities of polypeptide-containing substances so that the DNA concentration would be *less* in the tissues from the ballooned animals. Three explanations for our findings may be considered: (i) There may be an active flux of substances incorporating labeled leucine and acetate, e.g., glycoproteins, peptides, lipids, within the cells during incubation of the tissue segments. (ii) The increased uptake of radioactivity might represent increased synthesis of tissue constituents *in vivo;* however, the process might be too gradual to be distinguished from the controls by the criterion of decreased DNA concentration. (iii) Enhanced incorporation of radioactivity may be confined to relatively few cells of the neo-intima which might be observed radiometrically but not by a statistically significant increase in mass.

By 1 year postballooning, when essentially complete reendothelialization has occurred, there still may be increased progression toward plaque formation in injured areas surrounding the ostia of branch arteries which were reendothelialized early, after the proliferative response of the SMC had slowed. However, 1 year after deendothelialization may not be long enough to detect differences in biosynthesis of protein and lipids between newly covered and long-standing reendothelialized areas of ballooned aorta. It would be interesting to compare the incorporation of labeled lipid, protein, and DNA precursors in areas of aorta close to the ostia of branching vessels with more distant areas in rabbits which had been deendothelialized 2 years or more before sacrifice. Very recently, Ross *et al.* (32) have reported that SMC in lesions from human femoral arteries have a modified growth potential as compared with medial SMC.

Minick *et al.* (14) reported that 5 months after ballooning the aortae of rabbits maintained on lipid-poor diets the white, reendothelialized areas were thicker than the deendothelialized areas and appeared to accumulate more lipid. The same group further showed that free and esterified cholesterol accounted for a large portion of this increased lipid (15). Relating this to the metabolic activity of the ballooned vessel, they found that while acyl cholesterol acyl transferase (ACAT) activity was greater than in aorta from the nonballooned rabbits, it showed no difference between the blue and white areas in the experimental animals. However, acid cholesteryl esterase activity in the reendothelialized (white area) aorta was lower than that in the deendothelialized areas (16). This is possibly associated with the larger amount of cholesteryl esters found in the white areas. There are also data which suggest that white areas might show greater activity than blue areas in certain parameters: In growing, nonconfluent endothelial cell cultures, there appears to be more LDL receptor activity than in confluent cells (33) possibly related to increased lipid incorporation and/or biosynthesis at the edge of the advancing endothelial cover growing out from the branch vessels.

In rabbit aorta ballooned 3 months before sacrifice, we found that elevated protein and lipid biosynthesis persisted in the intima–media of the tissue as compared with the nonballooned controls whereas DNA synthesis had returned to baseline levels in accord with our previous study (13). However, we could demonstrate no significant metabolic differences between the blue and white tissue of the ballooned animals. In view of this apparent similarity in the rate of cellular proliferation in the deendothelialized tissue and in the control tissue, it is possible that the increased incorporation of labeled leucine, acetate, and mevalonate, occurring to the same extent in the blue and white areas, could be associated with increased production of connective tissue matrix. Although these observations may appear to disagree with those of Minick's group (34, 35) who found that white areas showed greater proteoglycan accumulation than blue areas, they are not, in fact, discordant, since incorporation of labeled precursors into total

tissue proteins and glycoproteins under *in vitro* conditions demonstrates the potential for increased metabolic activity and may not reflect the specific processes which take place *in vivo*.

Although there is little doubt that blood-borne lipids which enter the luminal surface of the aorta by receptor and nonreceptor mechanisms are, in the rabbit, a potential source of lipids present within the vessel wall as well as in atherosclerotic plaques, other factors may also be operative. It is our contention that injury to the luminal surface of the vessel, resulting from a variety of causes (1) and producing loss of endothelial integrity may induce aberrations of normal processes, as manifested by increased uptake of labeled lipid and protein precursors which, persisting for long periods of time, could also contribute to the appearance and development of atherosclerotic lesions.

We thank Mr. Jose Cintron and Mr. Jorge Won for their expert technical assistance and Miss Cipora Gruber for the preparation of the manuscript.

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Received December 28, 1982. P.S.E.B.M. 1983, Vol. 173.