Effects of Chronic Oral Consumption of Nicotine on the Rabbit Aortic Endothelium

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New Zealand white rabbits (10) were administered daily doses of nicotine (2.4 mg/kg/day) in their drinking water for 25 weeks. Nicotine-treated rabbits were compared with control rabbits (10) in terms of blood serum biochemistry and lipid profiles, blood cell counts, changes in aortic endothelial cell morphologic characteristics and distribution, and vessel wall permeability (Evans blue dye uptake). Fasting serum levels of glucose, triglycerides, total cholesterol, and LDL-cholesterol were elevated in nicotine-treated rabbits. No significant differences (nicotine vs control) were seen in leukocyte, erythrocyte and platelet counts, or hematocrit and hemoglobin. Control and nicotine-treated rabbit aortas showed similar focal areas of increased Evans blue dye uptake; staining was localized primarily to aortic arch areas. Endothelial cells (luminal surface) from non-Evans blue and Evans blue arch areas were examined by a combination of Häutchen preparation (silver-stained vessels) and scanning and transmission electron microscopy. Endothelial cells from nicotine-treated arch areas (Evans-blue-stained) showed extensive changes such as: increased cytoplasmic silver deposition, increased formation of microvilli, and numerous focal areas of "ruffled" endothelium (projections on cell surfaces). These data indicate that nicotine, administered orally to rabbits, has a demonstrable *in vivo* morphologic effect on endothelial cells in the aortic arch. (Am J Pathol 1981, 102:229–238)

CIGARETTE SMOKING appears to be a risk factor for diseases of the cardiovascular system. Smoking has been associated with an increase in arterial thrombosis,[1] ischemic heart disease,[2,3] myocardial infarction,[4,5] occlusive peripheral arterial disease,[6] high serum cholesterol, and hypertension.[7,8] The increased occurrence of atherosclerotic lesions in autopsied vessels from smokers has also been reported.[8-10]

Recent studies have shown that nicotine has a desquamating effect on endothelial cells, as measured by the increase in circulating endothelial cells in nicotine-treated rabbits.[11] Other studies have demonstrated that smoking or chronic nicotine administration resulted in changes in vascular resistance and tone in animals.[12-14] Also, nicotine in various combinations, with diet, vitamin D, and stress, produced extensive atherosclerotic lesions in animals.[15,16] The general sequence of events occurring in the development of atherosclerosis has been well documented; however, the initial events responsible for and/or involved in vessel wall changes are less well defined. Repeated endothelial cell injury has been suggested as an initiating factor in thrombosis and atherosclerosis.[17-21]

Although there is a growing body of evidence suggesting that nicotine may contribute to vascular damage and the ensuing complications prevalent in smokers, there have been no controlled studies that have focused directly on changes that may occur in the endothelium *per se* of animals chronically exposed to nicotine only.

The purpose of this communication is to describe the effects of chronic oral nicotine consumption on the morphologic characteristics of rabbit aortic endothelial cells and their distribution, vessel wall permeability (Evans blue dye uptake), serum biochemistry, serum lipid profiles, and blood cell counts.

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Materials and Methods

Animals and Diet

Twenty male New Zealand white rabbits weighing between 2.2 and 2.5 kg were used for these experiments. The test group rabbits (10) were fed a stock diet and administered daily doses of nicotine (2.4 mg/kg/day) in their drinking water for 25 weeks. The 10 control rabbits were fed a stock diet without the daily consumption of nicotine. Drinking bottles were covered with tape to prevent any light-induced decomposition of the nicotine. All nicotine used in these studies was in the form of nicotine hydrogen(+)-tartrate (Gallard-Schlesinger Chemical Manufacturing Corporation, New York).

The rabbits were given water *ad libitum* and found to consume, on the average, 160 ml/kg/day. Nicotine was added to the drinking water (15 μg/ml) of the test rabbits and represented an oral nicotine intake of 2.4 mg/kg/day. From previous human and animal studies on smoking and oral nicotine consumption, it was estimated that each rabbit consumed about as much nicotine per day (2.4 mg/kg/day) as a person smoking 1–2 packs of cigarettes per day.[13,22-24]

Serum Biochemistry, Serum Lipid Profiles, and Blood Cell Analysis

All 20 rabbits were bled 1 week prior to killing. Blood was drawn from the central ear artery using Vacutainer tubes (Becton, Dickinson and Co.). Cell counts and hemoglobin determinations were performed on blood collected in tubes containing tripotassium ethylenediaminetetraacetate as anticoagulant. Total erythrocyte, leukocyte, and platelet counts were obtained by direct counting in a hemocytometer after appropriate dilutions.[25] Differential leukocyte counts were performed on Wright's-stained, air-dried blood smears.[25] Hemoglobin (by cyanmethemoglobin method) and microhematocrit values were obtained by standard procedures.[25] Serum was obtained from 18-hour fasting animals by collecting blood in the absence of anticoagulant. Serum samples were analyzed for lipid by Upjohn Laboratory Procedures (Woodland Hills, Calif). Total cholesterol and triglycerides were determined on a Technicon SMAC and high-density lipoprotein cholesterol (HDL) on an Abbott ABA-VP with the use of appropriate respective methodologies. Low-density lipoprotein cholesterol (LDL) levels were calculated from the above values. Clinical biochemistry profiles on fasting serum samples were obtained on a Hycel Super 17 with the use of appropriate methodologies.

Evans Blue Dye Staining

At the conclusion of the nicotine consumption period (25 weeks), 2 hours prior to killing, each of the 20 rabbits was injected intravenously through the marginal ear vein with 2 ml of Evans blue solution as described by Sharma.[26] Evans blue was prepared as a 5% solution (w/v) in 0.85% saline and filtered through a 0.22-μ Millipore filter prior to use. After the animals were killed (as described below), the aortas were rapidly removed and sliced along the ventral surface, and the luminal surface from the heart to the common iliac arteries were examined for areas of Evans blue dye staining.

En Face Silver Staining

Silver-staining of the aortic endothelium was carried out on 10 rabbits (5 control and 5 nicotine-treated) by whole animal perfusion as described by Silkworth et al.[27] Briefly, each animal was injected intravenously with 1000 units of sodium heparin followed 1 minute later by a lethal dose of sodium Nembutal. The right common carotid artery was ligated and a cannula placed in the left artery. the femoral arteries were exposed quickly and transected to facilitate drainage of the perfusate. Perfusion commenced within 15 minutes following the dose of Nembutal. Control and nicotine-treated rabbits were each perfused successively with 300 ml of freshly prepared 5% glucose (w/v) in water, 100 ml of 0.25% silver nitrate (w/v) in water, 300 ml of 5% glucose, followed by 2 liters of 10% phosphate-buffered formalin solution (pH 7.3). All perfusions were carried out with solutions at 20 C at a pressure of 130 cm of water.[27] The aortas (5 control and 5 nicotine-treated), were rapidly removed, sliced along the ventral surface, pinned open as *en face* preparations, and stored in 10% phosphate-buffered formalin solution (pH 7.3) at 20 C. All 10 aortas were dehydrated through a graded alcohol series. Each aorta was cut into 2-cm segments and labeled to identify its position along the vessel. Häutchen preparations were prepared from segments (Evans-blue- and non-Evans-blue-stained areas) from each of the 10 silver stained aortas as described by Poole et al.[28]

Electron-Microscopic Examination

The remaining 10 animals (5 control and 5 nicotine-treated) were killed and prepared for perfusion as described above. Each animal was perfused under pressure (130 cm water) with 200 ml of Hanks' balanced salt solution (pH 7.3), followed by fixative consisting of 2% paraformaldehyde and 2.5% glu-

taraldehyde in 0.1 M cacodylate buffer (pH 7.3), at a pressure of 130 cm of water for 30 minutes.[29] The aortas were rapidly removed and fixed overnight in the same fixative. All procedures were carried out with solutions at 20 C, unless otherwise indicated. After washing in 0.1 M cacodylate buffer (pH 7.3), each fixed vessel was sliced lengthwise in half.

One half of each vessel was dehydrated through a graded alcohol series and cut into 0.5–1-cm segments. Dehydrated aortic segments (Evans-blue- and non-Evans-blue-stained areas) from each of the 10 rabbits were prepared for scanning electron microscopic examination by the critical point drying method of Anderson.[30] Mounted samples were coated with gold and examined with a Cambridge Mark IIA scanning electron microscope.

The remaining half of each vessel was washed with 0.1 M cacodylate buffer (pH 7.3) and postfixed in 2% osmium tetroxide and 0.1 M cacodylate buffer for 2 hours at 4 C.[29] Postfixed vessels were washed in 0.1 M cacodylate buffer (pH 7.3), dehydrated through a graded alcohol series, and cut into 0.5–1-cm segments. Evans-blue- and non-Evans-blue-stained segments from each vessel were embedded in Epon 812 and processed for transmission electron microscopic study. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a RCA Model EMU-312 electron microscope. It should be noted that transmission electron microscopic examination of Evans-blue- and non-Evans-blue-stained segments from each vessel was limited to the luminal surface (endothelial cells).

Results

Serum Biochemistry, Serum Lipid Profiles, and Blood Cell Analysis

Fasting serum biochemistry parameters for control and nicotine-treated rabbits are shown in Table 1. Glucose levels were higher (averaging 16%) in the nicotine-treated animals than in the control animals. All other measured serum biochemistry values were similar in control and nicotine-treated rabbits.

Fasting serum lipid profiles are shown in Table 2. Triglycerides, total cholesterol, and LDL-cholesterol were on the average 26%, 71%, and 127% higher, respectively, in nicotine-treated rabbits than in the control rabbits. HDL-cholesterol levels were similar in nicotine-treated and control rabbits.

Blood cell analyses are shown in Table 3. No significant differences were seen in the leukocyte, erythrocyte, and platelet counts or hematocrit and hemoglobin levels in nicotine-treated and control rabbits.

Table 1—Fasting Serum Biochemistry Data from Control and Nicotine-treated Animals

Serum components	Control* (N = 10)	Nicotine*† (N = 10)	P‡
Sodium (mEq/l)	140.1 ± 4.0	139.3 ± 3.5	NS
Potassium (mEq/l)	4.4 ± 0.4	4.4 ± 0.3	NS
Chloride (mEq/l)	103.3 ± 3.4	102.1 ± 4.4	NS
Calcium (mg/dl)	13.4 ± 0.3	13.3 ± 0.4	NS
Phosphorus (mg/dl)	4.4 ± 0.5	4.3 ± 0.3	NS
Glucose (mg/dl)	111.2 ± 3.7	128.8 ± 5.3	< .001
BUN§ (mg/dl)	24.6 ± 6.9	26.3 ± 7.0	NS
Creatine (mg/dl)	1.5 ± 0.3	1.6 ± 0.3	NS
Uric Acid (mg/dl)	1.0 ± 0.3	1.1 ± 0.7	NS
Bilirubin (mg/dl)	0.2 ± 0.05	0.2 ± 0.06	NS
Alkaline phosphatase (u/l)	11.7 ± 3.1	12.3 ± 2.7	NS
SGOT ¶(u/l)	28.9 ± 5.0	30.5 ± 4.8	NS
Total Protein (g/dl)	6.2 ± 0.4	6.1 ± 0.3	NS
Albumin (g/dl)	5.5 ± 0.2	5.6 ± 0.2	NS
Globulin (g/dl)	0.4 ± 0.2	0.3 ± 0.1	NS

* Values expressed as mean ± SD.
† Animals chronically treated with 2.4 mg/kg/day of nicotine for 25 weeks.
‡ The significance of differences between means were based on the Student *t* test. NS = not significant.
§ Blood urea nitrogen.
¶ Serum glutamic oxaloacetic transaminase.

Evans Blue Permeability

Control and nicotine-treated rabbits all showed similar focal areas of increased aortic endothelial permeability to Evans blue. The staining was localized primarily to areas of the arch of the aorta. Four of the 10 nicotine-treated rabbits showed more intense blue staining of the aortic arch than was seen in any of the 10 control rabbits. The remaining 6 nicotine-treated rabbits showed the same intensity of aortic arch staining as was seen in the control rabbit aortas.

Light-Microscopic Observation

Examination of Häutchen preparations from silver-stained non-Evans-blue areas, from both control and nicotine-treated rabbit aortas, showed an intact endothelium consisting of elongated cells oriented

Table 2—Fasting Serum Lipoprotein Data From Control and Nicotine-Treated Animals

Serum lipids	Control* (N = 10)	Nicotine*† (N = 10)	P‡
Triglycerides	33.1 ± 5.2	41.6 ± 8.3	< .001
Cholesterol, total	31.4 ± 5.8	53.7 ± 3.9	< .001
HDL cholesterol	23.4 ± 3.7	22.9 ± 3.1	NS
LDL cholesterol	10.4 ± 2.4	23.6 ± 3.4	< .001

* Expressed as mg/dl ± SD.
† Animals chronically treated with 2.4 mg/kg/day of nicotine for 25 weeks.
‡ The significance of differences between means were based on the Student *t* test. NS = not significant.

Table 3—Blood Cell Data From Control and Nicotine-treated Animals

Blood components	Control* (N = 10)	Nicotine*† (N = 10)	P‡
Hemoglobin (g/dl)	13.8 ± 0.9	13.6 ± 0.7	NS
Hematocrit (%)	40.1 ± 3.4	39.4 ± 4.0	NS
White cell count (cells × 10^{-3}/cumm)	10.1 ± 1.1	10.0 ± 1.2	NS
PMN (% WCC)§	56.7 ± 7.2	56.1 ± 5.9	NS
Lymphocytes (% WCC)	38.8 ± 9.1	36.5 ± 10.1	NS
Monocytes (% WCC)	2.3 ± 2.4	2.2 ± 1.7	NS
Basophils (% WCC)	2.7 ± 1.9	4.5 ± 3.4	NS
Platelets (cells × 10^{-5}/cumm)	4.9 ± 1.2	5.1 ± 1.1	NS

* Expressed as mean ± SD.

† Animals chronically treated with 2.4 mg/kg/day of nicotine for 25 weeks.

‡ The significance of differences between means were based on the Student *t* test. NS = not significant.

§ Polymorphonuclear leukocytes expressed as a percentage of total white cell count (% WCC).

with their longitudinal axis parallel to the direction of blood flow. Endothelial cell boundaries were continuous, with few gaps or breaks in the silver lines. Also, cytoplasmic silver deposition was not seen in any of these areas (Figure 1).

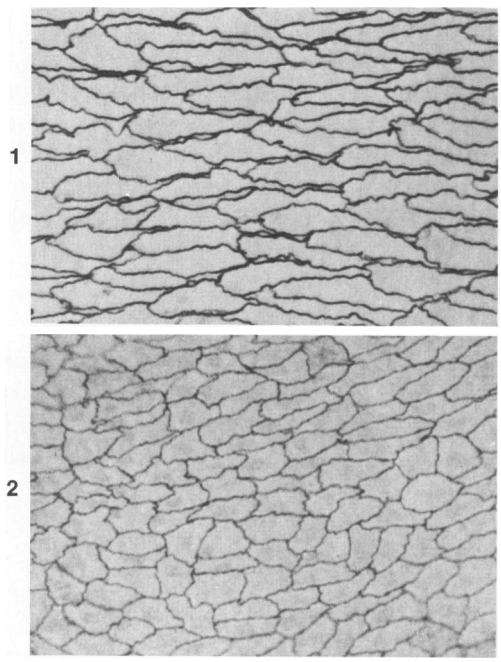

Figure 1—Control and nicotine-treated rabbit aortas (thoracic). Häutchen preparation of silver-stained endothelial cells from non-Evans blue area (nicotine-treated), showing elongated cells oriented with their longitudinal axis parallel to the direction of blood flow. The appearance and distribution of endothelial cells is similar in aortas from control and nicotine-treated rabbits. (×321) **Figure 2**—Control aortic arch. Häutchen preparation of silver-stained endothelial cells from Evans blue area showing irregularly shaped, more rounded, and polygonal cells. (×321)

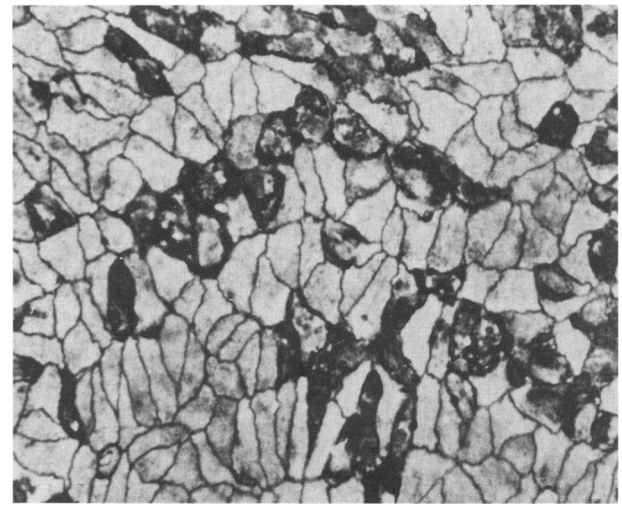

Figure 3—Nicotine-treated aortic arch. Häutchen preparation of silver-stained endothelial cells from Evans blue area showing rounded cells and randomly distributed areas of cytoplasmic silver deposition (*dark areas*) in the endothelium. (×321)

Examination of Häutchen preparations from silver-stained Evans blue arch areas from control rabbit aortas showed, in addition to the elongated endothelial cells described above, areas of irregularly shaped cells that were more rounded or polygonal in appearance (Figure 2). Silver-stained endothelial cell boundaries were continuous, with few breaks in the silver lines. Cytoplasmic silver deposition in the endothelial cells was rarely seen.

Evans blue arch areas from nicotine-treated rabbit aortas showed a distribution of elongated and irregularly shaped cells similar to that seen in the control rabbit aortas (Figure 3). Few gaps or breaks were seen in the continuous silver-stained endothelial cell boundaries. The most striking differences between Häutchen preparations from control and nicotine-treated aortic arch areas were the extensive cytoplasmic silver depositions apparent in the nicotine-treated preparations (Figure 3). Numerous focal areas, varying in size, of silver-stained endothelial cells (cytoplasmic silver deposition) were quite prevalent. The silver deposition ranged from a light granular appearance to intensely stained patches of cells (5–50 cells) that were randomly distributed throughout the Evans blue aortic arch areas of all 5 of the nicotine-treated rabbits examined (Figure 3).

Scanning Electron Microscopic Observations

Scanning electron-microscopic examination of the luminal surface of non-Evans blue areas of aortas from control and nicotine-treated rabbits revealed a smooth, intact layer of relatively flat endothelial

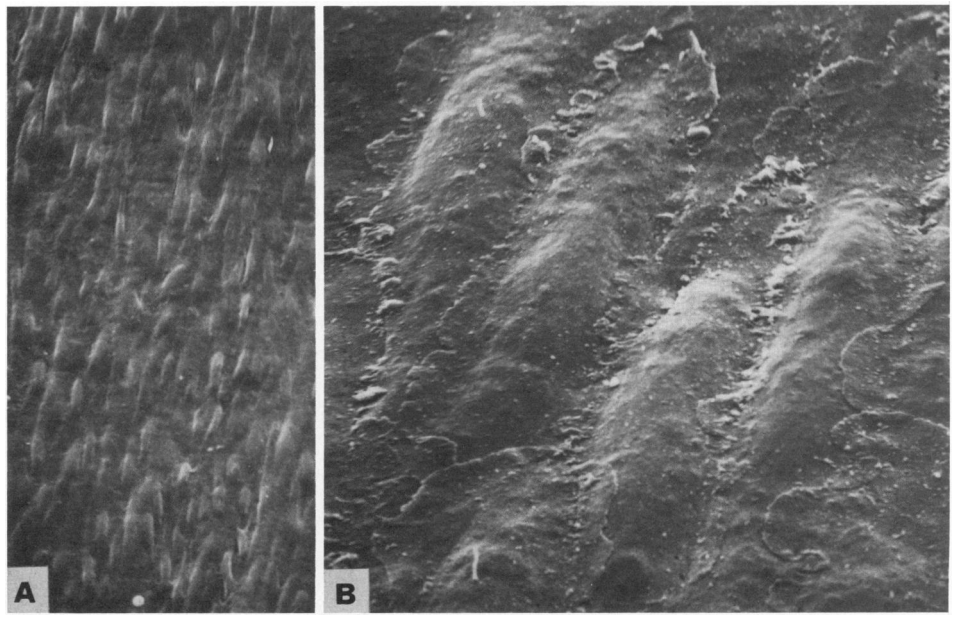

Figure 4—Nicotine-treated rabbit aortas (thoracic). Scanning electron micrograph of the luminal surface from non-Evans blue area (nicotine-treated) showing relatively flat endothelial cells, elongated in the direction of the blood flow. The appearance and distribution of endothelial cells is similar in aortas from control and nicotine-treated rabbits (**A**). (×300) Higher magnification of these cells shows essentially smooth surfaces with very few microvilli (**B**). (×3000)

cells, elongated in the direction of the blood flow (Figure 4A and B). Although most of the endothelial cells in this area had essentially smooth surfaces, a few cells were seen with a limited number of surface microvilli.

On the luminal surface of Evans blue arch areas of aortas from control rabbits there were few flat endothelial cells, most of the cells being raised or more rounded in appearance. These cells contained increased surface microvilli, compared with the cells in control non-Evans blue areas (Figure 5A and B). On the luminal surface of Evans blue aortic arch areas from nicotine-treated rabbits there also were few flat endothelial cells, most of the cells being raised or rounded. In addition, however, numerous focal areas of "ruffled" endothelium were seen in these areas (Figure 6A), as well as large numbers of microvilli, covering the surface of most of the cells (Figure 6B). These focal areas, of varying size, of "ruffled" endothelium usually encompassed about 5–50 cells and were irregularly distributed throughout the entire Evans blue arch areas. Examination of these areas of "ruffled" endothelium at higher magnifications suggested that the "ruffled" appearance of the cell sur-

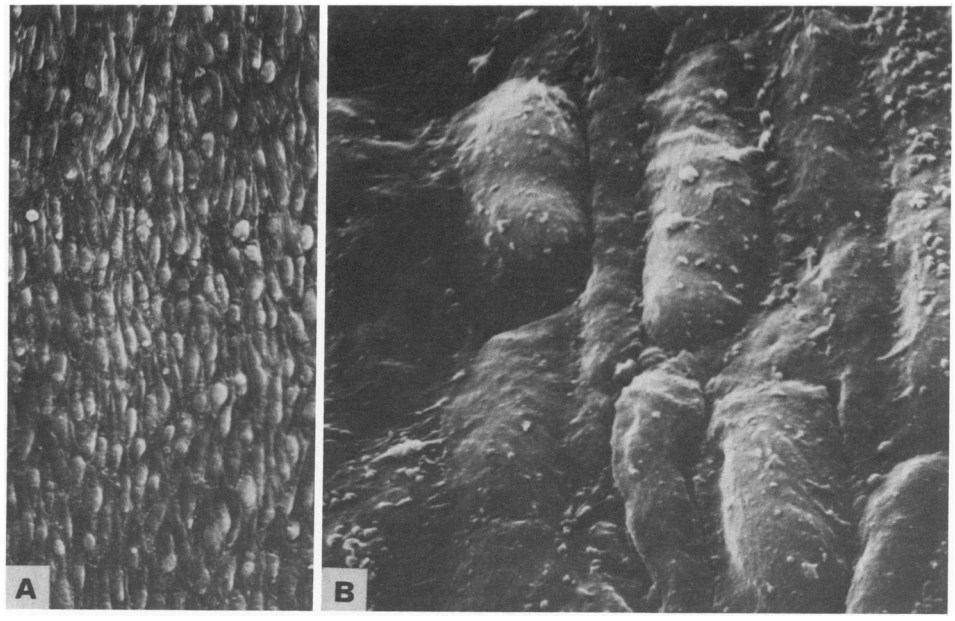

Figure 5—Control aortic arch. Scanning electron micrograph of luminal surface from Evans blue area showing few flat and some rounded and raised endothelial cells (**A**). (×300) Higher magnification of these cells shows few surface microvilli (**B**). (×3000)

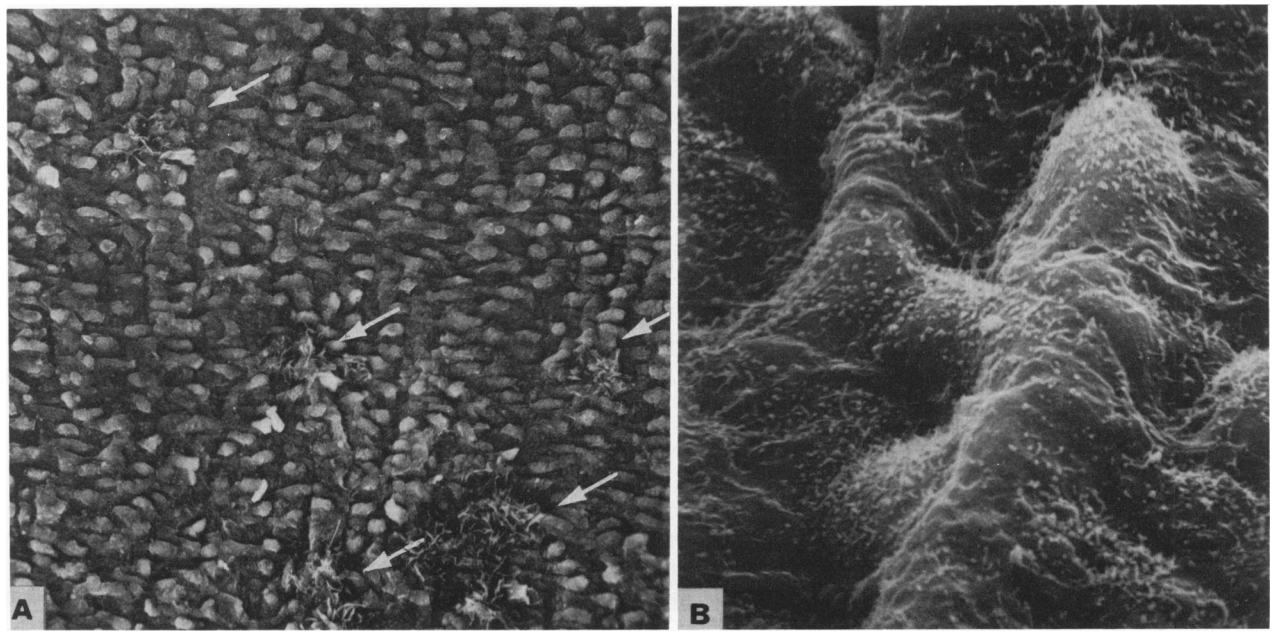

Figure 6—Nicotine-treated aortic arch. Scanning electron micrograph of luminal surface from Evans blue area showing extensive rounded and raised endothelial cells. Note numerous focal areas of "ruffled" endothelium (*arrows*) (**A**). (×300) Higher magnification of cells from an Evans blue area also indicates the presence of numerous surface microvilli. It should be noted, however, that cells containing numerous microvilli did not appear to coincide with focal areas of "ruffled" endothelium (**B**). (×3000)

face was due to varying degrees of plasma membrane alterations (Figure 7). These alterations consisted of what appeared to be extensive wrinkling or folding of the plasma membrane with distinct projections into the lumen (Figure 8). Areas of "ruffled" endothelium were seen throughout all of the Evans blue aortic arch areas from each of the 5 nicotine-treated rabbits examined. Cells containing numerous microvilli did not appear to coincide with areas of "ruffled" endothelium. It should also be noted that the general size (5–50 cells) and distribution of these focal areas of "ruffled" endothelium seen by scanning electron microscopy were similar in size and distribution to

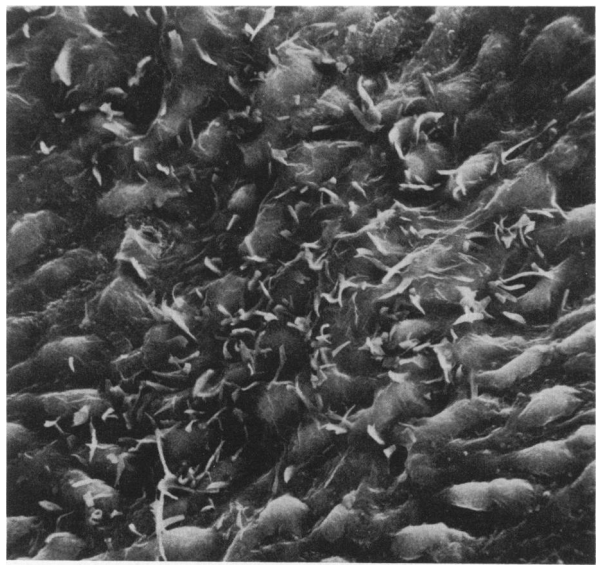

Figure 7—Nicotine-treated aortic arch. Scanning electron micrograph of a focal area of "ruffled" endothelium consisting of about sixty endothelial cells. (×800) (With a photographic reduction of 10%)

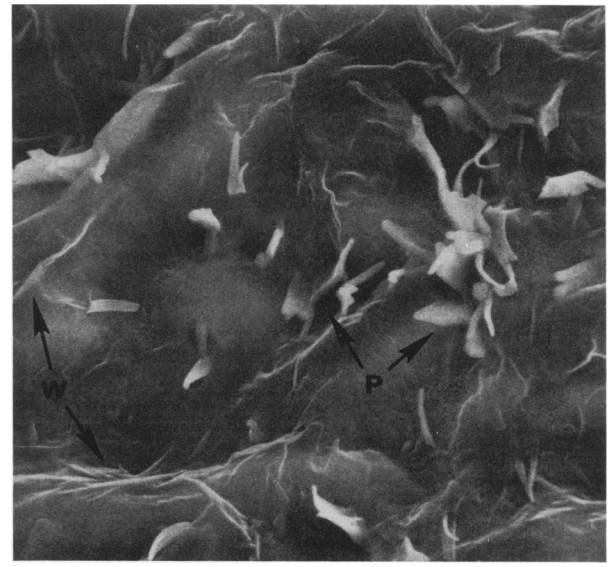

Figure 8—High magnification scanning electron micrograph of "ruffled" endothelium showing wrinkling or folding (*W*) of the endothelial cell plasma membrane with distinct surface projections (*P*) into the lumen. (×2000) (With a photographic reduction of 10%)

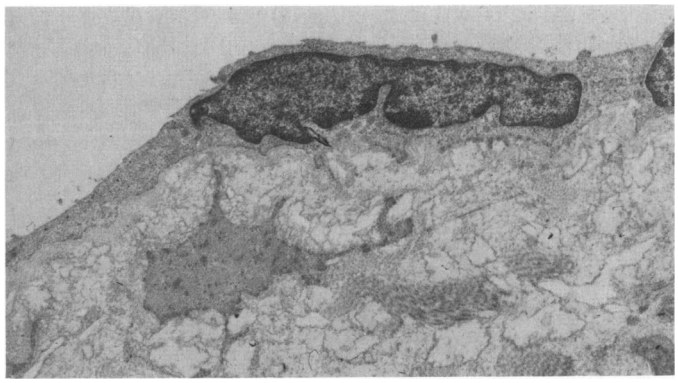

Figure 9—Control rabbit aortic arch. Transmission electron micrograph from Evans blue area showing the luminal surface consisting of a single layer of smooth-surfaced endothelial cells. (Uranyl acetate and lead citrate, ×5560)

the patches or areas of silver-stained cells seen in the Häutchen preparation and may well represent the same areas of altered endothelial cells in the aortic arch areas of nicotine-treated rabbits.

Platelets were not seen associated with the aortic endothelium of non-Evans blue or Evans blue areas from either the control or nicotine-treated rabbits.

Transmission Electron Microscopic Observations

The morphologic studies reported in this paper deal primarily with alterations in the luminal surface of the aorta, and therefore a detailed ultrastructural study of the endothelium and intimal regions of the aortas from control and nicotine-treated rabbits is neither intended nor included. Examination by transmission electron microscopy of the luminal surface of the aortas from control and nicotine-treated rabbits was used only as a means of confirming the presence of extensive alterations in the endothelial surface within the total areas of "ruffled" endothelium seen in the Evans blue arch areas of nicotine-treated animals.

The luminal surface of Evans blue arch areas from control animals was lined with a single layer of smooth-surfaced endothelial cells similar to that seen in the non-Evans blue aortas (thoracic and arch areas) from the same animals. A representative smooth-surfaced endothelial cell from an Evans blue area of a control aortic arch is shown in Figure 9. Smooth-surfaced, single-layered endothelial cells, similar to that seen in control aortas were also seen in non-Evans blue thoracic aortas of nicotine-treated animals. In contrast, the luminal surface of Evans blue arch areas of nicotine-treated rabbit aortas was lined with a single layer of endothelial cells showing many microvilli as well as frequent focal areas of "ruffled" endothelium, consisting of endothelial cells with numerous large surface projections extending into the lumen. A view through such a select focal area of "ruffled" endothelium (see Figures 7 and 8) is shown in Figure 10. Luminal surfaces consisting of

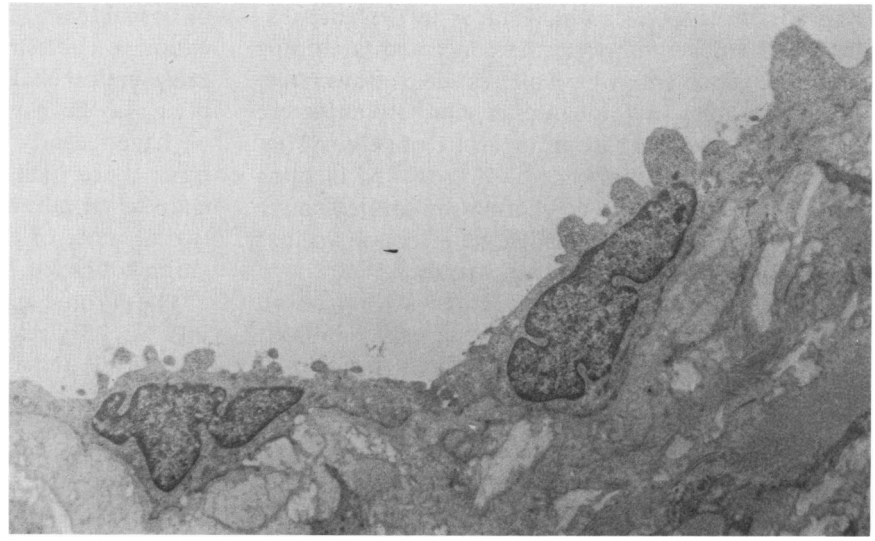

Figure 10—Nicotine-treated rabbit aortic arch. Transmission electron micrograph through focal area of "ruffled" endothelium from Evans blue area showing the luminal surface consisting of a single layer of endothelial cells with numerous surface projections extending into the lumen. (Uranyl acetate and lead citrate, ×5560)

endothelial cells with numerous large surface projections ("ruffled" endothelium) was seen in the Evans blue aortic arch areas in all 5 of the nicotine-treated rabbits examined.

Discussion

The correlation between nicotine intake and elevated levels of blood glucose and cholesterol has been well documented.[31,32] Epidemiologic studies have suggested a positive correlation between elevated LDL levels and the development of coronary heart disease.[33-35] Also, animals on hypercholesterolemic diets rapidly showed marked increases in LDL levels with concomitant changes in their endothelial cells.[15,36-38] Whether the changes in endothelial cells reported here are due solely to the increased levels of LDL in these nicotine-treated rabbits and hence the direct effect of LDL on endothelial cell morphology or some combination of effects remains to be established.

The importance of endothelial cell injury and the subsequent development of acute or chronic vascular disease has been suggested under a variety of experimental conditions.[17-21] It has been suggested that nicotine may be involved in and/or directly responsible for endothelial cell damage, which in turn could play a role in the occurrence of cardiovascular disease in smokers. Nicotine administration has been shown to have a desquamating effect on endothelial cells in animals treated with nicotine.[11] In addition, in animals treated simultaneously with both an atherogenic diet and nicotine there developed more extensive atherosclerotic lesions than in those treated with the diet alone,[16,17] presumably because of additional nicotine-induced endothelial cell damage. Our data are consistent with these observations that nicotine administration causes changes in the endothelium and further supports the concept that chronic nicotine exposure may have a direct deleterious effect on the vascular endothelium *in vivo*. Recently, we have shown that the chronic exposure of cultured bovine aortic endothelial cells to 10^{-7}–10^{-5} M nicotine caused increased giant cell formation and extensive cellular vacuolation and had some effect on various endothelial cell properties and functions.[39,40]

Several reports have described Evans-blue-stained areas in the aortic arch of normal control rabbits, dogs, and pigs.[26,41,42] Such foci are thought to result from endothelial cell damage induced by focal hemodynamic disturbances in the aortic arch. These arch areas have been shown to be more susceptible to further endothelial cell injury such as the additional damage caused by endotoxin,[43] immune complexes,[26] or conceivably nicotine administration as described in this paper.

The chronic administration of nicotine to rabbits caused extensive changes in the endothelium of the aortic arch of these animals, as demonstrated by a combination of silver staining and scanning and transmission electron microscopy. Endothelial cells from these Evans blue arch areas frequently showed increased numbers of microvilli similar to the increased microvilli that have been described in the umbilical cord arterial endothelium of smoking mothers.[44] However, the most extensive and striking changes in aortic endothelial cells were seen in the numerous focal areas of "ruffled" endothelium. Areas of "ruffled" endothelium contained cells with numerous surface projections and membrane foldings and were similar in appearance to the "ruffled" endothelium observed during the early stages of spontaneous atherosclerosis in White Carneau pigeons.[45] These investigators suggested that this endothelial cell change or damage was one of the earliest morphologically identifiable events in the genesis of atherosclerosis in these pigeons. The data presented in this paper provide direct evidence that nicotine administration causes rapid and extensive changes in the aortic endothelium *in vivo* similar to those seen during the early stages of spontaneous atherosclerosis in pigeons.[45] Nicotine may, therefore, be a prime factor in initiating the development of atherosclerotic lesions,[8-10] proliferative lesions in intramyocardial arteries, and other vascular abnormalities in smokers.[10]

The exact mechanism(s) by which the oral administration of nicotine to rabbits affects endothelial cell mophology remains to be established. Nicotine effects may be mediated, indirectly, through the release of vasoactive agents[46,47] and/or the subsequent elevation in cholesterol levels,[32,33] which in turn could cause endothelial damage.[15,36-38,48] Conversely, nicotine may have a direct effect on endothelial cells, as we have recently demonstrated in culture.[39,40] It appears more likely that nicotine-induced changes in endothelial cells *in vivo* are due to a combination of two or more of the direct and/or indirect effects of chronic nicotine administration.

The chronic oral administration of nicotine to rabbits for 25 weeks, increased blood cholesterol and LDL levels and also caused changes or damage to the endothelial cells in the aortic arch areas of these animals. These observations may therefore provide some insight into our understanding of the direct and/or indirect *in vivo* effect(s) of nicotine on aortic

endothelial cells and thus better define a possible role for nicotine in the development of thromboembolic complications and cardiovascular disease in smokers.

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