Diet-Induced Atherosclerosis in the Marmoset[1] (37505)

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Different species of New World monkeys differ markedly in their vulnerability to both spontaneous and experimentally induced atherosclerosis. Systematic surveys of wild caught specimens have demonstrated that naturally occurring atherosclerosis manifested by aortic sudanophilia is very prevalent in spider monkeys, fairly frequent in squirrel and woolly monkeys, uncommon in cebus monkeys and rare in marmosets (1). The striking differences in species specificity is also evidenced by comparative studies which show the order of susceptibility to diet-induced atherosclerosis to be squirrel monkeys > woolly monkeys > cebus monkeys (2).

The pronounced resistance of the marmoset to atherosclerosis in the wild prompted an attempt to produce the disease in captive animals restricted to a high cholesterol–high coconut oil diet. This effort was aborted when the marmosets developed a malabsorption syndrome characterized by jejunal lipodystrophy, steatorrhea and osteomalacia which necessitated their termination (3). Accordingly, the present study was undertaken to determine (a) whether replacement of coconut oil by lard in the experimental diet would prevent the onset of jejunal lipodystrophy and (b) whether the high cholesterol–high lard diet would be atherogenic for the marmoset.

Materials and Methods. Four adult, healthy, colony-conditioned cotton top marmosets (*Saguinus oedipus*) were restricted to the diet shown in Table I supplemented with 25.0 mg ascorbic acid, 4.9 mg nicotinic acid, 3.0 mg calcium pantothenate, 1.0 mg thiamine hydrochloride, 1.0 mg riboflavin, 1.0 mg pyridoxine hydrochloride, 0.1 mg folic acid, 25.0 μg biotin, 1.0 μg vitamin B_{12}, 1.1 mg α-tocopherol, 360 IU vitamin A acetate and 600 IU vitamin D_3/animal/day. The fat content was comprised of 5% cholesterol, 23% lard and 2% corn oil. The diet was compounded daily and offered at a level of 30 g/animal/day. Each marmoset was housed in a hanging wire cage containing an *ad libitum* supply of fresh drinking water in a room maintained at 80°F and a minimum humidity of 60%. Serum cholesterol levels were determined by the method of Schoenheimer and Sperry (4) on samples of femoral vein blood drawn immediately before the start of the experimental regimen and at monthly intervals thereafter. All animals were inspected daily and weighed weekly. They were killed by cardiac puncture exsanguination when the cholesterol concentrations approximated 4, 6, 8 and 16 times the pretest values. These levels were reached at 47, 52, 68 and 73 wk study, respectively.

At each necropsy, a 3 cm segment of the thoracoabdominal aorta was dissected free, denuded of adherent tissue, slit longitudinally along the anterior wall, pinned to a chipboard strip and fixed in 10% neutral formalin for 24 hr. The fixed specimens were stained for intimal fat with Sudan IV by the method of Holman *et al.* (5). All other structures removed at necropsy were also fixed in 10% buffered formalin. Representative samples were processed for paraffin embedding and cut at 6 μm. Alternate slides were stained with hematoxylin and erythrosin B and with Masson's trichrome. Selected specimens of the formalin-fixed hearts, aortas and tongues were embedded in gelatin, sectioned with a freezing microtome and stained with Oil red O.

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TABLE I. Composition of Experimental Atherogenic Diet.

Component	Amount (%)
Sucrose	40.6
Casein (vitamin free)	25.0
Lard	23.0
Cholesterol	5.0
Salts IV[a]	4.0
Corn oil (Mazola)	2.0
Choline chloride	0.2
p-Aminobenzoic acid	0.1
Inositol	0.1

[a] Salt mixture:

	(g)		(g)
$CaCO_3$	60.0	$Fe(C_6H_5O_7)_2 \cdot 6H_2O$	5.5
K_2HPO_4	64.5	KI	0.16
$CaHPO_4 \cdot 2H_2O$	15.0	$MnSO_4 \cdot 4H_2O$	1.0
$MgSO_4 \cdot 7H_2O$	20.4	$ZnCl_2$	0.05
NaCl	33.5	$CuSO_4 \cdot 5H_2O$	0.06

Observations. The high cholesterol–high lard diet was well accepted by the marmosets. Each had a progressive increase in body weight which peaked in the 15th study week. All retained a substantial part of the weight gain throughout the feeding periods. The mean initial, peak and final weights were 405, 487 and 440 g, respectively.

The average preexperimental serum cholesterol concentration in the four animals was 93 mg/100 ml with a range of 80 to 99 mg/100 ml. Ingestion of the high cholesterol regimen was accompanied by appreciable time-related increments in serum cholesterol levels. The longer the diet was consumed, the higher the elevation of serum cholesterol. Serum cholesterol values at the various study end points were 380 mg/100 ml at 47 wk, 550 mg/100 ml at 52 wk, 740 mg/100 ml at 68 wk and 1485 mg/100 ml at 73 wk.

Clearly demonstrable gross sudanophilia indicative of intimal fat deposition was found in the aortas of the marmosets followed for 68 and 73 wk but not in those killed after 48 and 52 wk. Involvement was manifested by reddish-orange, raised, discrete or coalescent streaks and patches on the intimal surface. The lesions were fairly evenly distributed throughout the length of the aortas. Histologically, they were comprised of collections of lipid-containing histiocytes (foam cells) confined to the intima (Fig. 1) or extending into the underlying media and disrupting the elastic lamellae and intervening smooth muscle cells (Fig. 2). Oil red O stained sections of the atheromas disclosed heavy intracellular fat accumulations (Fig. 2).

On microscopic examination, there were no abnormalities in the jejunal mucosa in any of the marmosets. Each had prominent atherosclerotic changes in the lingual arteries (Fig. 3) and arterioles (Fig. 4) and in the distal branches and arterioles (Fig. 5) of the coronary arteries. All of the arteriolar and the vast majority of the arterial lesions were of the histiocytic foam cell variety. In some of the affected small arteries and in all of the afflicted arterioles, the clumps of fat-filled cells bulged into the lumen, markedly reducing the size of the channel. In some of the medium sized branches of the lingual arteries, the lesions consisted of both intimal foam cells and lipid laden medial multipotential mesenchymal cells (Fig. 6).

As in the aorta there was a time-associated increase in susceptibility to the diet-induced atherosclerosis in many other parts of the marmoset arterial vasculature. Whereas only the lingual and coronary arteries were atherosclerotic in the animals given the atherogenic diet for 1 yr or less, foam cell atheroma formation was widespread and generalized in the marmosets terminated after 68 and 73 wk study. In these animals, atheromata were present in both the pulmonic and aortic circulations and were particularly prominent in the arteries supplying the lungs, kidneys, pancreas, spleen and gastrointestinal tract.

Discussion. A purified diet identical in composition to the present regimen, containing 20% corn starch and 10% corn oil in place of lard and cholesterol, has been used in this laboratory for many years to delineate the nutritional requirements of the marmoset. These studies (6) have involved detailed pre- and postmortem examinations of more than 100 animals. In no instance have there been any gross or microscopic manifestations of atherosclerosis in animals restricted to the

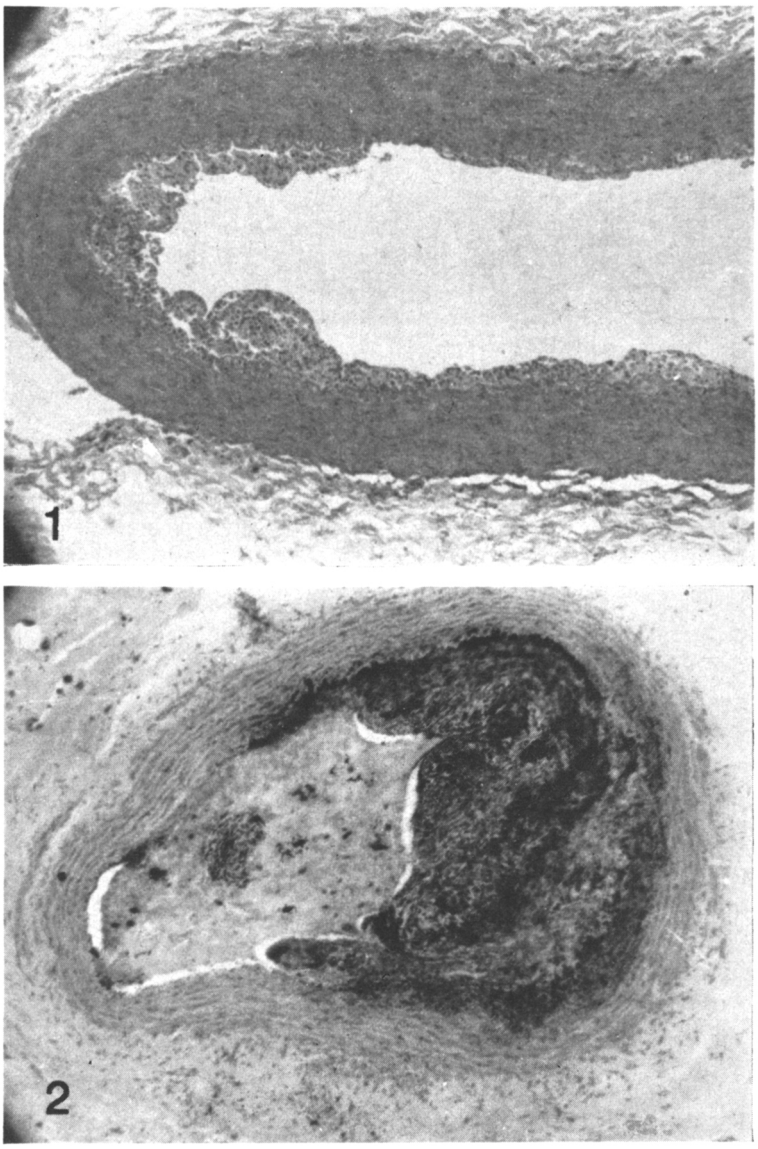

FIG. 1. Intimal atheromas in abdominal aorta of marmoset confined to atherogenic diet for 73 wk. Hematoxylin and erythrosin B stain. ×20.

FIG. 2. Atheromatous lesion in frozen section of abdominal aorta of marmoset fed atherogenic diet for 73 wk showing heavy intimal accumulation of fat filled foam cells. Oil red O stain. ×20.

chemically defined basic diet. Inclusion of lard and cholesterol in the diet, together with the long time ingestion, created an atherogenic challenge severe enough and prolonged enough to overcome the marmoset's resistance to atherosclerosis.

One of the most striking features of atherosclerosis in man and monkeys is the patchy distribution of the plaques in different parts of the vascular tree and in different segments of the same artery (7, 8). Localization of the lesions has been ascribed to favorable mechanical, hemodynamic and/or metabolic conditions. In man, the process usually begins in the aorta and extends to the muscular arteries in centrifugal fashion. An unusual

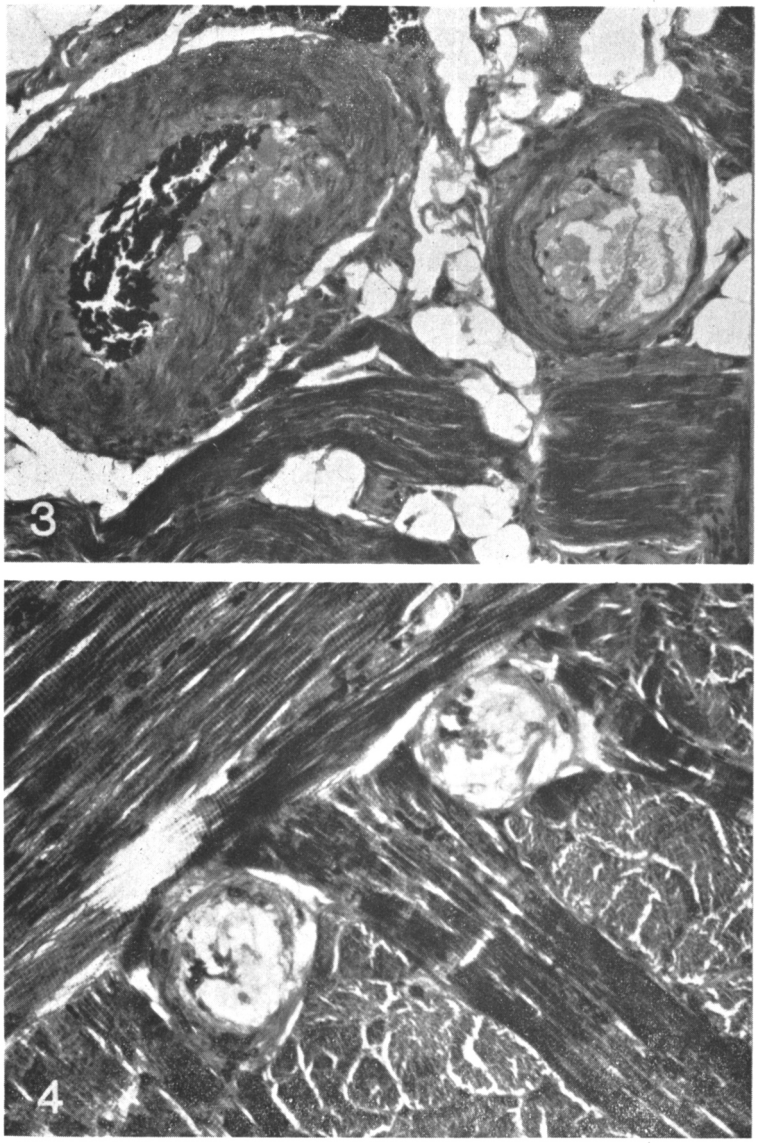

FIG. 3. Foam cell atheromas in major branches of lingual artery in marmoset given atherogenic diet for 47 wk. Masson's trichrome stain. ×50.

FIG. 4. Foam cell atheromas in lingual arterioles of marmoset ingesting atherogenic diet for 52 wk. Masson's trichrome stain. ×100.

and provocative aspect of the present study was that atheroma formation in the lingual arteries and distalmost sections of the coronary arteries preceded that in the aorta and contiguous major branches. The only apparent common denominator between the atherosclerotic lingual and coronary arteries was embodiment in firm muscle tissue. Except for the lingual arteries, the distribution of the experimentally produced atherosclerosis in the marmosets conformed with that reported by Strong *et al.* (1) for spontaneous lesions in free ranging primates. They found that aortic arterial lesions in primates are usually much less extensive and considerably less severe than in man, and that lipid-con-

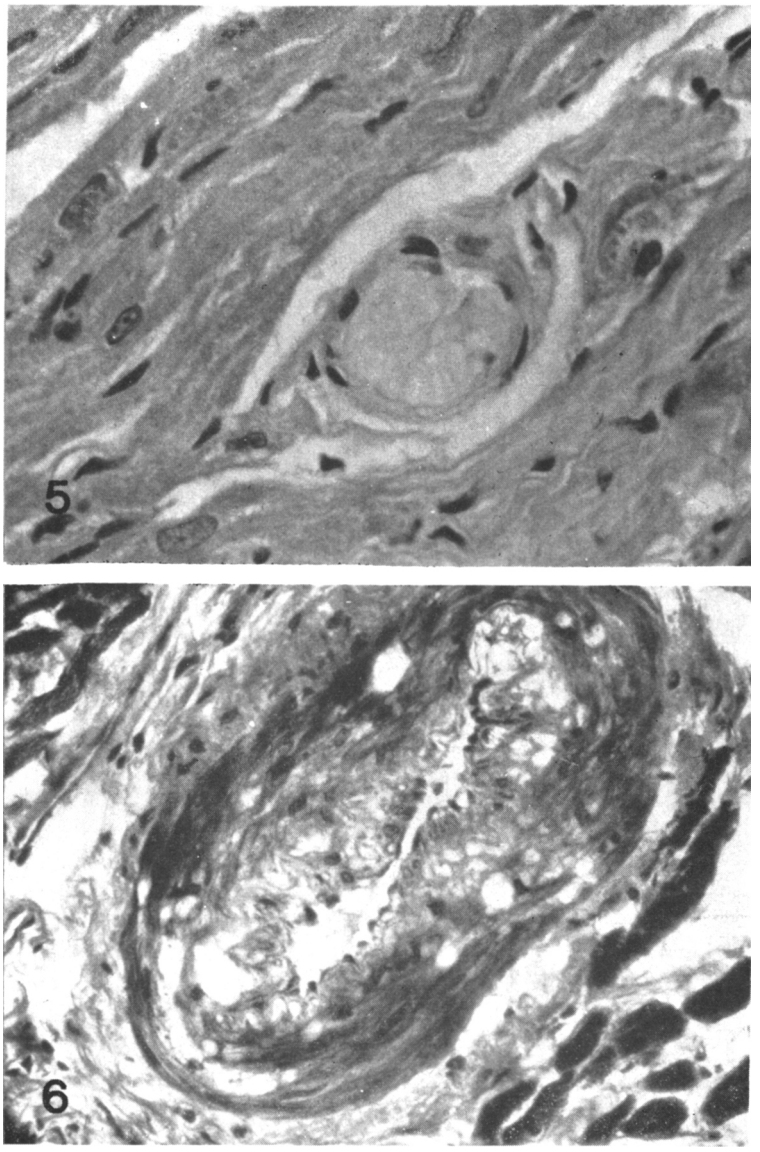

FIG. 5. Foam cell atheroma in myocardial arteriole of marmoset restricted to atherogenic diet for 47 wk. Hematoxylin and erythrosin B stain. ×200.

FIG. 6. Atherosclerotic changes in intima and media of major branch of lingual artery in marmoset relegated to the atherogenic diet for 68 wk. Masson's trichrome stain. ×50.

taining lesions in the major branches of the coronary arteries are rare even in those species in which aortic lesions are frequent.

The lingual arteries have been largely ignored in surveys of the vascular propensity to spontaneous atherosclerosis. Experimentally, lingual atherosclerosis has been produced in rabbits (9) and lingual medial arteriosclerosis has been generated in rats (10) by dietary means. The present data demonstrate that the lingual arteries may serve as an early target site of atherosclerosis which could, conceivably, be used clinically as a diagnostic marker.

Summary. Atherosclerosis has been produced in captive cotton top marmosets fed

a purified diet containing 5% cholesterol and 23% lard. The animals exhibited both site-specific and time-related differences in vascular susceptibility to the disease. Atherosclerotic lesions in the lingual arteries and distal extensions of the coronary arteries antedated those in the aorta and other vulnerable vessels. The tongue arteries were particularly prone to atherosclerosis under the experimental conditions as demonstrated by the extent and severity of atheroma formation throughout the course of these vessels.

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