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Myocardial Infarction in a Large Colony of Nonhuman Primates With Coronary Artery Atherosclerosis

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Relatively few cases of myocardial infarction associated with coronary artery atherosclerosis have been described previously in macaques. In this study the authors report the prevalence and characteristics of coronary artery atherosclerosis and myocardial infarction in 10 rhesus *(Macaca mulatta)* and two cynomolgus *(Macaca fascicularis)* macaques that were fed atherogenic diets for 16 months or longer. Our findings show clearly that myocardial infarction occurs in macaques with diet-induced atherosclerosis. The frequency seems to be related to the species, composition of the atherogenic diet, and length of time fed the atherogenic diet. The myocardial lesions are remarkably similar to those described in human beings in terms of location and gross and microscopic characteristics. The characteristics of coronary artery atherosclerosis, including the occurrence of thrombosis, severe stenosis, mineralization, atheronecrosis, and sterol clefts, especially in animals fed the atherogenic diets for longer periods of time, also closely resemble those of the arterial lesions found in human beings. The greatest prevalence of myocardial infarcts was found in rhesus monkeys fed a cholesterol-containing diet with 40% of calories supplied by peanut oil and in cynomolgus macaques from Malaya that were fed the same amount of cholesterol with 40% of calories from lard. Electrocardiographic abnormalities as well as the occurrence of unexpected and relatively sudden death in several of these nonhuman primates are also consistent with signs frequently observed in human beings. (Am J Pathol 1980, 101:675-692)

MACAQUES have been used with increasing frequency in research on atherosclerosis during the past several years. The usefulness of these nonhuman primates as models for the study of atherosclerotic heart disease as opposed to coronary artery atherosclerosis *per se* has been questioned because of the infrequently reported occurrence of myocardial infarction. Taylor et al [1] observed a case of myocardial infarction in a female rhesus monkey (*Macaca mulatta*) fed a high-fat, high-cholesterol diet for 40 months. Hamm et al [2] reported preliminary data on myocardial infarcts observed in three male rhesus monkeys fed atherogenic diets for 16 to 31 months. Kramsch and Hollander [3] have reported a *Macaca irus (Macaca fascicularis)* that was fed an atherogenic diet for 12 months and died suddenly during exertion. The myocardium showed fatty change and vac-

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Table 1—Composition of Atherogenic Diet

	Weight 100 g (g)	Protein (g)	Fat (g)	Carbohydrate (g)	Calories/ 100 g	Cholesterol (mg)
Lard	25.0	—	25.0	—	225.0	2.0
Wheat flour	20.0	2.10	0.2	17.0	78.2	—
Applesauce	7.3	0.01	0.007	1.43	5.82	—
Nonfat dry milk solids	30.0	10.68	0.003	15.6	105.14	—
Casein, USP	13.0	13.0	—	—	52.0	—
USP XIV salts mixture	2.0	—	—	—	—	—
Complete vitamin mixture (Devoid of vitamin D)	2.2	—	—	2.2	8.8	—
D_3 in corn oil (6.25 cc)	*	—	—	—	—	—
Cholesterol	0.5	—	—	—	—	500†
Total	100	25.79	25.21	36.23	474.96	502

* Equal to 250 IU/100 g diet.
† Equivalent to 1.056 mg cholesterol/kcal diet.

uolization consistent with ischemia. Maximum coronary artery lumen stenosis in that case was 75%.

The purpose of this report is to 1) describe the myocardial infarcts we have seen in 10 rhesus and two cynomolgus (*M fascicularis*) macaques fed atherogenic diets for varying periods of time and 2) report the prevalence of myocardial infarcts in a population of nonhuman primates with diet-induced coronary artery atherosclerosis.

Materials and Methods

The 12 male macaques that developed myocardial infarction were acquired either as juveniles or young adults.

After a routine quarantine period the animals were fed an atherogenic diet. The basic diet used in these experiments is presented in Table 1. The amount of cholesterol added to diet, the type and amount of fat, and the length of time each animal was fed the atherogenic diet is presented in Table 2. Total plasma cholesterol concentrations were determined for each animal at 60-day intervals according to the method of Rush [4] and are also presented in Table 2.

Four of the animals reported in this study were killed as part of the experimental design (Nos. 184, 242, 435, and 536). Of the remaining eight animals, six died suddenly and unexpectedly (Nos. 81, 117, 120, 507, 537, and 578). Animals that were killed had the coronary arteries fixed *in situ* by pressure perfusion (100 mm Hg) with either barium gel–sulfate mass [5] or 10% neutral buffered formalin. Hearts from the animals that died during the course of the experiment were fixed by immersion in 10% neutral buffered formalin.

Criteria for establishing the diagnosis of myocardial infarction were based on both gross and microscopic observations. The minimal size of the infarct in any dimension must have measured at least 1.0 cm, and the lesion must have involved the myocardium of the left ventricle or interventricular septum. Grossly, the lesions were characterized in terms of location and size. Microscopically, the infarcted areas were classified as either acute, healing, or healed. Acute myocardial infarcts had ischemic necrosis with or without acute inflammatory cell infiltration (Figure 1). Healing infarcts were composed predominantly of granulation tissue containing fibroblasts, collagen, numerous vascular channels, and macrophages (Figure 2). Healed myocardial infarcts had well-demarcated areas of relatively avascular fibrous connective tissue (Figure 3).

Table 2—Dietary and Plasma Lipid Data on Macaques with Myocardial Infarcts

Animal	Species	Cholesterol added to diet (mg/kcal)	Fat in diet (% of cal)*	Number of months fed diet	Total mean plasma cholesterol concentration (± SEM)
227†	*M mulatta*	1.0	40	16	876 ± 84
227†	*M mulatta*	1.0	40	16	923 ± 55
184	*M mulatta*	1.0	40	19‡	835 ± 34
242	*M mulatta*	1.0	40	19§	586 ± 49
81†	*M mulatta*	2.0	25 (butter)	31	744 ± 40
120†	*M fascicularis*	1.0	40	34	712 ± 30
117†	*M fascicularis*	1.0	40	34	818 ± 30
435	*M mulatta*	1.0	40	36	540 ± 25
537†	*M mulatta*	0.74	40 (peanut oil)	39	700 ± 15
578†	*M mulatta*	1.0	40	44	972 ± 46
536	*M mulatta*	0.74	40 (peanut oil)	48	673 ± 17
507†	*M mulatta*	0.64–1.1	40% lard or safflower oil or 36% butter	75	421 ± 24

* Expressed as a percent of total calories. Except where indicated, fat was lard.

† Animal died during the course of the experiment.

‡ Followed by the feeding of a diet for 24 months that contained enough cholesterol to maintain TPC between 280 and 320 mg/dl. The mean plasma cholesterol concentration (± SEM) during this period was 314 ± 19 mg/dl.

§ Followed by the feeding of a diet for 48 months that contained enough cholesterol to maintain TPC between 180 and 220 mg/dl. The mean plasma cholesterol concentration (± SEM) during this period was 175 ± 6 mg/dl.

Five transverse serial blocks, each 3 mm in length, were trimmed form the proximal left anterior descending (LAD), left circumflex (LCX) and right coronary arteries (RCA). When indicated, additional blocks were taken from marginal branches of the LCX and from the posterior descending branches. Two 5-μ sections were cut from each block and stained with hematoxlyn and eosin or Verhoeff van Gieson stain.

Results

The location, size, extent, and estimated age of the myocardial infarcts and the maximum lumen stenosis in epicardial and intramyocardial coronary arteries are presented in Table 3.

Rhesus monkeys 227, 277, 184, 242, and 435 were part of a large experi-

Table 3—Characteristics of Myocardial Infarcts and Coronary Artery Atherosclerosis

Animal and species	Characteristics of myocardial infarcts				Maximum coronary artery stenosis	
	Location	Size* (cm)	Extent	Age	Epicardial	Intramyocardial
227 (Rhesus)	Anterolateral	3.0 × 2.0 × 0.5	Transmural	Acute	95	95
227 (Rhesus)	Posterolateral	3.0 × 1.8 × 0.4	Subendocardial	Healing	95	90
184 (Rhesus)	Apical	1.4 × 1.0 × 0.2	Transmural	Healed	40	60
242 (Rhesus)	Posteroseptal Post-RV	3.0 × 7.2 × 0.2	Transmural	Healed	35	0
81 (Rhesus)	Anteroseptal	1.0 × 1.0 × 0.3	Transmural	Acute healing	100†	85
120 (Cynomolgus)	Anteroseptal	2.3 × 1.0 × 0.6	Transmural	Acute	99	75
117 (Cynomolgus)	Posteroseptal Post-RV	2.0 × 1.0 × 0.1	Transmural	Healed	100	90
435 (Rhesus)	Posteroseptal	1.7 × 1.6 × 0.3	Transmural	Healed	70	35
537 (Rhesus)	Anterolateral	2.0 × 2.0 × 0.2	Subendocardial	Healing	99†	100
578 (Rhesus)	Anteroseptal	1.0 × 2.0 × 0.2	Subendocardial	Acute healing	95	99
536 (Rhesus)	Posteroseptal Post-RV	2.0 × 1.0 × 0.1	Transmural	Healed	90	80
507 (Rhesus)	Posteroseptal Post-LV	3.0 × 3.0 × 0.5	Transmural	Healed	100†	75

* Length × width × thickness.
† Thrombus.

ment (N=256) to determine whether atherosclerosis would regress when total plasma cholesterol concentrations were reduced from 700 mg/dl to either 200 or 300 mg/dl for 24 or 48 months. The design of the experiment has been presented elsewhere.[6]

Four days prior to death rhesus monkey 227 was noted to have lost weight and was weak. His physical condition deteriorated and was characterized by hypothermia, dehydration, generalized weakness, and tachycardia. The animal died, and at necropsy a recent transmural myocardial infarct was found. Microscopically, the myocardium contained diffuse areas of ischemic necrosis, focal hemorrhage, and minimal cellular infiltrate. The infarcted zone was supplied by the LCX through several marginal branches (Figure 4).

Rhesus monkey 277 was in apparent good health until 10 minutes prior to death, when he was seen having convulsive seizures. A well-demarcated subendocardial infarct was present in the posterolateral and posterior wall of the left ventricle within the distribution of the posterior descending artery (origin from RCA). Microscopically, the lesion was characterized by loss of myocardial cells, prominent vascularity, macrophages, fibroblasts, and moderate collagen deposition. Few polymorphonuclear leukocytes were present. Although the typical appearance of the lesion was that of a healing infarct, the posterior papillary muscle was necrotic and contained multiple foci of mineralization.

Rhesus monkey 184 was fed an atherogenic diet for 19 months and then fed diets that maintained plasma cholesterol concentrations between 280 and 320 mg/dl for 2 years. The animal was killed at the end of the regression phase. A healed, transmural infarct was present in the apex and inferior lateral wall of the left ventricle (Figure 5). Microscopically, the infarcted region consisted of areas of dense fibrous connective tissue. Some areas also had hypertrophic myocardial cells interspersed within the fibrous areas. The coronary artery lesions in this animal contained relatively little intracellular lipid and were composed primarily of connective tissue.

Rhesus monkey 242 was fed the atherogenic diet for 19 months and then for 48 months diets that attempted to maintain plasma cholesterol concentrations between 180 and 220 mg/dl. A baseline electrocardiogram done on the animal prior to the feeding of the atherogenic diet was unremarkable. ECG tracings taken after the animal had been fed the atherogenic diet for 16 months indicated a large posterior infarct (Text-figure 1). The animal was killed at the end of the experimental period, and an extensive posteroseptal and right ventricular infarct was found (Figure 6). The infarcted area was composed of fibrous connective tissue without evidence of acute myocardial changes.

Table 4—Prevalence of Myocardial Infarcts by Diet and Species

Species	Diet: Cholesterol (mg/kcal)	Diet: Fat (% of calories)	Total animals	Number of infarcts	Prevalence
M mulatta	1.0	40% lard	262	6	1/43
M fascicularis	1.0	40% lard	5	2	1/2.5
M mulatta	0.74	40% peanut oil	6	2	1/3
M mulatta	2.0	40% butter	6	1	1/6
M mulatta	0.64–1.10	36–40%*	9	1	1/9

* This animal was fed different atherogenic diets that contained either safflower oil (40%), lard (40%), or butter (36%).

This same diet, however, when fed to cynomolgus macaques, resulted in a greater prevalence of myocardial infarcts. A similar prevalence of infarcts was observed in rhesus monkeys fed about twice the amount of cholesterol with butter substituted for lard. Among rhesus monkeys the highest prevalence of myocardial infarcts was in monkeys fed 1.0 mg/kcal of cholesterol but with peanut oil substituted for lard.

Discussion

Although Taylor [1] reported myocardial infarction with diet-induced atherosclerosis 20 years ago, a notion persists that experimentally produced atherosclerosis does not lead to the clinical sequela often seen in human beings. The cases presented here are evidence that experimental atherosclerosis in nonhuman primates is associated with myocardial infarction.

Two of the animals listed in Table 3 (rhesus monkeys 184 and 242) probably had infarction during the progression phase of a regression experiment and were killed at the end of the regression phase of the experiment. The maximum percentage of lumen stenosis (40%) of the epicardial arteries was considerably less than that of the other animals with myocardial infarcts. All of the other animals had percentage of lumen stenosis scores of 70% or more, suggesting that some reduction in the size of the coronary artery plaques did occur in the two "regression" animals. If regression does occur in people, and there is reason to believe that it does, the studies correlating the degree of atherosclerosis with myocardial infarction, particularly healed infarcts, might be misleading.

Organizing thrombi were found in 3 of the 12 cases. The relative paucity of thrombi is perhaps not surprising, considering the material available. The coronary arteries were not opened but were blocked serially. The small lumen size in most cases made detection of thrombi with the unaided eye quite difficult, and it did not seem to be an effective use of the resources available to serially section the entire coronary tree, partic-

ularly since not many of the cases were at the stage that others have described as most likely to have a thrombus.[7,8]

Another intriguing observation was that 2 of 6 rhesus monkeys fed a diet containing peanut oil had infarcts. No infarcts were found among an equivalent number of animals fed the same diet but with butter substituted for the peanut oil. This finding appears to be consistent with the observation of Gresham and Howard,[9] who fed peanut oil and butter-containing diets to rats and found that while butter appeared to be atherogenic, peanut oil appeared to be thrombogenic.

M fascicularis may be more susceptible to myocardial infarction secondary to diet-induced atherosclerosis than *Macaca mulatta*. Although the number of animals studied was small, the finding of two infarcts among 5 *M fascicularis* of Malayan origin seems high with regard to the number of infarcts among a much larger group of *M mulatta* fed the same or similar diets. *M. fascicularis* imported from the Philippines may be less susceptible to myocardial infarction.

The only infarcts of the right ventricle among the 12 monkeys were found in animals that had posterior infarcts involving both the left ventricle and the interventricular muscular septum. This observation is similar to that reported by Isner and Roberts,[10] who found right ventricular infarcts only in this pattern among people.

The observations reported in this study are consistent with the idea that myocardial infarction is common among nonhuman primates fed an atherogenic diet for 16 months or longer. Predicting which animals will have an infarct, however, remains a great challenge.

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