Morphologic Effects of Dietary Plant and Animal Lipids Rich in Docosenoic Acids on Heart and Skeletal Muscle of Cynomolgus Monkeys

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Cynomolgi (*Macaca fascicularis*) were fed diets containing 25% rapeseed oil (RSO), partially hydrogenated herring oil (PHHO), or a 3:1 mixture of lard and corn oil as control for 4 months. The RSO contained approximately 25% of the fatty acids as erucic acid; the PHHO contained a similar concentration of mainly cetoleic acid. The control diet did not include such fatty acids. At the time of necropsy, the RSO- and PHHO-fed monkeys showed myocardial and skeletal muscle lipidosis. Foci of mononuclear cell infiltration, although infrequent, occurred in all three groups and were thought to be nonspecific. The only significant intergroup difference in serum biochemical or hematologic parameters was an increase in serum glutamic-oxaloacetic transaminase activity in both RSO and PHHO groups. Ultrastructural studies confirmed the presence of lipidosis in cardiac and skeletal muscle and revealed mild mitochondrial degeneration, causing a depression of the P/O ratio of the RSO group and a State III respiratory rate depression of the PHHO group. The difference in the exposure/life span ratio represented by this experiment may account for the absence of clear intergroup differences such as are reported in rats used in similar studies, but a true species difference in regard to dietary oils containing docosenoic acids has to be considered as well. (Am J Pathol 90:551–564, 1978)

OILS from the seed of different *Brassica* (rapeseed) cultivars and from marine fishes have been used by humans in food for many years. These oils are almost the only natural source of long-chain, monounsaturated fatty acids, especially C 22:1 (22 carbon atoms:1 double bond). The C 22:1 of rapeseed oil (RSO) and partially hydrogenated herring oil (PHHO) differ isomerically, however. In RSO, the C 22:1 is almost exclusively erucic acid or *cis*-docos-13-enoic acid (22:1w9); in PHHO, the C 22:1 is predominantly cetoleic acid or *cis*-docos-11-enoic acid (22:1w11), partially altered to the *trans* form and containing other positional isomers. Although some species variation in response to these oils exists, a tentative conclusion has been reached from animal studies

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Supported by research grants from the Rapeseed Utilization Assistance Program of the Rapeseed Association of Canada and from Environment Canada, Fisheries and Marine Service, Halifax, Nova Scotia.

Accepted for publication November 8, 1977.

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that the C 22:1 fatty acids are cardiotoxic in animals and therefore also potentially toxic in humans.[1-5]

Most of the experimental work has been carried out on a short-term basis with laboratory animals such as rats [6] (including germ-free rats),[7] guinea pigs, mice, hamsters, gerbils, ducklings, and turkeys,[4] but there are also results from pigs [8,9] and squirrel monkeys (*Saimiri sciureus*).[10] Bonnet monkeys (*Macaca radiata*) fed a mustard oil (containing 40 to 44% C 22:1 from *B juncea*) for more than 1 year developed sarcoplasmic vacuolation; one half of these exhibited cardiac fibrosis.[11]

An experiment was designed to compare RSO and PHHO in cynomolgi (*M fascicularis*). Growth rates; clinical, physiologic, and biochemical findings; as well as mitochondrial activity studies have been reported elsewhere [12] and will be only summarized. Fecal, depot, and cardiac fatty acids showed differences in details of composition from the fatty acids in the diets. These findings are published elsewhere.[13] This report concerns detailed histopathologic and electron microscopic findings.

Materials and Methods

The three complete semisynthetic dietary treatments employed differed only in the experimental lipid used: lard and corn oil (LCO) in a 3:1 (w w) mixture (control), rapeseed oil (RSO), and partially hydrogenated herring oil (PHHO). Approximately 25% by weight or 50% of calories was supplied by the oils.[12,13] The refined RSO was from a *B napus* cultivar high (approximately 25%) in C 22:1, ie, erucic acid, which is no longer used commercially in Canada. The PHHO contained approximately 24% C 22:1, ie, the isomer described above.

The eleven monkeys (*M fascicularis*) selected for use were born and reared in the laboratory. They were caged and fed individually in stainless steel, screen-bottom cages and were randomized by sex, age, and parentage into three treatment groups. Their ages ranged from 7 to 15 months. Four monkeys were assigned to the lard-corn-oil (control), 4 to the RSO diet, and 3 to the PHHO group. Food and water (containing ascorbic acid) were available *ad libitum*.

At the end of the feeding period (120 days), 8 monkeys underwent function tests of cardiac muscle just prior to necropsy;[12] the other 3 monkeys (1 per treatment) were killed by overdose of pentobarbital, and complete necropsy was carried out immediately. Samples from all organs were fixed in 10% neutral buffered formalin for histologic examination. For the examination of cross-striated (skeletal) muscle, the following sample sites were chosen: musculus masseter, musculus longissimus dorsi, and diaphragm. Following fixation, portions of tissues were embedded in paraffin, sectioned at 6 μ, and stained with hematoxylin and eosin, hematoxylin–basic fuchsin picric acid,[14] allochrome, Masson's trichrome, and the periodic acid–Schiff (PAS) reaction. Three horizontal levels of sections (one close to the base of the heart, one approximately midline between base and apex, the third close to the apex) were obtained from each heart.

Portions of skeletal muscles and two sections of myocardium were cut on a cryostat and stained with oil red O. Myocardial and skeletal muscle tissues of the 3 monkeys on which necropsy was performed immediately after euthanasia were fixed in buffered glutaraldehyde. Specimens for electron microscopy were embedded in Epon resin. Staining *en bloc* was done with uranyl acetate and lead citrate. At least 20 different sections of each tissue were cut, and thin sections were examined on the HS–8 Hitachi electron microscope.

Results

General Findings

Growth rates, clinical biochemical findings, electrocardiograms, papillary muscle length–tension relationships, conductivity measurements in the freshly exposed heart, and hematology revealed no significant intergroup differences [12] except as follows: at Days 30 and 120, the RSO group had higher serum cholesterol values than the others. Serum glutamic-oxaloacetic transaminase activity (SGOT) was higher than our normal values in all groups. Serum triglyceride concentrations were roughly within normal range in all three groups. The results of five types of mitochondrial studies showed only two intergroup differences. The P/O ratio (number of molecules of phosphorus taken up per atom of oxygen) of the RSO group was depressed, while the State III respiratory rate (μ moles O_2/min/mg protein) of the PHHO group was decreased. No statistically significant intergroup differences in blood pressure, contraction strength, or electrical activity were observed. Details of these general findings were reported earlier.[12]

Gross Postmortem Findings

No remarkable findings were recorded at the time of necropsy. It was noted, however, that the myocardial tissue samples of the RSO and PHHO animals assumed a distinct ocher color after fixation in formalin.

Histologic Findings

All animals fed RSO and PHHO had a pronounced lipidosis of the myocardium and, to a lesser degree, of the skeletal muscles. Visible lipid deposits were fine and granular in the control animals (Figure 1A), but fat droplets of varying size were observed in myocardial fibers of the RSO and PHHO animals (Figures 1B and C). The subjective overall impression was that the RSO group had slightly larger and more numerous fat droplets than the PHHO group (Table 1).

Small foci of histiocytic infiltration were found in the myocardium in 2 of 4 monkeys of the LCO and RSO groups and in 2 of 3 monkeys of the PHHO group (Table 1). To quantitate the findings, the number of foci in all sections were counted and expressed as numerator. The total number of sections was taken as denominator, and the resulting value was called the "myocarditis index" (Table 1).

One animal of the PHHO group had one singular focus of histiocytes in the papillary muscle of the left ventricle (Figures 2A and B) and another focus in the left ventricular wall (Figure 2C). The second PHHO monkey had one focus in the left ventricular wall, close to the epicardial surface. In

Table 1—Summary of Myocardial Postmortem Findings

Group	Myocarditis index*	Lipidosis
LCO	0/18 = 0.00	++
LCO	0/18 = 0.00	+
LCO	1/18 = 0.06	++
LCO	1/18 = 0.06	++
Total	2/72 = 0.03	
PHHO	2/18 = 0.11	+++
PHHO	0/18 = 0.00	+++
PHHO	2/18 = 0.11	+++
Total	4/54 = 0.07	
RSO	1/18 = 0.06	++++
RSO	3/18 = 0.17	++++
RSO	0/18 = 0.00	++++
RSO	0/18 = 0.00	++++
Total	4/72 = 0.06	

* No. of foci of inflammation in all sections/No. of sections examined = myocarditis index. LCO = lard and corn oil (control); PHHO = partially hydrogenated herring oil; RSO = rapeseed oil; + = mild, ++ = moderate, +++ = severe, and ++++ = most severe lipidosis.

the RSO group, the histiocytes were more scattered (Figures 3A and B) in the left ventricular wall. A similar pattern was observed in the control animals.

The various special stains did not yield any further information; there was no evidence of scarring. No inflammatory changes were seen in any of the sections of the skeletal muscles. Findings in the other organs were regarded as incidental and not related to the high fat diets and, therefore, are not reported.

Electron Microscopic Findings

Ultrastructural studies of the control monkey heart revealed few, if any, fat globules (Figures 5A and B). In contrast, both in the PHHO monkey (Figure 6A) and in the RSO monkey (Figure 7A) numerous lipid globules gave the fibers a "moth-eaten" appearance. The lipid globules varied in size, up to 3 μ, and appeared to displace or distort mitochondria. No membrane was found around the lipid globules, but walls of dilated sarcoplasmic reticulum were found between globules (Figures 6 and 7). The size of the mitochondria in both the PHHO and RSO animals was increased compared with that in the control animals; the shape of the mitochondria was irregular in all instances. Higher magnification of the mitochondria revealed that in both PHHO and RSO animals the cristae were less clearly outlined and arranged and that amorphous or vacuolated material appeared to be located in the matrix (Figures 6B and 7B).

The examination of the skeletal muscle showed similar changes. Lipid

globules were infrequent in the control animal (Figure 8) but were numerous in both the RSO and PHHO animals (Figures 9 and 10).

Discussion

The cardiomyopathies of nonhuman primates have been reviewed by McNulty and Malinow.[15] In addition to myocarditis due to bacteria, viruses, and other infectious agents, there is a large number of reports of myocarditis of unknown etiology.[15,16] Photographic documentation of such cases displays findings similar to the discrete lesions in the heart of the cynomolgi of this report. Examples of primate cardiomyopathies specifically related to nutrition are few. Intracellular fatty deposition in the myocardium of squirrel monkeys on diets high in butter and cholesterol [15] and in rhesus monkeys fed alcohol [17] has been reported. Focal myocardial necrosis also has been observed in experimental thiamine deficiency,[18] insulin deficiency, hypothyroidism, and hypertension.[19] "Stress" situations have been incriminated in cardiomyopathy in rabbits and baboons.[20,21] Baboons appear to be particularly prone to such cardiomyopathies, since their normally low serum potassium level is said to be easily disturbed by the loss of electrolytes for a number of reasons.[22]

In humans and animals, myocardial damage has been associated with the administration of various chemicals and drugs.[23,24] Morphologic studies indicate that the so-called drug-induced lesions consist of multifocal areas of myocardial degeneration, necrosis, and subsequent inflammation and/or fibrosis. Most of these lesions, especially the so-called electrolyte–steroid–cardiopathy,[24] are not associated with vascular obstruction and fatty metamorphosis and are presumably caused by biochemical alterations, sometimes expressed in mitochondrial changes.[23] Similar lesions have been observed in rabbits intoxicated with seeds of coffee senna (*Cassica occidentalis*) [25,26] and in copper-deficient young rats.[27]

Although our light microscopic findings may be classified as belonging to the group of myocarditis of unknown etiology (previously discussed), the ultrastructural findings are in agreement with those described for rats [3,7,28–31] and pigs [8] fed diets containing lipids with erucic acid. The lipid droplets do not have a surrounding membrane as previously suggested,[8] unless one describes the walls of dilated channels of the sarcoplasmic reticulum as membranes. Our findings are somewhat inconclusive as far as the size of mitochondria is concerned since the enlargement of mitochondria does not justify the use of the term "megamitochondria." Megamitochondria and mitochondriosis have been mentioned by some authors [3,8,28,29,31] but were referred to as nonspecific phenomena. The mitochondrial changes observed in this study and the diminished mito-

chondrial ability to oxidize various substrates reported when animals are fed diets containing erucic acid [8,12,32] may explain the extramitochondrial accumulation of lipids, although it should be kept in mind that small lipid droplets are frequently found in normal myocardical cells.[33] The accumulated fat may also affect the inner membranes and cause a general inhibition of the mitochondrial respiration as a secondary effect. The inhibitory effect on the mitochondrial fatty acid catabolism as well as on the mitochondrial respiration and the energy supply of the heart due to docosenoic acids could explain those histologic and ultrastructural alterations described, which can be linked to the fat droplets. On the other hand there is no connection between the transient lipidosis in the rat and the later development of myocarditis and fibrosis or between the slow development of lipidosis in the pig and lesions which may or may not be observed in that species.[34]

Other dietary fatty acids and the ratio of fatty acids are suspect.[35,36] The relative amounts of various polyunsaturated acids in cardiac lipids markedly influence the development of myocardial necrosis following overstimulation with catecholamines.[37]

Long-chain polyunsaturated acids are capable of replacing the shorter and more saturated linoleic acid in phospholipids.[37] These observations were obtained from studies with polyunsaturated fatty acids, but Blomstrand and Svensson [38] have demonstrated that monounsaturated C 22 acids were incorporated into cardiolipin with a corresponding decrease of linoleic acid; thus, a common pathway is likely. Linoleic acid is the precursor for arachidonic acid by a series of desaturations and elongation. Arachidonic acid, in turn, is used for endogenous prostaglandin synthesis. Gudbjarnason and Hallgrimsson [37] have suggested that such prostaglandins may play an important regulatory role in cardiac muscle.

The relatively short feeding period (120 days) in relation to life span may not have been long enough to induce the supposedly characteristic foci of myocarditis observed in other animal species. On the other hand, one could conclude that the primate heart may respond differently from the rat heart to dietary oils containing approximatly 25% of the fatty acids, as C 22:1, although the feeding of mustard oil (*B juncea*) with even higher C 22:1 content (40 to 44%) has resulted in myocarditis and fibrosis after 1 year.[10] Modern varieties of Canadian *Brassica* now yield oils containing less than 1% erucic acid,[39] compared with the RSO used in this study, which contained 25%.

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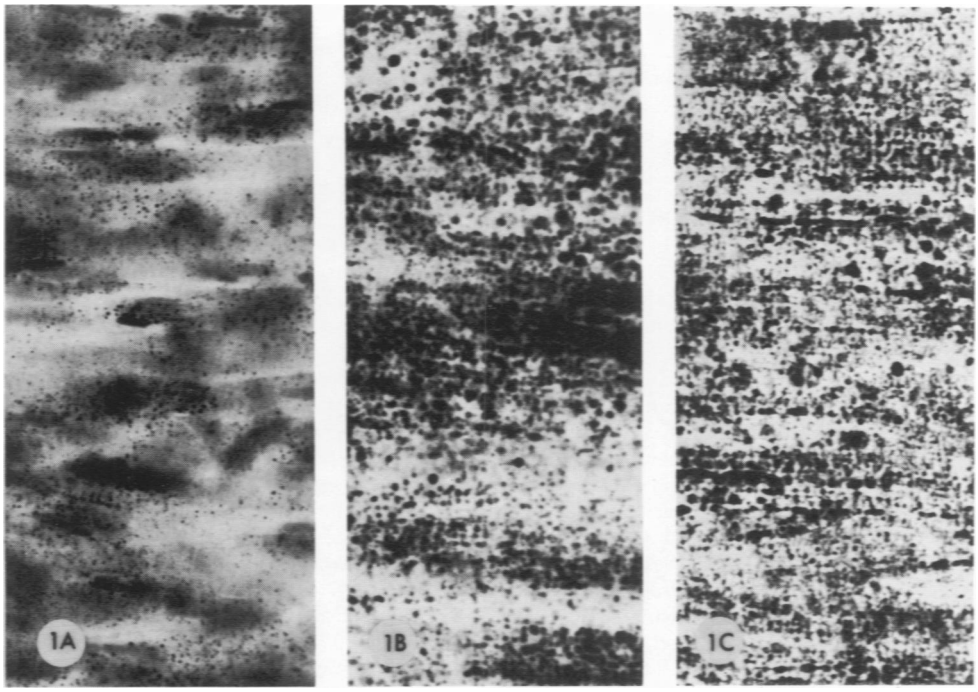
Acknowledgments

The skilled technical assistance of D. S. Hancock and I. Shirley is appreciated.



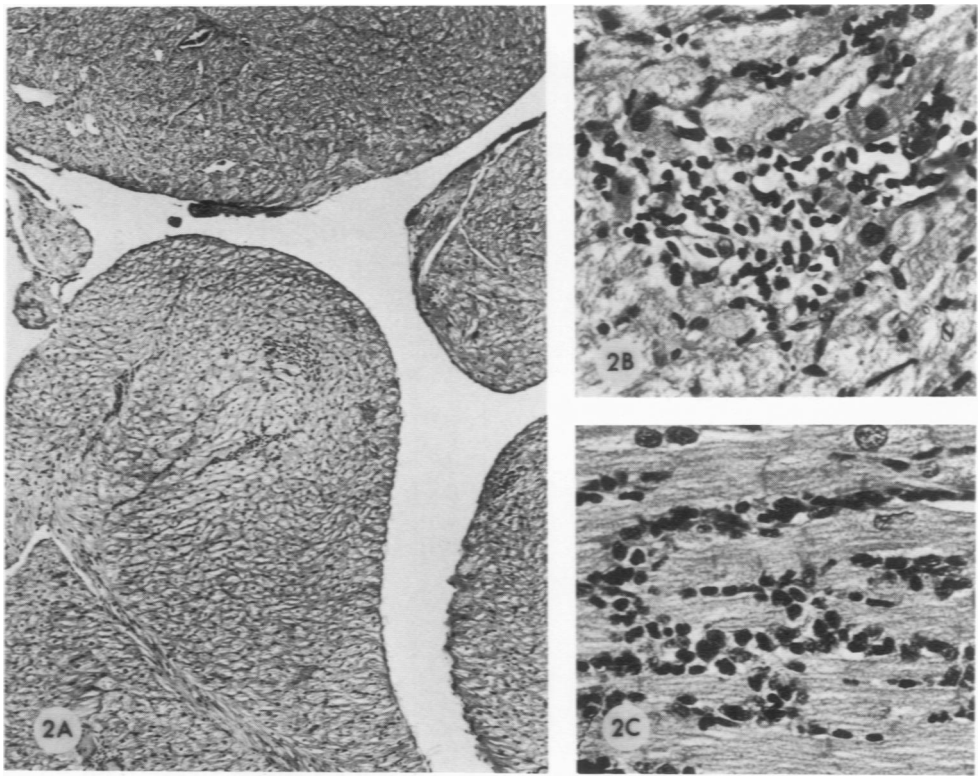
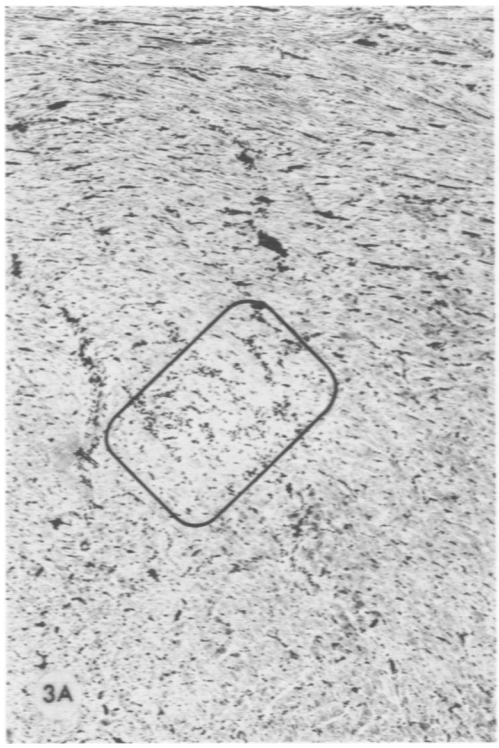
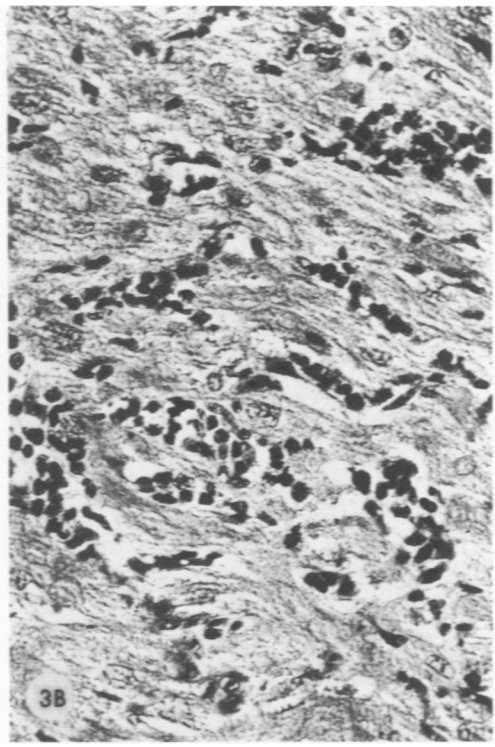
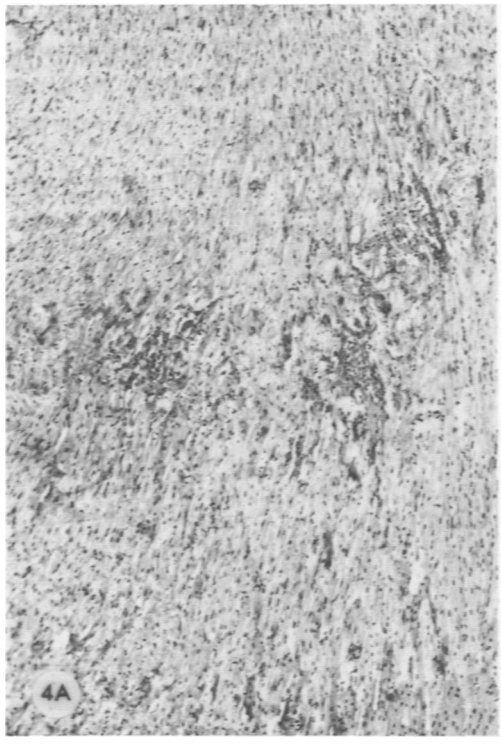


Figure 1A—Myocardium in control animal. **B**—Myocardium of PHHO animal. **C**—Myocardium of RSO animal. (**A, B,** and **C,** oil red O, × 400) Figure 2A—Focus of mononuclear cells in left papillary muscle in PHHO-fed monkey. (H&E, × 40) **B**—Higher magnification of **A**. (H&E, × 250) **C**—Second focus with mononuclear cells in same animal. (H&E, × 250)







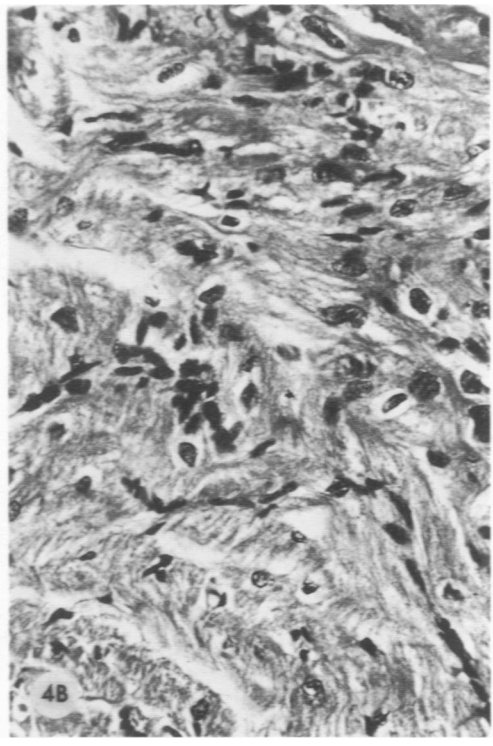


Figure 3A—Indistinct focus of mononuclear cells in myocardium in RSO-fed monkey. (Masson's trichrome, × 40) **B**—Higher magnification of **A**. (× 250) **Figure 4A**—Indistinct focus of mononuclear cells and partial acidophilic appearance of individual fibers. (H&E, × 30) **B**—Higher magnification of **A**. (× 250)

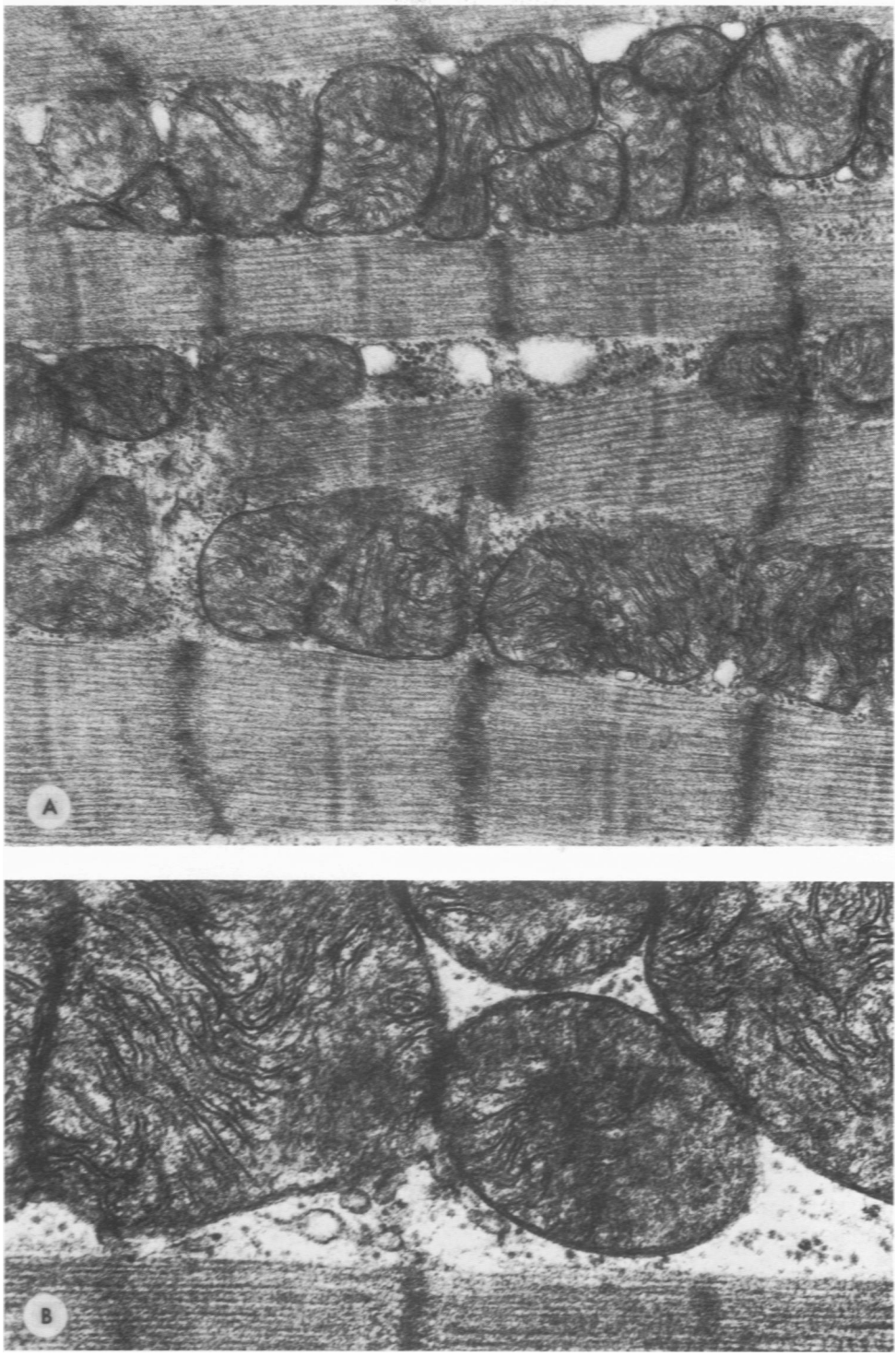


Figure 5A—Ultrastructure of myocardium of control animal. A few lipid vacuoles are present. (× 26,100) **B**—Higher magnification of **A**. (× 51,000)

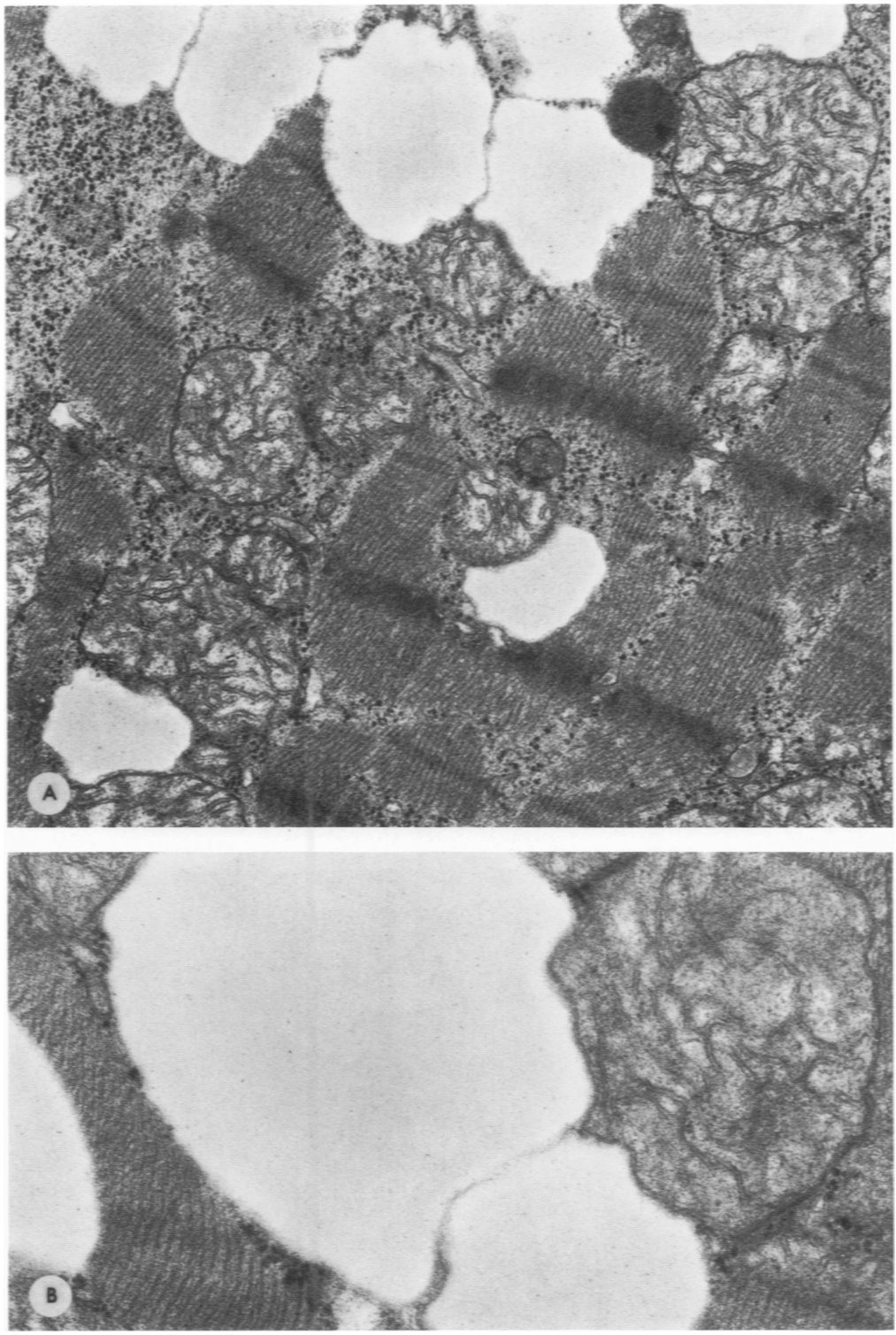


Figure 6A—Ultrastructure of myocardium of PHHO-fed monkey. Note lipid vacuoles and mitochondria of varying size. (× 26,100) **B**—Displacement of mitochondria by lipid vacuoles. Loss of cristae and appearance of homogenous masses in mitochondria. (× 51,000)

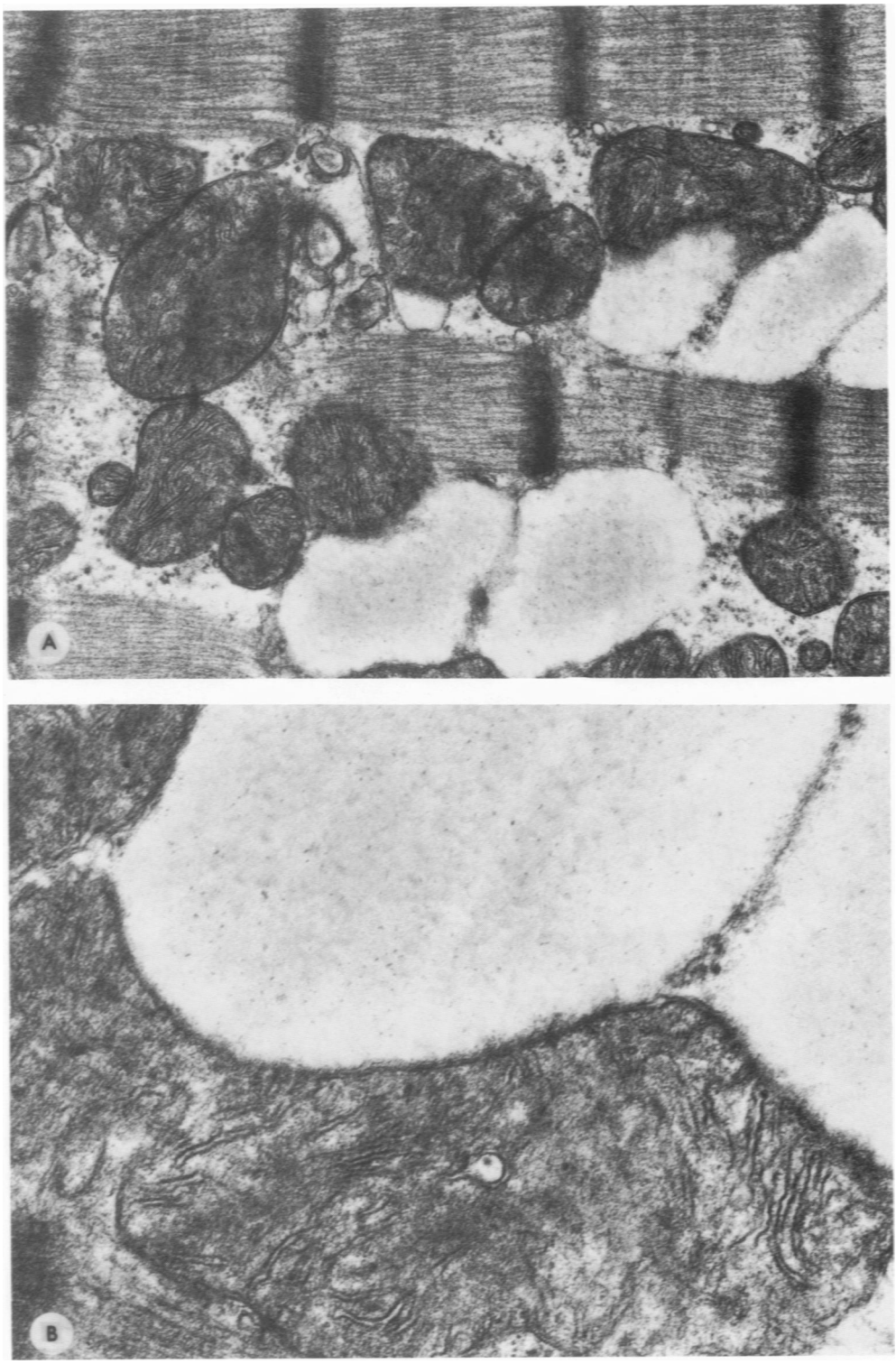
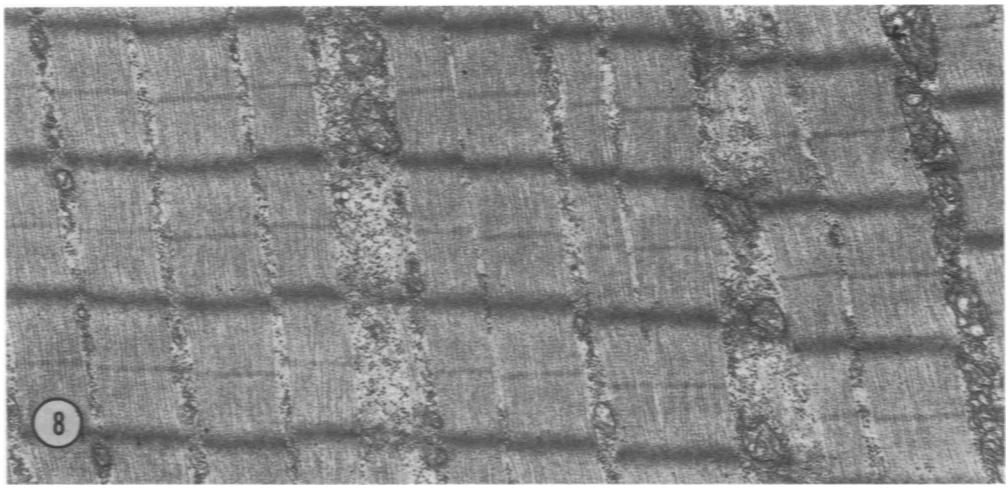
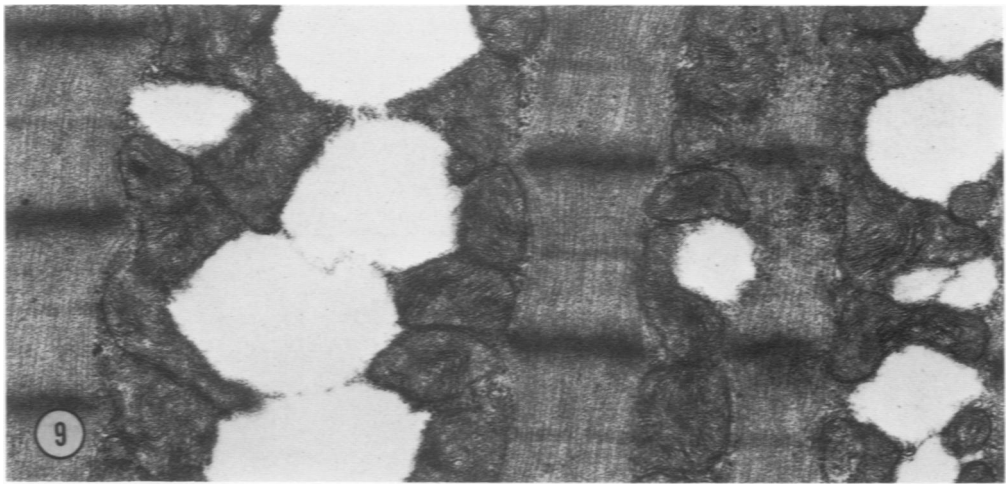


Figure 7A—Ultrastructure of myocardium of RSO-fed monkey. Varying size of mitochondria, partially displaced by lipid vacuoles. (× 26,100). **B**—On higher magnification, the loss of cristae and the inclusion of homogenous material in the mitochondria is visible, lipid appears to not be surrounded by a membrane, but walls of sarcoplasmic reticulum are visible. (× 51,000)





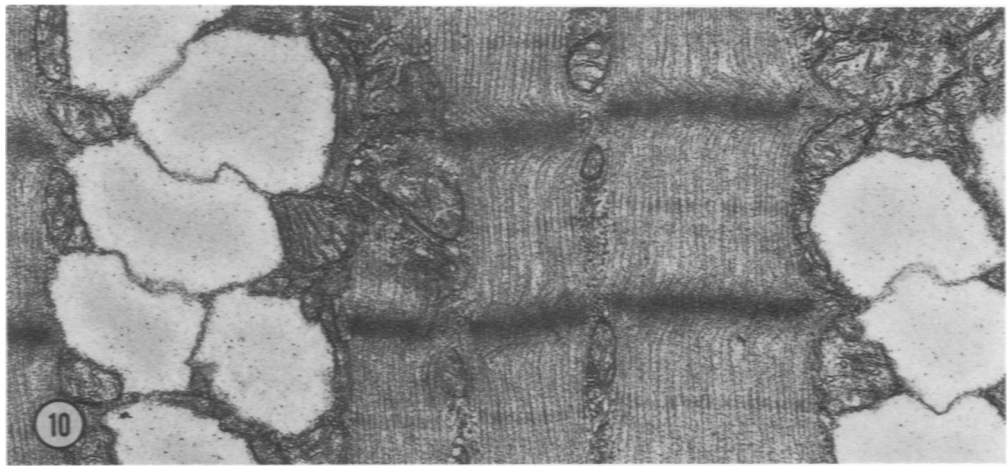


Figure 8—Masseter muscle of control animal. Note absence of lipid vacuoles. (× 11,400) **Figure 9**—Masseter muscle of PHHO-fed animal. Various lipid vacuoles displace enlarged mitochondria. (× 19,500) **Figure 10**—Masseter muscle of RSO-fed animal. Lipid vacuoles displace and distort irregularly enlarged mitochondria. (× 19,500)