Increased Myocardial Catalase in Rats Fed Ethanol

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The effects of chronic intake of dietary ethanol upon catalase, an enzyme capable of metabolizing ethanol, as well as upon myocardial morphology and hemodynamics, were studied in the rat. Ethanol, comprising 36% of dietary calories, administered to rats for 5 weeks, was associated with increased myocardial catalase of 45.9 ± 3.7 IU/mg protein, compared to 21.0 ± 1.8 IU/mg protein in pair-fed controls. The enzyme activity remained significantly elevated after 18 weeks of ethanol. Hepatic catalase did not differ in these groups. Parallel cytochemical studies confirmed the increase in myocardial catalase by demonstrating an increase in peroxisomes. Gross and light-microscopic examinations revealed no abnormalities at either 5 or 18 weeks. Remarkably few ultrastructural abnormalities were seen in this material fixed by vascular perfusion. Hemodynamic studies after 5 weeks of ethanol revealed decreased left ventricle systolic pressure and decreased mean arterial pressure but no change in ventricular filling pressure. The possibility of catalase playing a metabolic and potentially protective role in rat myocardium chronically exposed to ethanol is discussed. (Am J Pathol 96:373–390, 1979)

THE ACUTE ADMINISTRATION of ethanol to experimental animals causes structural and functional alterations in the myocardium.[1-5] Although there is circumstantial evidence that chronic ingestion of ethanol in man is associated with the clinical entity called "alcoholic cardiomyopathy,"[6] the inability to produce congestive cardiomyopathy in experimental animals by chronic ethanol feeding alone [3,7-9] leaves a gap in our understanding of the pathogenesis of alcoholic cardiomyopathy. If chronic ingestion of ethanol causes significant damage to the heart, there must be some direct effect of ethanol or its metabolites upon the heart. In the liver ethanol is metabolized by three enzymatic pathways: alcohol dehydrogenase (ADH), the microsomal ethanol oxidizing system (MEOS), and the catalase–H_2O_2 complex.[10] In the heart only trace amounts of ADH have been reported,[11,12] and the occurrence of MEOS is unknown. Recently, however, Herzog and Fahimi [13] demonstrated the occurrence of peroxisomes (microbodies) containing catalase in the myocardium of mice; and Hicks and Fahimi [14] showed the presence of per-

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oxisomes in the hearts of several mammalian species, including primates. Therefore, the present study was designed to assess the influence of chronic ethanol feeding upon the activity of catalase as determined biochemically and as visualized by ultrastructural cytochemistry. The data were also analyzed in relation to ultrastructural and hemodynamic indexes.

Materials and Methods

Animals

Male Sprague–Dawley rats, weighing 100–125 g, were purchased from the Charles River breeding Laboratories (Wilmington, Mass) and divided into three groups: ethanol-fed, pair-fed control animals, and *ad lib* control animals. Ethanol-fed and pair-fed animals were given Lieber, Jones, and DeCarli liquid diets [15] (Biomix #711, Bio-Serv, Inc., Frenchtown, NJ). An olive and corn oil mixture, which consisted of 16% amino acids and 41% sucrose, an amount of fat comparable to that of the typical American diet, made up 43% of the total calories of the pair-fed diet. In the alcohol diet sucrose was replaced isocalorically with ethanol to the extent of 36% of the total calories, approximately the same percentage reported in a population of human alcoholics.[16] Both pair-fed and ethanol diets contained adequate amounts of choline, vitamins, and salts and are considered to be nutritionally adequate for rats. The *ad lib* control rats, which served for obtaining baseline data on body and organ weights as well as on hemodynamic measures, were given water and a commercial rat chow *ad libitum* (rat/mouse/hamster formula, Charles River Laboratories, Wilmington, Mass). Ethanol-fed and pair-fed groups were caged individually, and their daily intake was closely monitored so that both groups consumed equal amounts of calories (approximately 60–70 cal/day). The *ad lib* rats were caged in groups. Body weights were determined twice a week. The animals were killed for study after a period of 5 or 18 weeks, ethanol having been withdrawn one day earlier.

For methodological reasons, three sets of animals were used, destined respectively for a) biochemical studies of catalase (46 rats), b) ultrastructural and cytochemical studies (16 rats), and c) hemodynamic studies (52 rats).

Biochemical Studies

Blood samples were obtained from selected animals. Ethanol levels were determined by gas chromatography.[17] Erythrocytic transketolase levels were measured according to Brin et al.[18]

To obtain tissue homogenates, animals which had been made to fast for approximately 12 hours were anesthetized with ether and perfused through the left ventricle for about 5 minutes with saline to wash out the erythrocytes. Both ventricles and the liver were minced and homogenized in ice-cold 0.25-M sucrose solution (1:10 w/v) containing 0.1% ethanol as a stabilizer of catalase.[19] The homogenates were filtered through double-layered cheesecloth and centrifuged at 4 C at 3500 g/min to remove unbroken cells, nuclei, and cell debris. Triton-X-100 was added to a final concentration of 0.1%, and the samples were sonicated for 5 minutes in an ice bath. Catalase was determined according to Lück,[20] using a Beckman DBGT recording spectrophotometer. Protein was determined according to the Lowry [21] technique, with bovine serum as the standard. Tissue creatine phosphokinase (CPK) was measured in the heart homogenates, using the Sigma Kit 45-UV (Sigma Chemical Company, St. Louis, Mo). Catalase and CPK activities are expressed as international units per milligram protein.

Morphologic Studies

A complete autopsy with gross examination of viscera was performed in each animal. For light microscopy the hearts, livers, and lungs were excised and weighed immediately after the completion of hemodynamic studies. Dry/wet weight ratios were obtained in all animals. Portions of the left ventricle, liver, and lungs were fixed with 10% buffered neutral formalin, followed by embedding, sectioning, and staining with hematoxylin–eosin or Masson stain or both. A total of 16 rats were used for ultrastructural studies. These were divided into two groups of 8 rats each, corresponding to the groups studied at the two time intervals of 5 and 18 weeks. Each group comprised 4 rats receiving the ethanol diet, 2 pair-fed animals and 2 *ad lib* control animals.

For electron microscopy and cytochemical studies, animals that had fasted for at least 12 hours were perfused through the left ventricle with physiologic saline for 1–3 minutes and then by 3% glutaraldehyde in 0.1 M Na-cacodylate buffer, pH 7.4, containing 0.05% $CaCl_2$ for 10 minutes at room temperature. Tissue strips from the supra-apical portion of the anterior wall of the left ventricle and from the left lobe of the liver were immersed in the same fixative at 4 C for an additional hour. These were sectioned and postosmicated with 2% aqueous OsO_4 and embedded in Epon. For cytochemical studies 50-μm chopper sections of heart and liver were incubated with diaminobenzidine (DAB) and H_2O_2 for the localization of catalase, as previously described.[14,22] Some tissues were stained en bloc with 2% uranyl acetate in 0.05 M hydrogen maleate buffer, pH 5.2, for 1 hour at 4 C. All tissues were dehydrated and embedded in Epon 812. A total of 60 blocks were processed from each animal. Sections 1–2 μ thick were examined under a light microscope, and ultrathin sections stained with lead or lead and uranyl acetate were studied in a Philips 201 and 301 electron microscope. From the heart of each experimental animal 5–7 blocks were carefully screened, and at least two dozen electron micrographs were made.

Hemodynamic Studies

These studies were performed only in the 5-week ethanol-treated rats. The animals were anesthetized with sodium pentobarbital, 50 mg/kg intraperitoneally. Electrocardiographic leads were connected to needles that pierced the extremities, fixing them to the operating table. The trachea was cannulated, and the animals were ventilated on a constant-volume rodent respirator (Model 680, Harvard Apparatus Co., Millis, Mass) to maintain arterial oxygen saturation and pH in the normal range. The right common carotid artery was isolated from the right vagus nerve and cannulated with polyethylene tubing (Intramedic PE-90, ID = .86 mm). The pectoralis muscle was incised, and the thorax was exposed. We introduced 26-gauge needles connected to PE-90 polyethylene tubes into the left and right ventricles by direct puncture. The carotid cannula was connected to a pressure transducer (Sanborn PS7, Hewlett Packard Co., Lexington, Mass). The left and right ventricular catheters were connected to another pressure transducer (P-26G, Statham Instruments, Inc., Hato Rey, Puerto Rico) via a three-way stopcock, allowing the transducer to be connected alternately to each ventricle. Polyethylene catheters were maintained patent by intermittent flushing with small volumes of heparinized saline. The pressure transducers were calibrated by the use of a mercury manometer; zero pressure was set at midthoracic level. Phasic tracings were made of arterial, left-ventricular, and right-ventricular pressures. Mean arterial pressures were also recorded. Ventricular end-diastolic pressure was recorded at sufficient sensitivity so that 1 mm Hg equaled 1 mm on the tracing. The first derivative of the left ventricular pressure pulse (dP/dt) was computed continuously by an active differentiating circuit, rolling off 12 dB/octave at 100 Hz, and converted into mm Hg/sec. The frequency response of the ventricular pressure measuring system was essentially flat to 100 Hz. The galvanometers reduced this to approximately 70 Hz. The differentiator was driven directly by the pressure preamplifiers, eliminating galvanometer-generated artifacts. At the same time, dP/dt was continuously monitored by an os-

cilloscope (model 422, Tektronix, Inc., Lexington, Mass), and the amplitude of each dP/dt on the tracing was confirmed. The tracings were recorded on a four-channel recorder (Sanborn model 964, Hewlett Packard, Inc., Lexington, Mass) at a chart speed of 100 mm/sec. After reaching a stable hemodynamic state, cardiac output was measured by the Fick method. All pressure tracings were recorded immediately before the cardiac output determination. Blood samples were obtained from left and right ventricles via the catheters for gas analysis and hematocrit.

The total procedure was completed within 2 hours after the induction of anesthesia in all experiments. Left ventricular stroke work was calculated from the formula

$$SV \times (MLVS - LVED) \times 13.6 \text{ g·mm}$$

in which SV = stroke volume in ml, MLVS = mean left ventricular systolic pressure in mm Hg, and LVED = left ventricular end-diastolic pressure in mm Hg. As an approximation of the contractility, we used dP/dt/PIP, where dP/dt = maximum rate of left ventricular pressure rise, and PIP = diastolic pressure in the carotid artery.[23] As an index of ventricular relaxation, −dP/dt was used at a maximum rate of left ventricular pressure fall.

Results

Animals and Organ Weights

The ethanol-fed animals often exhibited tremulousness, drowsiness, staggering gait, irritability, and grand mal seizures, resembling the symptoms observed in human alcohol addicts.

In the initial phase of this study it was noted that when rats were started immediately with a diet containing 36% ethanol, some of the animals developed convulsions and died suddenly. When the ethanol dose was increased gradually over a period of 5 days, however, the animals tolerated the diet and survived. Pathologic examination of animals that died suddenly did not reveal any changes in the heart.

Body and organ weights are presented in Table 1. Whereas the gain in body weight in the ethanol-fed rats was minimal, the pair-fed animals, consuming equal calories, gained more weight. The *ad lib* control animals gained weight rapidly, as had been expected. The heart/body weight ratio remained constant in all groups, with the exception of the 5-week ethanol-fed animals, which had the slowest weight gain. The liver/body weight ratio in ethanol-fed rats was greater than in pair-fed and *ad lib* groups.

Biochemical Observations

The catalase levels are presented in Table 2. Myocardial catalase was found to be significantly higher in the ethanol-fed animals than in the control groups, at both 5 and 18 weeks (Text-figure 1). In the liver, on the other hand, catalase levels remained approximately the same in all groups (Text-figure 1). Protein determination revealed no significant change in the level of protein per milligram homogenate in the heart of different groups. Tissue creatine phosphokinase was 18.2 IU/mg protein in pair-fed

Table 1—Body and Organ Weight in *Ad lib*, Pair-fed, and Ethanol-fed (EtOH) Rats* (Mean ± SEM)

	Pair-fed	Ethanol-fed	Comparison of pair-fed vs ethanol-fed	*Ad lib*
5 weeks				
Number	22	23		11
Body wt (g)	284 ± 5	214 ± 14	$P < 0.001$	411 ± 11
Heart wt (g)	0.90 ± 0.03	0.75 ± 0.03	$P < 0.001$	1.17 ± 0.04
Heart/body wt × 1000	3.2 ± 0.1	3.5 ± 0.1	NS	2.9 ± 0.1
Heart dry/wet × 100	23.7 ± 0.4	23.2 ± 0.3	NS	23.0 ± 0.4
Liver wt (g)	8.4 ± 0.3	8.8 ± 0.3	NS	15.0 ± 0.4
Liver/body wt × 1000	30.3 ± 0.4	40.9 ± 2.1	$P < 0.001$	36.5 ± 0.9
Liver dry/wet × 100	32.2 ± 0.7	35.1 ± 1.4	NS	32.3 ± 2.3
18 weeks				
Number	6	6		7
Body wt (g)	346 ± 8	347 ± 15	NS	454 ± 18
Heart wt (g)	1.05 ± 0.40	1.05 ± 0.02	NS	—
Heart/body wt × 1000	3.0 ± 0.1	3.1 ± 0.2	NS	—
Liver wt (g)	8.1 ± 0.3	9.5 ± 1.0	$P < 0.02$	—
Liver/body wt × 1000	23.3 ± 1.0	27.3 ± 0.8	$P < 0.02$	—

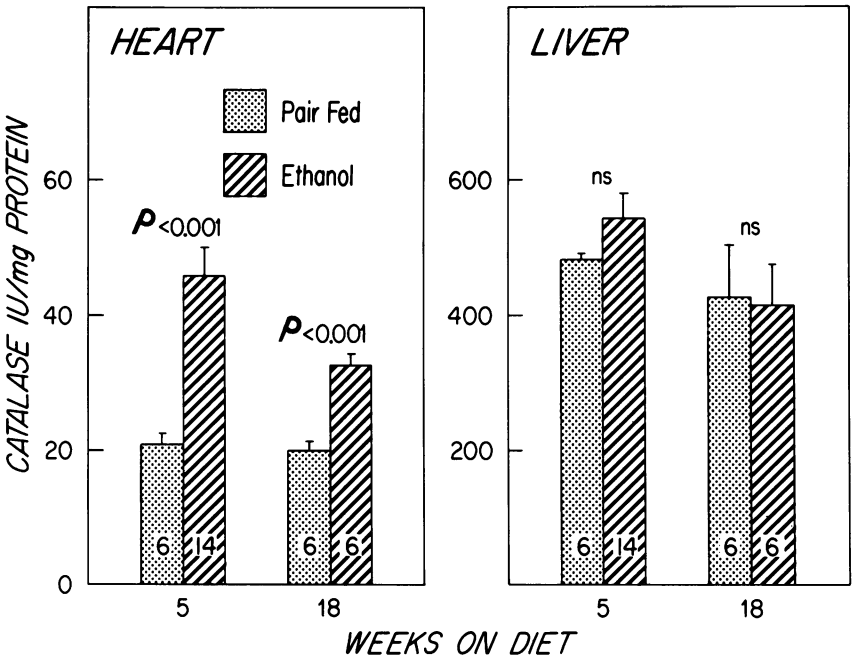
* Mean ± SEM.
NS = not significant.

control animals and did not change significantly in different groups. Random determination of serum ethanol in 9 animals in the 5-week experiment ranged from trace to 192 mg/dl. Serum ethanol levels taken on 10 additional animals directly after to 6 hours after feeding ranged from 163 to 275 mg/dl and averaged 207 mg/dl. Transketolase levels of the erythrocytes were normal in 14 pair-fed controls at 891.4 ± 59.3 units

Table 2—Catalase Activity in Heart and Liver*

	Pair-fed	Ethanol-fed	Comparison of pair-fed vs ethanol-fed	*Ad lib*
5 weeks				
Number	6	14		7
Heart	21.0 ± 1.8	45.9 ± 3.7	$P < 0.001$	13.3 ± 1.5
Liver	477.7 ± 7.5	536.1 ± 38.3	NS	583.5 ± 53.7
18 weeks				
Number	6	6		7
Heart	19.8 ± 1.3	32.8 ± 1.3	$P < 0.001$	15.1 ± 0.5
Liver	421.3 ± 78.0	410.2 ± 57.1	NS	574.6 ± 91.9

* Expressed in IU catalase/mg protein, mean ± SEM.
NS = not significant.



TEXT-FIGURE 1—Catalase levels in the heart (*left*) and liver (*right*) at 5 weeks and 18 weeks. Pair-fed and ethanol-fed groups are shown. Catalase level is expressed in IU catalase/mg protein (mean ± SEM). Myocardial catalase is significantly increased after ethanol feeding, whereas hepatic catalase remains unaffected (*ns* = not significant). The size of each group is indicated at the bottom of each bar.

and did not differ in 16 ethanol-treated animals in which this determination was made.

Morphologic Observations

In no instance did the postmortem examination reveal any evidence of cardiac dilatation, visceral congestion, or edema. By light microscopy no myocardial lesions were seen, and the lungs were not remarkable. Mild to moderate fatty infiltration of the liver was observed in all animals on the ethanol diet.

In the normal rat myocardium catalase is localized exclusively in peroxisomes.[14] In ethanol-fed rats also the enzyme remained confined to this organelle, and in agreement with our biochemical observations, the number of peroxisomes appeared increased in comparison to pair-fed or *ad lib* controls (Figure 1). This increase was visible in longitudinal sections (Figure 1C) as well as in cross-sections of myocardial fibers (Figure 3). Livers

stained for catalase showed essentially no change in the peroxisome population, although numerous fat vacuoles were observed in the ethanol-fed animals (Figure 2).

In material processed for ultrastructural cytochemistry of catalase the left ventricles of rats fed ethanol for 5 or 18 weeks were generally indistinguishable from those of their respective control groups, except for the increased peroxisome population. Figures 4–6 show material from rats fed ethanol for 5 weeks and demonstrate the normal ultrastructural appearance of the tissue with numerous peroxisomes. The mitochondria appear intact; and the contractile elements, as well as the sarcoplasmic reticulum, appear normal. The catalase reaction product is localized in peroxisomes. In well-oriented longitudinal sections peroxisomes are found typically at the junctions of A and I bands and in close association with the so-called junctional sarcoplasmic reticulum (Figure 6). Of particular interest was the finding of aggregates of free and membrane-bound ribosomes in some myocardial cells of ethanol-treated animals (Figures 7 and 8).

A careful search for ultrastructural abnormalities, especially those described in association with ethanol treatment and "alcoholic cardiomyopathy,"[3,4,24-26] was made. Remarkably few abnormalities were found, and these only in a few animals. Furthermore, some of the changes were also observed in pair-fed control rats. For example, in one instance a large mitochondrion ("megamitochondrion") was noted in one of the *ad lib* controls.

Hemodynamics

The hematocrit (Table 3) was 42.7 ± 1.0% in the pair-fed control group and was identical in the ethanol group. Similarly, pO_2 and pH were 89.7 ± 2.8 mm Hg and 7.39 ± 0.01, respectively, in the control group and were unchanged in the ethanol group. At normal levels of both right and left ventricular filling pressure, left ventricular systolic pressure and mean arterial pressure were lower, and left ventricular dP/dt and −dP/dt were decreased in the ethanol group as compared with the control group. Left ventricular stroke work was also decreased. When dP/dt, −dP/dt, and left ventricular stroke work were corrected for arterial pressure and body weight, respectively, however, none of these differences reached statistical significance. Oxygen consumption was lower in the ethanol group, compared with pair-fed control animals; when corrected for body weight, however, oxygen consumption did not differ between these groups. Arteriovenous oxygen difference and peripheral resistance did not differ among the various groups.

Table 3—Hemodynamic and Laboratory Data in Ethanol-fed, Pair-fed, and *Ad lib* Control Rats*

	Ethanol-fed (n = 25)	Pair-fed (n = 18)	Comparison of pair-fed vs ethanol-fed	*Ad lib* (n = 9)
Heart rate (beats/min)	380 ± 8	396 ± 6	NS	407 ± 10
Right ventricular end-diastolic pressure (mm Hg)	2.5 ± 0.5	2.6 ± 0.5	NS	4.4 ± 0.8
Left ventricular systolic pressure (mm Hg)	106 ± 3	124 ± 4	$P < 0.001$	133 ± 7
Left ventricular end-diastolic pressure (mm Hg)	4.8 ± 0.5	4.8 ± 0.4	NS	4.8 ± 0.6
Mean arterial pressure (mm Hg)	94 ± 3	115 ± 4	$P < 0.001$	132 ± 8
dP/dt (mm Hg/sec)	6488 ± 418	8548 ± 690	$P < 0.05$	8267 ± 611
dP/dt/PIP (sec^{-1})	85.0 ± 5.3	91.2 ± 6.5	NS	73.4 ± 6.2
−dP/dt (mm Hg/sec)	3830 ± 211	5519 ± 391	$P < 0.001$	6133 ± 377
O_2 consumption (ml)	3.37 ± 0.16	4.44 ± 0.30	$P < 0.001$	4.89 ± 0.19
O_2 consumption/body weight (ml/g × 10^3)	1.68 ± 0.08	1.91 ± 0.22	NS	1.12 ± 0.06
A-V O_2 difference (mg/dl)	7.80 ± 0.4	8.19 ± 0.6	NS	7.50 ± 0.57
Cardiac output (ml)	45 ± 3	59 ± 5	$P < 0.05$	69 ± 7
Cardiac output/body weight (ml/g)	0.22 ± 0.01	0.21 ± 0.01	NS	0.16 ± 0.02
Left ventricular stroke work (g mm)	134 ± 9	193 ± 17	$P < 0.005$	241 ± 37
Left ventricular stroke work/body weight (g mm/g)	0.66 ± 0.05	0.68 ± 0.06	NS	0.55 ± 0.09
Hematocrit (%)	42.8 ± 0.8	42.7 ± 1.0	NS	43.0 ± 1.2
pO_2 (mm Hg)	89.2 ± 1.7	89.7 ± 2.8	NS	100.2 ± 4.2
pH	7.4 ± 0.01	7.39 ± 0.01	NS	7.45 ± 0.02

* Mean ± SEM.
NS = not significant.

Discussion

Cardiac structure and function have been studied in rats fed liquid diets containing ethanol, which constituted 36% of the total calory intake, as described by Lieber, Jones, and DeCarli.[15] The serum alcohol reached levels corresponding to those encountered during moderate intoxication in man. Indeed, the animals often appeared clinically intoxicated and showed some of the signs seen in chronic alcoholic patients. The diet used was nutritionally balanced, and the one deficiency relevant to the cardiovascular system, namely, thiamine deficiency, was specifically ruled out by normal erythrocytic transketolase levels. However, animals on the experimental ethanol diet failed to gain weight and consistently remained below the pair-fed controls (Table 1), as has been witnessed also by other investigators.[15,27]

As expected, the livers of ethanol-fed animals exhibited fatty infil-

tration, whereas the pair-fed controls remained normal. These changes were evident after 5 weeks as well as after 4 months on the ethanol diet, with some evidence of progression. In this model of ethanol-induced fatty liver, the catalase levels remained unchanged in the liver. This result is in full agreement with previous observations of Lieber.[10] In keeping with this agreement, we found no significant change in the cytochemical appearance of peroxisomes in the liver.

In sharp contrast to the liver, biochemical determinations of catalase in the myocardium revealed a significant increase in animals fed ethanol for both 5 and 18 weeks, as compared with pair-fed and *ad lib* control animals (Table 2). This biochemical finding was also reflected in light- and electron-microscopic cytochemical preparations, which showed an increase in the number of peroxisomes in animals on the ethanol diet.

It should be emphasized that because of the paucity of peroxisomes in the heart and the variation in their occurrence in adjacent myocardial fibers, it was much easier to study the elevation of catalase by biochemical methods than by ultrastructural cytochemistry.

Of the three major enzyme systems involved in ethanol metabolism, catalase is the only one that has been shown unequivocally to be present in the heart.[13] It should be pointed out, however, that the activity of catalase in the heart is only a small fraction of its activity in the liver, a finding that is consistent with the rather low level of metabolism of alcohol in the heart. On the other hand, the rate of H_2O_2-mediated ethanol peroxidation by catalase is limited mainly by the rate of H_2O_2 generation rather than the amount of catalase itself.[28] Since heart mitochondria produce relatively large amounts of H_2O_2,[29] it is possible that even the small amounts of catalase could play a significant role in the myocardial metabolism of ethanol.

The cardinal finding of the present study is the elevation of catalase in ethanol-treated animals. This elevation could be due to increased synthesis or to decreased catabolism of this hemoprotein. The ultrastructural observation of several foci with free and membrane-bound ribosomes (Figures 7 and 8) in the hearts of *adult* animals treated with ethanol suggests significant protein synthesis. Indeed, such foci are rarely seen in the hearts of normal adult animals [30] but have been seen in patients with alcoholic cardiomyopathy[4] and in association with myocardial hypertrophy.[31] It is noteworthy that catalase induction has also been observed in microorganisms and yeasts after exposure to various alcohols, especially methanol,[32,33] and in mammalian liver after treatment with hypolipidemic agents,[34] thus demonstrating the ease of inducibility of this enzyme in association with changes in the metabolic conditions.

Schreiber et al found that ethanol did not interfere with the protein synthesis in the heart,[35] but, on the other hand, noted that acetaldehyde inhibited protein synthesis both in microsomal fractions and in isolated perfused guinea pig hearts.[35,36] Although acetaldehyde levels were not determined in our animals, the elevation of catalase activity and the ultrastructural evidence of active protein synthesis in adult animals suggest that no significant interference with the synthesis of this protein occurred in the hearts of our ethanol-treated animals.

A question which should be considered is whether or not the association of increased myocardial catalase with chronic administration of ethanol is a causal one. One possible alternative cause of increased catalase could be myocardial atrophy, since catalase has been reported increased in atrophic skeletal muscle.[37] Although the heart weight of animals fed ethanol for 5 weeks was somewhat less than that of the pair-fed controls, in the animals fed ethanol for 18 weeks the heart weights were equal in both groups. Furthermore, the heart/body weight ratios did not differ in either the group fed ethanol for 5 weeks or that fed for 18 weeks; and no morphologic or biochemical evidence of atrophy and muscle wasting was found in our ethanol-treated animals. Thus, it is unlikely that catalase elevations were related to any degenerative processes in the heart.

The significance of the elevation of catalase activity and the increased number of peroxisomes associated with chronic intake of ethanol is not known at present. However, in view of the possible participation of peroxisomes in lipid metabolism,[38] it could be speculated that they prevented the development of fatty infiltration in the myocardium.

The finding of the paucity of pathologic alterations in our ethanol-treated rats, seen both by light and electron microscopy, is in contrast with the findings of some of the earlier studies.[4] It is to be especially noted that no mitochondrial abnormalities were noted in our animals. In this respect, Alexander[26] recently described the occurrence of megamitochondria in the hearts of mice fed ethanol for 15–25 weeks. The only example of such mitochondria in our material, however, was seen in a control animal, thus suggesting the influence of dietary and environmental factors other than ethanol upon the myocardial ultrastructure. The other mitochondrial abnormalities described in association with ethanol treatment are focal swelling and vacuolization. None of our animals, which were properly fixed by vascular perfusion, showed this change. Ferrans et al, in a comprehensive review of the ultrastructural changes in alcoholic cardiomyopathy, pointed out the discrepancies between the various studies and indicated that differences in preparative procedures for electron microscopy could be responsible for such a divergence in findings.[4] Recently Vodovar

and Desnoyers [39] investigated the influence of methods of tissue fixation upon the ultrastructure of myocardium and noted that when normal rat hearts were fixed by immersion in glutaraldehyde, ultrastructural changes such as focal vacuolization and swelling of mitochondria were found that were indistinguishable from similar changes seen under pathologic conditions. Furthermore, when pathologically altered myocardium was fixed by immersion, it was impossible to distinguish between pathologic abnormalities and the fixation artifacts. Thus, it seems that perfusion fixation is obligatory for proper evaluation of myocardium under experimental conditions. This may explain the absence of significant ultrastructural changes in our animals. Indeed, Hall and Rowlands,[27] who also investigated the influence of ethanol upon the myocardial ultrastructure and who also used perfusion fixation, noted very few abnormalities in the hearts of their experimental animals.

The design of the present inquiry included hemodynamic assessment of cardiac function in order to permit characterization of pathophysiologic changes, if any, and their correlation with morphologic, biochemical, and histochemical data. *In vivo* hemodynamic studies of animals on chronic ethanol-containing diets have been reported only for the dog. Pachinger et al [8] administered ethanol to dogs for 14 weeks and observed no change in cardiac output, systemic arterial pressure, or left ventricular end-diastolic pressure. Studies after increasing left ventricular afterload with angiotensin did not differentiate between animals on the ethanol diet and control animals. Regan et al,[9] on the other hand, studied dogs after 18 months of dietary ethanol and found elevated resting left ventricular end-diastolic pressures that rose further and to a greater extent than those of controls upon increase in either preload or afterload. In the present study, the animals on the ethanol diet differed from the pair-fed controls in having significantly lower left ventricular systolic and mean arterial pressures by 15% and 18%, respectively. This decrease in systemic pressure was accompanied by an even greater decrease in cardiac output, namely, by 24%, implying that peripheral resistance did not rise sufficiently to maintain systemic pressure constant. The observed decrease in cardiac output, however, may not be interpreted as a direct hemodynamic effect of ethanol, inasmuch as both cardiac output and oxygen consumption per unit of body weight remained unchanged. Left and right ventricular filling pressures were identical in the ethanol and control groups, yielding no evidence of ventricular failure or altered ventricular compliance. Both positive and negative left ventricular dP/dt were found to be significantly decreased in the ethanol group. However, when these measures were related to the prevailing systemic pressures, they were not altered signifi-

cantly; hence, no impairment of ventricular systolic or diastolic function may be inferred. Thus, after chronic exposure to ethanol and under conditions corresponding to increased myocardial catalase, left ventricular preload and afterload were reduced, and there was no evidence of impairment of left ventricular function.

Since in animals fed ethanol chronically, increased levels of catalase were associated with neither morphologic nor functional cardiac alterations, one may raise the question as to whether catalase exerted a protective effect upon the myocardium. The present study does not permit an answer to this question. Future investigations under conditions of suppressed catalase activity,[40] however, could shed some light on this question.

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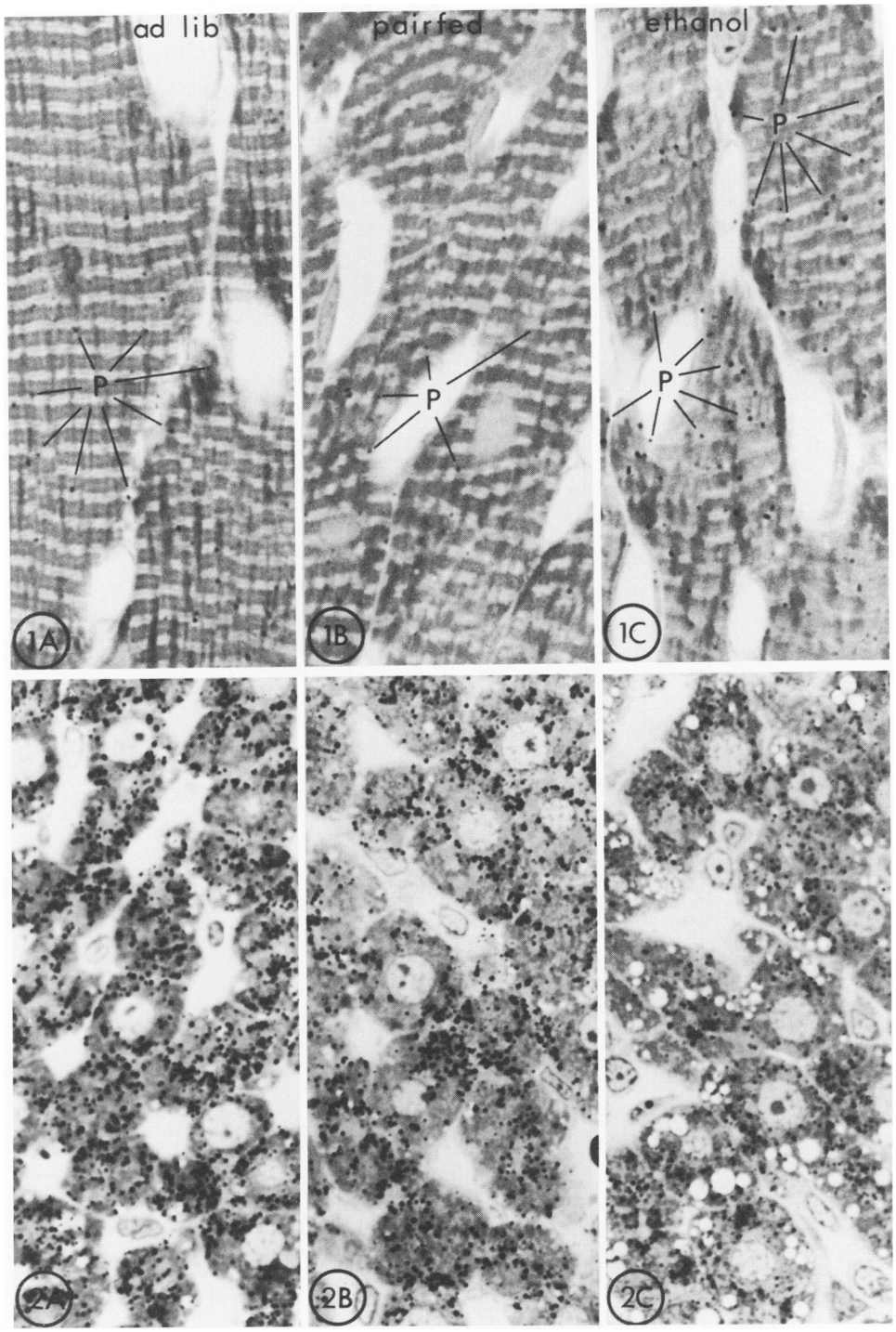
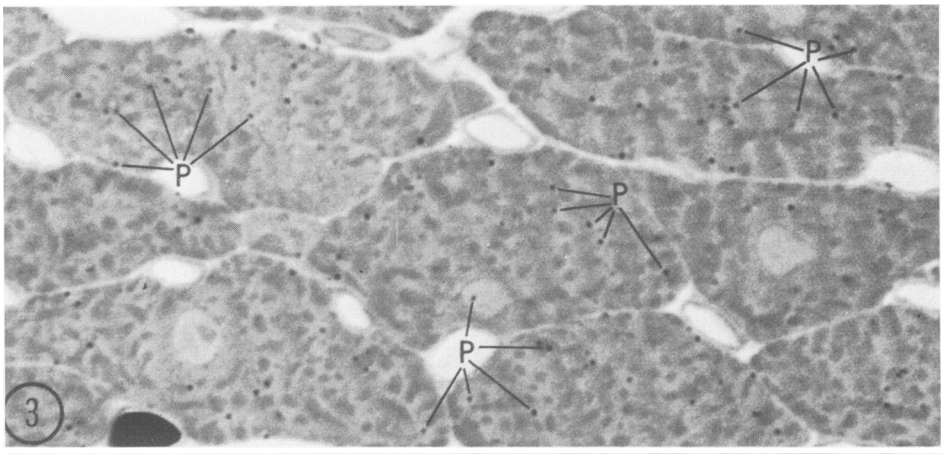


Figures 1 and 2—Light micrographs of 1-μ sections of rat myocardium (**Figure 1, A-C**) and liver (**Figure 2, A-C**). The tissues were fixed by vascular perfusion with glutaraldehyde and incubated for localization of catalase. The reaction product is found in small, distinctly stained peroxisomes (*P*). **Figures 1A** and **2A** are from *ad lib* control animals, **1B** and **2B** from pair-fed control animals, and **1C** and **2C** from animals that received ethanol for 5 weeks. Note the relative increase in the number of peroxisomes in the heart of the ethanol-treated animal (**Figure 1C**). There are numerous fat vacuoles in the liver cells of the ethanol-treated rat (**Figure 2C**) but no significant change in the localization of catalase and distribution of peroxisomes, in comparison with control animals (**Figure 1, A-C**, ×1600) (**Figure 2, A-C**, ×750)



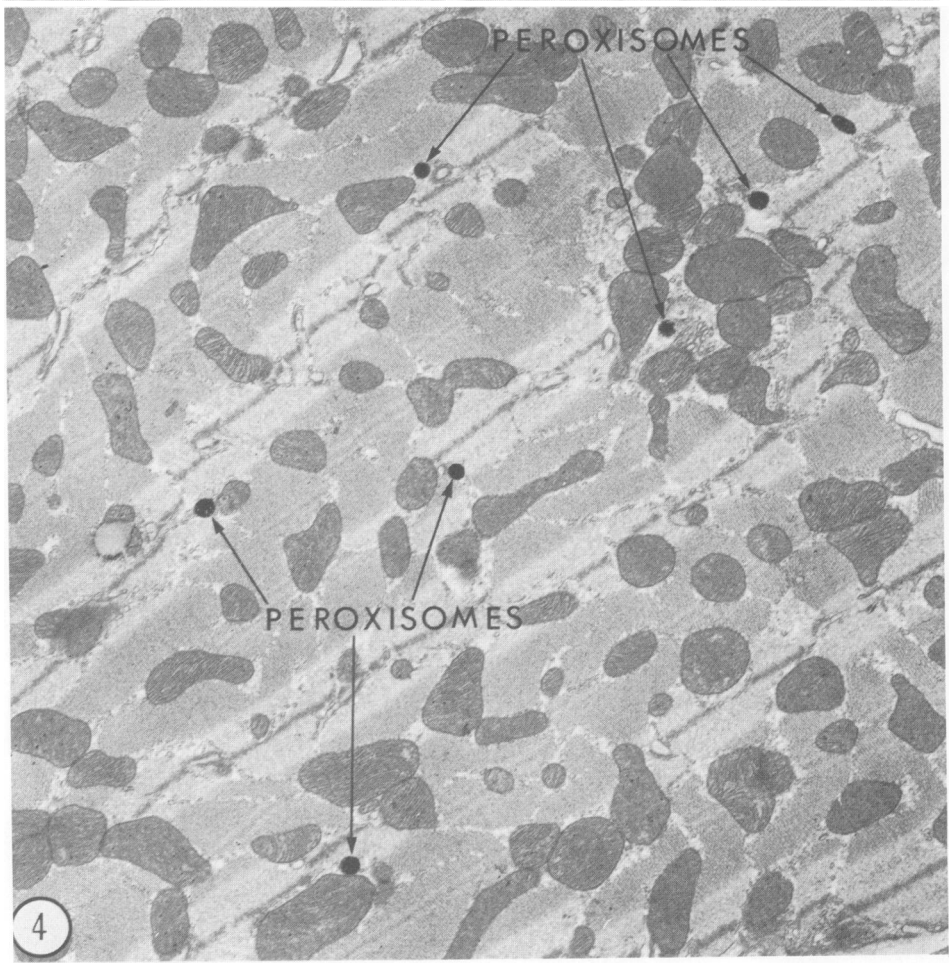
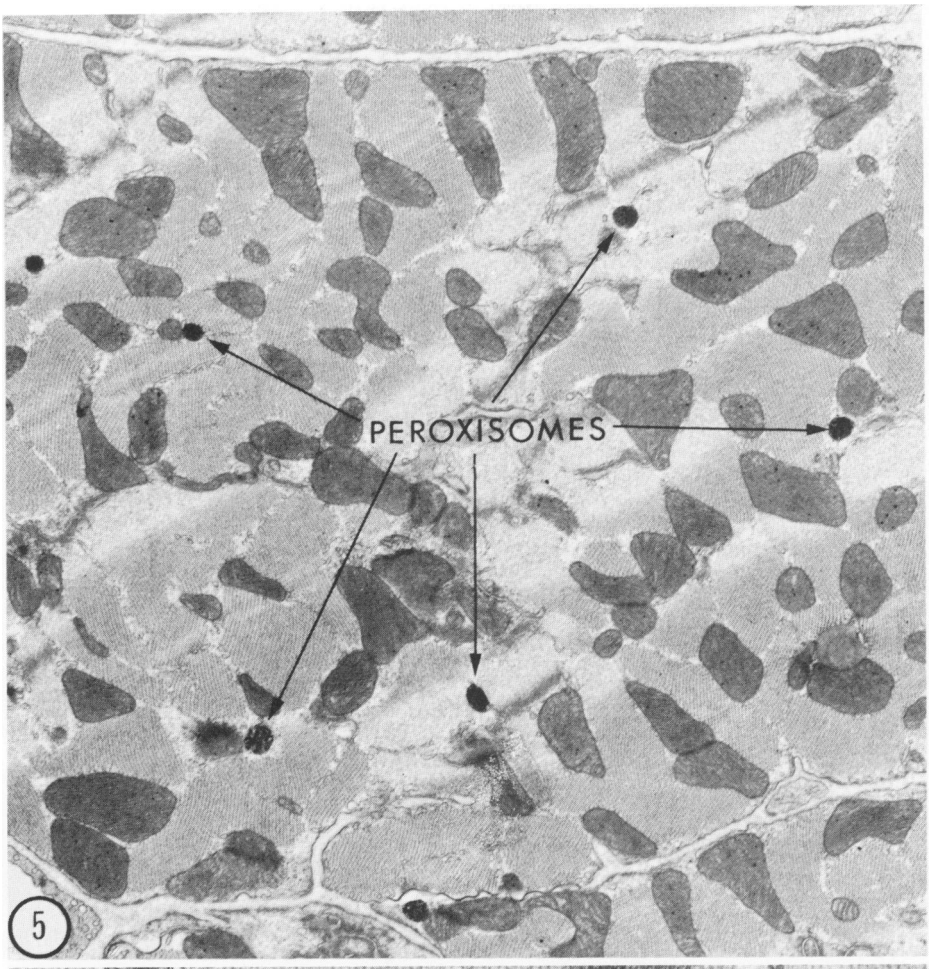
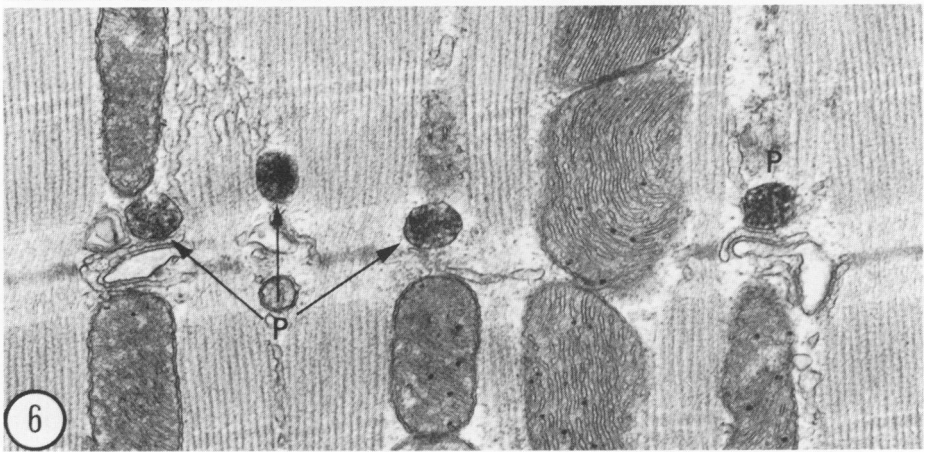
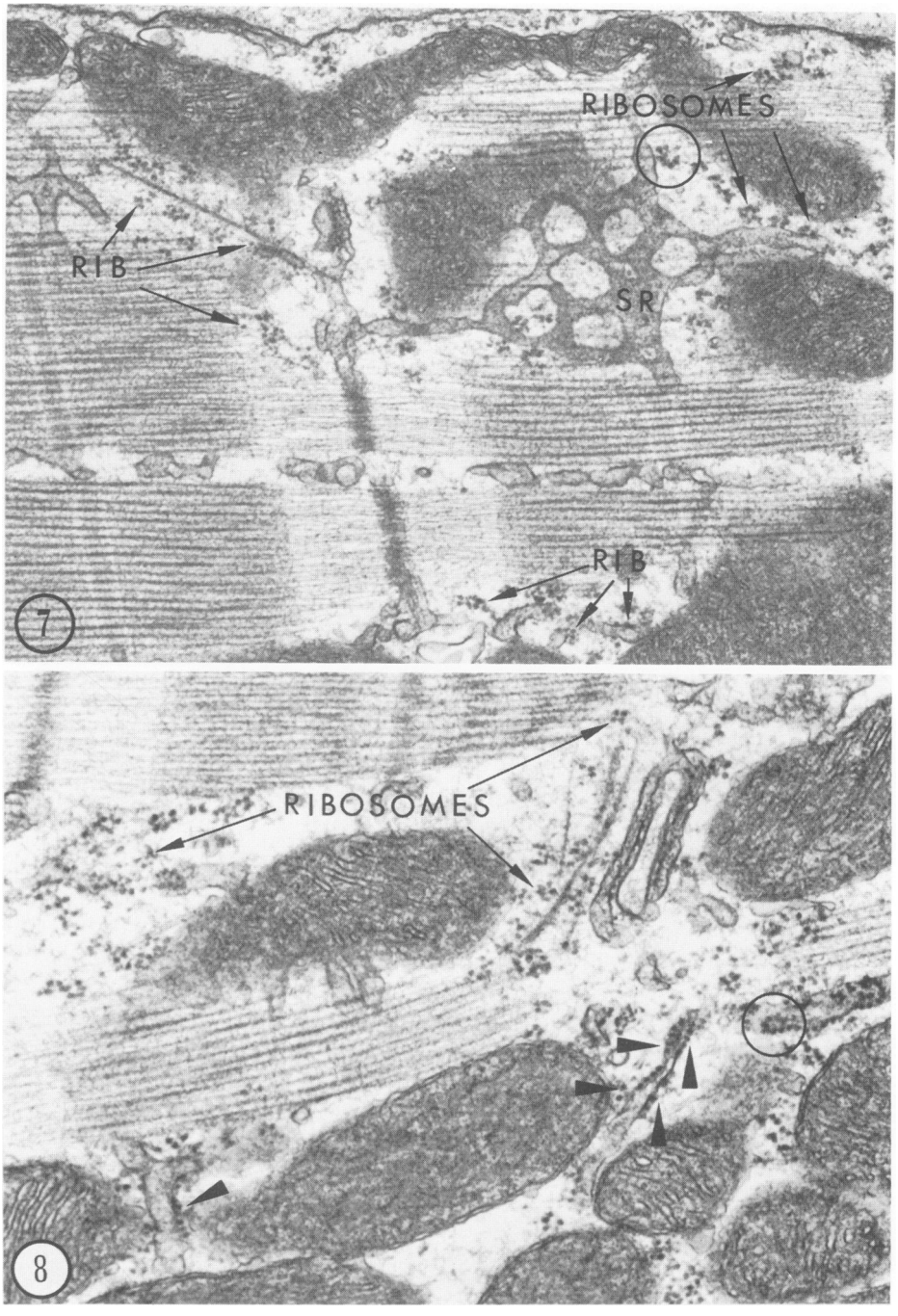


Figure 3—A transverse section of rat myocardium showing numerous peroxisomes (*p*) distributed in the cytoplasm of myocardial fibers. In comparison to cross-sections of untreated animals (eg, Figure 1 in Herzog and Fahimi[13]), the number of peroxisomes appears increased. (×1800) **Figures 4-6**—Electron micrographs of the myocardium of rats fed ethanol for 5 weeks, fixed by perfusion and incubated for catalase. Note the normal ultrastructural appearance of the heart. The mitochondria appear intact, and the contractile elements, as well





as the sarcoplasmic reticulum, appear normal. The slight extraction of glycogen is due to the incubation for cytochemistry. The catalase reaction product is localized in peroxisomes. In well-oriented longitudinal sections (**Figure 6**) these are typically found at the junctions of the A and I bands and in close association with the junctional sarcoplasmic reticulum. *P* = Peroxisome. (Lead citrate, **Figure 4**, ×18,000; **Figure 5**, ×19,000; **Figure 6**, ×26,000)



Figures 7 and 8—Electron micrographs of myocardial fibers from rats fed ethanol for 18 weeks, fixed by perfusion and processed for electron microscopy. Note the aggregates of ribosomes, some of which appear to be attached to the membranes of the sarcoplasmic reticulum (*arrowheads,* **Figure 8**). The circles in both figures point to polysome aggregates. SR = sarcoplasmic reticulum. (Uranyl acetate and lead citrate, **Figure 7,** ×42,000; **Figure 8,** ×45,000)