interacts specifically with a distinctive polysaccharide on the surface of the appropriate *Rhizobium* cell as a prelude to nodulation. These data demonstrate that the possibility is a real one which merits thorough investigation.

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6. The protein content of fraction II was adjusted to 1 percent with saline; to 10 ml of this solution was added 4 ml of 0.1*M* sodium phosphate buffer (*p*H 7.0) and then 4 ml of 0.1*M* sodium phosphate buffer (*p*H 8.0) containing 5 mg of fluorescein isothiocyanate (isomer I, Baltimore Biological Laboratories). The resulting solution was adjusted to *p*H 9.0, merthiolate (1 : 10,000) was added, and the reaction proceeded for 24 hours. Unreacted dye was removed by dialysis against saline buffered with 0.02*M* sodium phosphate to *p*H 7.2 until the dialyzate was completely colorless. The fluorescent lectin conjugate was distributed into tubes and held frozen at −20°C.
7. Microscopy was performed with a Zeiss Universal microscope equipped for double lighting with incident illumination from an HBO-200 (Osram) light source and transmitted dark-field tungsten illumination. Zeiss filter No. 1 was used in the path of the transmitted light and Zeiss No. 50 barrier filter was used throughout. Most observations were made by using the two lighting systems in combination.
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26 March 1974

Microbodies (Peroxisomes) Containing Catalase in Myocardium: Morphological and Biochemical Evidence

Abstract. *Microbodies characterized by a single limiting membrane and finely granular matrix occur in mouse myocardium and appear in close spatial relation to mitochondria and sarcoplasmic reticulum. The presence of catalase in the microbodies is revealed cytochemically and confirmed biochemically by direct measurement of its activity in myocardial tissue fractions. It is suggested that the microbodies may play an important role in myocardial lipid metabolism.*

Peroxisomes (microbodies) are cytoplasmic organelles, characterized morphologically by a single limiting membrane and a finely granular matrix (*1*). They contain catalase and a variety of H_2O_2-producing oxidases (*2, 3*). In germinating bean seedlings, peroxisomes [also referred to as glyoxysomes (*4*)] are abundant and play a key role in the conversion of lipids to sugars (*4, 5*). The exact biological function of peroxisomes in mammalian cells, however, remains unknown. Possible participation in gluconeogenesis has been suggested by de Duve and Baudhuin (*2*) and link to lipid metabolism was originally postulated by Novikoff and Shin (*6*). Indeed, application of a cytochemical method in which alkaline diaminobenzidine (DAB) is used to demonstrate the peroxidatic activity of catalase (*7*) has revealed large numbers of microbodies in animal cells actively involved in lipid metabolism, such as brown and white fat cells and steroid-secreting cells (*8*). The heart uses lipids and fatty acids as an energy source (*9*). To our knowledge, this is the first report of microbodies in this tissue, specifically in mouse myocardial fibers.

Hearts of male albino mice were fixed, either by vascular perfusion for 10 minutes or by immersion for 2 hours, with 2.5 percent glutaraldehyde in 0.1*M* cacodylate buffer (*p*H 7.4). Sections of the left ventricle (30 μm) were prepared with a tissue chopper (*10*) and were incubated for 2 hours at room temperature in a medium containing 1 mg of 3.3′-diaminobenzidine tetrahydrochloride in 1 ml of 0.1*M* NaOH-citrate-phosphate buffer (*p*H 10.5) and 0.02 percent H_2O_2 (*11*). After incubation, the tissue was fixed with OsO_4 and processed for light and electron microscopy.

Examination of unstained sections 1 μm thick by light microscopy revealed numerous distinct dark brown granules, which appeared smaller and darker than mitochondria. By electron microscopy, electron-opaque reaction product was localized in round and oval particles 0.2 to 0.5 μm in diameter and located in the sarcoplasm of myocardial cells (Figs. 1 and 2). The particles often appeared close to mitochondria and membranes of the sarcoplasmic reticulum and were mostly

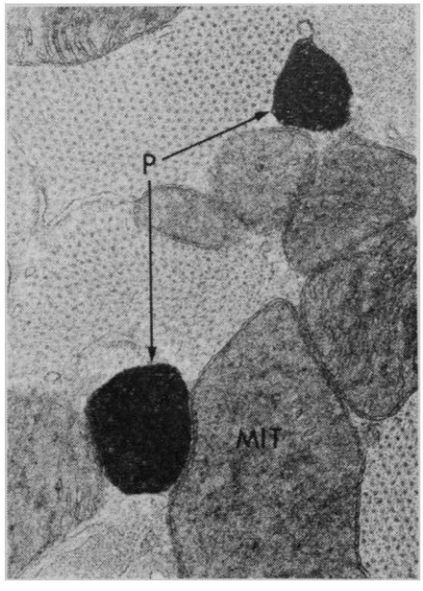

Fig. 1 (left). Electron micrograph of transverse section of mouse myocardial fiber. Tissue was fixed by immersion and incubated as described in the text for cytochemical demonstration of catalase (*11*). Electron-opaque reaction product is found in particles (*P*) 0.2 to 0.5 μm in diameter which appear in close association with mitochondria (*MIT*) (× 42,000).

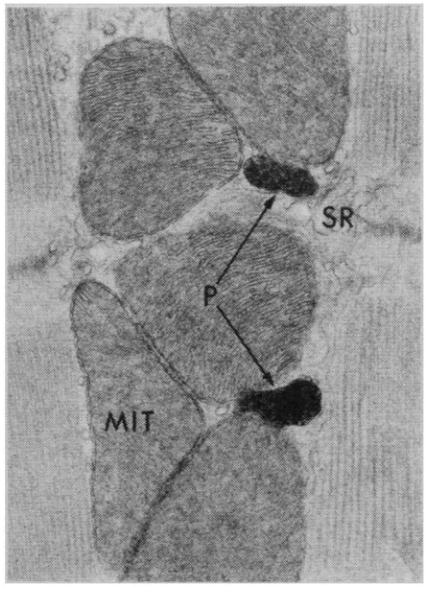

Fig. 2 (right). Longitudinal section of mouse myocardial fiber. Tissue was fixed by vascular perfusion and incubated as described in the text for demonstration of catalase (*11*). Reaction product is found in particles (*P*), which are mostly localized at the junction of I and A bands. The particles appear in close association with mitochondria (*MIT*) and elements of sarcoplasmic reticulum (*SR*) (× 30,000).

located at the junctions of I and A bands (Figs. 2 and 3). In lightly reacted preparations, a distinct limiting membrane was observed at the periphery of the particles, and the reaction product was confined to the matrix (Fig. 2). The number of particles seen in ultrathin sections varied in different cells, with some cells containing numerous particles (Figs. 1 and 2) while adjacent cells contained only a few. All particles lacked crystalline nucleoids.

In control preparations, the staining reaction was abolished by omitting H_2O_2 or DAB from the incubation medium; by adding $2 \times 10^{-2}M$ aminotriazole, $10^{-1}M$ KCN, or $10^{-1}M$ azide; or by boiling the sections. Partial inhibition of the reaction by $10^{-2}M$ dichlorophenolindophenol was also observed. The particles in the control preparations had the usual appearance of microbodies with a single limiting membrane and a finely granular matrix (Fig. 3). The results with inhibitors suggest that the staining reaction is due to peroxidatic activity of catalase in peroxisomes (*7*, *12*).

To confirm the presence of catalase in myocardium, mouse hearts were perfused with saline and erythrocytes were washed out. This was followed by homogenization and differential centrifugation according to the scheme of Stein and Stein (*13*). The catalase activity was determined in various fractions according to the method of Lück (*14*), and the protein was determined by the method of Lowry *et al.* (*15*). The results indicate that mouse heart contains catalase, and the highest specific activity of catalase is found in particulate form in association with the microsomal fraction (Table 1). The association of catalase with microsomes is in agreement with the electron microscopic finding that catalase-containing particles are smaller than mitochondria and approximately the size of large microsomal vesicles (Figs. 1 to 3).

These observations indicate that microbodies containing particulate catalase occur in myocardial fibers of mouse. The term "peroxisome" implies a particle which contains catalase and one or more H_2O_2-producing oxidases (*2*, *3*). Whereas the type and activity of oxidases vary markedly in different tissues, catalase occurs in high concentration in all animal and plant peroxisomes and can therefore be considered as a marker enzyme for this organelle

Table 1. Distribution of catalase activity in total homogenate and in subcellular fractions of mouse myocardium in a typical experiment. Catalase activity is given in international units per milligram of protein. The highest specific activity is associated with the microsomal fraction, which contains a large number of particles with the same ultrastructural and cytochemical properties as the peroxisomes in tissue sections.

Fraction	Catalase activity (units per milligram of protein)
Total homogenate	24.9
Cell debris nuclei	11.1
Mitochondria	53.3
Microsomes	164.0
Postmicrosomal supernatant	6.3

[for rare exceptions, see (*16*)]. We have as yet no evidence for any H_2O_2-producing oxidases in the particles of mouse myocardium. However, the biochemical and cytochemical localization of catalase in these particles and their ultrastructural appearance strongly suggest that they correspond to the group of small microbodies or "microperoxisomes" described recently in other mammalian cells (*8*, *12*).

Although the exact biological function of mammalian peroxisomes is not known, possible participation in lipid metabolism has been suggested by the observations of Svoboda and co-workers (*17*), who have noted proliferation of these particles in rats and mice after the administration of hypolipidemic drugs. Furthermore, Caravaca *et al.* (*18*) have shown that injection of

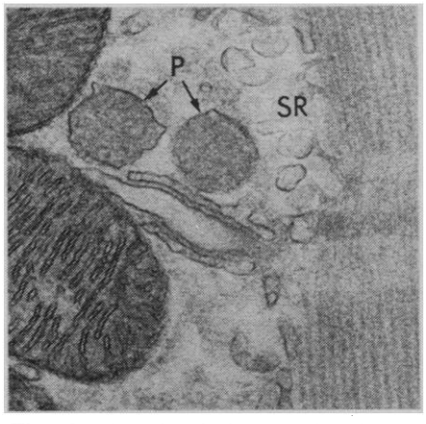

Fig. 3. Cytochemical control: electron micrograph of myocardial tissue after incubation in alkaline DAB medium in the presence of $2 \times 10^{-2}M$ aminotriazole. Reaction in the particles (*P*) is abolished. Note the distinct limiting membrane, and finely granular electron-opaque matrix of the particles, and their proximity to sarcoplasmic reticulum (*SR*) (× 41,000).

lyophilized catalase reduces the blood cholesterol level and prevents aortic atherosclerosis in cholesterol-fed rabbits. Further support for the possible involvement of peroxisomal catalase in lipid metabolism is provided by the observations of Goldfischer *et al.* (*19*), who noted low blood triglyceride and cholesterol levels in a strain of mice with abnormal and unstable catalase (*20*). The role of lipids as the respiratory fuel for continuous work of the contracting myocardium has been well established (*9*). Stein and Stein (*13*) have shown that elements of the sarcoplasmic reticulum are the intracellular sites of triglyceride synthesis in myocardium. The presence of catalase in myocardial peroxisomes and the close spatial relation of these peroxisomes to elements of the sarcoplasmic reticulum (Figs. 2 and 3) suggest that they may be actively involved in myocardial lipid metabolism.

Note added in proof: Since the submission of this report Hand (*21*) has described the occurrence of peroxisomes in various types of rat striated muscle including the cardiac muscle.

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Noise-Induced Reduction of Inner-Ear Microphonic Response: Dependence on Body Temperature

Abstract. *The rate of reduction of chinchilla cochlear microphonic response with exposure to steady noise is less at lower body temperatures and greater at higher body temperatures. Before exposure to noise, this auditory response is invariant within the range of temperatures employed. The mechanism of reduction of cochlear response appears to involve processes sensitive to body temperature.*

Carder and Miller (*1*) first showed by behavioral methods that continuous noise of sufficient intensity produces a progressive loss of auditory sensitivity in chinchillas until a steady state is reached in which auditory sensitivity is stable with continued exposure to noise. Recovery to normal auditory thresholds occurs within 3 to 6 days after cessation of the noise, but not without some risk of sensory-cell loss (*2, 3*). Similar behavioral results have been obtained for man (*4*). Such changes in auditory sensitivity are known as asymptotic threshold shifts and may include permanent as well as temporary components (*5*). Thus, these phenomena appear related to progressive, noise-induced hearing losses in man (*6*).

In the work reported here, the cochlear microphonic response (CM), which arises in the inner ear (*7*) and is correlated with auditory sensitivity (*2*), is progressively decreased at a rate dependent on body temperature during exposure of the ear to noise. Procedures used for surgical exposure of the cochlea and electrophysiological recording were those described by Tasaki *et al.* (*8*). Electrodes were inserted into the second turn of the chinchilla cochlea. Sound was delivered through a PDR-10 earphone and speculum (*2*) and consisted of either octave-band noise with center frequency at 1 khz at an overall level of 90 db referenced to 0.0002 μbar or short tone bursts at 200 hertz. A LINC computer controlled the signals and recorded the wave forms of CM on magnetic tape. Complete input-output functions for CM to tone bursts were obtained during 45-second interruptions in the noise. Body temperature of each anesthetized animal was rapidly adjusted to 29°, 37°, or 39°C by appropriate cooling or heating and held constant under control of a thermostat with thermistor probe. When the first measurements were taken the cochlear temperature was within about 1°C of body temperature.

In the absence of noise, CM showed no significant change with temperature or time for as long as the animal lived. When noise was presented, CM progressively decreased and approached an asymptote (Fig. 1). The rate of decrease of maximum voltage was significantly different for the three temperatures. The times for half-maximal loss of voltage were 5, 70, and 170 minutes at 39°, 37°, and 29°C, respectively (Fig. 1A). The CM values did not differ significantly at asymptote. In addition, maximum voltage at 29°C remained near initial values for almost an hour before decreasing. A different measure of CM, the sensitivity (in microvolts per microbar) obtained from sound pressure necessary to produce a criterion voltage in the region of linear output, showed a pat-

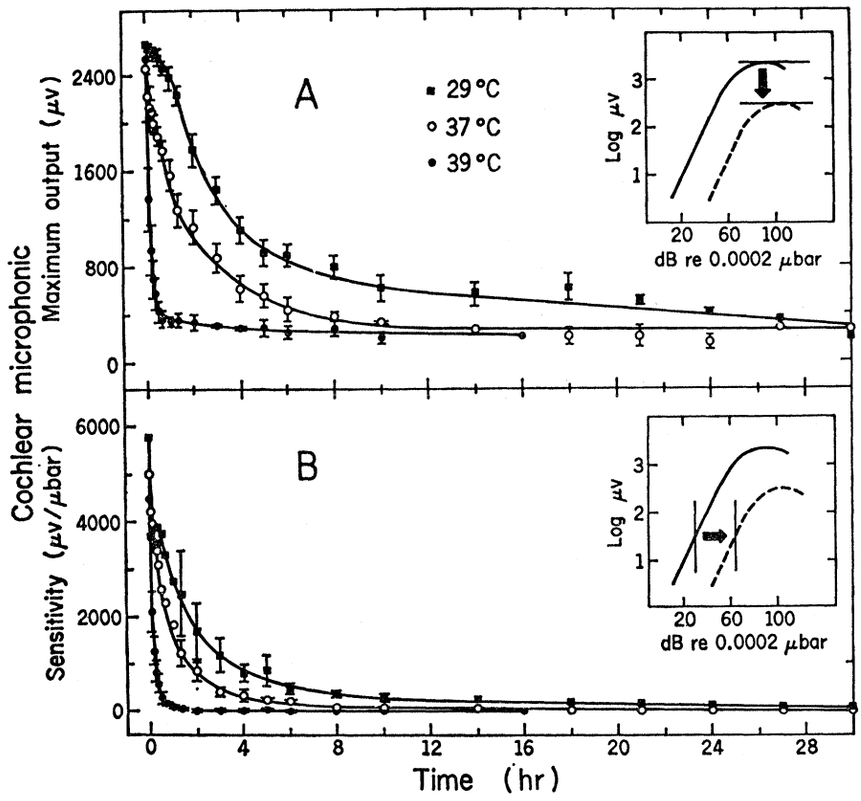

Fig. 1. Temperature dependence of the loss of (A) maximum voltage (peak to peak) and (B) sensitivity of cochlear microphonic response. The CM was recorded from the second turn of the cochlea of chinchilla during exposure to octave-band noise with center frequency at 1 khz at 90 db referenced to 0.0002 μbar. Body temperatures are listed. Significant differences are indicated with vertical bars denoting ± 1 standard error of the mean ($N = 3$, 4, and 4 for 29°, 37°, and 39°C, respectively). The insets are diagrams of the normal test input-output functions for CM (solid curves) and the corresponding functions after exposure to noise for an arbitrary period of time (dashed curves); reduction of (A) maximum and (B) sensitivity are illustrated.