cent of the cerebral oxygen consumption in the starving adult human patients studied by them. A similar adaptation may occur in regard to acetoacetate, but it is probably of lesser quantitative significance in that the concentration of acetoacetate in blood is only a small fraction of that of β-hydroxybutyrate during fasting (*1*).

The mechanism of the increased brain D(−)-β-dehydrogenase activity during fasting is still unknown.

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Giant Hepatic Mitochondria: Production in Mice Fed with Cuprizone

Abstract. *Giant mitochondria in hepatocytes that have the average size of nuclei can be consistently produced in the liver of weanling mice by feeding them cuprizone (bis-cyclohexanone oxaldihydrazone). The simplicity of the procedure and the consistency of the results make the feeding of cuprizone a new and useful experimental tool for the study of mitochondrial metabolism.*

Cuprizone (bis-cyclohexanone oxaldihydrazone) is a chelating agent used for quantitative determination of copper (*1*). It is also known as a strong inhibitor of amine oxidase of bovine hepatic mitochondria (*2*). When administered orally, this compound produced severe status spongiosus and enlarged glial nuclei resembling Alzheimer's glia in the cerebellum and the brainstem of mice (*3*). Alzheimer's glia are usually associated with hepatic damage. During the course of our ultrastructural study on cuprizone-induced encephalopathy, we examined livers of mice that had been fed cuprizone and found that the compound consistently produced extremely enlarged mitochondria in hepatocytes.

Weanling Swiss-Webster male mice were fed with a mixture of 0.5 g of cuprizone (*4*) in 100 g of Rockland Mouse/Rat Diet (*5*) from 3 weeks of age. Water was freely available. Compared to control animals that were fed with the same diet, except without cuprizone, experimental animals became inactive in 1 to 2 weeks, showing marked retardation of their growth and weakness of posterior limbs. Most of them died within 3 weeks when cuprizone feeding continued. All of them exhibited brain edema. Details of the neuropathological aspects of cuprizone intoxication will be reported separately (*6*). For electron microscopic examination of the liver, the tissue was fixed by perfusion with 5 percent glutaraldehyde, tnen post-fixed with osmium tetroxide or directly fixed with osmium tetroxide, and embedded in Epon; the sections were stained with uranyl acetate and lead citrate.

All hepatocytes contained extremely enlarged mitochondria that varied in size from 2 to 10 μm in diameter (Fig. 1). Some of the larger mitochondria measured up to 15 μm in diameter. The mitochondria had abundant, slightly osmiophilic granular matrix. Cristae were often arranged irregularly at the periphery, and they appeared to remain normal in size, but the intercristal space was enlarged, giving the relative appearance of short cristae. The outer mitochondrial membranes were in most instances intact. All mitochondria in all hepatic cells in all experimental animals were invariably affected. These giant mitochondria could be seen in preparations stained with hematoxylin-eosin by light microscope as well-defined pale areas in the cytoplasm, but they could easily be overlooked unless the observer was aware of the electron microscopic finding.

Although enlarged hepatic mitochondria have been reported in several other conditions (*7*), the magnitude of the change seen in cuprizone-fed mice appears to be unique. I believe that cuprizone should serve as a useful tool to study mitochondrial metabolism and its relation to their morphology. Since cuprizone binds copper strongly and deprives the tissue of copper required for its normal function, it is reasonable to suspect that mitochondrial copper metabolism may in some way be in-

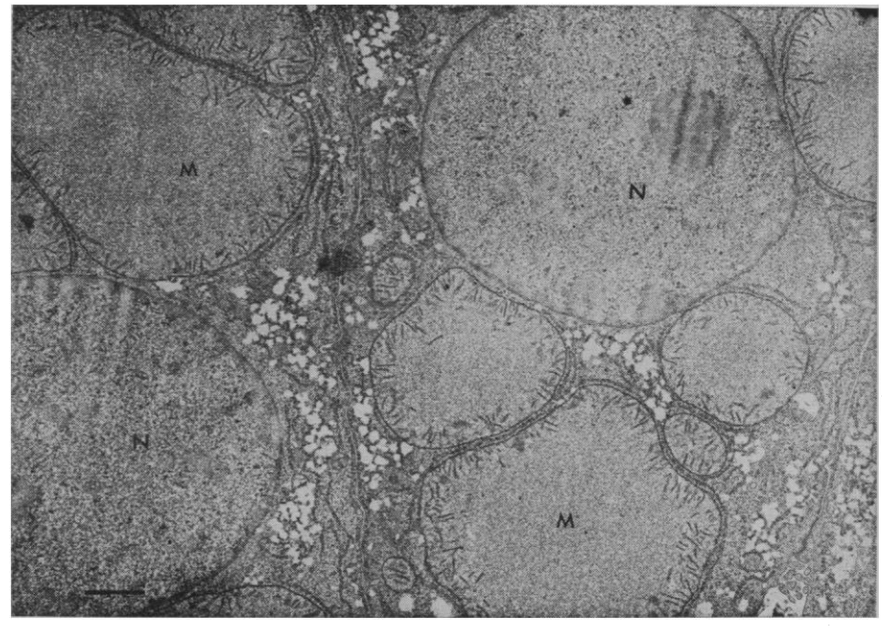

Fig. 1. Electron micrograph of the giant mitochondria in the liver of mice fed with cuprizone. The tissue was fixed by perfusion with 5 percent glutaraldehyde and then post-fixed with osmium tetroxide. *N*, nucleus; *M*, mitochondria. Scale is 1 μm.

volved in the formation of giant mitochondria. The production of these giant mitochondria with this compound is quite simple and the results consistent, a distinct advantage as an experimental tool. Study of isolated mitochondria from the liver and also of the effect of cuprizone on the isolated mitochondria in vitro provide interesting possibilities.

The mechanism by which cuprizone produces such changes in hepatic mitochondria, as well as severe edema of the central nervous system, remains an intriguing question. Despite extensive search, I have been unable to find similar giant mitochondria in the brain. This may be due only to the effect of the blood-brain barrier, but also could be due to the intrinsic difference in metabolic activities of these two organs.

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3 September 1968

Crayfish Muscle Fiber: Spike Electrogenesis in Fibers with Long Sarcomeres

Abstract. *Most of the muscle fibers in the walking legs of the crayfish* Procambarus clarkii *generate only graded electrical responses. However, some fibers in extensor muscles of the carpopodite have long sarcomeres, about 10 micrometers in length, and generate overshooting spikes that have conduction velocities of 0.3 meter per second. The spikes induce twitch contractions.*

Fibers of muscles in the walking legs of crustaceans usually generate only graded electrically excitable responses (*1, 2*). When a long-lasting depolarizing current is applied the response may be oscillatory. Rarely, however, fibers are observed that generate small, all-or-none action potentials, less than 50 mv in amplitude (*1, 3*). Graded responsiveness and long sarcomeres have been used as criteria for categorizing "slow" muscle fibers (*4*). I report here the rather frequent occurrence of fibers in the crayfish *Procambarus clarkii* which have long sarcomeres (about 10 μm), but which generate overshooting spikes that are associated with twitch contractions.

Extensor muscles in the carpopodite were prepared as described previously (*5*) and bathed in van Harreveld's crayfish saline (*6*). Superficial fibers were impaled with two glass microelectrodes. One, filled with 3*M* KCl, was for recording potentials; the other, filled with 2*M* potassium citrate, was for applying current.

The spike-generating muscle fibers were found in the posterior of the extensor muscle close to the propodite. In this region, too, most of the fibers normally produce graded responses (Fig. 1A). However, the record of Fig. 1B was from a fiber immediately adjacent to the one whose responses are given in Fig. 1A which produced a spike of 92 mv with an overshoot of 9 mv. Figure 1 (C and D) shows responses from another fiber to a short and a long pulse, respectively. The resting potential was 78 mv; the spike had an amplitude of 94 mv in Fig. 1C and an overshoot of 16 mv. The threshold depolarization to elicit spikes ranged from 25 to 40 mv. Conduction velocities, determined in two muscle fibers, were 0.33 and 0.34 m/sec and were similar to those reported for crab muscle (*7*). The sarcomeres of the spike-generating fibers were about 10 μm long, like those of fibers that produced graded responses (*3, 5, 8*). As already noted, the spikes induced twitch contractions.

Tetrodotoxin, which selectively blocks sodium activation in various cells (*9*), did not affect the spikes of the crayfish muscle fibers when applied in relatively high concentration (6×10^{-6} g/ml) for 30 minutes. The naturally occurring spikes are probably generated by calcium activation, since their amplitude was increased by about 4 mv when the external calcium was doubled (from 13.5 to 27 mmole/liter). However, the increase is less than half that expected from the Nernst relation: When calcium concentration in the saline is doubled, E_M increases approximately by 8.8 mv according to calculation from the equation

$$E_M = RT/2F \ln\Delta Ca$$

Fibers of the crayfish muscles which normally produce graded responses do not generate spikes when the calcium is increased by total replacement of NaCl with $CaCl_2$.

The form of the normally occurring spikes varied considerably. Several

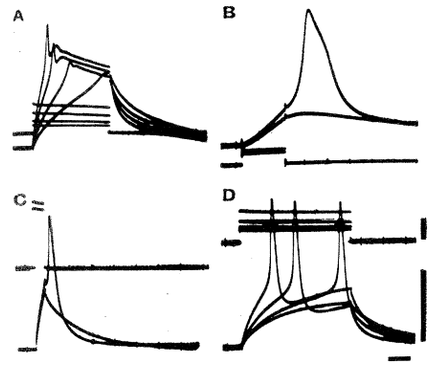

Fig. 1. Superimposed records of responses of crayfish muscle fibers to outward current pulses. (A) Oscillatory graded responses are evoked by strong pulses. (B) A graded response and an all-or-nothing action potential produced by just subthreshold and by threshold stimulation. (C and D) Spike recorded from another muscle fiber stimulated by a short pulse in (C) and by a long pulse in (D). Resting potentials were 78.5 mv in (A), 83 mv in (B), and 78 mv in (C) and (D). Calibrations: 50 mv; 1×10^{-7} amp for (A) and (B), 2×10^{-7} amp for (C) and (D); 20 msec for (A), (C), and (D), and 5 msec for (B).

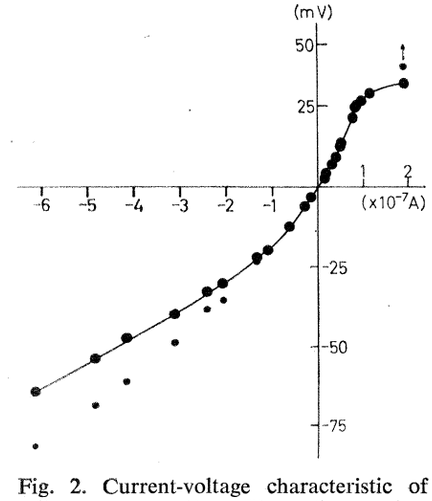

Fig. 2. Current-voltage characteristic of spike-generating fiber. The spikes evoked in the fiber are shown in Fig. 1 (C and D). Small symbols indicate the initial changes in membrane potential at the beginning of the applied current; large symbols, the potential at the end of 1-second pulses. Marked differences are seen in the time-variant hyperpolarizing chloride activation. The outward current of about 2×10^{-7} amp caused a depolarization of 37 mv (small symbol) which induced a spike (arrow). The steady-state potential after the spike is represented by the large symbol.