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

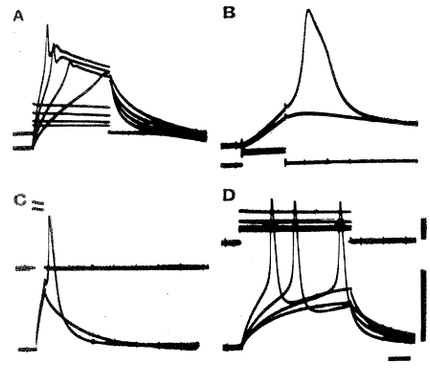

Fig. 1. Superimposed records of responses of crayfish muscle fibers to outward current pulses. (A) Oscillatory graded responses are evoked by strong pulses. (B) A graded response and an all-or-nothing action potential produced by just subthreshold and by threshold stimulation. (C and D) Spike recorded 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).

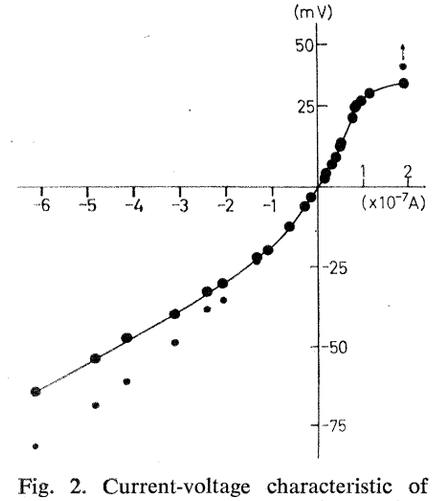

Fig. 2. Current-voltage characteristic of spike-generating fiber. The spikes evoked in the fiber are shown in Fig. 1 (C and D). Small symbols indicate the initial changes in membrane potential at the beginning of the applied current; large symbols, the potential at the end of 1-second pulses. Marked 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.

peaks, or a hump on the falling phase, were often seen (Fig. 1B), as in crab muscle fibers (*7*); but in other fibers the response was a smoothly rising and falling spike (Fig. 1, C and D). The irregularities resemble those seen in barium-induced spikes of lobster muscle fibers and are due to local inequalities in excitability (*2*).

The spike-generating fibers had characteristics of voltage and current (Fig. 2) that were essentially the same as those of fibers with graded responses (*10*). The effective resistance for small inward currents was about 2×10^5 ohms. For small outward currents it was about 3×10^5 ohms, indicating the occurrence of depolarizing potassium inactivation (*11*). Marked time-variant curvatures of the characteristics that were induced by inward as well as outward currents reflect decreases of membrane resistance that are due to depolarizing potassium activation and hyperpolarizing chloride activation, respectively (*11*), which are characteristic of crayfish muscle fibers.

Muscle fibers that generate spikes appear otherwise to have essentially the same characteristics as those that normally produce graded responses. The length of the sarcomeres is in the same range, they have essentially identical characteristics of voltage and current, and the depolarizing electrogenesis appears to be due to calcium activation in both varieties. The spikes induce twitch contractions, but that may be regarded as a consequence of the brief duration of a larger electrical response.

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24 October 1968

Progesterone Antagonism of Estrogen-Induced Cytodifferentiation in Chick Oviduct

Abstract. *Progesterone administered with estrogen to the intact female chick antagonizes the characteristic effects of estrogen to induce cell proliferation and cytodifferentiation of tubular gland cells in the oviduct, events that are accompanied by the appearance of lysozyme. Progesterone appears to exert its effect by preventing proliferation of cells destined to become tubular gland cells, but once such cells do proliferate, subsequent cytodifferentiation and appearance of lysozyme are not prevented.*

Repeated administration of estrogen to immature female chicks markedly increases the weight of the oviduct (*1*) and induces the synthesis of both lysozyme and ovalbumin in the magnum portion of oviduct (*2*). These effects can be influenced by other hormones; this estrogen-stimulated weight increase is augmented by testosterone (*3*) and inhibited somewhat by glucocorticoids (*4*). Although several workers have reported that progesterone antagonizes the increase in oviduct weight induced by estrogen (*5–7*), a synergistic effect has also been described (*3, 8*).

In this investigation we studied the antagonistic effect of progesterone on estrogen-induced growth of the oviduct. This effect can be ascribed largely to an inhibition of proliferation of characteristic tubular gland cells, and, as a result, progesterone prevents the estrogen-induced synthesis of lysozyme. Recently, Kohler *et al.* (*9*) showed that ovalbumin is also localized in tubular gland cells and indicated that cytodifferentiation of these cells was induced by estrogen.

Four-day-old White Leghorn female chicks were given estrogen (17 β estradiol benzoate, 1 mg per chick) with or without progesterone (1 mg per chick). The hormones, in 0.1 ml of sesame oil, were injected daily into left and right thigh muscles. At indicated days after the administration of hormones, chicks were killed and the magnum portion of the oviduct was removed. All studies were limited to this portion. The magnum was weighed and was then homogenized in 9 volumes (weight/volume) of ice-cold distilled water for analysis of lysozyme, nucleic acids, and protein. After nucleic acids were extracted by the method of Schneider (*10*), DNA was assayed by the diphenylamine reaction with calf thymus DNA as standard (*11*), and RNA was assayed by use of the orcinol reagent with yeast RNA as standard (*12*). Protein was estimated by the method of Lowry with bovine serum albumin as standard (*13*), and lysozyme was assayed as described by Litwack (*14*). Activity is expressed as micrograms of lysozyme and is based on the activity of purified egg-white lysozyme (Calbiochem) as standard. For histological examination, parts of the magnum were fixed in 0.1*M* sodium phosphate (*p*H 7.2) containing 4 percent glutaraldehyde (*15*) and then embedded in paraffin. Sections 7 μm thick were stained with Mayer's hematoxylin and eosin.

Figure 1 shows the pattern of increase in wet weight of the magnum resulting from daily administrations of estrogen for 10 days. In the absence of hormone treatment only a twofold increase in wet weight occurred. During the first 2 to 3 days there was a lag in the increase in weight, after which the increase was rapid, amounting to 500-fold by 10 days. Within 3 days of the beginning of daily administration of estrogen there were marked increases in total DNA, as well as in total RNA and in protein

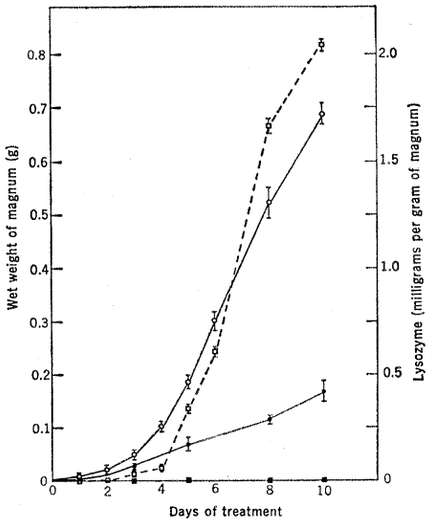

Fig. 1. Changes in the wet weight and lysozyme level in the magnum portion of oviduct during the daily administration of estrogen and its combination with progesterone for 10 days. Each point represents the mean ± standard error of the mean for four to seven chicks. ○, Magnum (wet weight) from estrogen-treated chicks, and ●, from chicks treated with estrogen plus progesterone; □, magnum lysozyme from estrogen-treated chicks, and ■, from chicks treated with estrogen plus progesterone.