between day 10 and 14 after the last dosing. For the '25 × 10 group', 3 out of 5 birds showed ataxia between day 8 and 10. On the other hand, only 1 of the '125 × 2 group' showed signs of ataxia on day 19, and no hen became abnormal in the '250 group'. Finally, 13 hens were attacked by ataxia during the observation period. Sooner or later all of them developed paralysis. 7 hens in a serious condition died by the 11th day after the onset of ataxia, probably as a result of respiratory failure. The results are summarized in table 1.

2nd experiment. Samples of fat were collected from the abdominal cavities of hens (adipose tissue of gastrocolic omentum). The concentrations of phosvel in the fat of hens on the 1st and the 8th day after the last treatment are shown in table 2. On the 1st day, the phosvel concentration was highest in the '125 × 2' and the '83 × 3 group', followed by the '50 × 5 group'. The mean value of the '250 group' was only half that of the '125 × 2' and the '83 × 3 group'. On the other hand, the '50 × 5 group' showed maximum phosvel concentration on day 8, and the level of the '250 group' was slightly less. The trend of the mean values on day 8 was not similar to that on day 1. Phosvel was not detected in the control group.

Concerning the toxicity of phosvel, it is necessary to consider the amount of a single dose, and the frequency of doses, as well as the total dosage, because, by the administration of the critical dose divided and administered at different times the toxicity was intensified in some groups and alleviated in other groups.

Phosvel was most absorbed by the fat with 3 doses of 83 mg/kg just after the final treatment but the residue in the fat was most pronounced with 5 doses of 50 mg/kg after the 'latent period (8 days)'. It may suggest that a large single dose tends not to cause effective absorption and retention.

Although the data of the 2nd experiment did not always explain the variation of neurotoxicity observed in the 1st experiment, it was suggested that the absorption of phosvel by fat and its concentration behaviour might depend on the treatment regimen; toxicity might be related not only to the level of phosvel in the fat but to the course of concentration during the 'latent period'.

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Embryonic development of an insect myocardium[1]

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Summary. Evidence from light and electron microscopic studies indicates that differentiation of myocardial cells in moth embryos begins at 6 days post-oviposition and is completed on eclosion. Fibrillogenesis, and development of cytoplasmic organelles and membranes are described. The heart is fully differentiated and functional at eclosion.

Cardiac cells that comprise the myocardium of the adult moth *Hyalophora cecropia* share many basic physiological properties common to cardiac muscle in phylogenetically higher forms[2], and indeed, fundamental phenomena common to all excitable cells and tissues have been studied on this preparation. Ultrastructural studies have likewise confirmed that the cellular matrix of the moth myocardium has both similarities and dissimilarities to vertebrate hearts. This paper describes investigations undertaken to study the development of this insect myocardium during the embryonic phase and to relate the findings to those reported for higher forms[3,4].

Material and Methods. The cecropia moth progresses through a 4-stage metamorphosis, the 1st stage being completed in the egg. *H. cecropia* eggs hatch into the larval forms in approximately 10 days at 23 °C and for this study embryos were removed at intervals of 3, 4, 6, 7, 8 days post oviposition (dpo) and fixed for examination in the electron microscope. Hearts of newly-emerged larvae were also fixed in situ and excised for the electron microscope.

Results. The adult form of the tubular heart consists of striated muscle arranged in a helical fashion with a total wall thickness of only a single cell. It is derived embryonically from ventrally located mesodermal cardioblast cells that migrate dorsally and assume a crescent shape as they fuse in the dorsal midline of the abdomen[5].

Fusion of the crescent-shaped, undifferentiated, mononuclear cardioblasts occurs at approximately six dpo, at which time they contain sparse amounts of rough endoplasmic reticulum, randomly scattered elongate mitochondria, measuring 6.5 µm × 1.5 µm, with ill-defined cristae, abundant free ribosomes and conspicuous basal lamina (surface coat). Irregularly shaped membranous vesicles, measuring 0.1–1.0 µm in diameter, which originate from the clearly defined Golgi bodies, are dispersed throughout the sarcoplasm. Microtubules lie parallel and just beneath the sarcolemma which faces the hemocoele. Microtubules contact and orient perpendicularly to the sarcolemma where 2 newly-fused myocardial cells contact each other. A significant amount of cellular projections containing numerous microtubules, presumably from undifferentiated pericardial cells, occur around the entire perimeter of the myocardium, and are in contact (fasciae adherentes) with the myocardial basal lamina (surface coat) and the sarcolemma (figure 1). The function of such an intercellular interaction is unknown at the present time; however, they may be responsible for the cardioblast assuming their crescent shape. These projections completely disappear by the time of eclosion. At 7 dpo Z-bands and thick and thin filaments can be identified just below the sarcolemma which faces the hemocoele (figure 1). The organized myofilaments and Z-bands are oriented in the longitudinal axis of the myo-

cardial cell and originate in the same area as the microtubules which appear to be responsible for myofilament organization and orientation. Nuclei and surrounding sarcoplasm initiate invagination into the lumen of the myocardium, while patches of organized myofilaments increase throughout the myocardium at 8 dpo. Complete sarcomeres are not present at this time. The sarcolemma between adjoining myocardial cells invaginate with adherent junctions appearing between the membranes, thus, giving rise to the initial stages of the intercalated disc. By eclosion the nuclei and surrounding sarcoplasm are fully invaginated into the lumen, while mitochondria proliferate in the perinuclear region and on both sides of the myofibrills at the periphery of the cell. The sarcolemma is folded and invaginated, the transverse tubular system is conspicuous, sarcoplasmic reticulum reduced to form dyads and triads and elastic fibres are embedded within the basal lamina. Distinct A, I and Z-bands comprise the now complete sarcomers, 2.2 μm in length (figure 2). Intercalated discs appear well-developed as evidenced by the tortuous nature of the sarcolemma and an increase in adherent junctions (figure 2). Alpha and beta glycogen particles are present, the former occur in localized regions near the sarcolemma, while the latter are interspersed among the myofilaments. Alary muscle fibres form a myo-muscular junction with the myocardium which is now completely formed and functional at eclosion. Nervous tissue was conspicuously absent from the myocardium and alary muscle. Subsequent morphological investigations will deal with neural innervation of the embryonic and larval hearts in conjunction with the heart's sensitivity to acetylcholine and epinephrine.

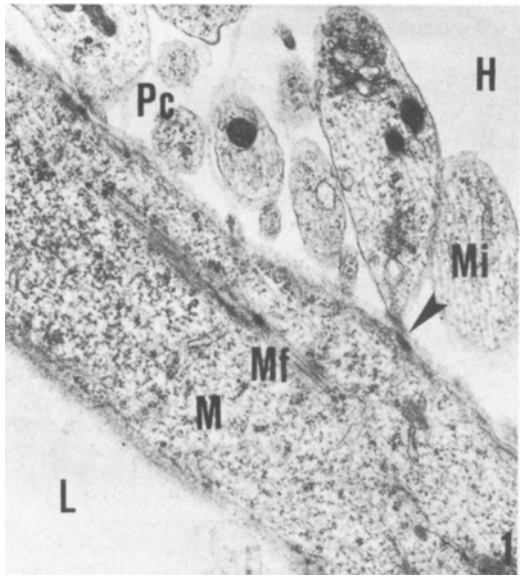

Fig. 1. Electron micrograph of myocardium (M) at 7 dpo with undifferentiated pericardial cells (Pc) contacting the heart (arrow). Z-band and organized thick and thin myofilaments (Mf) are visible in the myocardium. H, hemocoele; Mi, microtubules. × 15,000.

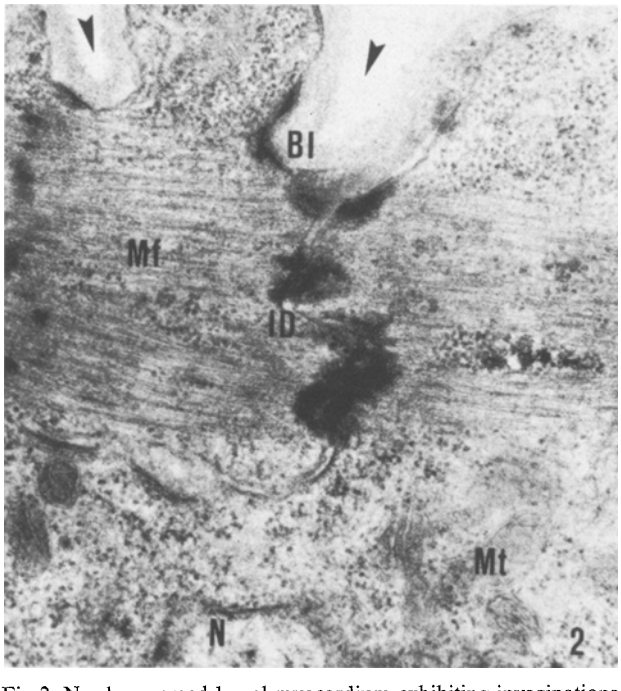

Fig. 2. Newly-emerged larval myocardium exhibiting invaginations of the sarcolemma (arrows), a single myofibril with complete sarcomeres (Mf), and intercalated disc (ID). Bl, basal lamina; Mt, mitochondria; N, nucleus. × 33,600.

Discussion. A significant number of similarities and differences exists between embryonic development in the moth myocardium and mammalian cardiac development. Perhaps the most interesting similarity involves the longitudinal orientation within the myocardial cell of the microtubules and subsequent myofilaments directly beneath the cortical regions of the sarcolemma. Other similarities include the development of myofilaments prior to the formation of the TTS. Intercalated discs develop in a corresponding manner. In contrast to embryonic mammalian cardiac cells, developing insect myocardia lack M and H bands, lipid, glycogen and abundant rough endoplasmic reticulum[6, 7]. The sarcoplasmic reticulum of the insect heart is extremely reduced and limited to dyads and triads in contrast to its abundance in higher hearts[7]. The surface coat (basal lamina) does not invaginate into the TTS in the moth myocardium, as it does in higher myocardia[7]. In the insect myocardium mitochondria are localized in the perinuclear region and adjacent to the myofibrils at the periphery of the cell rather than being randomly scattered throughout the myocardial cell[7]. The cellular development of the insect myocardium appears extremely useful as a paradigm for subsequent research into the basic processes of fibrillogenesis, DNA synthesis, cell fusion, and the development of the TTS and intercalated discs in cardiac embryology.

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