HORMONAL CONTROL OF REVERSIBLE DEGENERATION OF FLIGHT MUSCLE IN THE COLORADO POTATO BEETLE, *LEPTINOTARSA DECEMLINEATA* SAY (COLEOPTERA)

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ABSTRACT

In the hibernating (diapausing) Colorado potato beetle, *Leptinotarsa decemlineata* Say, the flight muscles show pronounced degeneration. The muscle fibrils are greatly reduced in diameter and the sarcosomes are virtually absent. Similar signs of degeneration could be produced by extirpation of the postcerebral complex of endocrine glands, the corpora cardiaca and corpora allata. Reimplantation of active postcerebral complexes resulted in a very rapid regeneration of the muscle fibrils and new formation of sarcosomes.

INTRODUCTION

In the life cycles of many insects a period of greatly diminished activity, called diapause, is observed. Often the diapause is a form of hibernation. It may occur in the egg stage, during larval life, in pupae and, less frequently, in the adult stage. An example of the latter type of diapause is found in the Colorado potato beetle, *Leptinotarsa decemlineata* Say. Extensive investigations have shown that the external factor which is responsible for eliciting this diapause is a photoperiod of 14 hours or less, acting *via* the neuro-endocrine and endocrine systems of the insect (1–4). The corpora allata, paired endocrine glands located in the head, appeared to be of especially great importance. Extirpation of these glands resulted in diapause even under conditions of a long photoperiod. Reimplantation of active corpora allata into allatectomized diapausing animals led to complete reactivation (2, 4).

In the course of biochemical studies on tissue respiration in the Colorado potato beetle, a preliminary account was given of the occurrence of a reversible degeneration of the flight muscles during diapause (5). The present paper gives a full account of electron microscope investigations of this phenomenon as it occurs in "normal" diapause, *i.e.* photoperiodically induced and spontaneously terminated, and in diapause induced by extirpation of corpora allata and c. cardiaca and reversed by reimplantation of active glands. Special emphasis is laid upon the mechanism of hormonal control of the process of muscle degeneration.

MATERIALS AND METHODS

The insect material was obtained from routine laboratory cultures kept at 23°C and either 18 or 10 hours of light per day. The short photoperiod induced diapause in the adult beetles in about 11 days. Diapausing beetles were kept in boxes filled with moist sand.

Extirpations and reimplantations of endocrine glands were performed as described by De Wilde and De Boer (4). In all cases the corpora cardiaca, which form the connection between the brain and the corpora allata, were left attached to the latter. Operated beetles received 18 hours of light per day.

Flight muscle preparations for electron microscopy were prepared as follows (6). The thorax of the insect was opened from the dorsal side and the flight muscles were exposed. These were subject to a preliminary 15 minutes' fixation *in situ* at room temperature with a 2 per cent osmium tetroxide solution in 0.32 M sucrose, pH 7.4. Then, small pieces of the dorso-ventral indirect flight muscles were carefully dissected and fixed for an additional 45 minutes at 4°C in a 1 per cent solution of osmium tetroxide in 0.32 M sucrose, pH 7.4. Subsequently the tissue fragments were washed five times, at 30 minute intervals, with 0.32 M sucrose, pH 7.4, at 4°C. After the final washing, the tissues were dehydrated in graded ethanols and embedded in methacrylate. Longitudinal ultrathin sections were made with a Servall Porter-Blum microtome using glass knives. The sections were examined in a Siemens Elmiskop I.

RESULTS

The first series of experiments was performed on beetles in which diapause was induced by a short photoperiod. Fig. 1 reproduces an electron micrograph of a section of a flight muscle taken from a fully active animal. The picture clearly shows the contracted muscle fibrils and between them the densely packed mitochondria. Moreover a few cross-sectioned tracheoles are to be seen.

Under "short day" conditions the animals become gradually less active and eventually stop feeding completely. By this time the metabolic rate has already decreased considerably. The most prominent feature of Fig. 2, showing a section of flight muscle from such an inactive animal, is the different appearance of the sarcosomes. They look as though they were undergoing a process of lysis. This would fit in with the observed lowered biochemical activity of these mitochondria (5).

Next, the animals enter true diapause; *i.e.*, they burrow into the soil and become completely quiescent. It is then that the most drastic changes in the flight muscles take place. Fig. 3 is an electron micrograph of a flight muscle section obtained from a beetle 8 days after it had entered diapause. The appearance of the muscle fibrils has distinctly changed. Their width has been reduced to one-third or less of the original and they are not contracted. Moreover, normal sarcosomes are virtually absent. Many osmiophilic bodies are found which are considered to be remnants of sarcosomes (*RS*). These bodies bear a strong resemblance to the weakly respiring particles isolated from diapausing beetles by differential centrifugation. These particles have been shown to contain still some of the components of the respiratory chain and were, therefore, considered to be degenerate mitochondria (5).

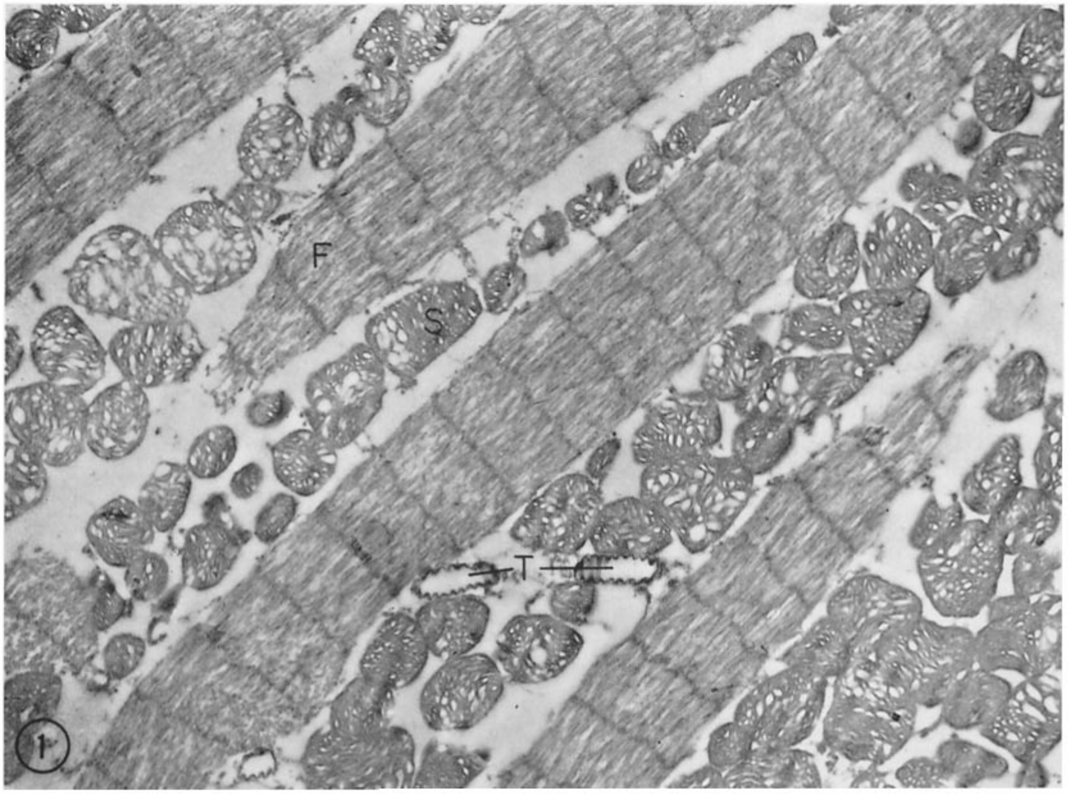
Noteworthy also are the numerous osmiophilic granules consisting presumably of lipid or lipoprotein. These granules bear a certain resemblance to lysosomes but, of course, a definite identification has to await their isolation and biochemical characterization. As a matter of fact, in this stage the muscles contain large amounts of fatty material which, for the greater part, was removed prior to fixation.

The situation depicted in Fig. 3 remains almost stationary for approximately 12 to 16 weeks. Six weeks after the onset of diapause, the fibrils appear still more reduced in diameter and even fewer sarcosomes are found.

After about 3 to 4 months, diapause is terminated and the animals reappear above the soil. When the flight muscles are examined shortly before the end of diapause, it appears that already during diapause a distinct regeneration has taken place. The muscles are much more compact than before, much fatty material has disappeared, and the muscle fibrils, which have grown considerably, appear contracted again (Fig. 4). Numerous sarcosomes are present again, although it should be noted that their size is still smaller than normal and also that their inner structure differs from

FIGURE 1 Longitudinal section of dorso-ventral indirect flight muscle of an active beetle, showing muscle fibrils (*F*), sarcosomes (*S*), and tracheoles (*T*). × 10,000.

FIGURE 2 Section of flight muscle of an inactive beetle, just prior to entering normal diapause. The sarcosomes appear in a stage of "lysis." × 10,000.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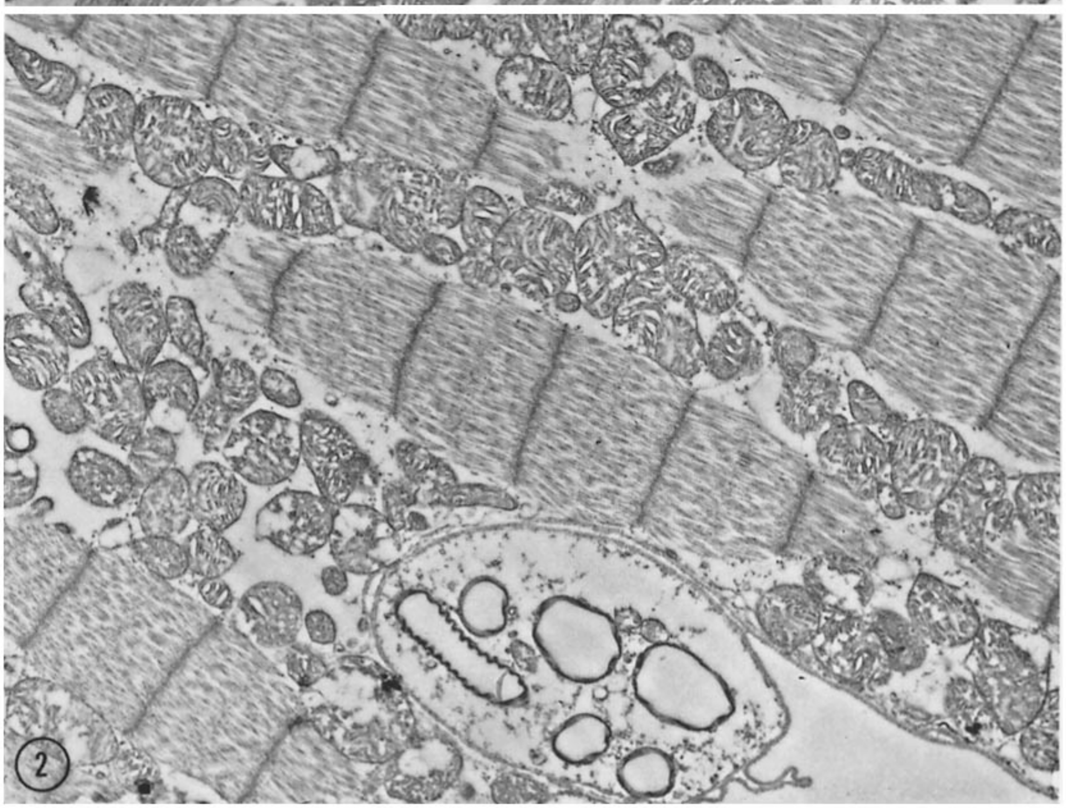
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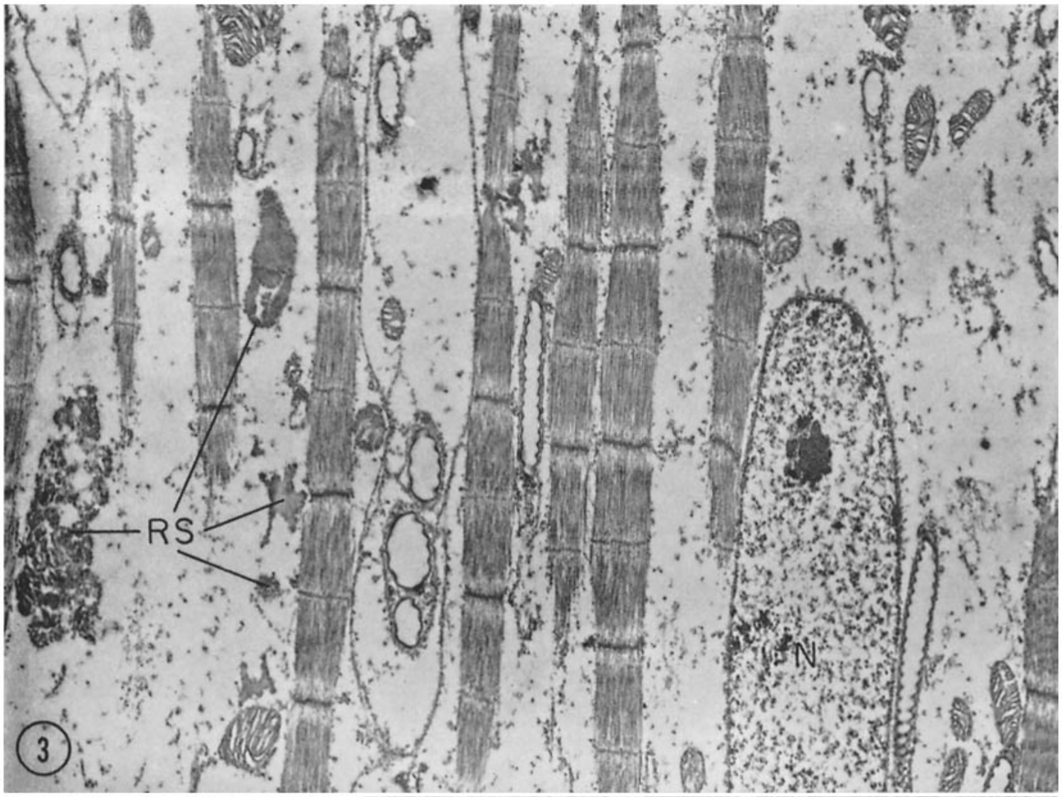
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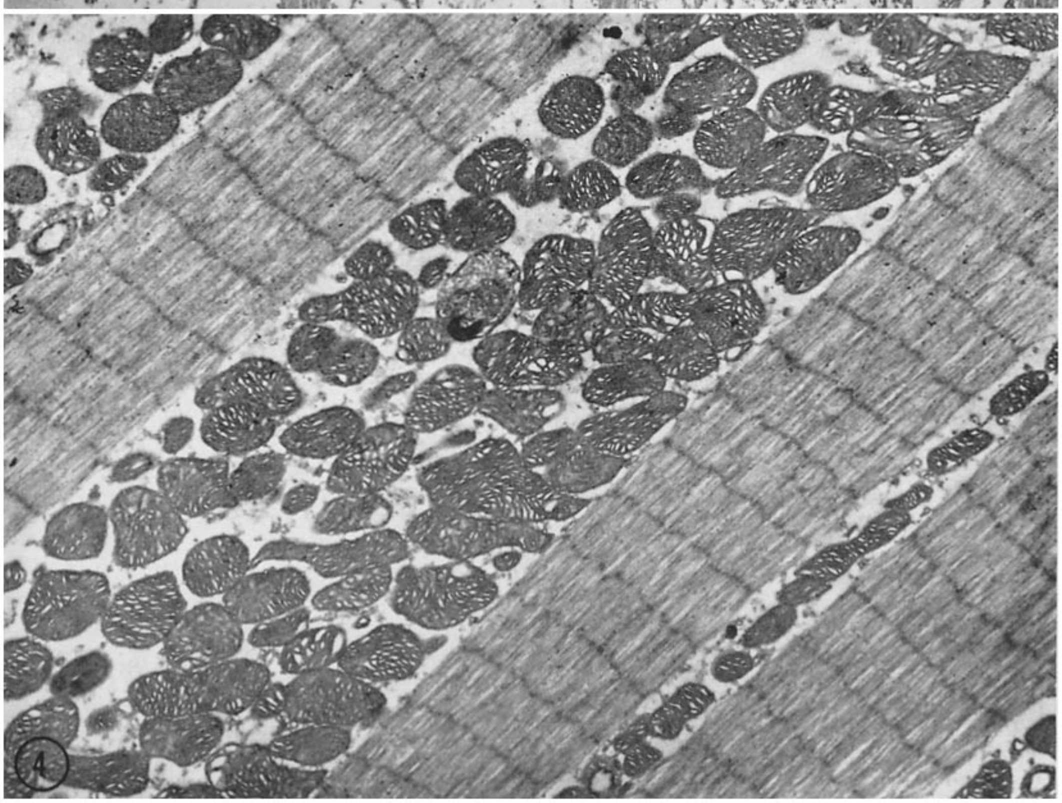
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that of normal sarcosomes. This is probably reflected in the low biochemical activity found in these particles (5).

An almost complete restoration of the original situation takes place as soon as the animals have left the soil. The section made from a muscle taken from an animal within 14 hours after the termination of diapause and presented in Fig. 5 can hardly be distinguished from the one shown in Fig. 1.

As was pointed out by De Wilde and De Boer (4), normal diapause in the Colorado potato beetle may be considered to be a case of "pseudo-allatectomy." That is, the symptoms can be ascribed, to a great extent, to corpus allatum hormone deficiency. It seemed obvious to study, in a second series of experiments, "real allatectomy" diapause, with respect to the muscle degeneration and regeneration found in normal diapause. This approach would also greatly facilitate the timing of the experiments. In the study of regeneration, it is especially difficult to tell beforehand how far normal diapause has proceeded in the individual animal. Thus it becomes almost impossible to put the pictures obtained in the right chronological order. If the corpus allatum plays a role in the process of regeneration, then in experiments with allatectomized beetles the moment of reimplantation of active glands could be taken as zero time.

Allatectomy (*i.e.*, removal of the postcerebral complex) was performed on eighteen female beetles, which were then kept under conditions of a long photoperiod. About 15 days later, the animals went into diapause. At 4 to 7 days after the onset of diapause, four active complexes of corpora allata and c. cardiaca were implanted into each of 8 beetles; the remaining 10 were kept as controls. Electron microscopic examination of the flight muscles was carried out at 0, 1, 3, 5, and 10 days after the implantation. Each time, 2 experimental animals and 2 controls were used.

The results of the experiments leave no doubt that after allatectomy a degeneration of the flight muscles occurs, quite similar to that found in normal diapause. Fig. 6 is an electron micrograph of a muscle taken from one of the control animals on the day of reimplantation of active corpora allata and c. cardiaca. This picture is representative of all those obtained on subsequent days from the control beetles.

At 1 day after the reimplantation, no significant changes could be detected. However, in both experimental animals examined on the third day after implantation, clear-cut signs of regeneration were observed. The electron micrograph in Fig. 7 illustrates this in a fascinating way. The muscle fibrils appear contracted again and are distinctly larger in diameter. The most remarkable feature, however, is the presence of numerous vesicle-like bodies of varying size and shape, many of them showing details of an inner structure which strongly reminds one of mitochondrial structure. It would seem that the mitochondria in this picture were fixed during an early stage in their formation. There is some agreement between these observations and those reported by Linnane (7) on the origin of yeast mitochondria. The latter, too, seem to be formed as small vesicular bodies that later take on the typical mitochondrial inner structure.

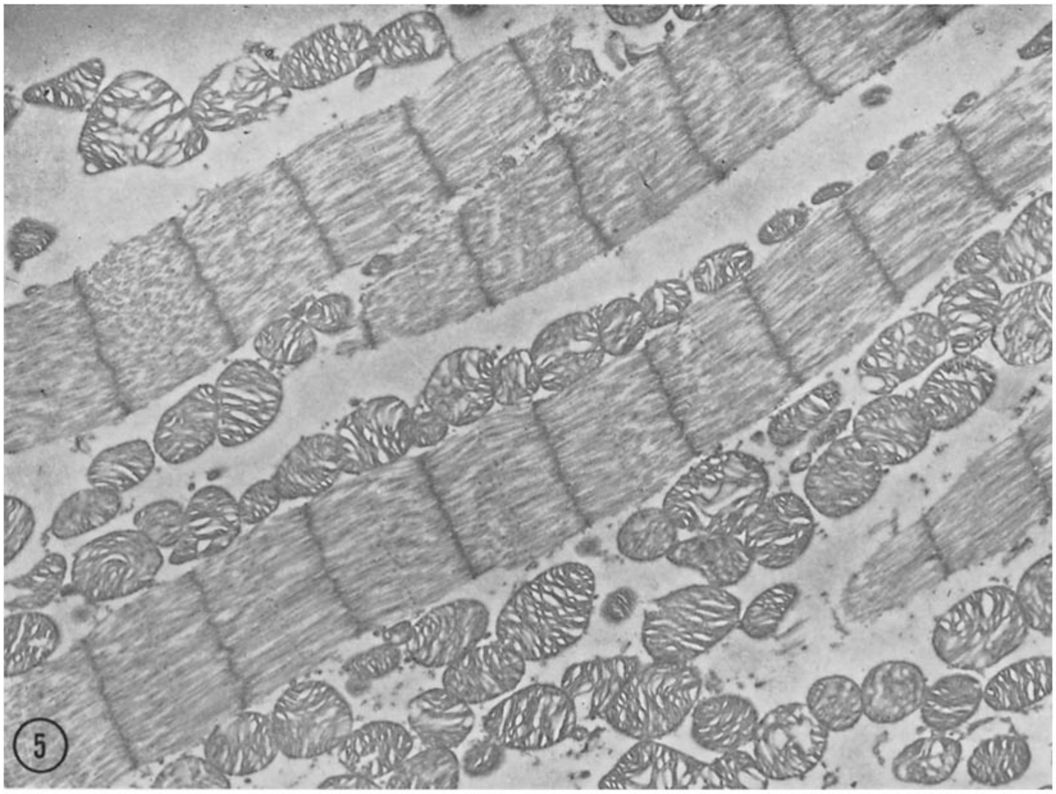
Two days later, *i.e.* 5 days after implantation of active corpora allata, the animals started oviposition, which can be considered a reliable criterion for complete reactivation (4). The picture of the flight muscles obtained at this time shows that they had completely regained their normal structure (Fig. 8). Numerous normal sarcosomes were present between the normal fibrils. Regeneration seemed complete, and this was confirmed by the pictures obtained at 10 days after the implantation, which did not show any significant differences.

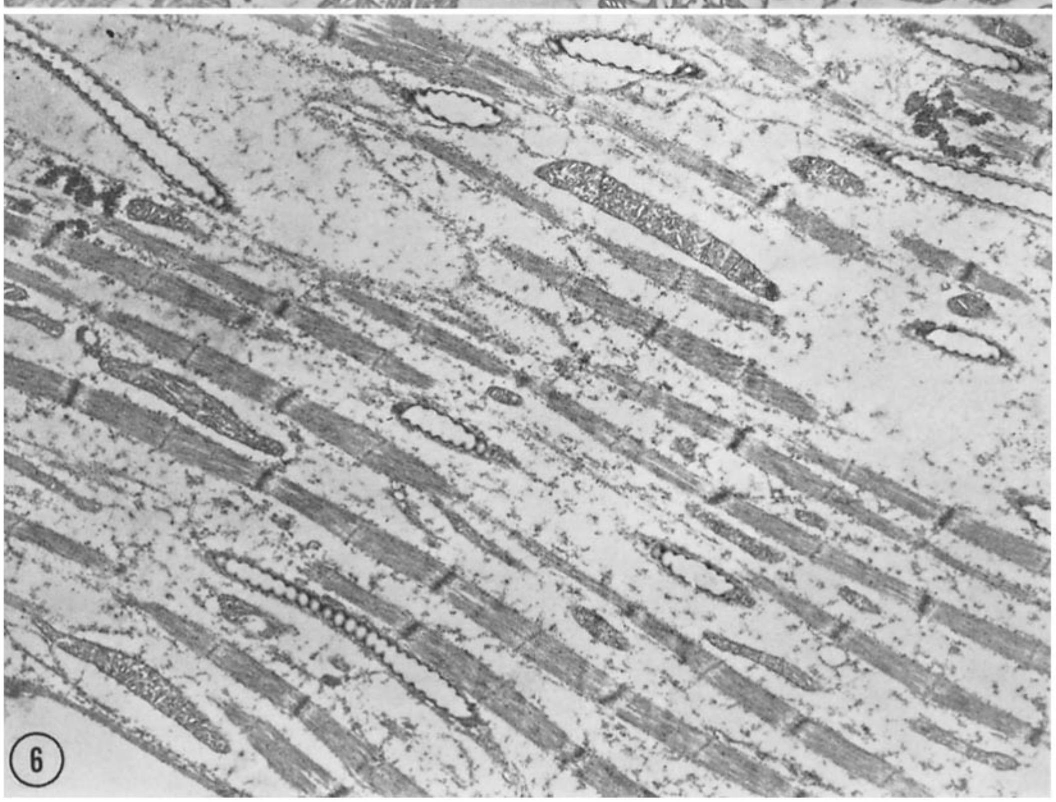
DISCUSSION

Muscle degeneration is not an uncommon phenomenon in insects. Flight muscle degenera-

FIGURE 3 Section of flight muscle of a beetle after 8 days of normal diapause. The muscle fibrils appear reduced in width and relaxed, only few small sarcosomes and dense particles considered to be remnants of sarcosomes (*RS*) are present. The large body at the right is a nucleus (*N*). × 10,000.

FIGURE 4 Section of flight muscle of a beetle at the end of normal diapause. Muscle fibrils and sarcosomes have regenerated. × 10,000.





tion is encountered in termites, water-bugs, aphids, flies, mosquitoes, ants, and in scolytid beetles (8, 9). Because very little is known about the factors controlling degeneration, it is very difficult to relate these cases with the present findings.

Striking examples of muscle degeneration have been found in higher diptera and in lepidoptera (for a review, see reference 10). In insects of these orders, during the transformation from larval and pupal stages into the adult, many muscles undergo complete histolysis. This degeneration seems to be irreversible.

Wigglesworth (11) described repeated cycles of degeneration and regeneration of the intersegmental abdominal muscles of larvae of the blood-sucking bug *Rhodnius prolixus* Stål. These muscles appear to be present only around the time of moulting.

The present paper offers evidence of a completely reversible degeneration in the muscle of an adult insect. For the purpose of this discussion it seems desirable to distinguish between the two processes, degeneration and regeneration. The central problem, of course, is whether these processes are under direct hormonal control.

As regards degeneration, the fact that in the thorax only the flight muscles degenerate presents a serious difficulty in correlating the process with hormonal activity or inactivity. It can easily be shown that the leg muscles are functionally completely unaffected, because diapausing animals, when disturbed, are fully capable of normal locomotion. The question arises as to whether the nervous system, which is known to have a trophic effect upon muscles in insects (12–15), has anything to do with the involution of the muscles. Finlayson (10) reaches no definite conclusion regarding this point, whereas Wigglesworth (11) gives evidence against a possible role of the nervous system. In the Colorado potato beetle, it is clear that extirpation of the corpora allata and corpora cardiaca results in degeneration of the flight muscles. It is tempting to think of a direct effect of hormone deficiency. However, in view of the drastic change of behavior, which is also caused by the removal of the endocrine glands mentioned, an effect of the hormone deficiency *via* the nervous system cannot *a priori* be excluded. If the nervous system were involved, the problem would become one of intrinsic differences in reaction between parts of the nervous system, as was already pointed out by Finlayson (10).

For the regeneration process, a similar line of reasoning can be followed. In this process, too, it is evident that hormonal control by the corpora allata and corpora cardiaca is involved, but a direct action of hormones has not been strictly proven. Such a direct action could well be visualized for at least part of the process, in view of the known stimulating effect of the corpora allata upon metabolism both *in vivo* and *in vitro* (2, 16–19). Although no experimental data are available, it seems obvious that during regeneration large amounts of protein are synthesized. Protein synthesis requires energy that could be made available by stimulation of metabolic processes yielding energy (18). In the Colorado potato beetle, these could very well be anaerobic reactions, as suggested by l'Hélias (18) for *Carausius morosus*, because oxidative metabolism remains very low in the last stages of diapause. It must be assumed that even the newly formed sarcosomes are biochemically almost inactive (5). It is hoped that further studies will provide a deeper insight into the relations between sarcosomal morphology and function and will give more information about the factors controlling them.

For the present, it can only be concluded that the existence and function of essential tissues and metabolic entities, such as the flight muscles of an insect and their sarcosomes, can be controlled by hormones, possibly in cooperation with the nervous system.

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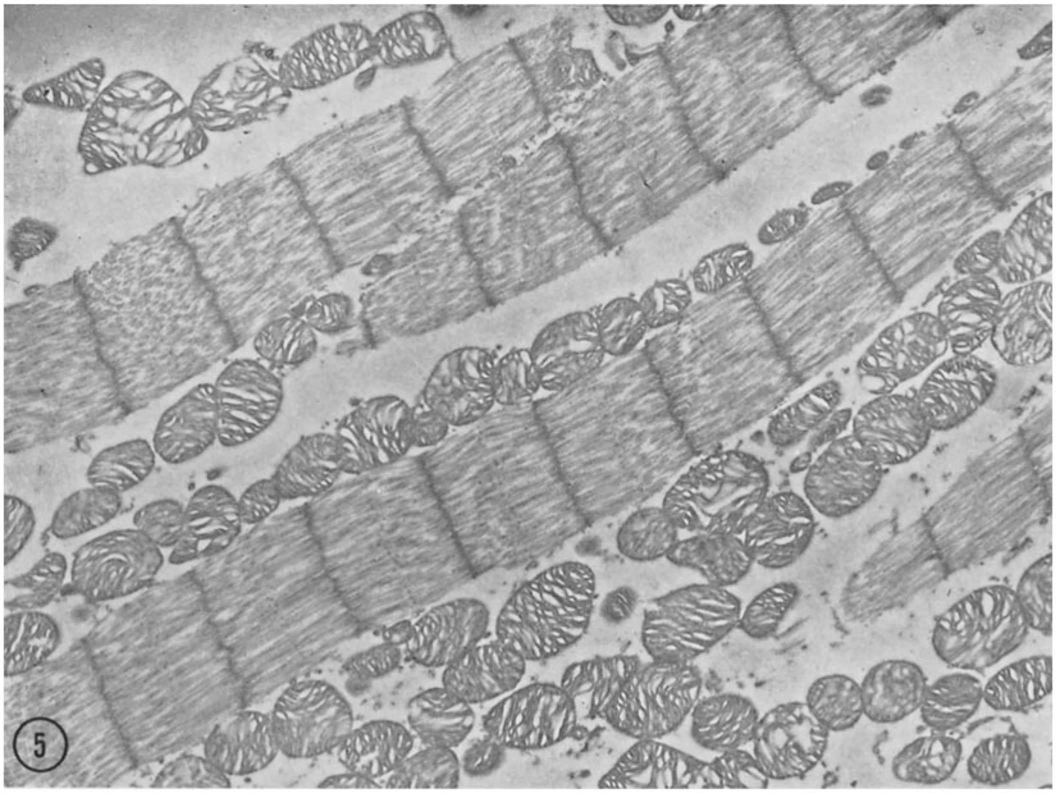
FIGURE 5 Section of flight muscle of a beetle 24 hours after the end of normal diapause. The situation of the prediapause stage has been restored. × 10,000.

FIGURE 6 Section of flight muscle of a beetle at 7 days after the onset of diapause induced by extirpation of the postcerebral complex of endocrine glands. Compare the signs of degeneration with those in Fig. 3. × 10,000.



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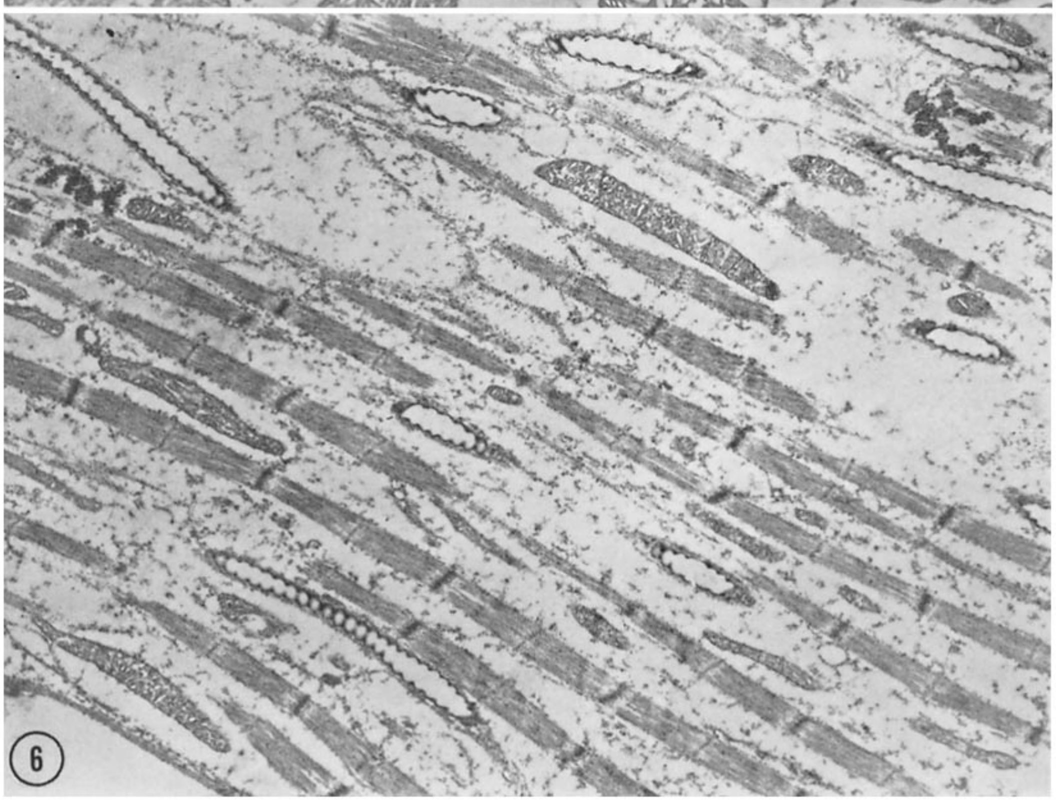

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Figure 7 Section of flight muscle of a beetle (in which diapause was induced by extirpation of the postcerebral endocrine complex) 3 days after reimplantation of four active postcerebral complexes. Note the occurrence of numerous vesicle-like bodies and the signs of regeneration of the muscle fibrils. × 10,000.

Figure 8 Section of flight muscle of a beetle, comparable to the section in Fig. 7, 5 days after reimplantation of active endocrine complexes. Section stained with a 2 per cent $KMnO_4$ solution. Complete regeneration of fibrils and sarcosomes. × 10,000.