Initiation of post-diapause development and reinstatement of diapause in Cephus Cinctus Nort.
by Norman Stanley Church

A THESIS Submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of Master of Science in Entomology
Montana State University

Abstract:
Sawfly larvae normally absorb contact water during the latter part of diapause and early prepupal development. Moisture thus absorbed is unnecessary for diapause development but is sometimes required for the initiation of post-diapause morphogenesis. Desiccation at 25° C. or higher had little effect on diapause reinstatement in undeveloped postdiapause larvae in their stubs. Moreover, previous dehydration at 0° C. did not enhance the diapause-reinstating activity of heat. Prolonged desiccation at 0° C. often prevented the initiation of post-diapause morphogenesis in larvae subsequently incubated moist; in other larvae it only delayed morphogenesis.

Ligation showed post-diapause morphogenesis to depend upon the secretion of a differentiation hormone from the prothorax. Its secretion, in turn, is prompted by a stimulatory substance from the head. Blood transfusion showed diapause to be primarily the result of a lack of differentiation hormone. It is proposed that the original stimulus releasing the endocrine mechanism is exerted by the nervous system, and that diapause development is a conditioning of the insect preparatory to the nervous system's becoming suitably activated.

In larvae just out of diapause, heat at first stimulates the endocrine mechanism, and thereby the initiation of morphogenesis. Longer heat exposure reinstates diapause, presumably by destroying the activated endocrine mechanism. Diapause can not be reinstated after the differentiation hormone from the prothorax has been released. That the developmental block induced by heat is a true diapause is confirmed by the fact that it can be eliminated by chilling. Chilling, however, was unsuccessful in eliminating the developmental block introduced by dryness. Because of this and other differences between the effects- of dryness and those of heat on the initiation of post-diapause morphogenesis, presumably the results of their influence on the endocrine mechanism, it is suggested that the developmental block caused by dryness is not a true diapause.

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TABLE OF CONTENTS

PAGE

ABSTRACT

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INTRODUCTION

Farstad in 1938 observed the failure of post-diapause larvae of wheat stem sawfly (Cephus cinctus Nort.) to develop during a hot dry spring. Salt (1947) confirmed that diapause, though once broken, could be reintroduced by the action of heat. Exposure to 35° C. for 25 days during the larva's transition from diapause development to post-diapause morphogenesis caused all larvae to revert to diapause. On the Great Plains heat and drought are usually closely associated, so the possibility presented itself that dryness, too, might influence the reinstatement of diapause.

Only one other indication of a possible similar reinstatement of diapause was found in the literature. Prebble (1941b) observed that a dry spring prevented the development of Diprion, which undergoes a prepupal diapause. In a bivoltine race development commenced if water was supplied later during the summer, but members of a univoltine race did not so respond. Possibly the latter had been returned to diapause.

Such a unique event in the life history of an insect as a reinstatement of diapause warranted further investigation. The possible influence of dryness in returning

C. cinctus to diapause was of particular importance and was chosen as the subject of this work.

REVIEW OF LITERATURE

A discussion of literature pertaining to diapause in general is presented in the first part of this section. It is followed by more detailed accounts of literature dealing with relationships between moisture and diapause, the "hormone failure" theory of diapause, and diapause in C. cinctus, each of which directly concerns the original work to be presented in this thesis.

Much has been learned about insect ecology and physiology since the phenomenon of diapause was recorded by Duclaux in 1869. Many diverse records on diapause have been reported, especially in relation to ecology. However, the real nature of diapause remains unknown.

Wheeler (1893) invented the term "diapause" to describe a stationary period during blastokinesis in Xiphidium. Henneguy (1904) extended the term to include an arrest of development at any stage of an insect's life. The word has frequently been used very loosely since then, even to include development temporarily retarded directly by cold, starvation, or drought. It is now most commonly and most satisfactorily restricted, as it was by Shelford (1929), to more or less spontaneously arrested development where further activity can not immediately be induced by providing the

kind of environment normally suitable. Duclaux's (1869, 1876) work on Bombyx eggs first pointed out this distinction. He found that their arrested development was brought to a conclusion by refrigeration but not by continuous rearing at room temperature.

This distinction between diapause and otherwise arrested development is an important one, but the division is arbitrary. Insects in which development is arrested directly by a sub-optimum external condition must adjust themselves physiologically to this state of inactivity. Though their development is resumed when the unfavorable condition is removed, this response sometimes becomes apparent only after considerable delay, during which they readjust themselves to activity. This behavior is intermediate between temporarily arrested development and a typical diapause.

General Characteristics of Diapause

Numerous workers have reported that diapause insects have a very low rate of respiration combined with a lowered respiratory quotient. The work of Bodine, Williams, and their associates proved that diapause respiration involved more than a mere quantitative change. Respiration of diapause Melanoplus eggs was unaffected by carbon monoxide, cyanide, and sodium azide, all of which affect the cytochrome-

cytochrome oxidase system. All but about one-fifth of the respiration of non-diapause eggs was affected by these agents (Bodine and Boell, 1934; Bodine, 1950). Williams (1947b) and Sanborn and Williams (1950), working with Platysamia, found that cytochrome respiration was broken down during diapause, except in the abdominal muscles, and life was maintained by a system of respiration involving flavoproteins.

The diapause state is also generally characterized by a lowered moisture content, increased food reserves in the form of an enlarged fat body, physical inactivity, and a pronounced decrease in mitotic activity as evidenced in grasshopper embryos (Carothers, 1924; Slifer, 1931). These characteristics have been noticed by many authors, most of whose papers on these and other aspects of diapause have been reviewed competently by Prebble (1941a), Simmonds (1948), and Lees (1950, 1952) and especially thoroughly by Andrewartha (1952).

Manifestations of diapause are not necessarily exhibited in all body functions. A few or many tissues or organs may be relatively unaffected by diapause. Muscular activity in diapause C. cinctus is relatively uninhibited (Salt, 1947). This is true also of the abdominal muscles of Platysamia (Williams, 1951). Imaginal diapause in many species affects only gonad development directly. On the

other hand, extensive cellular activity of the gonads continues in C. cinctus larvae kept at 16° C. (Mackay and Church, unpublished). Birch (1942) and Andrewartha (1943) observed visible growth and differentiation during diapause in Austroicetes embryos. It is wrong, then, to consider diapause simply as a state approaching "suspended animation". Although it is a useful term, "arrested development" can be and has been at times misleading.

Causes of Diapause

Sajo (1896) and Baumberger (1917) suggested that diapause was the result of "developmental fatigue" in which there was an accumulation of metabolic waste materials that inhibited development. Roubaud (1922, 1928, 1929) enlarged upon the hypothesis, concluding that excretion of these wastes had failed to keep pace with other physiological processes; a period of rest at a low temperature or low humidity was required in order that it catch up. When postulated, Roubaud's theory was readily compatible with the known developmental histories of the species of Diptera that he experimented upon, but more recent work has discredited the theory as an overall explanation of diapause.

Bodine (1932) suggested as the cause of the embryonic diapause in Melanoplus a similarly inhibitory "x-factor"

that could be eliminated best at low temperatures. Its existence has never received experimental support.

Although Roubaud's and Bodine's hypotheses do not completely explain diapause, inhibitory substances possibly do contribute to it. They may be the agents whereby external conditions can influence diapause. Generally unfavorable environmental conditions that commonly cause the rate of development to be reduced usually strengthen the tendency towards diapause in species having a facultative diapause, i.e., in species in which every individual of each generation is not obliged to enter diapause. The extensive literature on this subject was critically reviewed by Andrewartha (1952). Deficient or excessive moisture, food quality and its dryness, low or unusually high temperatures, and over-crowding and isolation have all been implicated. Unfortunately, in some of the literature in which the influence of these factors is described, no distinction is made between diapause and development arrested only temporarily.

There are other diapause-inducing factors that ordinarily are not associated with an unfavorable environment. Among them are declining, but not necessarily low, temperatures (Dawson, 1931). In many species of Lepidoptera, photoperiods in which "day" and "night" are roughly equal tend to increase diapause within a population. Wide

departures in either direction, but especially in the direction of a longer light period, tend to prevent diapause (Dickson and Sanders, 1945; Dickson, 1949; Way and Hopkins, 1950; Lees, 1952). In one species, Antheraea, the situation is reversed (Lees, 1952).

The effect of declining temperature and photoperiod show that diapause is not solely an undesirable delay in the life cycle arising from an unhealthy condition, as it is commonly considered to be (e.g., Simmonds, 1948). Diapause is an adaptation naturally selected for many species because of its value in winter survival. The ability of an insect to overwinter is often dependent on its being in a state resistant to cold and desiccation that is most easily attained during dormancy. Some of the previously mentioned environmental factors that tend to induce diapause, e.g., low or declining temperature, dryness or richness of food, and short photoperiod, are associated with the end of the growing season. They serve as signals to synchronize the onset of diapause with the seasons.

The distinction between obligatory and facultative diapause is arbitrary. According to definition, an insect that undergoes an obligatory diapause must enter it regardless of environmental influence, whereas entry into a facultative diapause depends laggely on external conditions.

In the latter case the insect may possess either a strong or weak tendency towards diapause. In some species where diapause at first appeared to be obligatory, upon investigation it has been found to be conditioned by environment (Cousin, 1932). Some insects may respond only to extremes not possible in nature (e.g., Danilyevsky, 1948).

The causative influence exerted by environment on the occurrence of ddiapause is through long and devious physiological processes. This is well demonstrated by the time lapse between cause and observed effect (Andrewartha, 1952). It is sometimes the next generation that is affected. The work of Kogure (1933) on Bombyx is particularly interesting. He found that light and temperature acting on incubating eggs partly determine whether or not eggs of the next generation will enter diapause.

Diapause Elimination

As previously implied, if the onset of diapause has been furthered by a certain environmental factor, the removal of or compensation for that factor does not break diapause. Low temperature is a common cause of diapause. However, almost invariably the best temperature for diapause elimination is 10° to 25° C. lower than the morphogenetic optimum.

Diapause-breaking is a progressive process. The rate-temperature curves for diapause-breaking and for normal non-diapause development are similar, except that the entire curve for diapause-breaking is displaced towards the lower end of the temperature scale. In both normal development and diapause-breaking, the rate of the process gradually diminishes as the temperature falls below optimum, and the rate falls off more abruptly if the temperature is raised. The similarity suggests that diapause-breaking is also a form of development (cf. Andrewartha, 1952). This "diapause development" must be completed before the more rapid post-diapause development and morphogenesis can begin.

Sometimes the rate-temperature curves for the two processes overlap extensively; sometimes not. In C. cinctus the optimum for morphogenesis is about 25° C. and for diapause development, 10° C. (Salt, 1947). An extreme overlap is evident where Matthée (1951) found that diapause in Locustana was eliminated at 35° C. Another is evident in Dickson's (1949) work on Grapholitha which showed diapause development to be more rapid at 26° C. than at lower temperatures.

There are numerous papers reporting the efficacy of various "shock" treatments such as pricking, singeing, oviposition by a parasite, and the action of acids and other chemicals in breaking diapause. But, as Andrewartha (1952)

has suggested, apparently these have been found effective only in insects with a "weak" diapause or in those nearing the end of a "stronger" one. In similar circumstances, sudden temperature changes have been found to have the same effect in Diprion by Gobeil (1941) and Melanoplus by Bodine and Robbie (1940). Browning (1952) demonstrated that Gryllulus eggs required shorter low-temperature exposures to break diapause if they were incubated at much higher temperatures afterwards. Some species require a final stimulus at the end of diapause development. In Locusta, Le Berre (1951) notices that even in eggs in which diapause had been broken at a constant temperature well above the threshold of development (17°, 21°, and 25° C.) a slight increase in temperature was necessary to stimulate post-diapause development.

Dryness and Diapause

Dryness of either the habitat or food has often been reported as inducing diapause. This has been best substantiated in Pectinophora (Squire, 1937, 1940; Fife, 1949) and Loxostege (Strelnikov, 1936). Less moisture in diapause than non-diapause forms has been recorded in Leptinotarsa (Fink, 1925), Lucilia (Mellanby, 1938), and Carpocapsa (Ushatinskaya, 1949).

It has been demonstrated in Leptinotarsa (Fink, 1925), Pyrausta (Babcock, 1927), Epiblema (Rice, 1937), Lucilia (Mellanby, 1938), Diprion (Prebble, 1941b), Melanoplus (Slifer, 1946), Carpocapsa (Ushatinskaya, 1949), and Locustana (Matthée, 1951) that, the water lacking during diapause must be replaced before post-diapause development can proceed. Squire (1937) found that the addition of contact water to the habitat of Pectinophora expedited its emergence from dormancy or facilitated its subsequent development, but that it was not essential. From such information has arisen the idea, often casually repeated, that an external moisture supply is necessary for diapause development. But as Andrewartha (1952) emphasized, data presented by most of the authors just mentioned have indicated that the provision of water is only essential at the end of or after hibernation. None showed water to be necessary during diapause development. The water deficit must be remedied before post-diapause development can take place, but an external water supply has not been proven to have much influence on diapause development itself. Readio (1931) found that Reduvius completed diapause even more successfully at low humidities. Similarly, Matthée (1951) specified that in Locustana diapause development is best promoted by a dry environment, though moisture is required at its completion.

An apparent exception to the generalization that contact moisture is unnecessary for diapause development is found in the work of Slifer (1946), who showed that diapause in Melanoplus differentialis eggs could be broken by treating them with zylol and other fat solvents. These presumably dissolved a waxy coat and permitted the entry of water into the egg, thereby breaking diapause. However, Andrewartha (1952) suggested that xylol may have eliminated diapause by way of other, direct effects on the egg contents. Andrewartha's suggestion is supported by the fact that xylol can break diapause in Myrmus eggs, which contain enough moisture when laid to carry them through to hatching (Woodward, 1952). Pepper (1937), too, found xylol and other chemicals effective in breaking diapause in Loxostege prepupae where impermeability to water is not likely a factor.

The Hormone Failure Theory of Diapause

Wiggleworth (1934), nearly twenty years ago, proposed that diapause may be primarily the result of a hormone failure. This viewpoint is gaining increasing support. It has been proved that molting, including that which produces a pupa or adult, depends for its initiation on a hormone secreted by a pair (usually) of diffuse organs, the thoracic glands. These have variously been called the prothoracic

glands, ventral glands, corpora incerta, suboesophageal glands, and hypostigmatic glands (Sellier, 1951). They are generally situated in the prothorax, but frequently extend into the head and/or mesothorax. These glands or their homologues have been seen in Lepidoptera, Hymenoptera, Orthoptera, Hemiptera, and Odonata. They exist in Diptera as giant lateral cells of the ring gland. Work on this subject has been reviewed by Williams (1948, 1949). Pflugfelder (1947) reported the presence of ventral or thoracic glands in a number of other, heterometabolous, orders. By ligation, gland extirpation and implantation, and blood transfusion experiments the control over molting exerted by the thoracic glands has been demonstrated in Bombyx (Fukuda, 1944) and Platysamia (Williams, 1947) among the Lepidoptera, in Diptera, Odonata, and Phasmida, and finally in the Hemipteran Rhodnius (Wigglesworth, 1952). Most of this work was reviewed by Wigglesworth (1951).

Kopec (1922) discovered in Lymantria that the brain controlled the initiation of molting, apparently by way of glandular action. Dependence of molting upon the brain has been revealed in other Lepidoptera, Hemiptera, Orthoptera, Phasmida, Coleoptera, and Diptera (reviewed by Wigglesworth, 1948, 1951), and in Hymenoptera (Schmieder, 1942). In some species it has been determined that the brain is the source

of the stimulation that activates the thoracic glands. A small number of neurosecretory cells in the protocerebrum secrete a hormone which, when it reaches the thoracic glands, causes them to begin secreting. This series of reactions has been traced in Rhodnius and Platysamia by the classic experiments of Wigglesworth (1934, 1940, 1951, 1952a) and of Williams (1946, 1947a, 1952), and in Calliphera by Possompès (1950) and in Bombyx by Bounhiol (1952a, 1952b) and Fukuda (1944). The sole function of the neurosecretory cells at this stage appears to be the stimulation of the thoracic glands. Once this has been done, the secretory activity of the thoracic glands and the molting process are no longer dependent upon the brain.

Protocerebral neurosecretory cells, since first found by Weyer (1935) in Apis, have been disclosed histologically in other Hymenoptera, Lepidoptera, Coleoptera, Diptera, Trichoptera, Orthoptera, Hemiptera, and Neuroptera (reviewed by Day, 1940, and Scharrer and Scharrer, 1945). Their homologues have been found in the Apterygota (Hanstrom, 1953). According to E. Scharrer (1952) neurosecretory cells are undoubtedly links of communication between the two physiological control systems, the nervous and the endocrine.

Platysamia experiences a pupal diapause before the endocrine mechanism controlling its imaginal molt and meta-

morphosis is set in motion. Williams (1946) demonstrated that during this diapause the implantation of brains from chilled, post-diapause pupae was enough to activate the thoracic glands and elicit normal metamorphosis. Active brain implants also proved to be effective in breaking diapause in Gryllus (Sellier, 1949).

Diapause seems to result from failure of the molting hormone mechanism to operate. In Andrewartha's (1952) opinion, since diapause (at least larval, nymphal and pupal diapause) occurs near the end of a stadium, the hormone failure theory of diapause is probably generally applicable. He believes the brain does not release the hormone mechanism because it is inhibited by the accumulation of reserve food in a form thatiis not immediately available to the tissues, and that diapause development is a process of food mobilization or processes prerequisite to it. The abundance of reports of marked differences in fat body and other tissues between diapause and non-diapause insects gives circumstantial support to this theory.

Diapause in Cephus cinctus

Ainslie (1929) noticed the inability of wheat stem sawfly larvae enclosed in their wheat stubs to develop indoors. It is now known that soon after the mature S-shaped

larva cuts off the wheat stem above the stub in which it overwinters, it enters an obligatory diapause. By spring diapause development is completed and post-diapause development can begin. Salt (1947) found that diapause development proceeds most rapidly at 10° C., and much more slowly at 15° and 5° C. He also found that, in the field in 1946, diapause was broken in every larva by January. It is invariably broken by spring, although, as already related, diapause can then be reintroduced.

Ainslie (1929) also observed that larvae in the field sometimes failed to develop in the spring though they were obviously viable. He attributed this to insufficient moisture. There seems to be little doubt that the larvae had been returned to diapause early in the spring. In extensive areas of Alberta and Saskatchewan a severe early drought in 1937 killed the sawfly's host plants and nearly annihilated the brood of offspring produced that year. In spite of this, there was a sawfly outbreak in 1938. Large numbers of larvae of the previous brood must have returned to diapause in the spring of 1937, and emerged in the spring of 1938 to cause the unexpected outbreak (Farstad, unpublished). In 1944 a similar carry-over occurred. In one infested field in eastern Alberta, 86% of the stubs still contained undeveloped S-larvae after the adult flight was over. Many

of the larvae survived, and in the spring of 1945 they emerged as normal adults about the same time as others of the next generation. Another observation of this kind was reported by Mills, Callenbach, and Reinhardt (1945) who wrote that undeveloped S-larvae were found in 50% of a stand of infested stubs on July 18, 1944. This "spring diapause" occurs frequently in small portions of a population whose microhabitats are especially hot and dry (Farstad, unpublished).

EXPERIMENTAL OBJECTIVES

This study was undertaken to establish and explain the influence of dryness on diapause reinstatement in C. cinctus. This objective may be subdivided into the following: 1) to determine the role of contact water at the end of the proceding "fall" diapause; 2) to measure the over-all effect of moisture lack in reinstating diapause and preventing post-diapause development; 3) to investigate the application of the hormone failure theory of diapause to C. cinctus; and 4) to find the relationship to diapause reinstatement of the hormone mechanism controlling post-diapause development.

In order to obtain a general idea of the importance of water to diapause development the role of contact water during a normal "fall" diapause was first investigated. The more important water is to diapause development, the more effect it should have on diapause reinstatement.

A series of experiments were then done on the influence of dryness and desiccation on diapause reinstatement. In some species of insects, dryness has been reported to induce the original entry into diapause. Possibly it could also re-induce diapause in C. cinctus. In this species high temperature is known to cause diapause reinstatement. Experiments were performed at a variety of temperatures

because the influence of dryness could not be studied without taking heat into consideration. In the course of the experiments it became apparent that the relations between dryness, temperature, and diapause reinstatement were more complex than at first anticipated. Dryness did not simply add to or subtract from the effect produced by heat.

The next steps were to find out whether post-diapause morphogenesis was initiated by an endocrine mechanism and whether a hormone failure was the immediate cause of diapause in C. cinctus. It was anticipated that the relationship of the hormone mechanism to diapause would suggest some explanation for the effects of heat and dryness on diapause reinstatement.

EXPERIMENTAL PROCEDURES

Because of the variety of experiments to be discussed, only the experimental procedures generally applicable to a number of them are outlined in this section. Procedures specifically relating to individual experiments are described in the sections on experimental results.

Source of Material

The mature sawfly larvae used in this work were collected from wheat fields near Lethbridge, Alberta. Roots and debris were removed from the infested wheat stubs, which were then packed loosely in moist garden soil in closed pint jars for storage.

Conditioning and Storage of Larvae

Nearly all the experiments reported here required larvae that were ready to begin post-diapause development but had not yet begun it. In a few experiments spring-collected post-diapause material was used, but generally it was not reliably uniform and undeveloped. Material for most experiments was gathered in the fall while still in diapause. Diapause was broken in the laboratory under strictly controlled conditions in order to obtain the desired material. Since

diapause development progresses most rapidly at 10° C., this temperature was used for the first part of their conditioning. Post-diapause development also will take place at 10° C. Therefore the stubs were removed from 10° to 0° C. as soon as the first few larvae were out of diapause. Several months storage at 0° C. broke diapause in the remainder, and permitted them to finish most of the development transitory between diapause and post-diapause morphogenesis. None of them could begin post-diapause morphogenesis at 0° C. In this way a stock was prepared of fairly uniform post-diapause larvae, ready to recommence morphogenesis as soon as they were incubated. This method of conditioning was essential to experiments concerning initiation of post-diapause development and diapause reinstatement. The ligation experiments reported in a later section would have been impossible without it.

A supply of diapause larvae for use in a few experiments was maintained by storing fall-collected stubs in slightly moistened soil at 25° C., a temperature too high to permit any diapause-breaking. This temperature and the 10° and 0° C. temperatures mentioned above were maintained in constant temperature rooms with an accuracy of ±0.5° C.

Techniques Used in Moisture Relationship Experiments

One experiment dealt with moisture absorption during and after diapause development. Others concerned the effects of dryness and heat on diapause reinstatement. In all of these experiments, larvae were treated and incubated while intact in their stubs because the initiation of development after diapause is very easily inhibited if they are removed. After a post-diapause S-larva has begun to develop and has transformed into a prepupa, or even a day or so before this has happened, it will continue to develop under most conditions until it either emerges as an adult or dies. At moderate temperatures, and if excessive drying is prevented, it develops normally and at about the same rate as usual. Removal from the stub at this time has little effect. Just before this, during the stage from the end of diapause until shortly before the larva is ready to become prepupal, removal from its stub usually inhibits further development, even when temperature, moisture, and light conditions are apparently favorable. The insect remains active and healthy for months, but will not develop. This inhibition affects a large and variable portion of any sample of naked larvae in this stage. Moreover, the only criterion available for determining the presence or absence of diapause in C. cinctus is whether or not it will develop

when incubated. Any attempt to measure the influence of dryness on diapause reinstatement using naked larvae is futile; they must be kept in their stubs.

In experiments where the humidity was controlled, it was done over sulphuric acid solutions or dry calcium chloride in glass desiccators. High temperature exposures (over 25° C.) were made in cabinets in which the temperature fluctuations were less than ±1° C.

Whenever it was desired to determine the number of larvae capable of development, or conversely, the number still in or returned to diapause, samples of stubs were covered with moist soil in closed pint or half-pint jars and incubated at 25° C. The minimum incubation period was three weeks, which was long enough to permit any larva that began development promptly to become adult. Wherever a treatment tended to delay the initiation of morphogenesis incubation was extended as much as three weeks longer.

The moist soil in which larvae were treated or incubated was sandy loam containing about 15% moisture. The dry soil used in some experiments was similar soil, air-dried at room temperature.

Moisture contents were determined on individual larvae. They were removed from the stub, weighed on a spring-torsion balance (150/0.1 mg.), oven-dried, and re-

weighed. Drying for one day at 95-100° C. was sufficient to bring the specimens to constant weight. Standard errors for the average ratios of water to dry material of the samples represented in Table 1 were small. Since other samples were similar in size and range, standard error calculations for them were omitted.

Techniques Used in Hormone Relationship Experiments

Ligation experiments were performed to resolve whether an endocrine mechanism controlled post-diapause development in C. cinctus, and, if a mechanism existed, to determine the source and timing of its hormone secretions. Larvae were removed from the stub and ligatures of fine silk thread, dipped in molten paraffin and beeswax, were tied tightly around them in the various positions to be described later. A single knot was sufficient because the wax held it firmly. The ligatures prevented seepage of fluids from one section to another. In ligated insects most organs thought to possess an endocrine function, e.g., the supra- and sub-oesophageal ganglia and associated structures, the corpora allata, and the corpora cardiaca, were contained in the head section. An isolated prothorax included the organs believed to be the thoracic glands, and an abdominal section contained the gonads.

In parabiotic experiments intended to investigate the effect of post-diapause larval blood on larvae still in diapause, larvae were joined in pairs, tail to tail. The tips of the abdomens of two larvae in different stages of development were severed and the ends of a glass microtube thrust into the wounds. Ligatures were applied just in front of the wounds, binding the larvae to the tube. The blood from one larva was continuous through the tube with that of the other. Finally, the operated parts were covered with a mixture of paraffin and beeswax.

Ligated larvae and parabiotic pairs were incubated at 25° C. in wax cells lined with blotting paper. Slot-shaped cells were melted into blocks of commercial paraffin wax, each quarter-pound block holding 25 slots. Each cell was 5 mm. deep and just wide and long enough to accommodate a sawfly or pair of joined sawflies. It was lined with a small folded rectangle of blotter. After an experimental larva was inserted, one end of the blotter was folded over and pressed in around the insect. The blotter was kept slightly moistened. This rearing technique did not completely solve the problem of inducing post-diapause larvae to develop when extracted from their stubs. However, enough did develop when reared this way so that useful data could be obtained.

EXPERIMENTAL RESULTS

Normal Moisture Absorption at End of Diapause

The water present in a sawfly larva's tissues during diapause is normally supplemented by absorption of soil moisture before pupation. This was shown by the following experiment. Diapause larvae obtained from a most field on September 19, 1952, were kept in moist soil at 10° C. to continue their diapause development. After 0, 50, 75, and 100 days at 10° C. samples of more than 70 of these larvae were tested for water content, and other samples, each containing more than 55 larvae, were incubated at 25° C. to determine the number in which diapause had been eliminated. Young prepupae developing from larvae given 110 days at 10° C. plus 9 days incubation were also tested for water content. The results are shown in Table 1. Some water was taken up during the latter part of diapause development. Some more was absorbed during the transition period between diapause development and active post-diapause development. In addition, moisture was probably absorbed during prepupal morphogenesis.

The transition between diapause and post-diapause development can be successfully accomplished at either a moderate (25° C.) or low (10° C.) temperature. The

TABLE 1

Water absorption as represented by ratio of water to dry matter in C. cinctus larvae during diapause, transition, and prepupal development

Number of days at 10° C.	Percentage of larvae out of diapause	Average ratio of water to dry matter
0	0	1.22 (± 0.02)
50	84	1.43 (± 0.02)
75	99	1.41 (± 0.03)
100	100	1.52 (± 0.02)
Prepupae	-	1.64 (± 0.03)

physiological processes occurring during this interval are unlike those occurring before or after. They do not depend upon low temperature as does diapause development, but neither is their activity as greatly increased by higher temperatures as is that of hormone secretion and morphogenesis. The transition period ends when hormone activity begins, less than a week before the visible change of the S-larva to a prepupa. This subject will be discussed more fully in a later section.

Additional sawflies from the same source as those mentioned above were enclosed in jars of dry soil for 50, 75, and 100 days at 10° C., after which they were incubated in moist soil. Each group comprised about 90 larvae. The percentage of each sample that developed upon incubation, and therefore in which diapause must have been eliminated, is compared with that of larvae chilled in most soil (Table II). It is evident that the differences are small. In the dry series there was no possibility of water absorption; probably a little dehydration occurred. Evidently diapause development is not dependent upon absorption of external moisture.

Other larvae were kept in dry soil at 10° C. for 90 and 110 days, and then incubated in dry soil, thereby receiving no moisture at any time. Only 37% and 19%,

TABLE II

Diapause elimination in *C. cinctus* at 10° C., with and without access to moisture

Number of days at 10° C.	Percentage developing when incubated moist after chilling at 10° C. in	
	dry soil	moist soil
0	0	0
50	83	84
75	97	99
100	92	100

respectively, developed. In the insects in which development ultimately did occur its beginning was delayed. Water absorbed near the end of diapause must be necessary for some of the insects, if they are to undergo post-diapause morphogenesis. But a moisture supply during diapause development is not a requisite. All of the 100-day dry series in Table II no doubt would have developed if the desiccation that accompanied chilling had not interfered with post-diapause development. That diapause development in the sawfly is independent of an external moisture supply offers additional support of Andrewartha's (1952) thesis that this relationship is general among insects.

Effect of Dryness on Diapause Reinstatement and Initiation of Morphogenesis

Although contact water is unnecessary for the elimination of diapause, it is still possible that dryness can cause diapause to return to C. cinctus. It has already been pointed out that dryness has been found responsible for the original initiation of diapause in many species of insects. However, the two situations are not identical. In C. cinctus, we are concerned with the action of dryness after a long period of relative inactivity, and long after feeding has ceased. In the other species, the influence of dryness is exerted during active growing and feeding stages.

If it is assumed that dryness helps to return post-diapause larvae to diapause, it could exert an effect under a variety of conditions: (1) an effect could be produced independently of the influence of temperature extremes, i.e., during incubation at 25° C.; (2) dry surroundings might also be expected to augment the action of heat in reinstating diapause if heat and dryness were combined in one treatment; (3) a decreased water content, previously produced by dehydration at a sub-developmental temperature, could induce a larva's return to diapause upon incubation; or (4) it could facilitate the action of high temperature in returning the larva to diapause. These four possibilities will be considered in turn.

(1) Influence of Dryness Applied During Incubation

The first possibility was easily eliminated. It was soon realized that in post-diapause larvae incubated at 25° C., lack of moisture was not an intensive enough factor to prevent morphogenesis. Larvae incubated from the beginning of their transition period in a current of dry air developed past the critical period for diapause reinstatement before dryness could exert its supposed influence. Even when 1952 material, which had a long transition period lasting about 2 weeks at 25° C., was subjected to this treatment practically all larvae developed. As a group the larvae lost only 9.6% of

their original water before the critical period was past. The average ratio of body water to dry matter was reduced from 1.36 to 1.26.

(2) Influence of Dryness when Combined with Heat

Sawfly larvae were subjected to drying and heat simultaneously. Post-diapause larvae were exposed for a number of days to 35, 40, or 45° C. in either dry or moist soil. They were then incubated, and developing forms and undeveloped S-larvae were counted. Each sample of 60 stubs contained about 50 living larvae. The data in Table III showing the percentages of living insects in which diapause was apparently reinstated suggested at first that reinstatement was more successful in the dry series. However, that is probably not true. Any S-larvae in which diapause had been reinstated, being somewhat more resistant to desiccation (Salt, 1946), would have a better chance of surviving in the dry soil than would developing insects. In the dry series, mortality tended to be greater among developing forms. This left a misleadingly high percentage of S-larvae among the survivors. Note that there is less consistent difference between the two series in the numbers of larvae that survived but did not develop than there is in the percentages. After allowance has been made for differential survival of S-larvae and developing insects in the two

TABLE III

Diapause reinstatement in post-diapause larvae
by exposure to heat in moist and dry soil

Temperature, degrees C.	Time, days	Treated in moist soil			Treated in dry soil		
		Number surviving	Number not developing	Percentage of survivors not developing	Number surviving	Number not developing	Percentage of survivors not developing
35	5	25	25	100	40	36	90
	10	42	42	100	41	41	100
40	2	52	6	12	38	10	26
	5	47	21	45	27	22	81
	10	8	8	100	1	1	100
45	1	19	18	95	13	13	100
	2	4	4	100	1	1	100
Control, no treatment		.....			46	3	7

series, this experiment offers no support to the hypothesis that dryness promotes return to diapause.

In the first of two other similar experiments, groups of 100 stubs each were given treatments shorter than those of the last experiment. They received exposures of six hours at 0 and 80% relative humidity and various temperatures between 37.5 and 50° C. They were then incubated in most soil at 25° C. Over 80% of the stubs contained larvae, most of which were in the critical period for diapause reinstatement when treated. The results of incubation are given in Table IV.

In the second experiment, similar critical period larvae were given six and eighteen hours at saturation deficits of 4 and 42 mm., and 35, 40, and 47.5° C., whereupon they were incubated as above. About 65 larvae were used in each sample. The results are shown in Table V. Moisture content samples of an equivalent series showed that the higher saturation deficit produced severe desiccation of the larvae, completely desiccating them in 18 hours at 47.5° C. At high temperatures, the lower saturation deficit caused slight desiccation.

The data of Tables IV and V show that, despite the brevity of the treatments, some larvae were successfully prevented from resuming morphogenesis. Humidity had no con-

TABLE IV

Diapause reinstatement in post-diapause larvae
by six-hour heat treatments at 0 and 80 per cent R. H.

Temperature, degrees C.	0 per cent R. H.		80 per cent R. H.	
	Percentage of survivors not developing	Total number surviving	Percentage of survivors not developing	Total number surviving
37.5	0	78	0	82
40	0	63	28	71
42.5	0	89	1	67
45	6	71	1	84
47.5	72	50	0	71
50	100	38	97	63
Controls, no treatment			0	74

TABLE V

Diapause reinstatement in post-diapause larvae
by short heat treatments at high and low saturation deficits

Time, hours	Temperature, degrees C.	High saturation deficit		Low saturation deficit	
		Percentage of survivors not developing	Total number surviving	Percentage of survivors not developing	Total number surviving
6	35	0	62	0	65
6	40	41	48	0	46
6	45	3	63	6	50
6	47.5	72	25	0	46
18	35	0	65	7	45
18	40	7	42	0	53
18	45	33	21	74	27
18	47.5	--	0	--	0
Controls, no treatment		.....		0	63

sistent effect. The influence of dryness on diapause reinstatement will depend on the exact stage of physiological development and existing internal state of the animal. One could expect, in the brief, severe treatments just described, that the influence of dryness would be greatly affected by the speed with which the physiological components of an insect reacted to temperature. These reactions, in turn, partly depend upon many other conditions that were not measured nor controlled. Complexities of this kind probably were responsible for some of the irregularities evident in the data above.

(3) Effect of Desiccation at 0° C.

Experiments described so far have shown contact moisture to be of little importance to the initiation of post-diapause morphogenesis and diapause reinstatement at temperatures suitable for incubation or higher. The larvae used had access to moisture before reaching the critical period for diapause reinstatement. It is important to know what would happen if similar larvae were dehydrated at a sub-developmental temperature before they were incubated or exposed to heat. By such treatment, water that had been absorbed during diapause could be eliminated before any development could begin. This procedure was tried in two experiments. In these experiments, conditions were equivalent to late

winter desiccation in the field, whereas previous experiments had simulated desiccation during the first heat of spring.

For the first experiment infested stubs were gathered in the fall and diapause was eliminated at 0° C. They were then dehydrated by a dry air current at 0° C. for 0, 30, and 60 days. During the first 30 days the larvae lost 9% of their original moisture; the loss in 60 days was 24%. The average ratio of water to dry matter was 1.51 before treatment, 1.30 after 30 days, and 1.16 after 60 days of desiccation. Moderately severe desiccation was achieved. After desiccation the larvae were incubated in moist soil. The number that failed to develop are listed in Table VI. The more severe desiccation inhibited morphogenesis in nearly one-third of them. In a control sample kept twice as long as 0° C., but in damp soil, most larvae developed. Thus most of the effect must be attributed to dryness and not to storage at 0° C.

In the other experiment, stubs from three sources were dehydrated for 5,20, and 50 days at 0° C. and 0% relative humidity. The pretreatment histories of the three groups of stubs were: (A) Collected in the field in the fall and stored nearly 20 months at 0° C. after diapause was broken. The latter part of this interval was in dry soil. (Upon incubation some would not develop.) (B) Collected in

TABLE VI

Inhibition of post-diapause morphogenesis by dehydration at 0° C.

Treatment	Number survived	Number not developing	Percentage not developing
Desiccated 0 days	85	3	4
Desiccated 30 days	82	5	6
Desiccated 60 days	73	23	32
No desiccation, 120 days at 0° C., moist	57	6	11

the fall, and stored at 0° C. to break diapause but not permitted to become dry. (C) Collected in early spring (April 10). (These were slightly ahead of (B) in development.) A sample of 70 stubs from each combination of treatment and pretreatment was then incubated for three weeks at 25° C. in moist soil to permit recovery of lost water. Another was incubated without soil in a covered jar to permit neither absorption of moisture nor further desiccation.

The results presented in Table VII show that two factors determined whether larvae developed promptly when incubated, developed after some delay, or did not develop at all. They were the persistent effect of desiccation at 0° C., and dryness during incubation at 25° C. Sawflies collected in early spring (C) had advanced far enough and had absorbed enough water to be practically unaffected by moderate amounts of desiccation. When incubated moist, development was uninhibited. In a few insects, however, dryness during incubation did delay the beginning of development. Fall-collected material (B) was both retarded in and prevented from developing to some extent by dry incubation, especially when this had been preceded by 50 days dehydration at 0° C. Desiccation at 0° C. followed by moist incubation failed to prevent development in any significant portion of the larvae. In fall-collected larvae, moderate desiccation with subsequent incubation in moist soil was very effective in

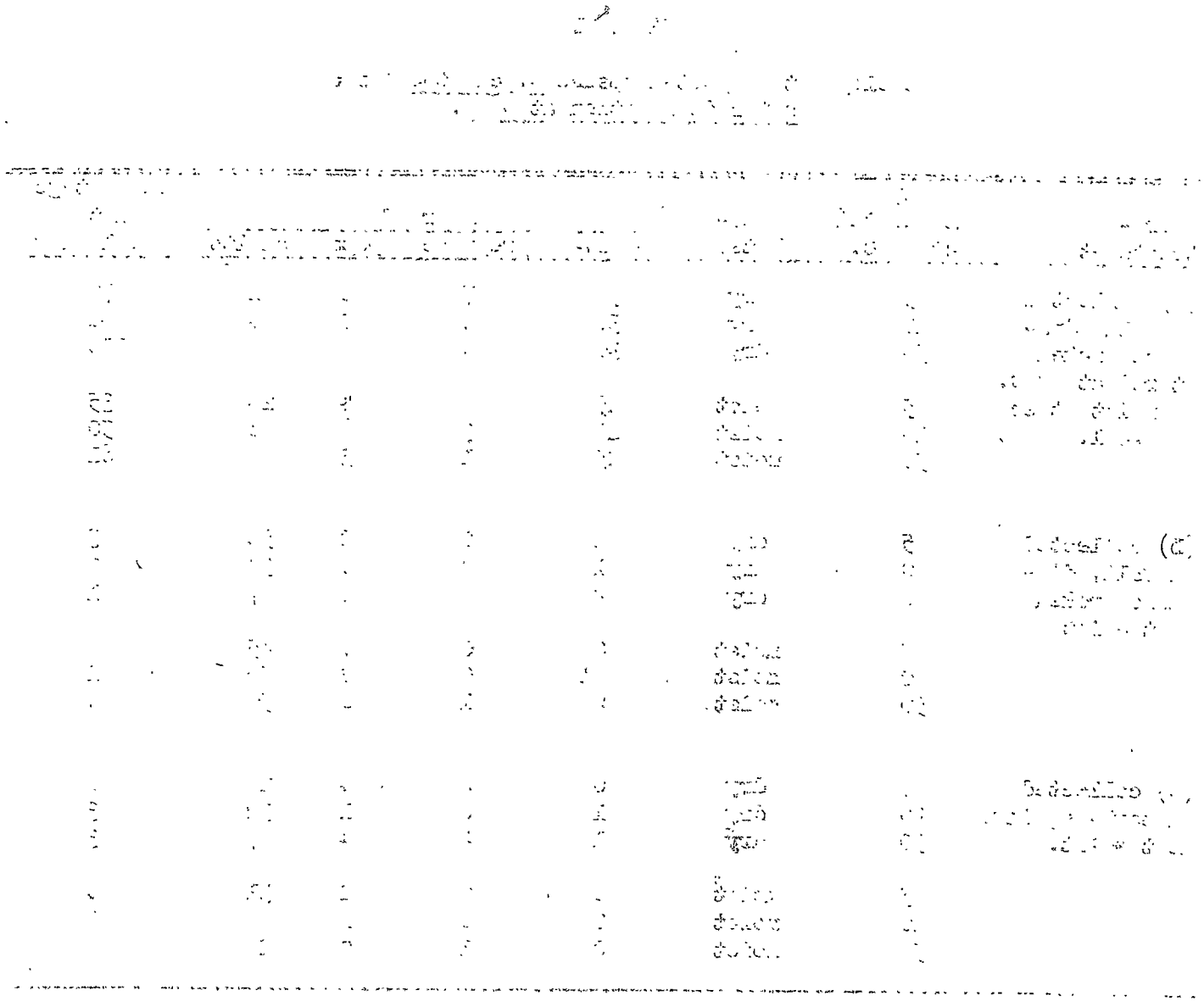

TABLE VII

Development of post-diapause C. cinctus larvae after desiccation at 0° C.

Pre-treatment	Days desiccated at 0° C.	Incu-bated	Number of S-larvae	prepupae	pupae	adults	Percentage not developing
(A) collected in fall; dia-pause broken; stored at 0° C. in moist, then dry soil.	5	dry	38	7	2	1	79
	20	dry	38	4	2	0	86
	50	dry	30	0	0	0	100
	5	moist	15	0	4	24	35
	20	moist	19	3	8	8	50
	50	moist	25	1	3	2	81
(B) collected in fall; dia-pause broken; kept moist	5	dry	2	2	2	56	3
	20	dry	1	0	4	37	2
	50	dry	9	4	2	27	21
	5	moist	0	0	0	65	0
	20	moist	1	0	2	56	2
	50	moist	2	1	0	50	4
(C) collected in early spring; kept moist.	5	dry	0	2	4	26	0
	20	dry	1	0	1	32	3
	50	dry	1	4	4	27	3
	5	moist	0	0	2	31	0
	20	moist	0	0	2	21	0
	50	moist	0	1	0	29	0

blocking development only if the larvae had already been subjected to dryness during their pretreatment (A).

Still longer desiccating exposures would have given more pronounced results. However, the exposures employed were already more severe than any that one would expect to occur naturally.

A long dry period at sub-developmental temperatures apparently can cause C. cinctus larvae to re-enter diapause. But the criterion used to indicate the presence of diapause, i.e., failure to develop when incubated, may not be adequate. Chilling has so far proved ineffective in activating larvae with a developmental block introduced by dryness, even though some have been chilled, moist, for as long as 150 days. If post-diapause development has been blocked by heating at 35° C., the developmental block can be removed by chilling (Salt, 1947; Church, unpublished). The developmental block instituted by heat is a real diapause. The developmental block instituted by dryness may be a pathological inhibition of development from which the larvae cannot recover, rather than a true diapause. In preventing post-diapause development, dryness appears to reinstate diapause, but possibly does not.

(4) Influence of Reduced Moisture Content on Reinstatement of Diapause by Heat

In the following experiment, larvae that had been desiccated at 0° C. were given various exposure to 35° C. in order to test the effect of a moisture content previously established at a low level on diapause reinstatement by heat. One series of post-diapause larvae was dried 60 days at 0° C. in a dry air current. Moisture content samples showed that the average ratio of water to dry material was reduced from 1.93 to 1.24, a substantial reduction. As a group they lost 36% of their original water. A second series of the same stock meanwhile was kept moist at 0° C. Then two groups of about 25 stubs each from each series were subjected to 0, 2, 4, 6, and 8 days at 35° C. and 95% relative humidity in attempts to reinstate diapause. Treatment was followed by moist incubation at 25° C. Table VIII shows that in almost one-fifth of the insects development was precluded by the desiccation alone, even in the absence of any exposure to 35° C. However, a comparison of the results of equivalent heat exposures on desiccated and non-desiccated larvae shows that when desiccated larvae received a heat treatment, their having been desiccated did not encourage diapause reinstatement at all. Rather, previous dehydration appeared to hinder the diapause-reinstating efficiency of heat, but this trend was not statistically significant. The experiment supported

TABLE VIII

Influence of moisture content of post-diapause larvae on reinstatement of diapause by heat

Time at 35° C., days	Number of S-larvae	Prepupae and pupae	Adults	Total	Percentage not developing	Average percentage not developing
Series not desiccated:						
0	1	0	15	16	6	3
0	0	0	21	21	0	
2	14	0	4	18	78	74
2	14	0	6	20	70	
4	17	0	0	17	100	94
4	17	0	2	19	89	
6	15	00	0	15	100	100
6	13	0	0	13	100	
8	12	0	0	12	100	100
8	18	0	0	18	100	

TABLE VIII a.

Influence of moisture content of post-diapause larvae on reinstatement of diapause by heat

Time at 35° C., days	Number of S-larvae	Prepupae and pupae	Adults	Total	Percentage not developing	Average percentage not developing
Series desiccated 60 days:						
0	4	0	18	22	18	19
0	5	0	20	25	20	
2	5	0	3	8	62	68
2	9	0	3	12	75	
4	10	0	3	13	77	85
4	16	0	1	17	94	
6	10	0	1	11	91	95
6	11	0	0	11	100	
8	12	0	0	12	100	100
8	16	0	0	16	100	

the idea suggested in the preceding section that moisture deficiency and heat may prevent development in quite different ways.

Hormonal Control of Initiation of Post-Diapause Morphogenesis

Physiological studies were thought necessary to explain how desiccation can block development while it fails to help reinstate diapause. The possibility was investigated that a two-stage endocrine mechanism, of the kind Williams (1946, etc.) found in Platysamia, controls the initiation of post-diapause development in C. cinctus. Experiments regarding the possible direct control of prepupal differentiation and molting by a hormone from the thorax are described in the first section below. The second section concerns whether a hormone from the head sets the mechanism in motion by stimulating secretion of the thoracic hormone.

(1) Differentiation and Molting Factor Produced in Thorax

Larvae from which chilling had eliminated diapause were each ligated in two places: 1) between the head and prothorax, and 2) between the metathorax and abdomen. They were incubated in the wax cells previously described, and were examined periodically. The chilling had lasted long enough so that most larvae had passed through the transition period and

were ready to begin post-diapause development promptly. Other larvae were similarly treated but were first permitted to develop at 25° C. in their stubs for 2, 3, and 4 days.

The majority of larvae ligated on the fourth day exhibited some development of all their parts. In six of the group ligated on the third day, only the thorax developed, while the abdomen and head underwent no change from the original S-larval form (see figure 4). In other specimens in this 3-day group in which all three parts ultimately did develop, development in most of the abdomens and heads lagged behind the thorax by a few days. Few larvae ligated earlier than the third day of incubation developed at all. Complete results are presented in Table IX. Abdomens and heads in which development was slower than in the accompanying thoraces are described as "retarded" in the table.

Control larvae from this stock were incubated in their stubs. Approximately one-third became prepupal on the fifth day, and one-half of the sixth; in other words, five-sixths were prepupae by the sixth day of incubation.

Anterior sections of ligated larvae, especially isolated heads, did not survive as well as more posterior ones, perhaps because of an oxygen shortage. Yet, frequently a developing thorax or even a head lived long enough to become a mature pupal section (see figures 4 and 7).

TABLE IX

Post-diapause larvae ligated behind head and metathorax

Number of days incubation before ligated	Total number ligated	Total number survived	Number in which the following sections developed: Head	Thorax	Abdomen	None
0	25	19	0	2	2*	17
2	25	16	0	3	0	13
3	25	22	10 (9*)	17	11(8*)	5
4	25	20	13	15	13	5

* retarded

Fig. 1 - C. cinctus: (a) normal S-larva, (b) prepupa, (c) unpigmented pupa (d) partially pigmented pupa, and (e) fully pigmented pupa. x9.

Fig. 2 - Normal S-larva. x15.

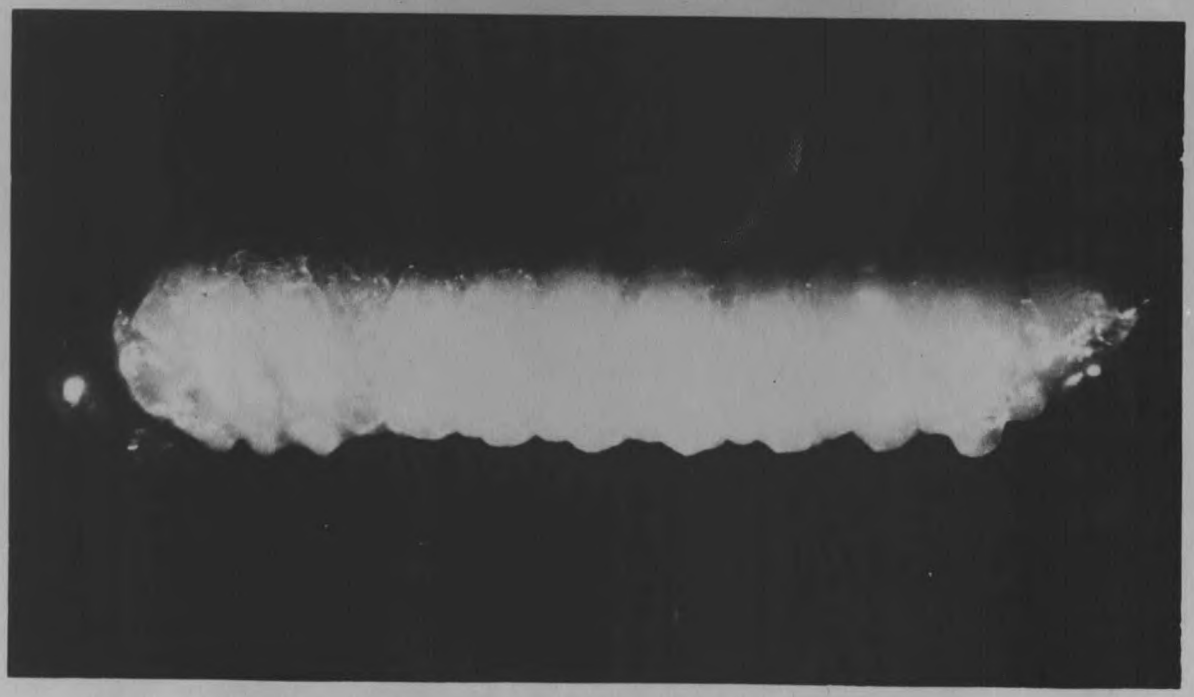

Fig. 3 - Normal prepupa. Note pigmented compound eye and fine wrinkles in the loosened larval skin. x15.

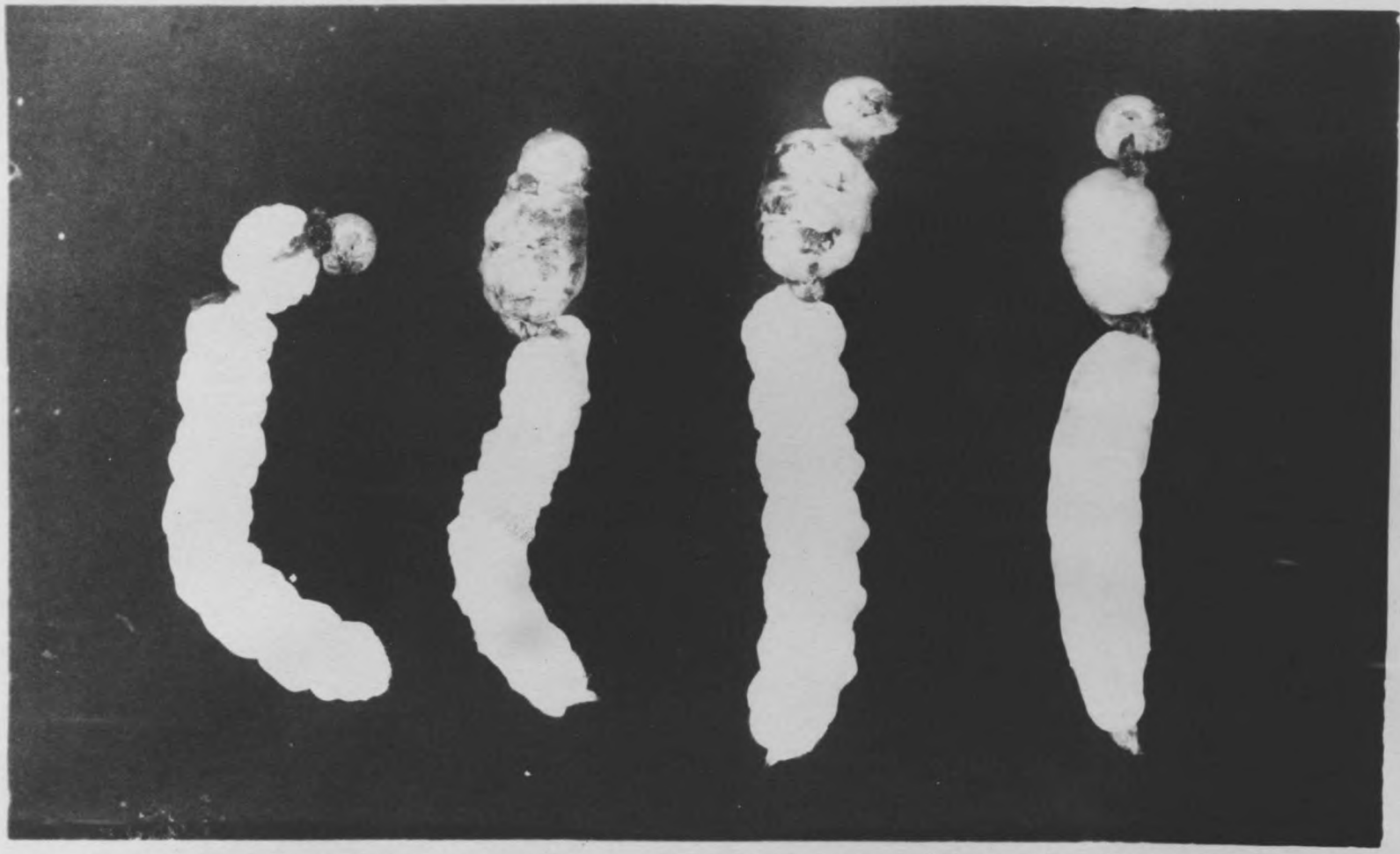

Fig. 4 - S-larvae ligated behind head and metathorax: (a) no development; (b) thorax pupated and pigmented, head and abdomen undeveloped; (c) thorax pupated, abdomen developed only to prepupa, head undeveloped; (d) all sections pupal and becoming pigmented (note pigmented compound eye).

Compound eyes became pigmented, mouthparts, legs, and wing buds formed, and the larval exuvium was loosened, though it could not be shed. Sometimes pigmentation of the pupal integument beneath the exuvium occurred. Other visible changes employed in deciding whether or not a section of insect had developed were the formation of optic lobes on the brain, straightening out of the abdomen (see figures 1, 3, and 4), the fine crinkling of the integument that indicated the digestion of its inner layers preparatory to molting (figures 3 and 6), and the digestion of the fat body, all of which are prepupal characters.

It may be concluded that a factor produced in the thorax is the promoter of pupal differentiation. In most individuals it has been produced and dispersed in the hemolymph in sufficient concentration to complete its work two days before the transformation to prepupa. At this time, if the head or abdomen is isolated from the thorax its metamorphosis will not be stopped.

Three days before the prepupal transformation the "thoracic center" has apparently been activated but has not yet secreted the minimum effective amount of its product. A thorax isolated at this time develops, but the rest of the larva does not. A day or so still earlier, the center has not yet been activated and even an isolated thorax will not metamorphose.

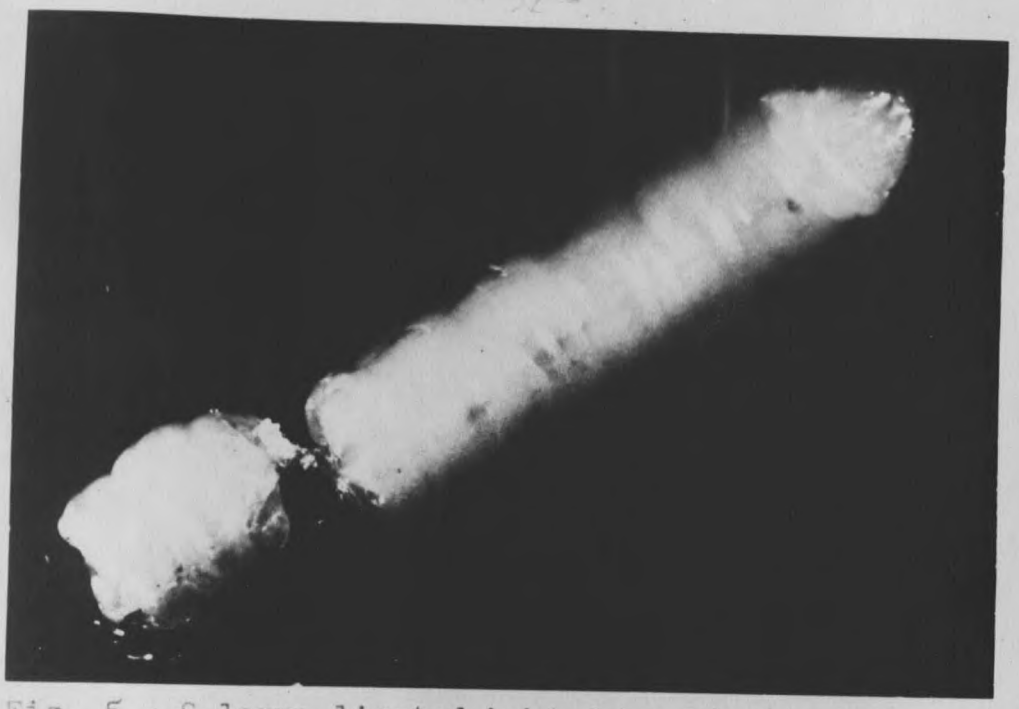

Fig. 5 - S-larva ligated behind head and metathorax, head removed: no development.

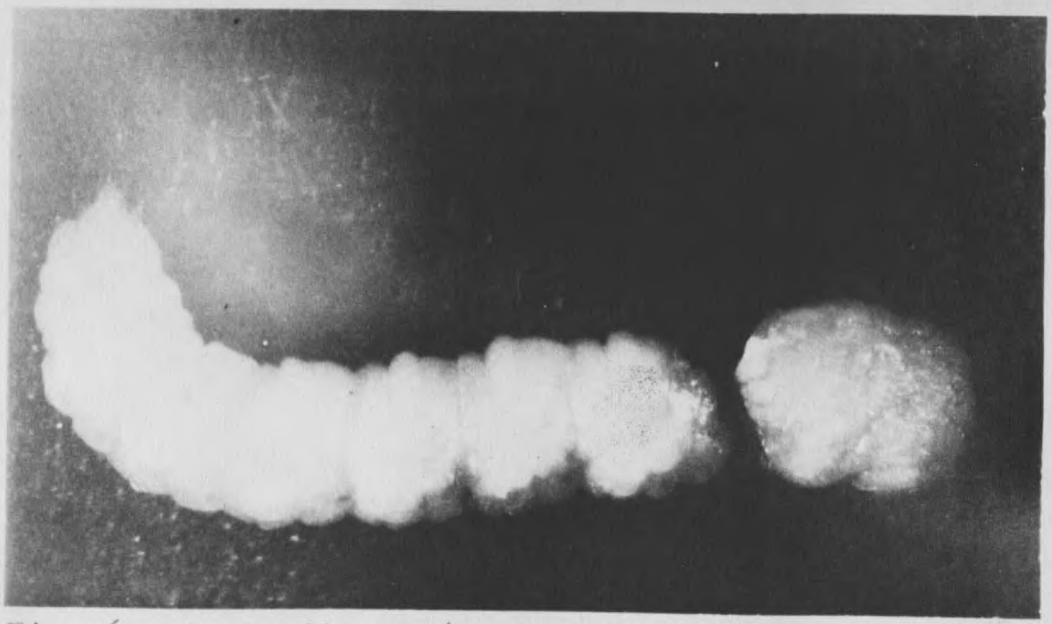

Fig. 6 - As in figure 5: thorax only has become pre-pupal. Note fine wrinkles in skin of thorax, showing here as small highlights.

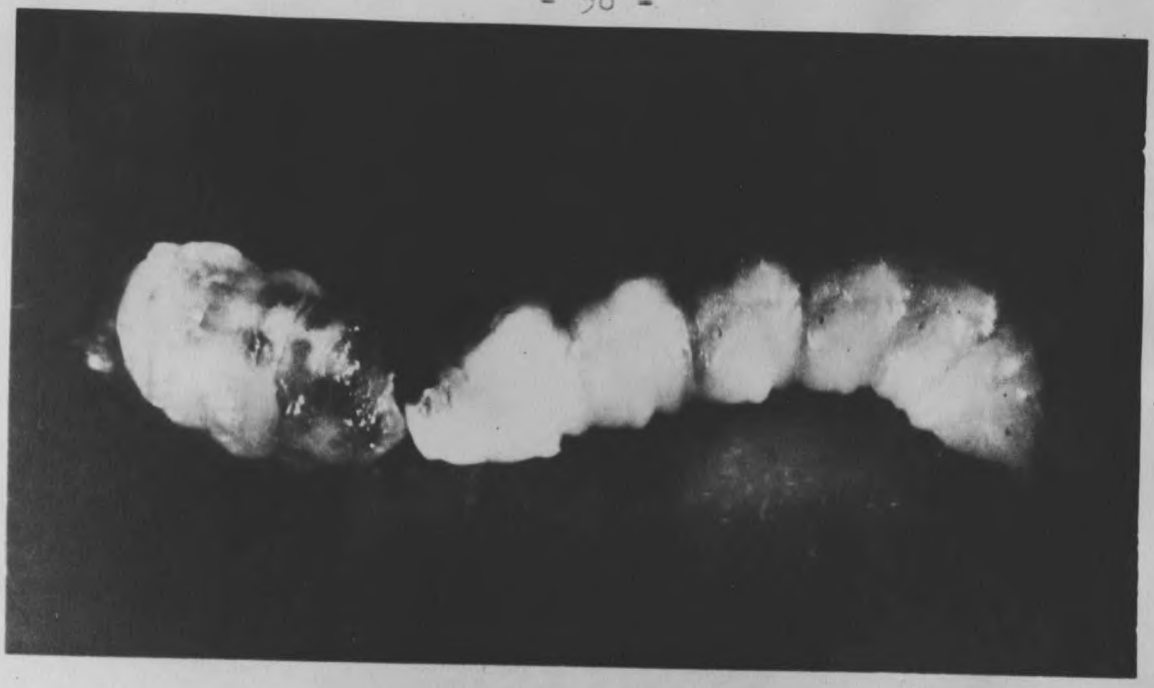

Fig. 7 - As in figure 5: thorax has become pupal and partly pigmented, abdomen not developed.

Fig. 8 - S-larva ligated behind metathorax: head and thorax only have become pupal beneath the unshed larval exuvium.

An attempt was made to further localize the source of this differentiation factor. Larvae presumed to be near the critical period for its secretion were ligated behind the head and through the mesothorax. To obviate the possibility of seepage from the head, it was cut off just in front of the first ligature. Most of the isolated prothoraces soon died. Nevertheless, in addition to several specimens in which positive results were questionable, two prothoraces did begin to molt while their attached posterior sections remained unchanged. Therefore the source of the factor must be in the prothorax.

Holmes (unpublished) has recently found a strand of tissue attached to the spiracle and lower main tracheal trunk on either side of the sawfly larva's prothorax. It closely resembles the descriptions of the prothoracic glands that produce the molting or growth and differentiation hormone in other insects.

(2) Function of Head in Stimulating Production of Differentiation Factor

Single ligatures were applied between various segments of sawfly larvae. The first group so treated was of the same stock as the larvae referred to in Table IX, and were ready to begin post-diapause morphogenesis promptly upon incubation. They were ligated just behind the head, prothorax, mesothorax, or metathorax after 0 or 3 days of

incubation in the stub. In Table X results from larvae ligated behind any of the thoracic segments are combined because the exact position of the ligature was not critical.

As before, some abdomens were capable of development when isolated from the thorax, or more precisely, from the prothorax, on the third day of incubation. When larvae were constricted behind the prothorax before any incubation had been permitted, their abdomens did not develop.

As long as there was no constriction between the prothorax and head, the section that included the prothorax was usually capable of development (figure 8). In some larvae ligation behind the head after three days incubation did not prevent development of the section containing the prothorax. Constriction behind the head at 0 days incubation, however, did block development of the section containing the prothorax. These observations are generally consistent with the data presented in Table IX. Two 0-day specimens that developed (Table IX) may have already passed the critical period before the operation, despite precautions taken to prevent it. In all, these data give strong support to the idea that the differentiation center in the prothorax is dependent for stimulation upon a second endocrine center in the head, which functions four to six days before the pre-

TABLE X

Post-diapause larvae ligated behind the head
or behind the prothorax, mesothorax or metathorax

Number of days incubation before ligated	Position of ligature	Total number ligated	Total number survived	Number of insects in which the following parts developed:		
				Anterior	Posterior	Neither
0	Behind head	25	16	0	0	16
0	Behind pro-, meso-, or metathorax	75	54	28	0	26
3	Behind head	25	17	0	4	13
3	Behind pro-, meso-, or metathorax	75	69	46	32(18*)	23

* retarded

pupal transformation. The fact that a two-stage mechanism of this type has been discovered in other insects strengthens the idea.

In another ligation experiment, less advanced sawfly larvae were used. They had not yet gone through the transition stage between diapause development and post-diapause morphogenesis, and if incubated at 25° C. would not have become prepupal until after the eleventh day. They were constricted after 0 or 6 days incubation in their stubs, as indicated in Table XI. A ligature behind the head prevented development even when applied after 6 days of incubation. This fact would indicate that during the transition period, which in this case included the first week or more of incubation, the endocrine mechanism is inactive. Hence, transition development does not involve a sluggish functioning of the mechanism, beginning immediately upon incubation; rather it encompasses processes preceding endocrine activity. These processes are preparatory to the stimulation of the "head center".

Temporary Failure of Endocrine Mechanism as Cause of Diapause

In Parabiotic experiments larvae were joined in pairs as previously described so that the hemolymphs of the two were confluent. In five pairs of developing prepupae

TABLE XI

Transition period larvae ligated behind the head and metathorax

Number of days incubation before ligated	Position of ligature	Total number ligated	Total number survived	Number of specimens in which the following parts developed:		
				Anterior	Posterior	Neither
0	Behind head	45	35	0	0	35
0	Behind metathorax	20	20	5	0	15
6	Behind head	25	22	0	0	22

joined to diapause S-larvae, the blood from the prepupa caused the S-larva to commence pupal development. In two other pairs this result was probably achieved but they died before the S-larva was distinctly prepupal. Thirty-four such parabiotic pairs out of 61 survived for more than 5 days. In 27 the diapause larva underwent no visible development. The surgery and the possibility of some prepupae being unable to produce enough differentiation factor for a pair of insects could account for many S-larvae failing to develop. Controls showed that the surgical treatment itself was never effective in breaking diapause.

The diapause-hormone relationship that Williams found in Platysamia pupae can be assumed to apply to wheat stem sawfly larvae. Essentially, all that is restraining the morphogenesis of a diapause sawfly is a lack of differentiation factor. If this is artificially supplied, the diapause tissues are quite competent to develop. The primary problem in inducing post-diapause development is merely one of initiating the two steps in the endocrine mechanism. Diapause development entails the preparation necessary for these reactions to occur naturally.

Relation of Dryness and Heat to Endocrine Mechanism and Diapause Reinstatement

Short exposures to heat were suspected of stimulating the activation of the endocrine center in the head. To test this idea, larvae that were still in the transition period were heated in their stubs in moist soil for 0, 1, and 2 days at 35° and 40° C. They were then incubated exactly 21 days at 25° C. and examined. Table XII shows that each brief heat treatment had a marked effect, causing development to begin much earlier than ordinarily. Since prepupal and pupal morphogenesis at 25° C. is nearly constant, it can be assumed that the period before morphogenesis began was reduced by more than a week. Had the high temperature treatments been extended some days longer, the larvae would have failed to metamorphose and would have re-entered diapause as S-larvae instead (Salt, 1947). Most likely high temperature first stimulates the "head center", presumably the brain, to activity. Then, further heating has a deleterious effect on the hormone produced in the head, or on the activity of the prothoracic endocrine center.

Two experiments were performed to determine whether this destructive action of heat affects the hormone from the head or a later stage of the endocrine mechanism. In the first experiment, 20 larvae that had begun post-diapause

TABLE XII

Stimulatory effect of short heat exposures
on the initiation of post-diapause development in transition period larvae

Heat treatment	Number of the following forms produced by 21 days incubation: Adults	Pigmented pupae	White pupae	Prepupae	S-larvae	Total
None	0	0	14	11	1	26
None	0	0	13	11	0	24
1 day at 35o C.	0	8	12	0	0	20
1 day at 35° C.	1	10	12	0	1	24
1 day at 40° C.	0	9	18	0	0	27
1 day at 40° C.	2	10	11	0	0	23
2 days at 35° C.	8	11	3	0	0	22
2 days at 35° C.	4	15	7	0	0	26
2 days at 40° C.	1	17	8	0	0	26
2 days at 40° C.	6	18	3	0	0	27

development were ligated at the metathorax. In 16 of them, the abdomen developed despite the ligature, thus showing that the prothoracic product had already been secreted in four-fifths of the sample. Fifty-five similar larvae were exposed to 35° C. for 12 days in their stubs, and then were incubated. In only two of them was diapause successfully reinstated. Two developed to adults, and 51 died, most of them after having exhibited abnormal prepupal development during incubation.

In the second experiment, two groups of post-diapause larvae were used. One group had completed a few days of post-diapause development in the field, and the other had not. Some of each group were ligated at the metathorax to determine the approximate proportion of them in which the prothoracic factor had already been secreted at the time of treatment. Others were subjected to a diapause-reinstating treatment of two weeks at 35° C. in their stubs, after which they were incubated. The two-week 35° C. treatment had previously been tested and found ample to return to diapause all larvae that were capable of it. Results of this experiment are given in Table XIII.

The two experiments demonstrated the same point; if a portion of a group of larvae has secreted the differentiation hormone, as large a portion has passed the

TABLE XIII

Diapause reinstatement by heat
before and after secretion of the differentiation factor

	Larvae ligated at metathorax		Larvae heated two weeks at 35° C. in stubs		
	Number in which differentiation factor			Number that	
Condition of larvae	Had been secreted	Had not been secreted	Number in which diapause was reinstated	developed wholly or partially	Percentage in which diapause was reinstated
Not developed	0	20	181	5	97
Partially developed	4	11	59	42	58

critical period for diapause reinstatement. By the time the differentiation factor has been released from the prothorax, a larva is no longer able to return to diapause. In reinducing diapause, heat must act on the first part of the endocrine mechanism. Probably the high temperature disrupts the action of the hormone from the head before it can activate the prothoracic endocrine center. If the "head hormone" is not already being secreted when the insect is subjected to heat, the heat first stimulates the hormone's secretion and then exerts its destructive action.

Experiments have shown that a severe moisture deficiency can prevent or delay post-diapause development. This effect might be brought about either by blocking the endocrine mechanism at its very inception or by disrupting it while in progress. The former alternative seems the more likely. The retarding action of dryness is in contrast to heat's stimulatory effect on the endocrine center in the head. By preventing or hindering the activation of the hormonal center in the head, dryness could be antagonistic to the reinstatement of diapause by heat. If the interpretations given here are correct, heat, in order to reinstate diapause in desiccated larvae, would first have to overcome the inhibitory action of dryness and stimulate the endocrine mechanism into activity. Then its destructive action could begin.

Heat and dryness have been discussed here as if their action on the endocrine mechanism were direct, but their influence on the mechanism may actually be exerted indirectly, by way of other body functions.

Whatever may be the validity of the hypothesis outlined above, this work has more closely defined the sensitive period during which external influences may stop the initiation of post-diapause morphogenesis and reinstate diapause. This sensitive period has been correlated with endocrine activity. It begins with the stimulation to activity of the endocrine center in the head, and has ended before the prothoracic endocrine center has secreted its differentiation hormone.

DISCUSSION: ROLE OF NERVOUS SYSTEM IN DIAPAUSE

In insects studied thus far the final stimulus to begin molting and differentiation is carried to the tissues by the hormone from the thoracic glands. The secretion of this hormone is brought about by the action of a stimulatory hormone from neurosecretory cells embedded in the nervous system. These or similar neurosecretory cells have also been found to provide the stimulus for other endocrine systems in invertebrates and vertebrates (Scharrer and Scharrer, 1944, 1945). Histological examination indicates that neurosecretory cells, although they have acquired a capacity for hormone secretion, have not lost their original nervous function. Consequently, Scharrer (1952) believes that these peculiar cells serve as connecting links between the nervous and the endocrine systems, thereby co-ordinating the control exerted by the two systems over the organism's physiology.

It is likely that strictly nervous elements control, or leastwise greatly influence, the activity and timing of secretion of the neurosecretory cells. A number of facts support this concept. Bounhiol (1952b), working with Bombyx larvae, found that at a certain stage of development the brain provides a hormonal stimulus for differentiation.

At that time removal of the brain prevents development, whereas removal and immediate re-implantation of the brain allows development to proceed, despite the fact that all the brain's nervous connections with the rest of the body have been severed. About 24 hours earlier, however, presumably before the secretory cells in the brain have become activated, severance of the brain's nervous connections prohibits development, though the brain be re-implanted as before. It seems that the brain itself must receive the proper impulses through its connective nerves before it can release the endocrine mechanism. Furthermore, Wigglesworth (1934) demonstrated that in Rhodnius the brain triggers the molting process in response to a stimulus provided by distension of the abdominal wall accompanying ingestion of a large blood meal. Probably the ultimate control of molting lies in the nervous system, being effected by way of the neurosecretory cells and thoracic glands.

It was related in the Review of Literature that the molt and differentiation normally occurring after diapause can often be induced somewhat prematurely by various physical and chemical shocks. Such shocks presumably must stimulate the nervous system violently. An insect undergoing a strong diapause apparently does not respond to shock treatments. Once the main part of diapause development has been completed,

or if diapause was originally weak, and "the stage is nearly set" for further development, a strong stimulus will invoke the nervous-hormonal mechanism and development will begin immediately. It was shown that C. cinctus larvae in the transition period can be induced to pupate if given a stimulus such as a short exposure to heat. If the stimulus is not forthcoming and the larvae are kept at a moderate temperature, in due course they do develop. Maybe their threshold of response is gradually lowered during transition development until the mechanism is released in response to such stimuli as are always in or around the insect. In some species, however, this presumed lowering of the threshold does not occur to the same extent as in C. cinctus. Locusta will not commence post-diapause development unless stimulated by a rise in temperature (Le Berre, 1951).

There is good evidence that, essentially, only a hormone lack keeps diapause insects from developing, for the tissues of a diapause insect are competent to develop if provided with molting and differentiation hormone. The processes essential to diapause development must then be concerned with preparing the insect to produce and release the hormone. These processes may perform one or both of two possible functions, viz., 1) the actual manufacture of a supply of the hormones to be secreted, and 2) the condition-

ing of the insect to enable the nervous system to receive an appropriate stimulus, and respond by releasing the endocrine mechanism. The second function would probably be the more critical and sensitive. The conditioning could conceivably take place in the nervous system alone, particularly the brain, but more likely it involves other parts of the organism. A physiological condition may be built up in the insect, which makes the nervous elements responsive to an appropriate stimulus, or which itself serves as the stimulus necessary to release the endocrine mechanism. In diapause the reactions that hasten the provision of an internal environment suitable for neuro-endocrine activity have been diverted or are delayed by other reactions. In non-diapause instars this does not happen.

Andrewartha (1952) thinks that is is lackkof food in an available form that keeps the hormonal mechanism inactive during diapause. His theory derives some support from the changes that have been noted in the fat body or yolk of various insects during diapause development. He believes that as soon as the inaccessible food reserves are made available or "mobilized", or enzyme systems have been set up to do this, then the differentiation hormone mechanism is released. Perhaps the condition of food reserves affects the threshold of response of the nervous system. But other factors comprising

the physiological state of the animal may be more important. Activation of the brain may depend on the presence of minimal amounts of one or a number of essential materials, the production of which is inhibited or promoted by countless internal and external conditions.

In the evolution of many insects the tendency towards diapause has been selected because of its winter survival value. Other characteristics have simultaneously been selected because of their value in surviving the same adverse conditions. For example, an extra amount of food material is stored to nourish the insect while it is unable to feed during the winter. A reduced water content may be an aid to winter survival, or it may be a secondary adaptation of the insect to the dormant state. The presence of large food reserves and low water content would natrually be correlated with the occurrence of diapuase if they all ultimately served the same purpose, that of winter survival. The frequent co-existence of these characteristics with the diapause state does not prove that they and diapause are interdependent. Their influence on the developmental block and its elimination may be small.

SUMMARY AND CONCLUSIONS

Sawfly larvae were found to absorb contact moisture during the latter part of diapause and during the first part of post-diapause development. Moisture thus absorbed was not necessary for normal diapause development. However, some specimens required some of this supplementary water if they were to begin post-diapause morphogenesis.

Desiccation at temperatures of 25° C. or higher had little effect on reinstating diapause in post-diapause larvae in their stubs. Moreover, reductions in body moisture previously created by prolonged dehydration at 0° C. did not enhance the diapause-reinstating activity of heat.

Prolonged desiccation at 0° C. did, however, prevent many post-diapause larvae from commencing morphogenesis when they were subsequently incubated in moist soil. In other larvae this treatment only delayed morphogenesis. Attempts to eliminate by chilling the developmental block caused by dryness have proved unsuccessful.

By means of ligation it was demonstrated that post-diapause morphogenesis is controlled in C. cinctus by a differentiation factor produced in the prothorax. About two days before the S-larva transforms into a prepupa this factor has been secreted into the blood in sufficient

quantity to initiate pupal development. Henceforth, further activity by the prothoracic center is unnecessary. Additional experiments indicated that the secretion of this differentiation factor is in turn prompted by the action of a stimulatory substance produced in the head, presumably by the brain.

Diapause is primarily the result of a lack of differentiation hormone. A diapause larva can be caused to develop if it is provided with post-diapause blood by joining it to a developing prepupa.

When a larva just out of diapause is subjected to heat, a short exposure stimulates the endocrine mechanism, thereby hurrying the initiation of morphogenesis. However, a longer exposure apparently destroys the mechanism in its early stages of activity, since it returns the larva to diapause. Because this developmental arrest produced by heat can be eliminated by appropriate chilling it therefore must be a true diapause. Diapause can be reinstated only before the differentiation factor from the prothorax has been secreted.

Partly because of the fact that heat at first stimulates the initiation of development, whereas dryness retards it, it is suggested that, in preventing post-diapause development, heat and dryness act in quite different ways.

Because of the failure of chilling to eliminate the developmental block introduced by dryness, it is further suggested that dryness may not reinstate diapause at all, but produces, rather, a pathological condition inhibiting development.

On the basis of experiments described here and others reported in the literature, it is proposed that the ultimate control of the initiation of post-diapause morphogenesis is exerted by the nervous system. Diapause development, which culminates in the elimination of the developmental block, then, embodies a conditioning of the animal so that the nervous system can become suitably activated.

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