

THE
PHYSIOLOGY OF DIAPAUSE
IN ARTHROPODS

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CHAPTER I

INTRODUCTION

THE possession of a definitive 'resting stage' is a common feature in organisms that inhabit inconstant environments. In such forms of life the dormant state is usually characterized by the temporary failure of growth or reproduction, by the reduced metabolism and often by the enhanced resistance to adverse climatic factors such as cold, heat and drought. The appearance of the 'resting stage' may sometimes be evoked by just those conditions which it is adapted to survive. But the relationship is often more complex, for dormancy may supervene before the milieu becomes unfavourable for growth and sometimes persists after these conditions have vanished.

Although the occurrence of this state of arrested growth or diapause (*διάπαυσις* = rest, interruption of work) is particularly frequent in the Arthropoda, it is by no means confined to this group. In the plant kingdom many seeds, bulbs and the buds of woody plants display a similar type of dormancy. The gemmules of sponges, the yolky winter eggs of Cladocera and the drought-resisting eggs of *Artemia* and other branchiopod Crustacea, are all familiar examples of resting stages. Among the Vertebrata an excellent instance of diapause is afforded by the delayed implantation of the blastocyst in mammals such as the American marten and armadillo. Dormancy also occurs in the adult vertebrate. Sometimes the suspension of physiological activity is general, as in the hibernating marmot or aestivating *Protopterus*; sometimes, as in many birds and mammals, the growth failure is limited to the reproductive organs. Nevertheless, despite the generality of the phenomenon, it is perhaps not without significance that it is among the Insecta—a group in which normal growth is essentially discontinuous—that examples are most numerous and varied.

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The ecological aspects of diapause have been stressed in the recent reviews of Bonnemaison (1945) and Andrewartha (1952). This condition has been rightly regarded as an important adaptation for preserving the species in regions where seasonal climatic conditions are unfavourable for continuous multiplication. However, from the standpoint of physiology the central issue concerns the problem of growth and its control by intrinsic and extrinsic factors. It is now well known that both the arrest of growth itself and the accompanying metabolic adjustments are governed by the organs of internal secretion. These endocrine centres are in turn responsive to certain definite stimuli from the environment. Indeed, it is this link that permits the diapause mechanism to function as a timing device, synchronizing the periods of dormancy and active growth with the rhythm of the environment in general. These subjects will furnish the principal themes in the present monograph.

DEFINITION OF DIAPAUSE AND QUIESCENCE

The term diapause was introduced by Wheeler (1893) to describe a stage in the embryogenesis of the grasshopper *Xiphidium ensiferum*. The complicated pendulum-like movements of the embryo round the posterior pole of the egg were divided into an ascending stage (*anatrepsis*), when the embryo was moving tail-first through the yolk, and a descending stage (*catatrepsis*), when the direction of movement was reversed. All the movements were collectively referred to as *blastokinesis*. The phase intervening between *anatrepsis* and *catatrepsis* when the embryo remained poised with the head directed towards the posterior pole was called 'the diapause'.

The term was subsequently given an entirely different meaning by Henneguy (1904), who applied it not to a *stage* of morphogenesis but to the *condition* of arrested growth, whether in the developing or in the adult insect. Many authors have followed Henneguy in extending the term to cover all forms of arrested growth, even simple inhibition by cold. Yet as early as 1869 Duclaux had shown in experiments on the hibernating egg of the silkworm that the condition differs fundamentally from one of cold torpor. Duclaux observed that the egg batches of

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Bombyx mori invariably failed to hatch at room temperatures, and eventually died, whereas similar batches which were first chilled for 40 days in an ice box hatched successfully when returned to the higher temperature.

In view of these special attributes, attempts have been made from time to time to frame a narrower definition of diapause. Thus Shelford (1929) suggested that the use of the term should be restricted to instances where development or activity is arrested 'spontaneously', whereas an interruption of growth directly controlled by unfavourable conditions could be referred to as quiescence. The terms 'diapause vrai' and 'pseudo-diapause' coined by Roubaud (1930) were based on similar distinctions.

Shelford's definition is perhaps no longer wholly appropriate, for in many cases the causes for the onset of diapause can be traced back to the action of the environment, although it is 'spontaneous' in the sense that the response may later become independent of the primary stimulus. However, the immediacy of the response to the environmental factor remains a valid basis for distinction, particularly if this factor is temperature. In the absence of a full experimental analysis, the type of arrest is usually classified more simply by reference to the termination of dormancy. For example, one insect will develop without delay when the temperature is 'favourable' for morphogenesis, whereas a second fails to develop (as in *Bombyx*) or grows slowly and irregularly. The recognition of these states as 'quiescence' and 'diapause' serves as a useful reminder that certain physiological mechanisms are brought into action in the diapausing insect which are absent in the quiescent—this is the implication of the delay which ensues before growth is resumed. The retention of these terms is further justified on the grounds of generality, since the termination of diapause in the vast majority of insects is controlled by temperature.

The distinction, however, is less clear when stimuli other than temperature govern the resumption of growth. The following example illustrates some of the difficulties. Researches by Gayspitz (1949, 1953) have shown that the larvae of the moth *Dendrolimus pini* (Lasiocampidae) will feed and grow without interruption at 19–20° C. provided they are continuously

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illuminated. But if the day length is reduced to 9 hours per day the larvae moult once and finally become dormant within 30 days. In this state of dormancy, which persists for at least a month if the conditions are unchanged, feeding is spasmodic and the growth of the batches slow and uneven. However, the arrest can be ended at any time by exposure to continuous illumination; feeding and growth are then resumed after a delay of about 14 days.

Photoperiod is a stimulus which, unlike temperature, cannot be regarded as immediately favourable or unfavourable. Further, larval development in *Dendrolimus* is evidently more or less directly controlled by photoperiod; yet the definite time lag separating the institution of the new day-length régime and the response suggests that physiological changes of some complexity are set in motion by the stimulus. On these grounds it would be more justifiable to regard this as an instance of diapause.

There are other borderline examples. The ichneumonid parasite *Diplazon fissorius* will develop no further than the 1st instar until the syrphid host forms the puparium. By injecting larvae into developing hosts Schneider (1950) has proved that the parasite is activated almost instantly—probably by some change in the host's blood associated with pupation. This arrest is therefore more appropriately regarded as quiescence. In many insect larvae the absorption of water is a necessary prerequisite for the resumption of growth (see Chapter 5). Since the arrest is usually ended in a few days when water is made available, this too can be regarded as quiescence. However, it may well be that hydration influences growth through the endocrine system rather than by activating the tissues directly. In this event the final cause of the arrest, and of the resumption of growth, is internal to the insect, as it is in diapause. It is clearly inadvisable to press these distinctions too far. Even exposure to low temperature may influence the growth of the insect both directly, by inducing torpor, and indirectly by causing subtle changes in the hormone balance (Wigglesworth, 1952).

In classifying undoubted examples of diapause, the terminology introduced by Steinberg and Kamensky (1936) has proved

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of considerable utility. In many insects the potentialities for diapause are not realized in each generation. Diapause is then said to be facultative. The onset of the arrest in these species seems invariably to be influenced by the environment and can either be induced or averted by the appropriate external conditions. Other insects possess an obligatory diapause. When they are reared under varied conditions virtually every individual enters diapause in each generation regardless of the environment. Insects with facultative diapause commonly complete two or more generations annually in nature, while those with obligatory diapause exhibit a strictly univoltine* life cycle.

THE DIAPAUSE STAGES

Diapause may occur in any of the major stages of the life history. In the egg, larva or pupa it takes the form of an arrest of development; in the imago the state is associated with the failure to enlarge the reproductive organs, particularly the ovaries, and with the compensating hypertrophy of the fat body and other storage tissues. This process has been termed 'gonotrophic dissociation' by the students of mosquito physiology, but it is equally characteristic of all insects with an imaginal diapause.

The precise morphogenetic stage of the growth arrest in the egg or larva is also characteristic of the species. Growth may be halted when the embryo is still rudimentary, when it is half-grown, or when the young larva is fully formed but still unhatched (see p. 7). Larval arrests are perhaps most frequent in the last instar after the feeding period is ended; but many insects (for example, the Lepidoptera *Arctia caja*, *Lasiocampa quercus*) hibernate in the middle instars. In a few species such as the ermine moth *Hyponomeuta padella* (Thorpe, 1929) and the spruce budworm *Cacoecia (Choristoneura) fumiferana* (Graham and Orr, 1940) diapause supervenes immediately after the 1st-instar larva has hatched from the egg.

Although a general trend can be traced in some groups, the stage of arrest, even among closely related species, is often

* The term 'voltinism' derives from Italian sericulture: *volta* = time, in the sense of 'occasion'.

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notably inconstant. For example, the tortricid *Cacoecia rosana* enters diapause as an egg, *C. fumiferana* as a newly emerged larva. The silkmoth *Antheraea yamamai* has an egg diapause, *A. pernyi* a pupal diapause. The multivoltine syrphid *Epistrophe balteata* possesses a facultative imaginal diapause, whereas the univoltine species *E. bifasciata* enters diapause at the close of the last larval stadium (Schneider, 1948).

These considerations suggest first, that diapause has often been evolved independently, and secondly, that in short-lived insects the selective value of this character is little affected by the stage in which it occurs. It is noteworthy that in insects with an imaginal life extending over several years, such as the beetle *Dytiscus marginalis*, diapause is of the reproductive type.

Although the point of occurrence of diapause within the life cycle is normally a very constant specific feature, larval arrests are sometimes influenced by temperature. If the Japanese beetle *Popillia japonica* is reared at 25° C. the 3rd instar is of relatively long duration, lasting for 126 days instead of the 92 days required at lower temperatures. At 20° C. growth is delayed in the 2nd or 3rd instar and at 17.5° C. in the 1st instar (Ludwig, 1932). Similarly, in *Orgyia gonostigma* (Lymantriidae) dormancy supervenes in the 5th and last instar at 22–25° C., in the 4th instar at 14–18° C., and in the 3rd instar at 10° C. (Kozhantshikov, 1948). High temperatures also cause the stage of arrest to be postponed to a later instar in the bug *Reduvius personatus* (Radio, 1931).

Although diapause is usually confined to one stage in the life cycle, there are a few exceptions. Both the pupa and egg of the winter moth *Operophtera brumata* are said to possess an obligatory diapause (Kozhantshikov, 1950b). In some insects with a facultative diapause, growth may be suspended in more than one instar. Development can be arrested in both the 3rd and the 6th instars of *Dendrolimus pini* if the 2nd and 5th instars are exposed to short photoperiods; indeed, the larva of this species sometimes overwinters twice in the northern part of its range (Gayspitz, 1949). In *Reduvius* all nymphs diapausing in the 3rd instar also experience a lengthy arrest in the 5th instar; and one-quarter of the nymphs which enter diapause in the 4th instar again become dormant in the 5th. In this insect it seems

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that the onset of diapause to some extent prevents the recurrence of the arrest in the succeeding instar (Readio, 1931).

The grasshopper *Pardalophora apiculata* has a two-year life cycle in western Canada, spending the first winter in the egg stage and the second as a late nymph. The eggs are of the diapause type and probably the overwintering nymphs also exhibit this condition as they fail to grow even at high temperatures (Pickford, 1953). Other insects requiring two years for development have a definitive diapause stage only in the last larval stadium, the first winter being passed in quiescence as a half-grown larva. Two examples are the alder fly *Sialis lutaria* (Rahm, 1952) and the dragonfly *Anax imperator* (Corbet, 1954).

THE STAGES OF ARREST IN EMBRYONIC DIAPAUSE

In the post-embryonic stages of insects the cyclical nature of ecdysis and growth may well favour the evolution of diapause mechanisms. This 'predisposition' is less apparent in the embryo where morphogenesis is continuous. Nevertheless, a closer examination suggests that the arrests are confined to certain definite periods in the growth and differentiation of the embryo.

From this point of view it is convenient to divide embryonic development into three stages. The first phase sees the successive divisions of the cleavage nuclei and ends with the formation of the blastoderm. The second includes the early growth of the embryo up to the beginning of the formation of the mesoderm. This phase, which corresponds approximately to anatrepsis, is one of growth but not of differentiation. The number of cells in the embryo is increased by mitosis, the somites are blocked out and the rudiments of the appendages appear, but all the cells remain alike in form. During the third phase, which commences at the beginning of catatrepsis, active growth and cellular differentiation proceed side by side. By the end of catatrepsis all the main organ systems have been differentiated. Fig. 1 illustrates the changes in the external morphology of an insect with embryonic diapause, namely, the cricket *Gryllulus commodus*.

No species are known in which diapause supervenes before the formation of the blastoderm. The arrest occurs immediately

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after this point in the winter egg of the fruit-tree red spider mite *Metatetranychus ulmi*, for no definitive germ band has been distinguished with certainty. In the grasshopper *Austroicetes*

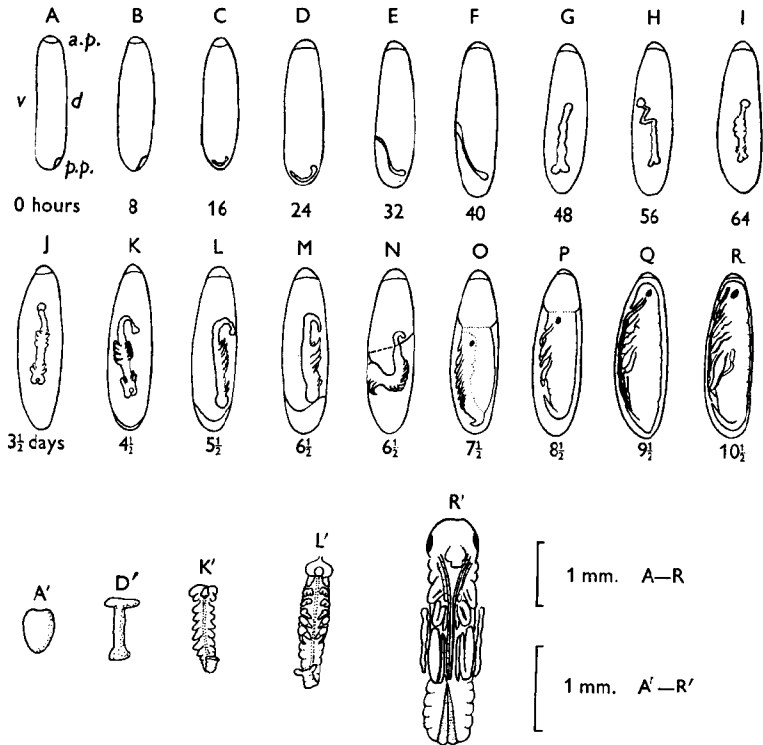


Fig. 1. Embryonic development in *Gryllus commodus* (after Brookes). A-R, stages reached during blastokinesis at an incubation temperature of 25° C. A'-R', changes in the form of the embryo at corresponding time intervals. A-K, anatrepsis. L-R, catatrepsis. From A to F the embryo is moving tail-first round the posterior pole of the egg and sinking into the yolk. If the egg is affected by diapause the arrest occurs at stage J. Rupture of the amnio-serosal membrane and the revolution of the embryo occur at stages M and N. Dorsal closure is complete by stage Q. a.p., p.p. anterior and posterior poles; d, dorsal; v, ventral.

cruciata development is only a little further advanced when diapause sets in. The first rudiment of the embryo is nevertheless visible as a small cap of cells formed by the condensation of the blastoderm (Steele, 1941). The eggs of some Lepidoptera

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(*Notolophus thyellina*, *Dendrolimus undans excellans*, *Archips xylosteanus*) and a cricket *Homeogryllus japonica* are also known to enter diapause at this early stage of embryogenesis (fig. 2 A, B) (Umeya, 1950).

In some species growth may cease at a slightly later stage when the embryo is dumbbell-shaped but as yet unsegmented. This is the diapause stage in *Bombyx mori*, *Theophila mandarina* and

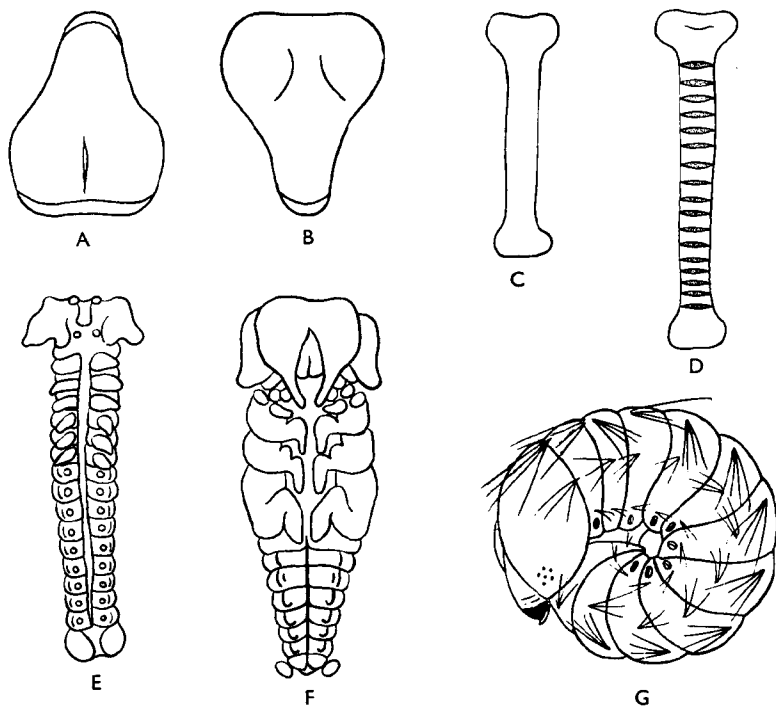


Fig. 2. Diapausing embryos of various insects (after Umeya). A, pyriform embryo—some Lepidoptera. B, reversed pyriform embryo—some Orthoptera. C, dumbbell-shaped embryo—*Bombyx mori*. D, segmented embryo—*Rhopobata naevana* (Lepidoptera). E, embryo with appendages—some Lepidoptera. F, ditto—*Locusta migratoria* and other Orthoptera. G, prelarval embryo—*Lymantria dispar* and other Lepidoptera.

other Lepidoptera and in the cricket *Gryllus mitratus* (fig. 2 C) (Umeya, 1950). In the eucosmid moth *Rhopobata naevana* the growth arrest seems to come slightly later as the somites are forming (fig. 2 D).

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In many species morphogenesis is suspended at the close of anatrepsis when the somites and other axial structures are present and the embryo is apparently about to undergo revolution. The eggs of several Orthoptera (*Gryllulus commodus*, *Melanoplus differentialis*, *Locusta migratoria*) and those of the lymantriid *Orgyia antiqua* are examples (fig. 2E, F) (Browning, 1952a; Slifer, 1932; Le Berre, 1953; Christensen, 1937).

Finally, development may also be halted very late in embryogenesis when the larva is fully formed and seemingly ready to hatch (fig. 2G). This is the stage of the arrest in *Antheraea yamamai* (Saturniidae), *Lymantria dispar*, *Malacosoma testacea* and *M. disstria* (Lasiocampidae), also in certain Orthoptera (*Campsocleis buergeri*, *Melanoplus bivittatus*) (Umeya, 1950; Salt, 1949a) and Coleoptera (*Timarcha tenebricosa* and *T. violacea-nigra*) (Abeloos, 1935, 1941).

This survey suggests that development cannot be arrested during the phase of intense mitotic activity which accompanies blastoderm formation or during the later phase of active differentiation; these seem to be processes which, once begun, must proceed to completion. On the other hand, different species have evolved mechanisms for halting development at almost all stages during the early growth of the embryo when some cell multiplication is taking place but no cellular differentiation.

The extent of the growth arrest in the egg is often virtually complete, even at high temperatures. The rarity of mitoses in the diapause embryos of *Melanoplus differentialis* contrasts with the veritable mitotic 'explosion' which follows the resumption of growth (Slifer, 1931). But in some eggs which enter diapause at an early stage of embryogenesis the arrest is not absolute. The egg of *Austroicetes*, when exposed to moderate temperatures, will continue to develop slowly, eventually reaching the end of anatrepsis in about two months (Andrewartha, 1943). Embryogenesis in *Dociostaurus maroccanus* follows a similar course. In Iraq the egg pods of the Moroccan locust are deposited in the soil in May. The egg enters diapause within a few days when the embryo consists of a small triangular cap of cells. Slow and irregular growth takes place during the hot summer months, from 2 to 5 months being required for the completion of

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anatrepsis. After wintering at this stage the eggs eventually become free of diapause in the spring; the embryos then revolve round the posterior pole of the egg, active growth is resumed, and hatching finally takes place in March (Bodenheimer and Shulov, 1951). The eggs of the Lepidoptera *Notolophus thyellina* and *Dendrolimus undans* mentioned by Umeya (1950) evidently belong to the same category. In all these species there is of course only one *period* of diapause, although this condition affects several different stages of embryogenesis.