On the Role of the Integument in Acarine Development and its Bearing on Pupa-formation

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With one plate (fig. 7)

Summary

Typical development in mites begins with the retraction of the epidermis from the cuticle. It is accompanied by a decrease in size and a change in shape. Atypical development also begins with a moult. But it is distinguished by regrowth, a change in shape, and an extra moult.

Each moult is unique in that the epidermis divides into an outer and an inner layer. The outer layer adheres to the old cuticle. Its cells are provisionally regarded as being the source of the moulting enzymes. The inner layer retracts and persists as the true epidermis.

The components of mite cuticle, according to histochemical tests, resemble those of insect cuticle.

The formation of a provisional cuticle during atypical development produces the condition generally recognized as a pupal state. The moult of this provisional cuticle is primarily aimed towards synchronizing cuticle deposition with muscle development. Integration of the intermediary connecting processes, the fibrillae, with both the muscle ends and the cuticle is thus made possible.

Introduction

THE main purpose of this communication is to describe the unique role of the acarine epidermis during moulting, and to define the essential nature of pupa-formation on the basis of observations on acarine development.

The structure of the arthropod integument and the process of moulting have been described in the decapod Crustacean (*Homarus*) by Yonge (1932), in the Insecta (*Rhodnius* and *Tenebrio*) by Wigglesworth (1933, 1948), and in the Araneida (*Tegenaria*) by Browning (1942). Lees (1947) pointed out that the structure of the cuticle of a tick resembled that of an insect. However, attention had not hitherto been properly focused upon either the degree of variation or the process of moulting in acarine development.

It so happens that some mites in their development produce a pupal condition. In view of this it is somewhat surprising that no suggestion, as far as I am aware, had been made, that a clearer understanding of the pupal state in arthropods might be elicited from an investigation of this condition in mites. The beautifully executed figures of *Trombidium fuliginosum* by Henking (1882) gave a reasonable clue. However, it was the close resemblance between the pupal state expressed in the development of the British harvest-mite (Jones, 1951) and that of insects which led to part of the present work.

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Material and Methods

A tyroglyphid mite *Histiostoma polypori*, an oribatid mite *Scheloribates laevigatus*, and the British harvest-mite *Trombicula autumnalis* were used for study. A method for rearing mites has been described (Jones, 1951). Much of the success of sectioning these minute animals with their sometimes extremely hardened cuticle depended upon a judicious use of the microtome blade. Hot alcoholic Bouin was used for fixation, and the hardening effects of absolute alcohol and xylene were eliminated by substituting amyl acetate. Paraffin wax of m.p. 56° C. or Peterfi's celloidin-paraffin was used for embedding. Mayer's haemalum, Heidenhain's haematoxylin, and eosin were the principal stains used.

The Interposed Phases of Development

It seems reasonable to suggest that mites fall into two natural groups according to their development. In one group each phase of development is accom-

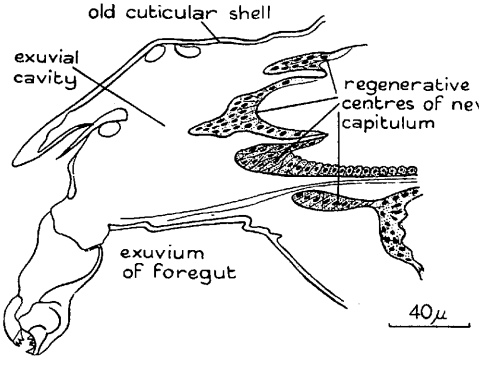

Fig. 1. Section of a typically developing mite showing the spaciousness of the exuvial cavity and the epidermal regenerative centres moulding the capitulum of the next stage.

panied by a typical single moult. In the other, each phase of development is punctuated by two moults. The extra moult makes it atypical. It also involves the condition generally recognized as pupa-formation. Each atypical phase of development therefore assumes a pupal state (Jones, 1950).

Of the mites examined in this work, the tyroglyphid and oribatid mites develop typically. Each phase of development is initiated by the retraction of the epidermis from the cuticle. The retraction is considerable. The mite not only shows a marked decrease in size, but also a change in its spatial relations (fig. 1).

The first instar or hexapod stage is conveniently referred to as the larva. The succeeding stages (the number varies with the species) which intervene before metamorphosis to the adult are termed nymphs. A stage has two well-

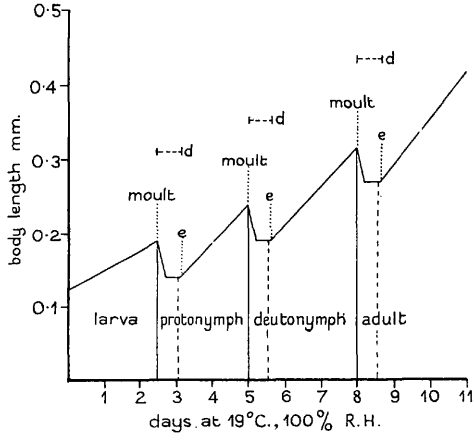

FIG. 2. A diagram illustrating periodic growth in a mite, *Histiostoma polypori*, which has typical developmental phases. d, phase of development; e, emergence from cuticle of previous stage.

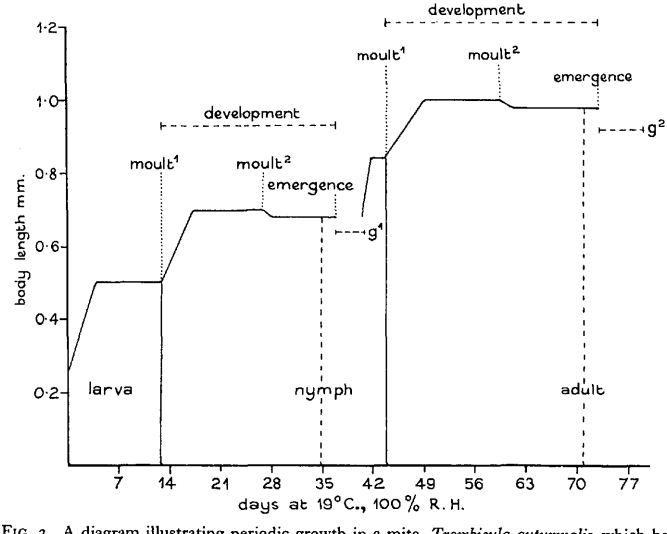

FIG. 3. A diagram illustrating periodic growth in a mite, *Trombicula autumnalis*, which has atypical developmental phases, each being distinguished by a moult of provisional cuticle. g^1 and g^2 indicate the free-living existences of the nymph and adult respectively; in nature they may extend considerably to make the life-span from egg to adult last about 6 months.

defined phases: one the immobile developmental phase; the other, the mobile growth phase. Any recognizable advance or change in development may best be termed a step.

In general, it is convenient to regard the moulting cycle as beginning with the retraction of the epidermis from the cuticle. It continues with the dissolution of the separated cuticle. The discarding of the exuvium is merely incidental to emergence which often takes place some time after the moult.

As shown in fig. 2, the retraction of the epidermis from the cuticle in *Histiostoma polypori* marks the end of a stage and the initiation of the next. In fig. 3 the beginning of a stage in *Trombicula autumnalis* is likewise marked by the retraction of the epidermis from the cuticle. However, the succeeding phase of development is further punctuated by a second moult. Because this occurs the mite assumes a pupal condition (fig. 7). It also became apparent that regrowth (fig. 3) and a change of shape accompanied pupa-formation.

The Component Layers of Mite Cuticle

The results of histochemical tests on the lines devised by Wigglesworth (1933) demonstrated that the nature of the cuticles of the mites chosen for study resembled that of a tick (Lees, 1947) and that of insects (Wigglesworth, 1933, 1948).

The epicuticle of *T. autumnalis* is a uniform limiting membrane, which is amber in colour. It is less than 1μ thick. When this mite was pretreated with cold chloroform and immersed in silver hydroxide solution the entire surface of the cuticle stained brown. The argentaffin reaction demonstrated that a 'polyphenol' layer had been exposed after treatment with the cold chloroform. Cold chloroform is capable of dissolving away an outer layer of wax provided a 'cement layer' is not present. Treatment with hot caustic potash first dissolved the endocuticle before leaving finally a layer which broke up into oily droplets. This layer of the epicuticle termed the cuticulin layer has been provisionally regarded as consisting of a lipo-protein (Wigglesworth, 1948). It was therefore evident that the epicuticle of this mite was composed of a wax layer, a polyphenol layer, and a cuticulin layer.

The epicuticles of both tyroglyphid and oribatid mites could be readily differentiated histologically into two layers. In sections treated with haematoxylin and eosin the sclerotized exocuticle stained reddish. But the cuticle of certain parts such as the capitulum, the legs, the genital plates, the porous plates of large dermal glands, and the bases of setae has no affinity for stains. It retains its natural uniform amber colour. These amber-coloured regions in fact represent the hardest parts of the general cuticle.

The epicuticle of these mites, like that of the harvest-mite, was composed of an outer wax layer, a polyphenol layer, and a cuticulin layer according to the tests already mentioned.

The lamellated structure of the endocuticle was best seen in sections of these mites either before, or after, they had emerged. Sections of cuticle in wax

treated first with xylene before being immersed in silver solution demonstrated the presence of delicate pore canals (fig. 4).

The provisional cuticles of *T. autumnalis* formed during each phase of development possessed, according to the tests described, a true epicuticle. They were also resistant to concentrated acids and alkalis, another property of the epicuticle.

Before the emergence of either a tyroglyphid or an oribatid mite from its old cuticle or exuvium, the exocuticular region of the new cuticle was already established and tanned. A part of the inner soft endocuticle had also been laid down. The muscles became effectively attached to the cuticle at this time. Most of the endocuticle was secreted after emergence.

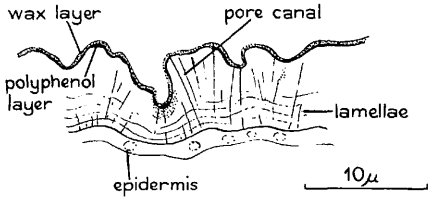

FIG. 4. Section of cuticle of a mite before emergence, stained with silver hydroxide solution.

On the basis of these tests the cuticles of the mites studied were each composed of two essential layers, namely, a thin outer epicuticle, and a relatively thick inner layer, the endocuticle, capable of becoming tanned on the outside to form an exocuticle.

CHANGES IN THE INTEGUMENT DURING MOULTING

The only cellular elements of the epidermis other than the sensilla, which may be regarded as complex, are the dermal glands. In the harvest-mite *T. autumnalis* a pair of obscure dermal glands are situated midway along the dorso-lateral surface of the idiosoma. They are relatively large sac-like structures having a neck region and they protrude well below the epidermal layer. The contents consist of spheres of acidophil cytoplasm and indistinct nuclei. In *Scheloribates laevigatus* there are three kinds of large dermal glands. Eight pairs of multiple glands in the nymph are arranged along the dorso-lateral region of the idiosoma. Each communicates with a sunken multi-perforated oval plate, and each pore is connected by a duct to one of the large pear-shaped cells with its acidophil cytoplasm, a large nucleus, and usually a vacuole. The mass of closely packed pear-shaped cells projects some way below the epidermis into the haemocoel (fig. 5, F). In the nymph these multiple glands, with their sunken porous plate and accompanying seta, closely resemble the pit glands of *Tenebrio* (Wigglesworth, 1948). In the adult, a relatively deep pit with porous walls is substituted for the plate.

In the same plane as the multiple glands, situated posteriorly, is a pair of dermal glands of quite different appearance which may be termed the brown glands, because on deterioration they produce a brown exudate. The gland is oval, and consists of large cells, with acidophil cytoplasm, and weakly basiphil nuclei, which surround a cuticle-lined lumen communicating with the exterior through a pore of a raised tubercle (fig. 5, G). A pair of contiguous dermal glands are closely pressed against the anterior face of the perforated cuticle of the body.

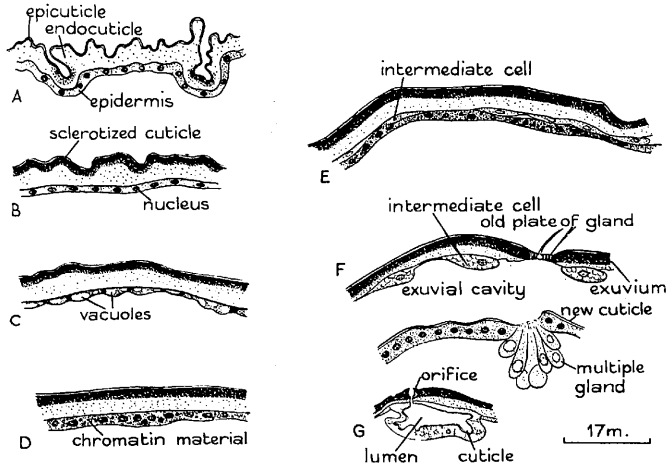

FIG. 5. Changes in the integument of the final nymph of an oribatid mite from the time of emergence to the metamorphic moult. A, pre-emergence; B, early growth phase; C, mid-growth phase; D, late-growth phase; E, beginning of moult showing the epidermis in the process of dividing; F, intermediate cells lying against the old cuticle, and the persistent epidermis laying down fresh cuticle; G, 'brown' gland.

In *Scheloribates laevigatus* the histological changes that take place in the integument have been observed particularly during metamorphosis to the adult stage, and in the harvest-mite *Trombicula autumnalis*, during the development of the fully engorged larva to the newly emerged nymph. Individuals were fixed in hot Bouin and stained with Mayer's haemalum and eosin, at early, mid, and late periods of growth and developmental phases.

The epicuticle and endocuticle of the tritonymph of *Scheloribates laevigatus* on emergence are deeply folded, the exocuticle is tanned, and the relatively thick, colourless endocuticle is clearly lamellated. The epidermis is thickened and its nuclei are distinct and evenly spaced. The cytoplasm contains basiphil granules giving the general epidermis an appearance in keeping with its

continued function of secreting new layers of endocuticle after emergence (fig. 5, A).

As the mite grows, the integument distends to accommodate the increased bulk. Midway in the growing phase the folds have been almost flattened out. The exocuticular layer is completely tanned and loses its former distinctly lamellated appearance. The epidermis is reduced to about half its former depth when this mid-growth period is reached. The nuclei are still distinct and evenly spaced, but there is a noticeable reduction of the chromatin granules in the cytoplasm (fig. 5, B).

There is little change in the integument from the mid to late period of growth. The epidermis becomes attenuated and vacuolated and the endocuticle fully extended (fig. 5, C).

The prelude to the pre-adult developmental phase is marked by a thickening of the epidermis and accompanying mitosis and chromatolysis. Great numbers of nuclei are produced, far in excess of those needed, and there is an accompanying profusion of deeply staining chromatin granules (fig. 5, D).

During the early period of a developmental phase the epidermis differentiates into two distinguishable regions—an inner one, containing most of the nuclei, in all stages of mitosis and chromatolysis, and the chromatin granules; and an outer one next to the endocuticle, distinguished from the inner one by its clear acidophil cytoplasm and the absence of basiphil granules. The outer region further differentiates into a layer of flattened cells, each with a single nucleus and non-granular cytoplasm (fig. 5, E). These flattened cells, spindle-shaped and attenuated in side view and rounded in surface view, separate from the underlying epidermal region. Whereas these cells adhere to the old endocuticle, the persistent epidermis retracts and leaves in its wake an exuvial cavity (fig. 5, F).

The persistent epidermis, with equally spaced nuclei, thickens, and two distinct, superficial, epicuticular layers are secreted. These outer and inner layers of the epicuticle in sections are probably the wax and polyphenol layers respectively. The flattened cells, still adherent to the endocuticle, change from spindle-shape to oval, and in side view become more attenuated. The old endocuticle is generally dissolved away. It is significant that these cells, called by former observers the intermediate cells because of their presence in the intervening space between the old and new cuticle, can often be observed with their boundary wall having slightly migrated into the endocuticle (fig. 5, F).

During the late period of development, comparatively few of these cells persist and lie against the exuvium. This consists, finally, of the exocuticle and the epicuticle and some unabsorbed moulting fluid adhering to the inner surface. Before the eventual degeneration of these intermediate cells, an intense vacuolization of their cytoplasmic components accompanies the dissolution of the endocuticle. By the time the intermediate cells have virtually disappeared and when the dissolution of the endocuticle has been completed,

the epidermis has already laid down the potential adult exocuticle. It is amber-coloured and distinctly lamellated before emergence.

On emergence, the exuvium is shed in a dry state; it is therefore reasonable to suppose that, as in insects (Wigglesworth, 1933), the exuvial fluid is re-absorbed by the mite. The epidermis, at least in some regions, begins to secrete the endocuticle before emergence, but the remaining bulk of this part of the cuticle is secreted afterwards.

In a harvest-mite, the epidermis of the unfed larva is very attenuated and the epicuticle is minutely folded. The distension of the integument of the engorged larva results in a flattening of the endocuticle.

The ensuing process of changes in the integument follows on the lines of that described for *S. laevigatus* except that only the epicuticle resists dissolution and remains as the larval exuvium. When the developing mite assumes the shape of the nymph, an epidermal splitting takes place and the intermediate cells separate with the shed cuticle (the provisional cuticle). This provisional cuticle, which persists, is thin and the intermediate cells lie against it, in most regions, as a thick well-formed layer until just before emergence (fig. 6, c). Histological evidence suggests that the intermediate layer of cells secretes enzymes in the moulting fluid which bring about the granular disintegration of the provisional cuticle when the nymph emerges. It is equally interesting that the active enzymes which are released, are capable of dissolving the epicuticular larval exuvium (Jones, 1951).

The deposition of a provisional cuticle by the epidermis, the persistence of the intermediate cells as a layer until up to the time of emergence of the next stage, and the granular disintegration of this intermediate cuticle at the time of emergence when the larval cuticle is shed in a dry state, are features which distinguish the moulting process of a harvest-mite from that of tyroglyphid and oribatid mites.

The changes that take place in the multiple glands are cyclical. During each growth phase these glands appear to attain a ripeness and compactness of structure which suggest it is the time when they are fully functional. When the epidermis differentiates and retracts, the ducts of these glands separate from the pores of the old plate and they are exposed to the active enzymes of the moulting fluid which dissolves the endocuticle (fig. 5, F). If we accept the view that living tissue is unaffected, it follows that the open ducts and exposed cells have the capacity to resist the enzymes of the moulting fluid.

The glands deteriorate, the cytoplasm of most cells becomes increasingly vacuolated, and the cytoplasmic components of some cells completely disintegrate as they drift into the haemocoel, where only the chromatin material persists for any length of time. These multiple glands, although they lose their compactness, do not break down completely, since some cells appear to persist and divide to form a new gland; the process being principally one of rejuvenation. When the next stage emerges, these glands are completely formed.

The 'brown glands', like the multiple glands, retain their identity during

the developmental phases. As the epidermis retracts, the duct of the gland breaks away from the tubercle. The cuticle lining the lumen of the gland is shed and the general deterioration of the cells is accompanied by mitosis and chromatolysis; a golden brown exudate is ejected into the haemocoel. The anterior dermal glands also undergo a process of rejuvenation and, like the other dermal glands, they are completely reformed by the time the mite is ready to emerge. The function of these glands is very obscure. Similar glands in beetles are thought to be aphrodisiac (Wigglesworth, 1948), but since these glands, when present in mites, appear to be equally functional in all the juvenile stages, it is doubtful if they perform the function ascribed to the glands in beetles. It is more reasonable to suggest, in the case of mites, that the exudate of the multiple glands is an attractant, for promoting aggregation within the community, the function being thus comparable with that of the scent glands of social insects.

The Relation of Cuticle Deposition to Muscle Development

The almost complete loss of segmentation in mites has resulted in the muscles being distributed as simple systems. However, there is a considerable development of dorso-ventrally placed integumentary muscles. Other integumentary muscles, each attached at two sites upon the cuticle, extend in an antero-posterior direction. These are quite well developed, but scattered in their distribution.

A need for describing the different systems of integumentary muscles does not arise in this paper. The changes in the muscle systems illustrated in fig. 6 have also been thought sufficient to clarify the intelligibility of the views expressed because the process of differentiation was apparently similar in all the integumentary muscles.

In functional muscle the sarcoplasm stains pink and the chromatin of the large oval nuclei stains dark blue with Mayer's haemalum and eosin. Its connexion with the cuticle is usually obtained through intermediary processes known as fibrillae. These are derived from the epidermis (fig. 6, A and B). Tendons are also present in mites.

When muscles obtain effective attachment to the cuticle their transverse striations show up clearly. Their disappearance in old muscles is a sign that the connexion with the cuticle has been severed. Thereafter the old muscles differentiate.

The muscles of the larval stage of a harvest-mite do not sever their connexions with the cuticle or begin to disintegrate until about 6 to 12 days after complete engorgement (fig. 7). The leg muscles became reduced to clumps of myoblast tissue at the bases. The degenerate integumentary muscles retained their shape with regions of myoblast tissue connecting them up to the epidermis (fig. 6, B).

When the mite becomes immobilized owing to the severing of the muscles from the cuticle, it enters the phase of development of the next stage. In the

harvest-mite this state of immobilization presaged the beginning of the nymphal stage.

The initial period of development was marked by regrowth (fig. 2). This increase in size was accompanied by a change of shape. The mite became elongated. This was followed by the epidermis moulding itself into the shape of the nymph (fig. 7). The mould, having the same spatial relations as the future nymph, now operated as a guide for the developing muscles. Hinton (1949) reviewed theories on this problem in insect metamorphosis.

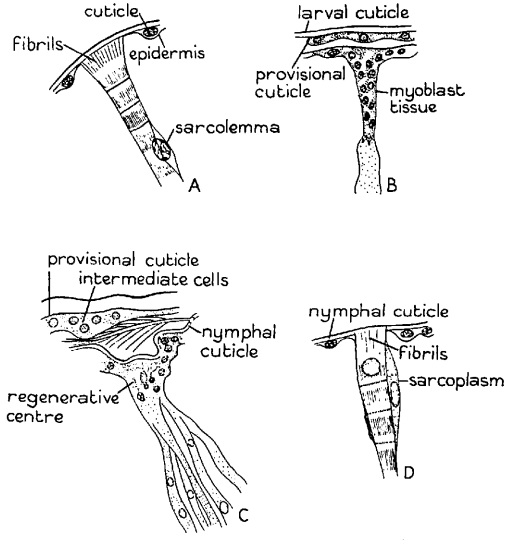

FIG. 6. Changes in skeletal muscle during the moults accompanying an atypical developmental phase in a harvest-mite. A, striped larval muscle attached to the larval cuticle; B, active region of myoblast tissue intimately connected with the epidermis; C, regeneration of muscle tissue nearing completion; D, striped nymphal muscle attached to nymphal cuticle.

The unfolding muscles are destined to become attached to the integument at predetermined points. They must, of necessity, be regenerated from the centres of myoblast tissue (fig. 6, B and C) and bridge the distances between these predetermined points. Now it so happened in the harvest-mite, that these distances increased considerably during the early part of the phase of development, when new muscle tissue had already begun to form.

FIG. 7 (plate). Changes in form and the expression of the pupal state at each phase of development in the growth of a harvest-mite. All figures are at the same magnification. The larval stage shown in A is 0·25 mm. long. A, unfed larva. B, larva extended after engorgement. C, nymphal 'pupation'. D, nymph. E, adult 'pupation'. F, adult.

When the second moult occurred, the proliferation of new tissues by the different regenerative centres was still in progress. By this time the different systems, for example the gut and the various glands, were composed entirely of new cells. But despite their having obtained their proper form and dimensions, differentiation of the various systems had not yet been completed. The muscles were unstriped and the myoblast centres were still active (fig. 6, C).

The epidermis on shedding the first-laid cuticle, redeposited fresh cuticle with which the muscles gained effective attachment (fig. 6, D). It therefore seemed reasonable to assume that the moult was aimed towards the achievement of this attachment of the muscles to the cuticular skeleton. It was also reasonable to suppose that since the other internal systems, at the time of the moult, were progressing towards the final phase of their development, they would not appear to need or to gain specifically from the induction of this extra moult.

Towards the end of the period of regrowth the epidermis fashioned a guiding mould for the developing internal systems. The fashioning of the mould would therefore appear to be postponed until this process of regrowth had been completed. It is also legitimate to assume, in the case of the harvest-mite, that this first mould was imperfect, because the pliability of the integument could have been restricted by the presence of cuticle initially deposited soon after the beginning of the moult.

The important point that calls for comment is that the developing muscles must perforce extend over distances which were substantially increased during the period of regrowth. This task, laid upon the muscles, probably puts their development out of relation to the redeposition of cuticle. The implication is, that the regenerative centres must continue to proliferate tissue, beyond the time when the first-laid cuticle would have reached the threshold for allowing the attachment of the muscles. This conception would certainly appear to be valid on the basis of the muscle tissue being still proliferated while the moult of provisional cuticle occurred (fig. 6, C).

When development is typical, the formation of the muscle-ends coincides with the development of the fibrillae, which in turn become integrated with the first-laid cuticle. Only the one moult is therefore necessary. However, in the harvest-mite the development of the muscles takes relatively longer, which puts it out of relation to the rate of deposition of cuticle. To counteract this disharmony between the systems the first-laid cuticle was shed. Thereafter the rate of deposition of fresh cuticle was attuned to the final steps in the development of the muscles and the fibrillae.

During metamorphosis the same sequence of events takes place. The nymph assumes an elliptical shape on engorgement and growth takes place once again when the adult is first developing (fig. 7). In fact the same set of conditions that induce an extra moult of provisional cuticle were present during metamorphosis.

A provisional cuticle is also formed during embryogenesis. It is similar and corresponds to the provisional cuticles of the post-larval stages. The occurrence of this embryonic provisional cuticle suggests that pupa-formation is

expressed within the egg. It is noteworthy that the embryo of a harvest-mite swells considerably and breaks through the egg. However, the embryo invested with its provisional cuticle remains in place (Michener, 1946). This period of growth would appear to correspond to the period of regrowth which takes place during the early part of a post-larval phase of development. The set of conditions which appear to induce pupa-formation in later development are therefore apparent during embryogenesis.

Discussion

In the light of the observations described in this paper, it seems reasonable to suppose that the moulting process in mites is unique. The production of a provisional cuticle in some mites would also appear to be the critical factor for distinguishing pupa-formation from a typical phase of development.

The provisional cuticle and the intermediate cells in the harvest-mite are homologous with the 'apoderma' and the isolated cells respectively, noted in *Trombidium fuliginosum* by Henking (1882), and the 'Zwischenhaut' and 'haemamoebae' of *Atax bonzi* observed by Claparede (1868). But hitherto the significance of the cells was not understood. Moreover, indefinite speculation on their origin and function only led to confusion.

In the decapod Crustacea, Yonge (1932) noticed nuclei in the exuvial cavity during the shedding of the old cuticle of the oesophagus of *Homarus*. But he was unable either to distinguish associated cytoplasmic components, or to discover the origin of the nuclei. He suggested, however, that they appeared to play a part in softening the 'chitin' of the old cuticle. The moulting process in mites probably explains the origin and function of the nuclei detected in the exuvial cavity of *Homarus*.

Browning (1942) in his work on the spider *Tegenaria atrica* came to the conclusion that the cells found in the exuvial cavity were discarded 'granulocytes' which had functioned at the previous moult in laying down the new 'exocuticula'. The figures of Browning are very definite, but it seems unusual that a discarded cell should have to maintain itself from one moult to the next in the epidermis before migrating into the exuvial cavity to undergo disintegration. These 'granulocytes' in the spider bear a striking resemblance to the moulting cells described in this paper. It is therefore tempting to suggest that they may have a similar origin and function.

In view of the observations on the process of moulting in mites it would appear that the intermediate cells are capable of excavating the cuticle. They also become vacuolated and enlarged, thus assuming the appearance of secretory cells. It is therefore not amiss to suggest that they secrete the chitinase and protease enzymes responsible for the dissolution of cuticle.

So far, I have failed to locate, either histologically or chemically, dermal glands equivalent to those which, in insects, are both the possible source of the enzymes and the definite source of the 'cement layer' of the epicuticle (Wigglesworth, 1948). Since a cement layer is not present in the epicuticle of the mites studied in this work, the absence of such glands is understandable.

The reasons for the unique moulting process in mites are not easy to fathom. The clean separation of the cuticle from the epidermis would appear to be a simpler operation. In view of the exuvial cavity in mites being relatively very spacious (fig. 3), it is tempting to suggest that in these small animals the dissolution of the sometimes extremely hard cuticle is made more certain by the moulting cells being placed against the surface of the cuticle. The effusion of enzymes from these cells would ensure greatest activation at the exposed surface of the cuticle.

The reasons for the production of a provisional cuticle during a phase of development in some mites have been tentatively put forward in the last section of this paper.

Atypical development or pupa-formation in mites implies that the mite during its development becomes enclosed by a provisional cuticle, having more or less the spatial relations of the final mould of the stage concerned. Strip the mite of its provisional cuticle and pupa-formation ceases to exist. It would also be legitimate to translate this proposition in terms of holometabolous insects. Poyarkoff (1914) postulated that only the developing muscles would appear to be in need of the extra moult which produced a surrounding provisional cuticle. In mayflies, for example, the provisional cuticle (the cuticle of the sub-imago) is shed in order that a few not quite fully formed thoracic muscles only may gain proper attachment to the cuticular exoskeleton. Pupa-formation in these insects at least is therefore even more direct than it is in the harvest-mite.

The production of an embryonic provisional cuticle is not uncommon in arthropods. It is probably universal among insects (Wigglesworth, 1950). Some spiders, and the nauplii of certain Crustacea on emergence from the egg, are, like a harvest-mite, invested with a provisional cuticle. Whether or not this condition during embryogenesis can be regarded as a form of pupation is open to question.

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