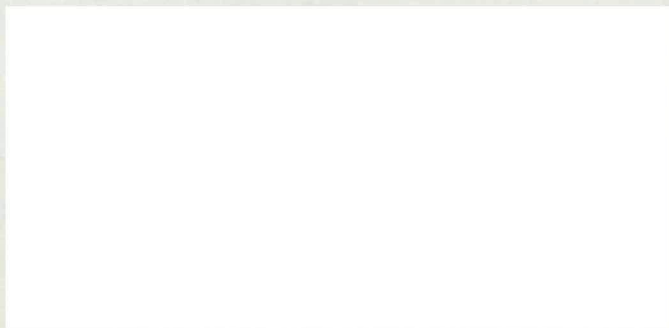


STUDIES ON THE HYPOPIUS FORMS
OF MEMBERS OF THE ACARINA



Thesis presented for
the Degree of Ph.D.
in the University of Edinburgh.



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MADE IN GREAT BRITAIN

INTRODUCTION.

The spontaneous development of the peculiar hypopus forms in the Acarina is mainly confined to members of the Tyroglyphoidea. The normal sequence of stages in the life-cycle of these mites consists of the egg, the larva and two nymphal stages, which intervene before the adult state is reached. But, this sequence is liable to be complicated by the spontaneous occurrence, between the nymphal stages, of a peculiar arrest in normal development expressed as the so-called hypopus form.

When this hypopus form does occur, it invariably does so at this juncture in the life-cycle, but, the actual form in which it is expressed varies according to the species. In fact, it has been possible to grade them into a linear series. At one extreme, the hypopus condition assumes a cyst-like state, at the other, a shield-shaped well sclerotised mobile form, utterly unlike the normal form of the mite that produced it. A range of intermediate forms link these two extreme conditions (Oboussier 1939).

It has recently been pointed out that mites appear to fall into one of two natural groups according to their type of development. Those of one group are distinguished by exhibiting typical ecdyses, those of the other, by atypical ecdyses involving pupa-formation (Jones 1950). Tyroglyphid mites belong to the former group, thus they moult typically as each stage is developing. The same worker has also proposed that each stage consists essentially of a developmental phase and a growth phase (mobile).

It will now be clear that in tyroglyphid mites the developmental phase immediately following the protonymph stage is /

is aimed towards the realisation of either the normal deutonymph or the hypopus form. The production of either is accompanied by a single moult.

The hypopus condition has been accepted by acarologists as an extra nymphal stage, when it occurs, either in the cyst-like state, or as the mobile shield-shaped form. Reuter (1909), for example, regards the hypopus form as being equivalent to the deutonymph. Interest, in fact, had been centred upon whether or not the form was equivalent to the deutonymphal stage when it occurred, thus querying the validity of regarding the second nymphal stage which followed, as the deutonymph. Opinions differed, but, the view most upheld, was that the condition was a spasmodically produced stage occurring between the protonymph and the deutonymph. Its main function appeared to be concerned with distributing the species, since the hypopus forms were adapted for attachment to larger arthropods and other animals, that provided them with an opportunity of hanging on. The bulk of scattered evidence on factors which induced mites to develop the condition, left much to be desired with regard to detail and care in experiments. But, the indication was that harsh conditions in the environment effected a change from the normal state to this hypopus condition. A genetical explanation had also been put forward, as a speculation, to account for it. Solomon (1943) in his review of tyroglyphid mites made it clear, consciously or otherwise, that much confusion existed over the modulating effect of the environment, and that the hypopus form was still something of an anomaly to the acarologist. It was certainly true that the exact nature of the hypopus condition had not been satisfactorily defined.

The chief aim of the present thesis has therefore been an attempt to define the essential nature of the hypopus condition by investigating the morphogenetic movements which are directed towards the achievement of the hypopus form. Before this work was begun, there seemed a possibility that, at least the cyst-like hypopus state, was the result of arrested development. In other words, there was a halt during the developmental phase of the deutonymph and the mite persisted in the cyst-like condition. Whereas this view was the mainspring for carrying out investigations, it by no means explained the development of the shield-shaped form, which was so utterly unlike the normal form, and obviously so well adapted for carrying out its specialised task of distributing the species.

The second complementary aim has been to investigate the influence of the environment upon hypopus development. But it has been done only to an extent sufficient to find out if there is a first causal connexion between external factors and the arrest of development, and, furthermore, if such factors break the persistence of the hypopus condition once it has been established.

There is perhaps little or no need to emphasise at the outset, that the hypopus forms of Histiostoma polyperi Oud. (mobile form), Glycyphagus destructor Schr. (intermediate form), Glycyphagus domesticus de Geer (cyst-like form), which were chosen for investigation, being minute in size and equipped with a tough or hard cuticular exoskeleton, provided a difficult medium for histological studies. It is perhaps reasonable to suggest that the disadvantages they presented as a medium for study may have been /

been responsible for little previous investigations of this kind.

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MATERIALS AND METHODS.

Rearing Arrangements.

This section is concerned primarily with these methods, which were adopted for providing mite material (H. polyperi, G. destructor and G. domesticus) at known steps in the development of protonymphs into hypopi, and, of the hypopus forms back to the normal state. Descriptions of the arrangements for carrying out appropriate experiments have been included in those sections of this paper dealing exclusively with the exact nature of the experiments and the results obtained.

Stock colonies of G. destructor and G. domesticus were housed in shallow dishes (3 $\frac{3}{4}$ " x 2" x 1" deep), having ground edges and fitted glass covers. To provide the appropriate humidity requirements, the glass cover was sealed to the edges of the dish with vaseline, and the underside of the cover was wetted with a drop of water once a week or as required to retain a relative humidity sufficiently high, but without encouraging excess fungus growth. A thin slice of cork was placed in the dish to encourage hypopi, which show a predilection for collecting in crevices, to aggregate in the holes and grooves of the cork itself. This attraction of hypopi to the cork was useful, because removal of the piece of cork with attached mites, eliminated the laborious task of hunting for scattered individuals among the colony. It was customary to introduce a small quantity of flour, infested with the required species, into the dish, when setting up a fresh stock. The atmosphere inside the dish was aerated by simply removing /

removing the cover at regular intervals.

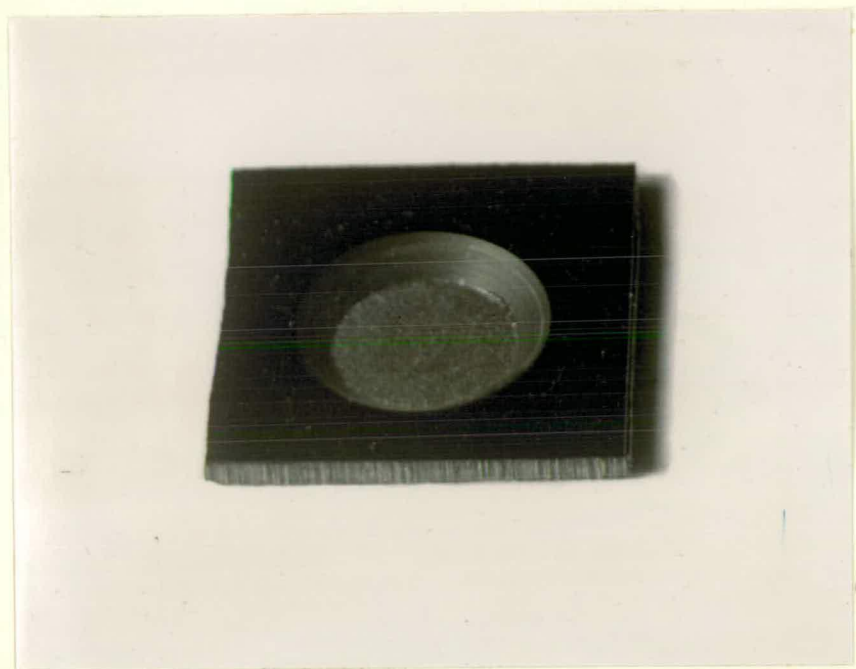
The humidity and food requirements for colonies of H. polypori were different. The colonies were kept in Petri dishes. A Petri dish for housing H. polypori was first prepared by placing a moist layer of cotton wool on the floor of the dish; a piece of black filter paper was then pressed on to the surface of the cotton wool. The colony fed and multiplied best on cut portions of dead earwigs; small quantities of decaying vegetable matter were added intermittently.

Now, it so happens, that even when a colony is thriving under the influence of a favourable food supply, and a high humidity, small numbers of hypopi are produced. Should the colony become exceptionally crowded, the hypopus production is liable to increase. But, for experimental purposes, large numbers of hypopi were often required, and the necessary numbers were produced by modulating the environmental conditions. This was done by simply neglecting the culture. The atmosphere became slowly drier, and consequently, the food lost its moisture content. It was precisely these conditions which, at least indirectly, promoted the development of a colony dominated by large numbers of hypopus forms. As long as the harsh conditions were maintained the colony consisted almost entirely of hypopus forms.

In order to examine the progress of the development and growth of mites under the microscope, sub-cultures were kept in filter paper cells. Each cell consisted of a Perspex framework (with 4 cm. sides, 0.3 cm. thick and with a central hole with bevelled sides). Filter paper was fixed over the narrower diameter of the hole with Durofix; a cover glass was placed over the wider opening to enclose the rearing chamber (Plate I).

Rearing /

PLATE I. The perspex cell (see text for details).



Rearing cells containing H. polypori were housed in Petri dishes prepared as described above. Those containing G. destructor and G. domesticus were likewise placed in the type of dish used for rearing a stock colony. The appropriate humidity was maintained inside the cell by placing the filter paper floor of the rearing chamber either upon, or immediately above, the thin layer of flour in the dish.

The Distinguishing of Mites in Different States of
Growth and Development.

It is legitimate to assume that a hypopus form begins when the protonymphal cuticle loosens from the epidermis. But it was necessary to examine carefully protonymphal stages to try and discover at a much earlier time, discriminating changes, by the examination of whole organisms, that would point to the subsequent development being directed towards, either a normal deutonymph, or a hypopus form. Fortunately, it was possible to identify without much difficulty, growing protonymphs, except those of H. polypori, that were destined to change into hypopi. Moreover, the identifiable changes began to show about three quarters of the way through the growth phase of the protonymph. Consequently, changes linked with differentiating processes aimed towards the realisation of the hypopus form could be followed from the last quarter of the growth phase of the protonymph, and further changes could be observed up to the time of emergence of a normal deutonymph from the cuticle of the hypopus. In order to obtain histological information on the progressive sequence of events concomitant with the differentiation of a protonymph into a hypopus, and of the hypopus to a deutonymph, the following growth and /

and development phases were chosen for histological examination, 1, protonymph midway through the growth phase before any sign of the change to deutonymph or hypopus could be detected, 2, protonymphs about to change into hypopi, 3, hypopi in early and late steps of the developmental phase, 4, stable hypopi, 5, hypopi showing signs of change to the next stage, 6, deutonymphs in early and late steps of the developmental phase and 7, newly emerged deutonymphs. Serial sections of the chosen material were cut in sagittal, horizontal and transverse planes.

The varying steps in the progress of development and growth of G. destructor were identified in the following ways. Protonymphs, fixed three days after emergence at 20°C., corresponded to the mid-way mark of the growth phase. Late protonymphs, destined to change to hypopi, were easily distinguishable because their shape resembled that of the hypopus case and because of the characteristic net-like pattern of the cuticle (Fig. 1A). The early developmental phase of the hypopus was detectable owing to the mite's immobility, and it would not move even when stimulated. The net-like pattern on the cuticle was also more definite and there was a slight retraction of the soft body from the anterior end. In the late developmental phase of the hypopus the soft mite had assumed the definitive oval shape of the hypopus (Fig. 1B). The stable hypopus was oval in shape and the whitish, somewhat opaque, protonymphal cuticle now possessed an even more definite net-like pattern. When the hypopus was removed from its case and viewed from the ventral surface, a large number of globules could be seen in the haemocoel (Fig. 1C). When the hypopus was about to change to a deutonymph the globules broke up and the anterior part of the mite became more transparent, and the /

FIGURE 1. External views of whole mites of G. destructor
and of the cuticle of G. domesticus.

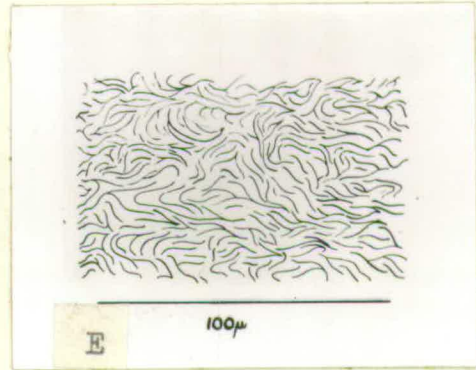
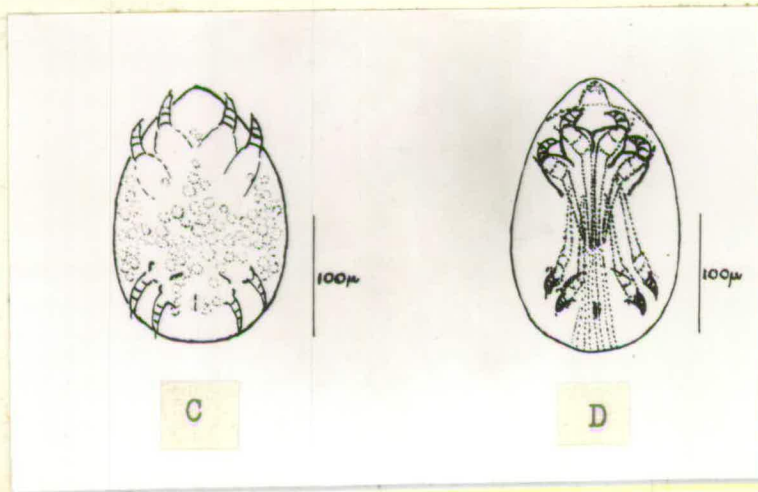
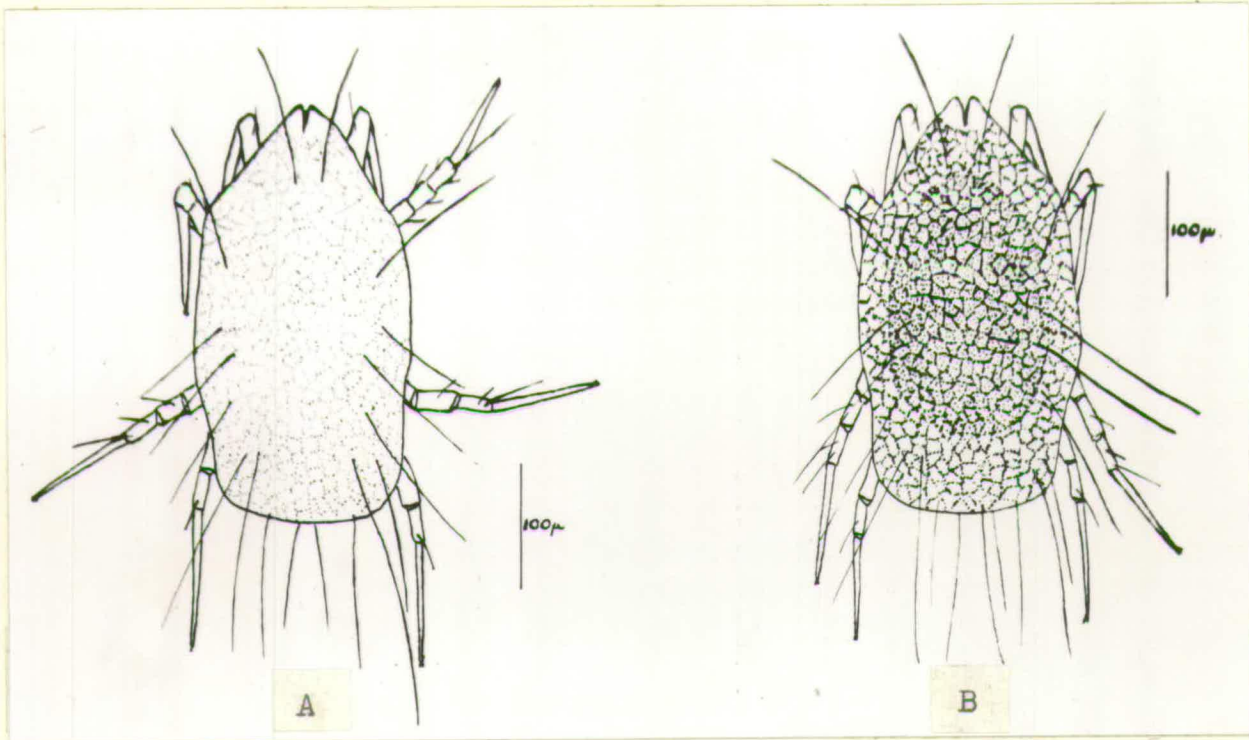
A, Dorsal view of a late protonymph.

B, Dorsal view of a late developing hypopus mite.

C, Ventral view of a hypopus mite.

D, Ventral view of a mite changing from its
hypopus condition to the deutonymph.

E, The surface of the cuticle of a late protonymph
of G. domesticus.



the posterior part darker. But the mite at this juncture responded to touch by moving its legs. The early developmental phase of the deutonymph was recognised by the complete absence of globules, a further increase in the density of the posterior region and the inability to react to stimulation. Late development of the deutonymph was recognised by the outline of the deutonymph visible inside the hypopus cuticle (Fig. 1D). Finally, deutonymphs were fixed within six hours of emergence from the hypopus cuticle.

The steps in development and growth of G. domesticus were identified in much the same way as those of G. destructor, except that the appearance of the protonymph cuticle was different and the hypopus lacked legs. The pattern of the protonymph cuticle, for example, is due to conchoidal markings (Fig. 1E) and, when it forms the outer hypopus case, it is more transparent than that of G. destructor. The absence of legs in the hypopus eliminated the possibility of using a stimulus to produce leg movement as an indicator to help in distinguishing the late hypopus from the early developing deutonymph.

Protonymphs of H. polypori mid-way through the growth phase were obtained by fixing protonymphs about two days after emergence from the larval cuticle. As already mentioned, late protonymphs about to change into hypopi could not be distinguished from those changing to deutonymphs. Developing hypopi could be recognised, only when the outline of the hypopus had become visible inside the protonymphal cuticle, the earlier stages being indistinguishable from developing deutonymphs. Newly emerged hypopi were fixed within two hours of emergence before the cuticle had hardened completely, to minimise damage to the sections by the cuticle tearing. Hypopi about to change to deutonymphs

were identified by a slight swelling of the hypopus and the appearance of excretory crystals in the haemocoel. Considerable swelling of the hypopus with the legs pointing straight out at right angles to the body indicated the start of the change to deutonymph. In the late developmental phase of the deutonymph the outline of the deutonymph was visible inside the hypopus cuticle. Deutonymphs were fixed within six hours of emergence.

Histological Techniques.

Fixation of the soft tissues of arthropods, encased, as they are, with a hard exoskeleton, always presents the problem of employing a method which will enable the fixative agent to first penetrate the covering cuticle. The small size of mites, with its accompanying high ratio of surface to volume, adds to this difficulty of cuticle penetration especially in the case of hypopi with their hard brown cuticle (H. polypori) or resistant thick cuticle (G. destructor and G. domesticus). My colleagues in the Department have previously obtained the required success of fixing mite material by simply heating the fixative, a necessary prerequisite for breaking down the resistant property of the cuticle; cold fixative fails to penetrate.

The mite material used in the present work was fixed in hot alcoholic Bouin. H. polypori and nymphs of Glycyphagus spp. were fixed by immersion in fixative at 50°C., the fixative being thereafter kept in a small tube at 48°C. for twenty four hours. Developing hypopi, hypopi, and developing deutonymphs of Glycyphagus spp. were first immersed in cold fixative, and only afterwards, was the fixative slowly heated in an oven kept at a temperature /

temperature of 48°C. This treatment for these stages of Glycyphagus spp. was essential, because immediate immersion in Bouin at 50°C. caused individuals to burst.

The alcohols used in the dehydration of the material were also kept at 48°C. Two hours in 90% alcohol, with one change, were followed by one hour in 96% alcohol. Since the material became almost completely transparent by this time, it was further treated with a solution of 0.5% eosine in 96% alcohol, the material usually taking up the stain sufficiently in about half an hour. It is imperative that the material is coloured with the stain at this stage, because otherwise, when it comes to orientating the mites in the molten wax, it is difficult even to see them, and therefore, almost impossible to move them into appropriate positions for subsequently obtaining sections in the required plane. Nymphs in general, but hypopi and developing deutonymphs of H. polypori only, were then washed in 96% alcohol and cleared in amyl acetate for half an hour before a further change to fresh amyl acetate in which the material was left overnight. Developing hypopi, hypopi and developing deutonymphs of Glycyphagus spp. and the developing hypopi of H. polypori were not cleared in amyl acetate owing to the detrimental effect of this agent upon them. Instead, this material was subjected to treatment with various clearing agents, before a somewhat specific technique was elaborated to prevent irretrievable damage due primarily to collapse and shrinkage of the material. It was reasonable to assume that this collapse could be accounted for by the differential rates of diffusion of alcohol on the one hand and the clearing agent/

agent on the other, the alcohol diffusing out through the cuticle more rapidly than the denser clearing agent could diffuse into the material. Support was given to this assumption when it was found that, on testing various clearing agents, for example, cedarwood oil, xylene and benzene, the one having the highest density caused the most rapid and extensive collapse of the material. Prevention of shrinkage of the soft tissues was immensely important, but to some extent apparent collapse was unavoidable if the treated mites possessed relatively large unsupported exuvial cavities at the time, or even a haemocoelic space in a state suitable for accommodating a relatively large volume of liquid agent.

Benzene has a density value nearer to that of the alcohols than the other clearing agents that were available, and so it was used in the treatment of material which had this tendency to collapse and shrink if exposed to a change from alcohol to a clearing agent as normally carried out. The material was rinsed in absolute alcohol after staining in the eosine solution, before being left in absolute alcohol for half an hour. The material was transferred to a solid watch glass, containing about $1/3$ cc. of alcohol, which was placed under a binocular microscope. Benzene was added to the alcohol drop by drop from a pipette, the mites at the same time being observed through the microscope. Each drop of benzene was thoroughly mixed with the alcohol before the next drop was added. A brown coloration formed at the interfaces of the newly added benzene drop and the alcohol-benzene mixture. This was due to a reaction between the pure benzene and the water taken up by the alcohol on exposure to the air and it indicated the near completion of the clearing process.

When /

When this coloration appeared, a mixture of benzene and alcohol was prepared in a separate watch glass by adding a few drops of absolute alcohol to about 1/3 cc. of benzene. In order to prevent shrinkage of the mites when they were transferred to the new mixture, the density of the mixture had to be approximately the same as that of the liquid containing the material. If the density of the drop of mixture was correct it produced no reaction when added to the liquid. The drop spread rapidly over the surface of the liquid if the density of the mixture was too low, in which case benzene was added to the mixture until the reaction of a drop of mixture added to the liquid containing the material indicated that the density was correct. If the density of the mixture was too high the drop sank through the liquid, then spread over the bottom of the watch glass before mixing with the liquid. When this occurred alcohol was added to the mixture until the density was correct.

The material was transferred to the new mixture of the correct density, to which benzene was added, two or three drops at a time, until the mixture was practically all benzene. Transfer-ence to pure benzene contained in a glass tube at 48°C. was followed by a change of benzene in which the material was kept overnight at the high temperature.

Wax impregnation was carried out by placing the material in 56°C. melting point paraffin wax contained in a solid watch glass. Three changes, each of two hours duration, were made to ensure thorough penetration of the cuticle and proper impregnation of the soft parts.

Embedding was carried out under a binocular microscope
so /

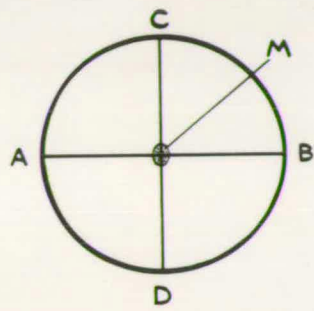
so that the mite could be appropriately orientated in the block to provide sections cut in the desired plane. Owing to the small size of the mites embedding was actually carried out in a glass ring, $\frac{1}{2}$ inch in diameter, which rested on a glass slide. The ring was filled with molten wax and the material, still in some wax, was transferred from the watch glass through the medium of a warm pipette. The material was manoeuvred to the centre of the ring, with a warm needle heated to a temperature slightly above that of the melting point of the wax. At the centre, it was allowed to settle on the surface of a solidifying wax layer, which first forms near to the slide at the bottom of the ring. Care was taken to ensure that a relatively thick layer of wax separated the material from the bottom of the block to protect the wax close to the material from melting when the bottom of the block would be sealed to the microtome chuck. The warmed needle was used to orientate the mite so that sagittal, horizontal, or transverse sections would be obtained when the flat bottom of the block, that is to say, the surface next to the slide would be sealed to the chuck. Four sets of scores at right angles were fashioned at the edge of the hardened block to indicate the two axes of the mite. The slide and the ring containing the wax were placed in water at a depth less than the height of the ring, to prevent a possible overflow on to the wax. On contraction, the upper surface of the wax usually subsided and more wax was added to make the surface flat and so simplify the trimming of the block. When a solid film of wax had formed at the surfaces the whole arrangement/

arrangement was completely immersed in the water.

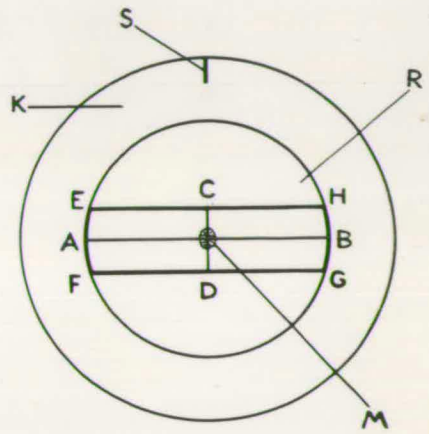
Diameters were scored on the block through the pairs of orientation marks at the edge of the block. The intersection of the diameters lay above the position of the mite in the block and the diameters were parallel to the axes of the mite (Fig. 2A). The block was trimmed and mounted so that in cutting the knife edge would be parallel to the shorter axis AB of the mite. The block was trimmed parallel to and on both sides of the diameter AB until about 1/16 inch of wax remained on each side of the diameter.

A raised platform of wax was fashioned upon the chuck and the vertical surface of it was trimmed flat by the microtome knife. The flat bottom of the block was placed on the platform with the intersection of the scores over the centre of the chuck and the axis AB of the block at right angles to a mark at the edge of the chuck (Fig. 2B). This mark on the chuck was used to aid the correct orientation of the chuck on the microtome.

The wax at the base of the shorter pair of sides EF and GH of the block was sealed to the platform with the aid of a warm needle. The sides EH and FG were trimmed cautiously parallel to AB until the mite was just visible as a dense patch in the wax of each side. Next, the top of the block was trimmed until the dense patch of the mite was visible from the top. Sides EH and FG were trimmed further so that the mite was placed centrally between them, care being taken to ensure that the sides were parallel when trimming was complete, as the cutting edge of the knife would be parallel to these sides. The bases of the sides EH and FG were sealed to the platform with the aid of a heated needle, the needle being /



A



B

being pushed deep into the wax at the base of the block below the region with the mite, to make certain the block was sealed securely to the platform. The sides EF and GH were trimmed parallel to the axis CD until a suitable amount of wax remained on either side of the mite, then the bases of these sides were sealed to the platform.

The chuck was placed on the microtome with the mark at the edge of the chuck on top, and adjusted to get the bottom edge of the block parallel to the knife edge, so that the mite would be correctly orientated for cutting.

Sections of all material except the hypopi of H. polyperi were cut at a thickness of 4μ , the hypopi of H. polyperi being cut at a thickness of 6μ , because the cutting of thinner sections of hypopi of this species only resulted in the cuticle becoming badly torn.

Ehrlich's haematoxylin was used to stain the sections after the eosine had been removed by 70% alcohol. Material of H. polyperi stained properly in twenty to thirty minutes, that of Glycyphagus spp. required an hour. Counterstaining with eosine in 96% alcohol was followed by rapid dehydration and mounting to prevent excess loss of eosine from the sections.

HYPOPUS DEVELOPMENT.

About fifty years ago when zoologists made elaborate excursions into the field of descriptive morphology, Michael (1901) in his monograph on Tyroglyphid mites gave an account of the anatomy of the adult mite. Whereas the descriptive style of Michael made one confident that his observations were carried out with meticulous care, it was not until Oboussier (1939) and Hughes and Hughes (1938) produced their accounts of the anatomy of various tyroglyphoid mites that one could really feel confident about the details of such attempted investigations.

As already emphasised in the introduction of this paper, one of the chief aims has been to investigate the nature of hypopus development and of the reversion or further development to the normal or regular morphological pattern of the mite.

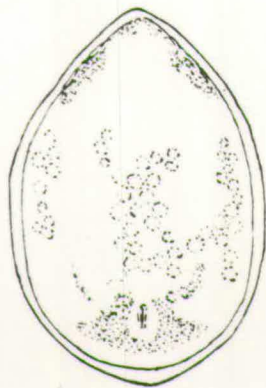
This section has therefore been concerned with examining sequences of histological pictures provided by sectioned material of the mite at different steps in its development to the hypopus form, and also during its further development to the normal state. From these histological pictures it has been possible to trace morphogenetic movements, and elicit from them the true nature of this bizarre condition obtained in tyroglyphoid mites. In hypopus development the fate of the various systems of organs and tissues has therefore been followed, and the state they have obtained in the various types of hypopi, selected for study in this work, has been carefully examined. For convenience, the morphogenetic movements of the different systems of organs and tissues have been treated separately. The external appearance of the three hypopus forms /

FIGURE 3. Ventral views of different hypopus mites.

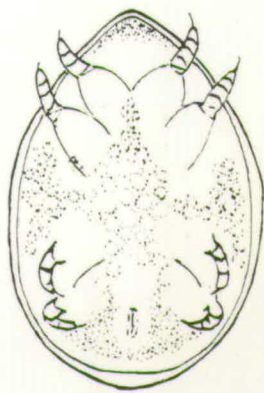
A, G. domesticus; B, G. destructor;

C, H. polyperi.

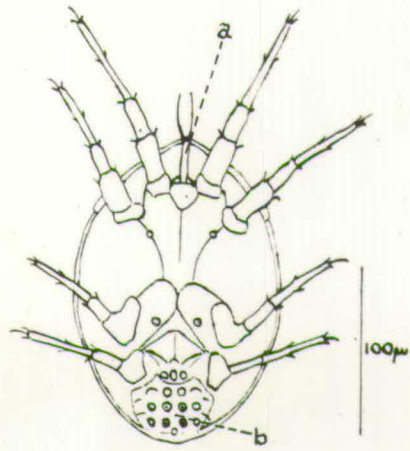
a, atrophied rostrum; b, battery of suckers.



A



B



C

forms selected for study are shown in Figure 3A, B, and C. It will be noticed how the hypopus form of G. destructor, with respect to outer appearance, lies midway between those of G. domesticus and H. polyperi.

Morphogenetic Movements Aimed Towards the
Achievement of the Hypopus Condition.

The behaviour of the integument. When the protonymph of Glycyphagus spp. was due to change to the hypopus form, the surface of the cuticle acquired a distinctive pattern. The epidermis of the late protonymph thickened slightly and this was accompanied by a thickening of the cuticle. A general thickening of the cuticle also occurred in G. domesticus and conchoidal markings appeared on the cuticle surface. In G. destructor the thickening of cuticle was not uniform. It was confined only to a series of ridges which were responsible for the reticular pattern of the surface of the cuticle. These ridges were, in fact, small projections which developed inwards from the endocuticle (Fig. 4A). When fully formed they pushed the epidermis into an undulating layer (Fig. 4B).

At the end of the protonymphal stage the epidermis had thickened further, and budded off the intermediate cells which remained between the epidermis and the cuticle. In G. domesticus the intermediate cells formed a continuous layer surrounding the epidermis. In G. destructor they appeared to be distributed as separate regions between the ridges of the endocuticle (Fig. 4C).

In the early developing hypopus the cuticle loosened from the epidermis and the intermediate cells remained attached to the old protonymphal cuticle which, for convenience, is termed the /

FIGURE 4. Changes in the integument of G. destructor during hypopus development.

- A, Section of the integument of a protonymph showing initial development of the cuticular ridges.
- B, Section of the integument in the late protonymph showing the fully developed cuticular ridges.
- C, Section of the integument showing the intermediate cells prior to the moulting of the protonymph cuticle.
- D, Section of the integument of a mite during early hypopus development and its surrounding hypopus case.
- E, Section of the integument of a hypopus mite.
 - a, protonymph cuticle; b, epidermis;
 - c, cuticular ridge; d, intermediate cell;
 - e, hypopus cuticle.

FIGURE 5. Section of the integument of G. domesticus when in its hypopus state.

- a, hypopus cuticle; b, epidermis;
- c, food granules.

Fig. 4

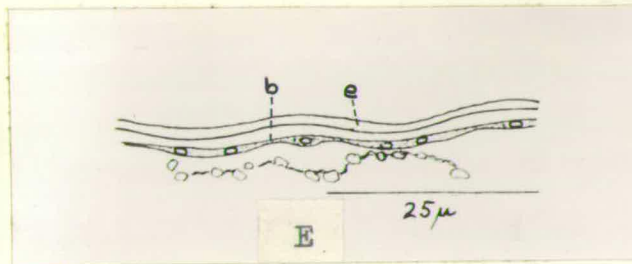
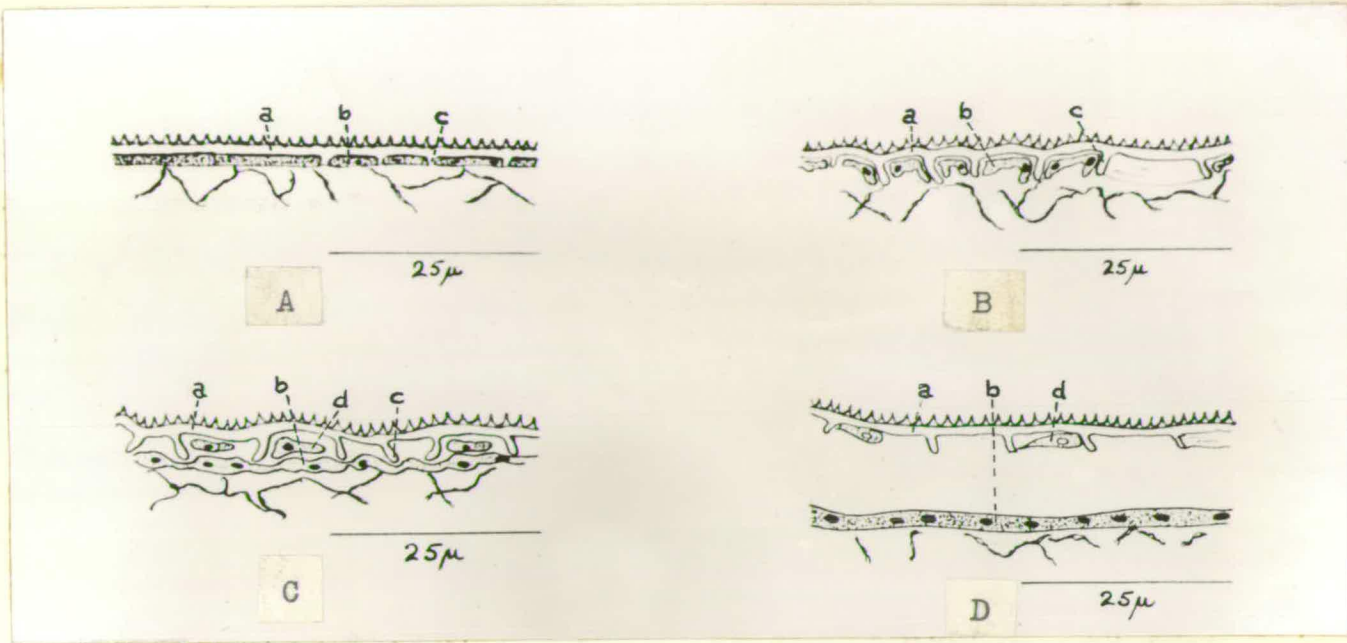
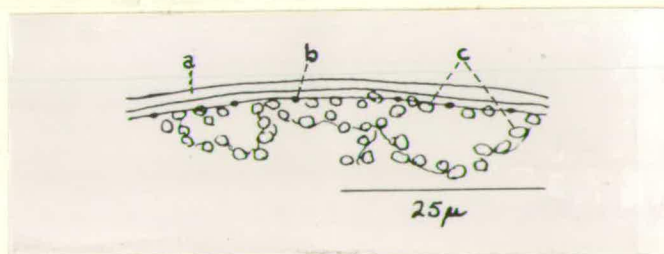


Fig. 5



the hypopus case. (Fig. 4D). The hypopus case of a living specimen was, at this stage, relatively pliable and transparent, the exuvial cavity between the case and the developing hypopus being filled with fluid.

After the cuticle had sloughed off, a retraction of the epidermis, along with the other tissues of the mite, occurred. At this time the epidermis thickened prior to laying down the cuticle of the hypopus, and an epidermal fold arose in a horizontal plane around the hypopus (Fig. 10A). In G. domesticus the mite assumed an ovoid shape and the hypopus cuticle was laid down by the epidermis at this time. Very slight projections developed at the positions corresponding to those where one would expect limb buds to appear. The limbs developed further in G. destructor and by the time the hypopus cuticle was laid down over the general surface, distinctive but short legs were formed.

On the completion of cuticle deposition, the epidermis changed to the thin strand of tissue which persisted throughout the hypopus condition assumed by the mite. In G. destructor the epidermal nuclei stained lightly, but, the cytoplasm retained its normal affinity for the stain (Fig. 4E). In G. domesticus the epidermis became extremely attenuated^(Fig.5). Observations seemed to imply that the fluid in the exuvial cavity evaporated after the hypopus cuticle had been formed. The hypopus case became dry, changed to a whitish colour and assumed a rigid property.

It has already been mentioned that in H. polypori a developing hypopus could first be detected in a culture only at the time when the cuticle was being secreted. At this time it was possible to see the peculiar form of the mite, through the transparent protonymphal cuticle. The striking feature, as already /

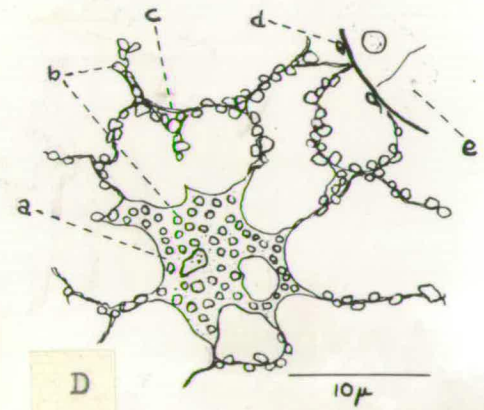
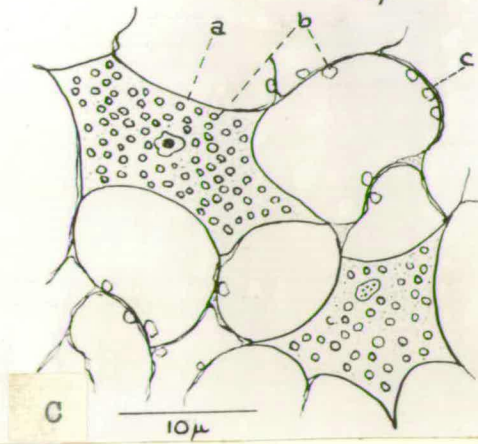
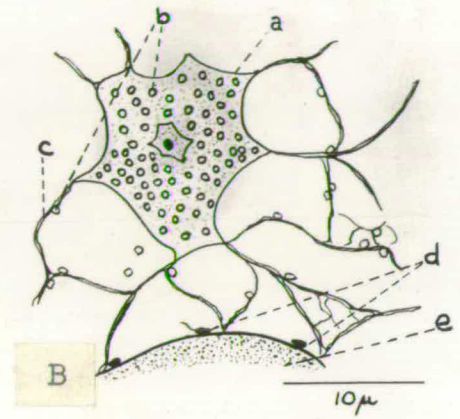
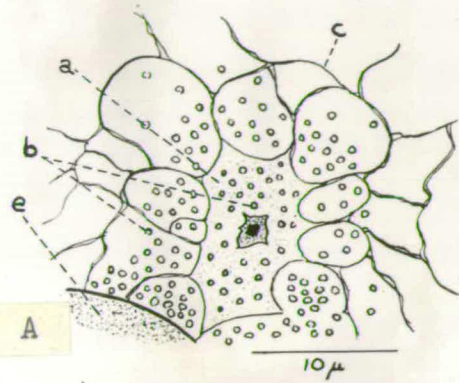
already mentioned, was that the integument had moulded the mite into a shape utterly unlike that of the normal individual. Moreover, the epidermis had given rise to integumental structures, for example, the sucker disc, which were specific to the hypopus mite. The epidermis was very thick in H. polypori and a large number of epithelial cells assembled in the region of the sucker disc. The epidermis of the mite in its hypopus condition retained a normal thickness.

The fate of the parenchyma, the cells, and other bodies in the haemocoel. The main changes which occurred in the haemocoel during the hypopus development were principally, the partial destruction of the parenchyma network of the protonymph, the production of the new parenchyma forming cells and the accumulation of food reserves.

In Glycyphagus spp. the parenchyma network of the protonymph had been partially destroyed at the end of the feeding phase and the stellate phagocytes had become charged with waste, hence increasing their size. In the late protonymph the gut contents were ejected from the lumen of the degenerating gut, and in the haemocoel they appeared in the form of small granules which had an affinity for eosine stain. Large numbers of these granules aggregated around the gut itself and also near the phagocytes which engulfed them (Fig. 6A). The phagocytes became even larger in the early developing hypopus. Their nuclei assumed a more irregular appearance, while their cytoplasm became increasingly filled with the pink granules. The parenchyma network appeared to undergo no further disintegration as the hypopus developed, but numerous circular gaps appeared with the result that /

FIGURE 6. Changes within the haemocoel during hypopus development in G. destructor.

- A, Phagocyte and food granules in the haemocoel of a late protonymph.
- B, Phagocyte and parenchyma-forming cells during early hypopus development.
- C, Phagocytes during late hypopus development.
- D, Phagocyte and parenchyma-forming cells of a hypopus mite.
- a, phagocyte; b, food granules;
c, parenchyma network; d, parenchyma-forming cells;
e, wall of gut.



that the remains of the network became concentrated into islands of tissue. The new parenchyma forming cells were small and had deep staining nuclei. They tended to aggregate against the epidermis, and around the gut and the other organs. These cells were particularly numerous around the gut, and some cells had sent out short strands of cytoplasmic material (Fig. 6B).

In the late developing hypopus, the nuclei of the phagocytes tended to become rounded and they lost their affinity for stains (Fig. 6C). When the hypopus was fully formed the nucleus was rounded, lightly stained and a number of granules replaced the deep staining nucleolus. The pink granules both in the haemocoel and in the phagocytes increased in size. The parenchyma forming cells were still contiguous with the epidermis and the various organs. The nuclei of these cells stained lightly, and they contained deep staining granules (Fig. 6D).

The changes occurring in the haemocoel in the late developing hypopus and the hypopus of H. polypori were similar to those in the corresponding stages of Glycyphagus spp., except that the pink staining granules are far less numerous in H. polypori.

The circular spaces in the haemocoel in serial sections of developing hypopi and hypopi correspond to the globules visible in the haemocoel of the living developing hypopi and hypopi. These globules stain blue-black with Sudan black indicating their fat content.

The central nervous system. The central nervous system underwent no change during hypopus development in Glycyphagus spp. or H. polypori. The ganglion complex and the main nerve branches remained unaltered in the hypopus. However, owing to the hypopus being /

22.
being smaller than the protonymph from which it developed, the ganglion complex in the hypopus was relatively much larger.

Differentiation of the muscle systems. The muscles which are present in the hypopus can be divided into two main groups; (a) muscles which are present in the protonymph and are represented in the hypopus either by groups of myoblasts in various steps of development or by fully developed muscles; (b) muscles which are present only in the hypopus, and are not found in either the protonymph, or the deutonymph.

The muscle systems present in the protonymph were all developed in varying degrees in the hypopus form. Some of the muscle systems were represented in the hypopus by groups of myoblasts only, while others were in the form of a thin strand of myoblast tissue composed of cells with visible nuclei, but whose cytoplasm had a poor affinity for eosine. A third group of the protonymphal muscles was fully developed in the hypopus.

In Glycyphagus spp. and H. polyperi the muscles of the mouth appendages except the cheliceral retractor muscles, the pharyngeal muscles and the anterior oblique body muscles degenerated as the hypopus developed. During early hypopus development new myoblasts appeared among the degenerating muscles and they persisted throughout the hypopus condition (Fig. 7).

The leg buds of the developing hypopus and the hypopus of G. domesticus contained groups of undifferentiated myoblasts (Fig. 8). In G. destructor and H. polyperi the leg buds unfolded into definitive leg appendages and this was accomplished by the myoblasts converting themselves into proper muscles. Short, not properly developed legs were formed in the hypopus of G. destructor /

FIGURE 7. Differentiation of anteriorly placed muscles during hypopus development, as seen in sagittal section.

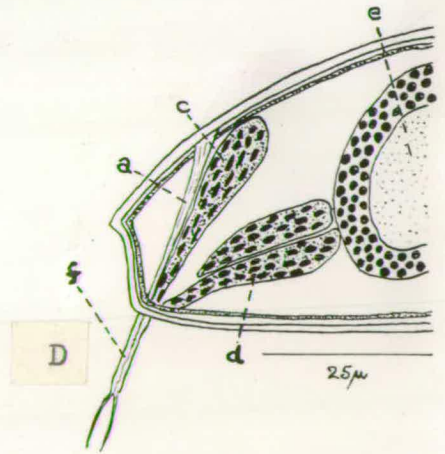
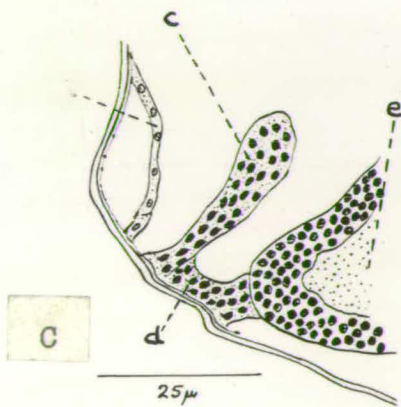
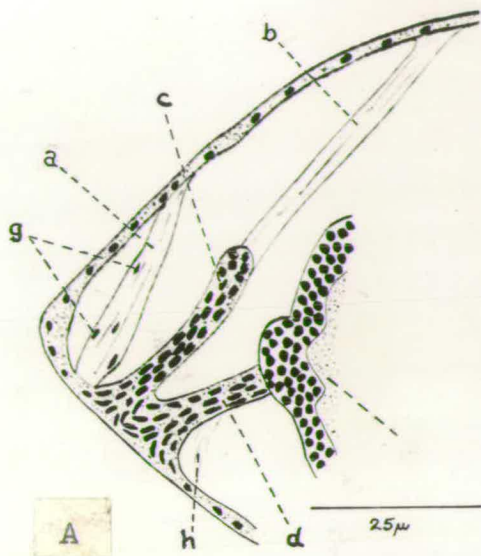
A, The state of the muscle tissue during early hypopus development in G. destructor.

B, Muscle tissue of the hypopus mite, G. destructor.

C, Muscle tissue of the hypopus mite, G. domesticus.

D, Muscle tissue of the hypopus mite, H. polyperi.

a, cheliceral retractor muscle; b, degenerating anterior oblique body muscle of protonymph; c, myoblasts of new anterior oblique body muscle; d, myoblasts of pharyngeal muscles; e, nerve ganglion; f, atrophied rostrum; g, new myoblasts in tissue of muscle; h, degenerating pharyngeal muscles of protonymph.



G. destructor. In H. polypori the legs, especially the first pair, became efficient locomotory appendages. The anterior legs were longer than the corresponding ones of the succeeding deutonymph. The third and fourth pairs of legs of H. polypori were relatively short and poorly developed.

The dorso-ventral muscles, the transverse anal muscles and the dorsal muscles underwent similar changes in the three species during hypopus development. In the early developing hypopus the old muscle fibres degenerated and new myoblasts appeared among the fibres. In the late developing hypopus the myoblasts had developed into muscle fibres which showed a better affinity for stain than the old muscles.

The development of the ventral muscles and the cheliceral retractor muscles in G. destructor and H. polypori was on similar lines to that of the dorso-ventral muscles mentioned above. In H. polypori, however, the ventral muscles were stouter and better developed in the hypopus than they were in the protonymph. The ventral and cheliceral retractor muscles in G. domesticus did not develop very far. Myoblasts were visible in the deteriorated protonymphal ventral and cheliceral retractor muscles during early hypopus development. In late hypopus development, the myoblasts formed a strand of tissue in which the nuclei of the cells were visible. These muscles remained in this partly developed condition in the hypopus form (Fig. 8).

The usual pair of dorso-ventral muscles supporting the hindgut of the three species of mites were, in the hypopus, represented by a pair of strands of partly developed muscle, not unlike the ventral muscles in the hypopus of G. domesticus.

FIGURE 8. The incompletely differentiated ventral muscle and the myoblast tissue of a potential posterior limb bud in the hypopus of G. domesticus seen in horizontal section.

a, ventral muscle; b, myoblast tissue of potential limb bud;

c, nerve ganglion; d, nerve tissue at base of limb.

FIGURE 9. Sagittal section showing the differentiating posterior dorso-ventral muscles during hypopus development in G. domesticus.

a, posterior dorso-ventral muscle; b, hindgut.

FIGURE 10. Transverse section of dorsal oblique muscles differentiating during hypopus development in A, G. destructor, and B, H. polyperi.

a, dorsal oblique muscle; b, ventral muscle;

c, dorso-ventral muscle; d, nerve ganglion;

e, gut; f, fold of epidermis; g, myoblasts.

Fig. 8

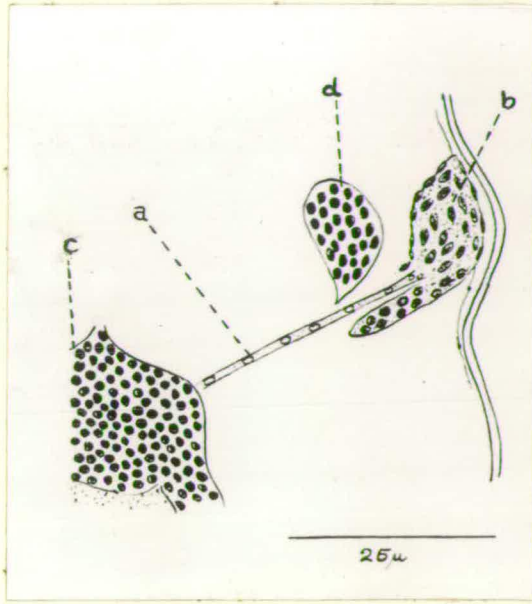


Fig. 9

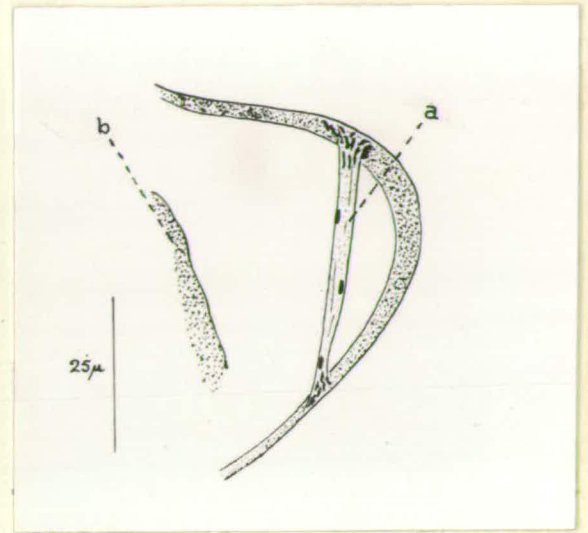
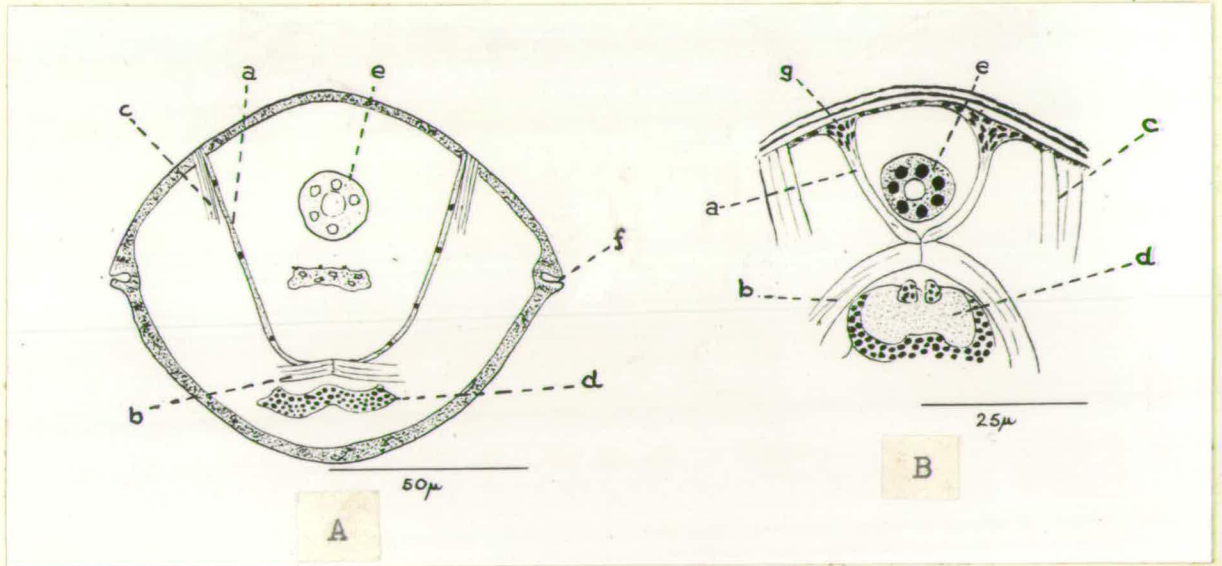


Fig. 10



Two systems of muscles, however, were produced in the mite only at the time it assumed the hypopus condition. During hypopus development they appeared first as groups of myoblasts, which gave rise to fully formed muscles. In Glycyphagus spp. and H. polypori groups of posterior dorso-ventral muscles developed on each side of the hypopus, in a position posterior to the hindgut (Fig. 9). In H. polypori these muscles were more extensively developed than in Glycyphagus spp. and they were associated with the ventral sucker disc (Fig. 12).

The second system of muscles which made their appearance exclusively in the hypopus forms, consisted of a pair of dorsal oblique muscles. Dorsally they were attached to the cuticle close to the attachment of the dorso-ventral muscles. Ventrally they were attached to the point where the ventral muscles were attached to the nerve ganglion. In G. destructor and H. polypori these potential muscles appeared as myoblasts during early hypopus development (Fig. 10). At a late step in hypopus development these muscles were fully formed. In G. domesticus these muscle systems unfolded only as far as the step in development when they assumed the form of a pair of strands of myoblast tissue.

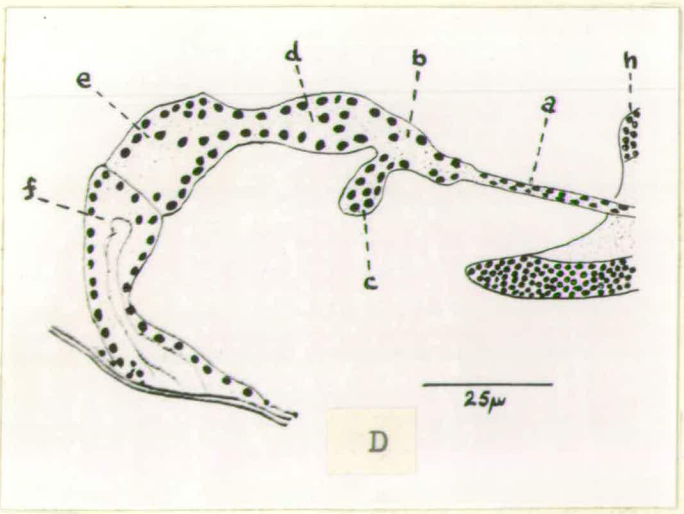
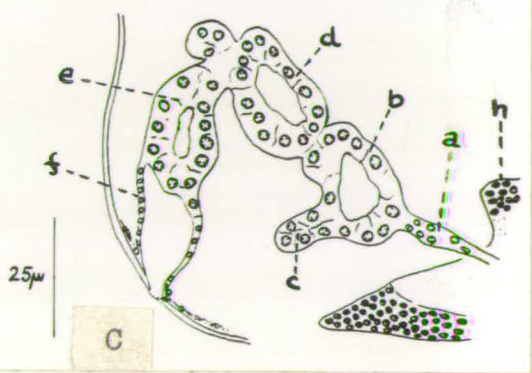
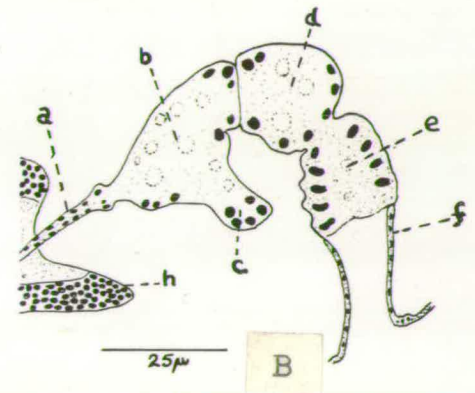
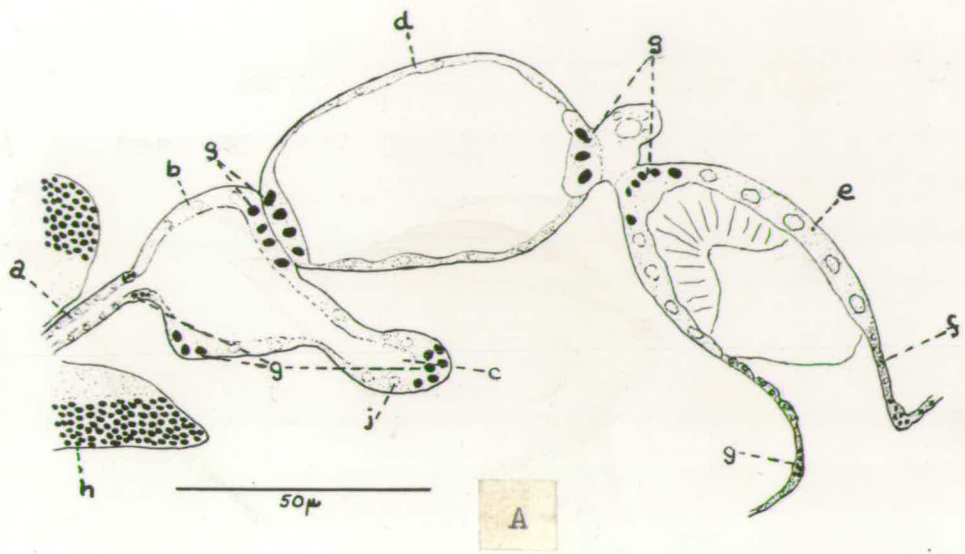
Changes in the digestive system and the salivary glands. In the late protonymphal stages of Glycyphagus spp. the gut became greatly distended with food and the gut wall itself became relatively thin, except for certain thickened regions. These regions were the predetermined regeneration centres, which gave rise to a new gut. They were composed of cells with deep staining nuclei (Fig. 11A). The regeneration centre for the oesophagus was a ring of tissue at the junction of the oesophagus and the foregut. The foregut had /

had a lateral pair of regeneration centres, one on each side just posterior to the oesophageal ring, and a posterior centre at the sphincter leading to the midgut. Each caecum possessed a regeneration centre at its posterior extremity. The two regeneration centres of the midgut were situated near the anterior and posterior sphincter valves. The dorsal portion of the hindgut was regenerated from a ring of cells situated at its junction with the midgut. The ventral region of the hindgut of epidermal origin was regenerated from a ring of epidermal cells near the anus.

When hypopus development took place the gut cells of the protonymph degenerated, the cytoplasm became intensely vacuolated, the nuclei lost their definition and the cell walls disintegrated. Deterioration seemed to commence at the anterior end of the gut and continue posteriorwards (Fig. 11A). Food material passed out into the haemocoel during this period when degeneration had set in; this was accompanied by a general shrinkage of the gut. The new gut cells were proliferated from the regenerative centres, the cells in G. destructor, increasing in size slightly as they migrated away from the centre. In G. destructor regeneration occurred rapidly in the hindgut region. This resulted in new cells with their deep staining nuclei, being in their proper position about halfway through hypopus development (Fig. 11B). The cells of the posterior region of the hindgut had secreted a layer of cuticle, the anal opening was present and remained in the hypopus. In both G. destructor and G. domesticus the epidermal type of cells of the pharyngeal region appeared to secrete a thin layer of cuticle, but, no mouth opening was present in the hypopus mite.

In G. domesticus the new gut cells in the anterior regions
did /

- FIGURE 11. Changes in the gut during hypopus development in Glycyphagus spp. as seen in sagittal section.
- A, The state of the gut in the late protonymph of G. destructor.
- B, The state of the gut during early hypopus development in G. destructor.
- C, The gut of the hypopus mite, G. destructor.
- D, The gut of the hypopus mite, G. domesticus.
- a, oesophagus; b, foregut; c, caecum;
d, midgut; e, dorsal region of hindgut;
f, epidermal region of hindgut;
g, proliferation areas; h, nerve ganglion;
j, degenerating nucleus.



did not increase in size as they migrated away from the proliferation centre, but instead they remained in an embryonic condition. The cells in both regions of the hindgut had increased in size to a certain extent, and a thin layer of cuticle had formed in the ventral region. The gut of the hypopus mite consisted of a shrunken strand of cells with deep staining nuclei. (Fig. 11D).

In the late developing hypopus condition of G. destructor the nuclei and cytoplasm of the new gut cells had lost their affinity for the stain so that the gut of the hypopus consisted of a light staining mass of cells. Since the arrest in development of these new cells occurred relatively later than it did in G. domesticus, the shape of the gut during the hypopus of G. destructor more nearly approached the condition it obtains in the normal stages (Fig. 11C).

It was not possible to follow the changes in the gut during early hypopus development in H. polypori for the reasons which have already been mentioned (see page 9). But, during late hypopus development the hindgut had completely regenerated, and the cytoplasm of the cells in the dorsal region had become vacuolated, while the cells of the ventral region had secreted cuticle. The mouth opening persisted in the hypopus mite and a thin layer of cuticle had formed inside the atrophied rostrum, in a position corresponding to the pharynx of the protonymph. The oesophagus, the foregut and its caecae, and the midgut had been reformed but their cells, with their deep staining nuclei and cytoplasm, remained small. The cells of the anterior parts of the gut remained unaltered in the hypopus condition, but, the vacuolation of the cytoplasm of the cells of the upper portion of the /

the hindgut had visibly increased (Fig. 12).

In the late protonymph of Glycyphagus spp. the nuclei of the salivary gland cells were stained more lightly and the nucleolus was replaced by deep staining granules. The cytoplasm stained unevenly and vacuoles appeared in it (Fig. 13A). During hypopus development cells possessing deep staining nuclei, with a large nucleolus, appeared in that region close to the duct of the gland. The cytoplasm had deteriorated considerably, it contained numerous vacuoles and it had lost the striations characteristic of these cells when functioning normally (Fig. 13B). By the time hypopus development had been completed, the cells of the old protonymphal salivary glands had entirely disappeared and they were replaced by new cells forming a strand of light staining tissue (Fig. 13C).

The differentiation of the salivary glands during late hypopus development in H. polyperi was difficult to follow owing to their small size at this time. However, as far as could be seen, their development was similar to that of the glands in Glycyphagus spp.

The reproductive system. This system changed only slightly during hypopus development in both Glycyphagus spp. and H. polyperi. Shortly before the protonymphal stage of Glycyphagus spp. enters the quiescent state, the genital strands in front of the rudiments of the gonads, increased their size. During hypopus development the strands grew stouter, and they took up a position more vertical than normally owing to the retraction of the body of the mite at this time. The gonadial rudiments themselves remained unchanged during /

FIGURE 12. Sagittal section of the gut of the hypopus of H. polypori.

- a, oesophagus; b, foregut; c, caecum;
- d, midgut; e, dorsal region of hindgut;
- f, epidermal region of hindgut;
- g, nerve ganglion; h, posterior dorso-ventral muscle;
- j, sucker plate.

FIGURE 13. The fate of the salivary glands during hypopus development in G. destructor, seen in horizontal section.

- A, The salivary glands of a late protonymph.
- B, The salivary glands during late hypopus development.
- C, The salivary glands of the hypopus mite.
 - a, degenerating cytoplasm; b, degenerating nucleus;
 - c, new nucleus; d, duct of gland.

Fig. 12

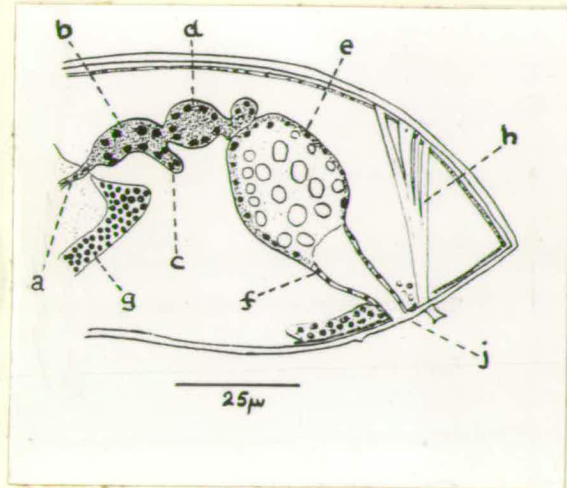
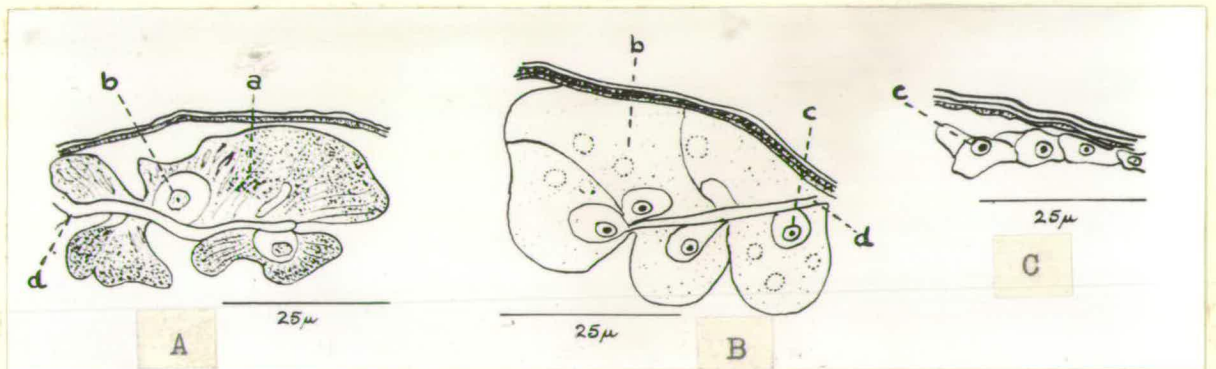


Fig. 13



during hypopus development.

During late hypopus development in H. polypori the genital strands attained the same appearance as those of the corresponding hypopus condition in Glycyphagus spp. Neither did the gonadial rudiments of H. polypori undergo any change during hypopus development.

Further Development from the Hypopus Condition to
the Regular Morphological Pattern.

The role of the integument. The series of changes culminating in the emergence of the deutonymph were preceded by a moulting of the hypopus cuticle. In the late hypopus of Glycyphagus spp. and H. polypori which was about to develop to the deutonymph, the epidermis thickened and this was followed by the separation from the epidermis of the intermediate cells and the moult of the hypopus cuticle. However, the exuvial cavity was very small, since there is very little retraction by the epidermis.

The epidermis after the moult increased its thickness, and when it had moulded the form of the deutonymph, it secreted the definitive cuticle of the deutonymph.

Changes within the haemocoel. When the hypopus condition of Glycyphagus spp. was about to change to the normal deutonymph, the fat globules in the haemocoel broke up into small particles and these became uniformly distributed in the haemocoel. This was shown by staining them with Sudan black. The remains of the parenchyma network and the pink granules were also more uniformly dispersed in the haemocoel. The amount of excretory crystals had also increased. The changes that took place in the haemocoel of H. polypori /

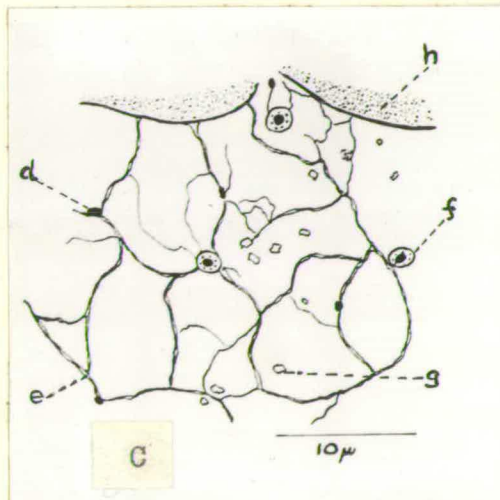
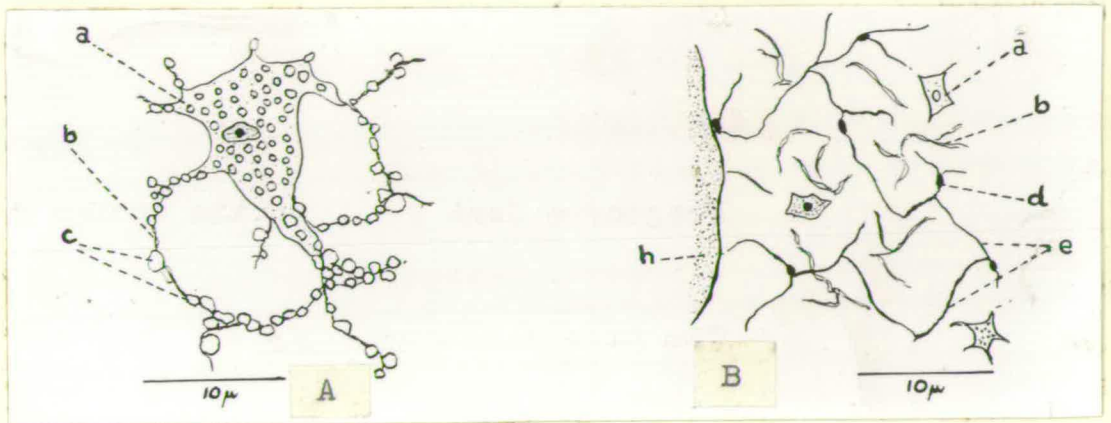
H. polypori at this time were somewhat similar. However, the fat content was relatively less and the excretory crystals arranged themselves postero-laterally.

An attempt was made to analyse the chemical nature of these excretory crystals in Glycyphagus spp. and H. polypori, but one could not arrive at a confident conclusion owing to the small amounts present. Hughes (1950) maintained that guanine was the chief excretory constituent in Tyroglyphus farinae. But uric acid has been shown to be in some mites. Lison (1936) stated that uric acid and guanine can be distinguished owing to the insolubility of the latter when incubated in a solution of piperazine. The excretory crystals of hypopus mites of Glycyphagus spp. and of H. polypori were insoluble in piperazine, thus suggesting that they are composed of guanine.

In all three species of mites examined, when the hypopus condition was nearing its end, the nuclei of the phagocytes stained more deeply, and a nucleolus was visible. The nuclei of the parenchyma forming cells also stained deeply (Fig. 14A). When the mite developed from the hypopus condition to the deutonymph the remains of the parenchyma network of the protonymph disintegrated completely. At the same time the nuclei of the phagocytes became irregular in shape and the cells themselves showed the usual signs of being active phagocytically. Later, when the deutonymphal systems were taking shape, the cytoplasm of these phagocytes could not be distinguished. The new parenchyma network of the deutonymphal stage was formed by the small parenchyma forming cells, as the remnants of the old protonymphal network were destroyed (Fig. 14B). /

FIGURE 14. Changes in the haemocoel when a mite develops from the hypopus condition to the deutonymph in G. destructor.

- A, Phagocyte just prior to the change from the hypopus condition.
- B, The haemocoel during development to the deutonymph.
- C, The haemocoel of the newly emerged deutonymph.
- a, phagocyte; b, parenchyma network;
 - c, food granules; d, parenchyma forming cell;
 - e, fibres of new parenchyma network;
 - f, new phagocyte;
 - g, excretory crystal; h, wall of gut.



(Fig. 14B). The pink staining granules of food material in the haemocoel were gradually used up as the deutonymph developed. In the newly emerged deutonymph were a few food globules, a certain amount of excretory crystals, phagocytes with rounded, deep staining nuclei each with a large nucleolus, and a completed parenchyma network with numbers of its intimately associated connective tissue cells (Fig. 14C).

Development of the internal systems to their normal pattern. The nervous system remained unchanged during this further or delayed development to the deutonymphal stage.

The posterior dorso-ventral muscles and the dorsal oblique muscles, which were specific to the hypopus mite, degenerated on development to the deutonymph. Groups of myoblasts situated in the anterior region regenerated the muscles of the mouth parts and pharynx. In G. domesticus the myoblasts of the hypopus leg buds gave rise to the muscles of the legs of the deutonymph. In G. destructor and H. polyperi the muscles of the legs of the hypopus mite degenerated, and myoblast regions formed the new muscles of the legs of the deutonymph. In all the three species muscles which were partly developed in the hypopus mite but were still present as strands of myoblasts, unfolded into proper muscles. Other muscles which were fully developed in the hypopus were rejuvenated by the migration of myoblasts into their tissue.

In Glycyphagus spp. the nuclei and cytoplasm of the cells of the gut stained deeply when the potential gut cells of the deutonymph began to enlarge. Cuticle was deposited in the hindgut by the epidermal type cells. The gut cells had enlarged completely on emergence of the deutonymph. In H. polyperi the cells of the oesophagus /

oesophagus, the foregut and caecae, and the midgut, which remained in an embryonic state in the hypopus mite, enlarged to form the definitive gut of the deutonymph. The hindgut which had been fully developed in the hypopus did not alter in size but became more spherical in shape as the anterior regions of the gut enlarged. The vacuoles present in the cells of the dorsal portion of the hindgut disappeared when the deutonymph was formed.

The salivary glands, in all three species, were non-functional and somewhat embryonic during the hypopus condition. But, when the hypopus condition had come to an end and the mite developed towards the deutonymphal state, the cells of the salivary glands enlarged and unfolded fully into functional organs.

The reproductive system at this time developed a little further to reach a state equivalent to that which one expects in any deutonymphal stage.

The Significant Features of Hypopus Development.

The findings which have been presented in this section have been discussed at the end of this paper in the appropriate Discussion section. But it will be useful here to outline briefly the noteworthy features that can be lifted out from the foregoing observations on the morphogenetic movements which take place in the different systems of organs and tissues, when the mite develops through a hypopus state.

First, hypopus development was invariably accompanied by a specific moult. Secondly, despite the interception of the hypopus condition, the changes that took place in the haemocoel, apart from their arrest at a particular time, were no different from /

from those observed when a protonymph changes to a deutonymph. The nervous system and the reproductive system also developed normally, except as already mentioned, for the intervention of a pause accompanying the hypopus condition. The development of the gut and salivary glands also comes into this category, there being merely a halt in their unfolding when the mite assumes the hypopus state.

On the other hand, in H. polypori, the singular behaviour of the integument and the differentiation of the various muscles was most striking. The integument moulded the mite into a new hypopus form, utterly different from that of the normal form, and integumental structures appeared which were specific to the hypopus mite. However, this was not the case in G. domesticus and it happened only very slightly in G. destructor. With respect to the muscles in H. polypori and Glycyphagus spp., those formed specially during hypopus development underwent de-differentiation before the definitive muscles of the deutonymph were regenerated.

HISTOCHEMICAL ANALYSIS OF HYPOPUS CUTICLES.

The waterproof properties of the insect cuticle reside in the outermost layer or epicuticle. Beament (1945) and Wigglesworth (1945) have shown that the property of impermeability to water depends upon the nature of the thin outer film of wax of the epicuticle. In some cases the wax is protected from mechanical damage by a so-called "cement" layer. This cement layer is extremely thin and appears to be composed of hardened polyphenol substances which have undergone a change in consistency. Lees (1947) found that ticks inhabiting moist environments possess a thin cuticle with a layer of soft low melting point wax. On the other hand, ticks living in dry conditions possess a thicker cuticle, with a layer of hard wax, and in some cases a cement layer. Hence it is possible to deduce the humidity conditions of the environment in which the ticks live by examining the structure of their cuticle.

Many workers (including Michael (1901), Schulze (1924a) and Hora (1934)) observed that tyroglyphid hypopi survived for long periods in dry conditions which otherwise proved quickly fatal to the eggs and the normal post-embryonic stages. The differences in texture and shape between the cuticle of the mobile hypopus stage of H. polyperi and that of the normal stages are certainly spectacular. The cuticle mould is peculiarly shield-shaped and it is more heavily sclerotised, a property indicated by its brown coloration which is absent in the normal stages.

Histochemical tests were employed to determine the different component layers of the cuticle of the hypopi and the normal stages /

stages. Since the hypopus case (old protonymphal cuticle) which surrounds the hypopus of Glycyphagus spp. has been shown to help this stage in resisting desiccation (see later p.48), it was also subjected to the various chemical tests.

Hypopi of H. polypori occur commonly on earwigs, hence they naturally become exposed to the humidity conditions of the environments into which they are taken by the earwig. Since earwigs are capable of remaining in niches where the conditions are dry, the hypopus in order to survive will have to resist desiccation in these conditions as successfully as the earwig. It was therefore thought worth while to examine the structure of the cuticle of the earwig, especially the wax layer, in order to gain some measure of its resistance.

The histochemical tests used by Wigglesworth (1933) and Lees (1947) for identifying the different component layers of cuticle were adopted in this work. The small size of the mites however called for much modification in technique.

Briefly, the principle of the histochemical testing is one of elimination. The epicuticle of hardened cuticle may consist of the full complement of layers, an outer cement layer which protects the wax layer, a polyphenol layer, and an inner cuticulin layer. The cement layer unlike the wax layer resists dissolution in cold chloroform, but this layer and the wax layer can be removed by hot chloroform. The exposed polyphenol layer gives the argenteaffin reaction, turning chestnut brown after immersion in ammoniacal silver nitrate. The inner cuticulin layer when treated with hot caustic potash, breaks up into oily droplets, suggesting a lipo-protein consistency. It will now be clear that treatment of /

PLATE II. Arrangement for testing the effect of caustic
potash on the cuticle of a mite (see text for details).

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of the epicuticle with cold chloroform and a subsequent negative test after immersion in silver solution would indicate the presence of an outer cement layer. If, on the other hand, after pretreatment with cold chloroform, the silver test gave the argenteaffin reaction, it presages the absence of a cement layer which can be checked by making serial sections of the cuticle.

The chitosan test for identifying chitin in cuticle was carried out in the following way. An evaporating basin was filled with glycerol, and on the surface of this liquid floated a metal disc with a raised rim (Plate II). The temperature of the glycerol was kept constant at 140°C. A piece of cuticle was placed in the cavity of a slide containing two or three drops of saturated caustic potash and a coverslip was placed over the cavity to prevent evaporation. The slide was heated on the metal disc for five minutes. It was afterwards cooled and placed on the stage of a dissecting microscope. The coverslip was removed and the cuticle was washed in water several times to remove the alkali. The cuticle was treated with a 0.2% solution of iodine in 1% sulphuric acid. A blue-violet colour in the inner layers of the cuticle indicated the presence of chitin, while the epicuticle was stained bright yellow.

The preparation of serial sections for the application of the chitosan test according to a method devised by Browning (1942) was attempted. However, since the rest of the cuticle loosened away from the epicuticle and at the same time partly disintegrated, when treated with caustic potash, it was impossible to embed the material satisfactorily.

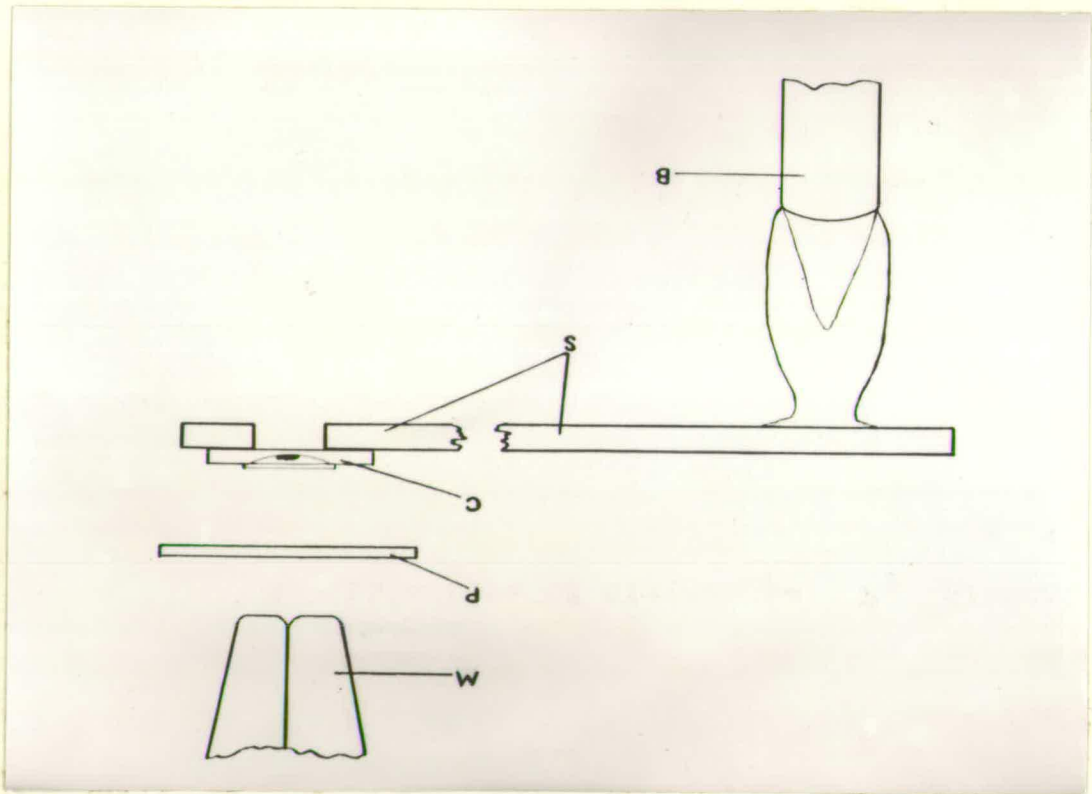
Treatment /

FIGURE 15. Arrangement for testing hot concentrated acids on the cuticle of mites.

B, Bunsen burner; C, cavity slide containing cuticle and reagent;

M, objective of microscope; P, protective glass plate;

S, metal strip.



Treatment of cuticle with concentrated acids, for isolating and identifying the epicuticle, was also carried out upon a cavity slide. A metal strip (12" x 1½" x 1/12") having a ½" diameter hole near one end, was placed under the objective of a dissecting microscope with the hole centred within the field of vision (Fig.15). The cavity of the slide containing the cuticle and liquid agent, with a coverslip on top, was placed over the hole of the metal strip. A glass plate was clamped in position between the slide and the objective, to prevent damage to the microscope. The other end of the metal strip was gently heated with a bunsen and the reaction of the piece of cuticle to the hot reagent was watched closely through the microscope. Slow cautious heating of the small amount of acid was essential in this test. Hot concentrated nitric, sulphuric, or hydrochloric acid dissolves the inner parts of the cuticle, the epicuticle alone being unaffected by these agents.

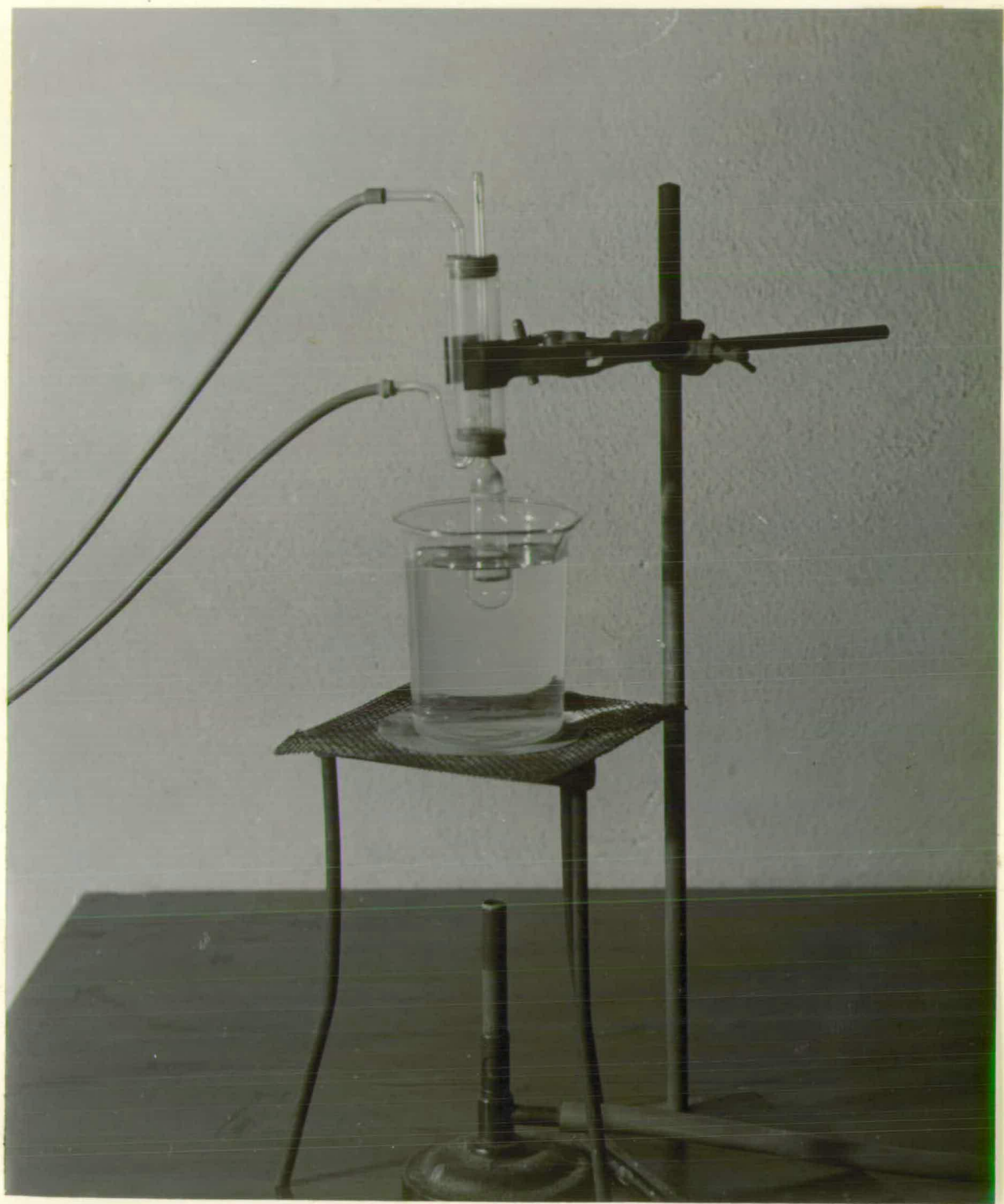
The following techniques were adopted for testing various reagents on the epicuticle for detecting, if present, the cement, wax and polyphenol layers. Immersion of cuticle in cold chloroform was carried out in a solid watch glass covered by a glass plate. Plate III shows the arrangement for immersing cuticle for long periods in boiling chloroform. The cuticle and chloroform were placed in a round bottomed tube, appropriately immersed in a water bath kept at the temperature at which chloroform boils. The tube containing the material was connected by a male and female joint to a narrow condenser tube, open at the top. The condenser tube was surrounded by a jacket through which passed a continuous flow of cold water.

After /

PLATE III. The arrangement for treating the cuticle of a mite in hot chloroform (see text for details).



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After pretreatment in chloroform the cuticle was placed on a cavity slide which contained a 5% solution of ammoniacal silver nitrate. Care was taken to see that the cuticle was totally submerged and that no air bubbles were in contact with it. Air bubbles were also removed when a coverslip was placed over the cavity and sealed down with paraffin wax to prevent evaporation of the silver solution. Two or three days usually elapsed before the argentaffin reaction showed clearly. The reaction of the cuticle was observed at intervals, and, when the argenteffin reaction was well shown the coverslip was removed and the cuticle was rinsed in distilled water before being examined in detail. This sometimes entailed embedding the treated cuticle in wax in the normal way. The sections after removal of the wax were mounted in balsam.

Before a piece of cuticle was tested for the presence of a cuticulin layer it was pretreated with chloroform to remove the wax layer. The cuticle was afterwards gently heated in 10% caustic potash to remove the polyphenol layer of the epicuticle, and the exo- and endocuticle. The procedure for heating the cuticle in caustic potash was the same as that described above for heating cuticle in a concentrated acid. The cuticulin layer, if present, is momentarily left as a thin layer, but it too finally succumbs to the caustic treatment by breaking up into oily droplets.

Results.

The cuticle of the normal stages of Glycyphagus spp. The component /

component layers of the cuticle were the same for the larvae, the nymphs and the adults of both G. destructor and G. domesticus. The chitosan test indicated the presence of chitin in the endocuticle and the exocuticle regions and the presence of an epicuticle. The separate staining reactions were readily distinguished because the blue-violet stained part of the cuticle became swollen, disintegrated slightly, and loosened away from the yellow stained epicuticle. Attempts to apply the chitosan test to serial sections of the cuticle were unsuccessful. It was therefore not possible to discover the exact distribution of chitin in the hardened and non-hardened regions of the endocuticle.

The epicuticle was also isolated after treatment in concentrated mineral acids.

Cuticle, pretreated in cold chloroform, and immersed in silver solution gave the argenteaffin reaction, thus showing the absence of a cement layer and the presence of a wax and a polyphenol layer. The argenteaffin reaction was produced more rapidly if the cuticle was pretreated in hot chloroform. Examination of both pieces of cuticle and serial sections showed concentrated patches of silver deposit upon the tips of the tubercles and occasionally they also occurred upon the areas between the tubercles. Otherwise the silver deposit was more or less evenly deposited over the surface of the body and legs.

The presence of a cuticulin layer was indicated by treatment with 10% caustic potash.

The cuticle of the hypopus form of Glycyphagus spp. The results of the chitosan test on the hypopus cuticle of Glycyphagus spp. were the same as those obtained on the cuticle of normal stages. The hypopus cuticles also reacted in the same way to concentrated acids /

acids and 10% caustic potash as those of the normal stages.

Cuticle pretreated with hot or cold chloroform and immersed in ammoniacal silver nitrate showed the argentaffin reaction. But the silver deposit, unlike that upon the cuticle of normal stages, was uniformly spread over the whole surface of the cuticle. The hypopus cuticle when immersed in the silver solution without previous treatment with chloroform remained unchanged.

The hypopus case of Glycyphagus spp. The chitosan test, applied to the hypopus case of G. destructor, indicated the presence of chitin in the endocuticle and exocuticle regions and the presence of an epicuticle. The reticulated markings on the cuticle were stained a deeper blue violet colour than the intervening areas. The epicuticle loosened from the inner layers of the cuticle to a greater extent than in the normal stages.

Treatment with concentrated mineral acids isolated the epicuticle.

The hypopus case pretreated in hot or cold chloroform gave the argentaffin reaction after immersion in the silver solution. A silver deposit formed more slowly if the hypopus case was immersed in the silver solution without prior treatment in chloroform. The hypopus case of early developing hypopi did not give the argentaffin reaction when placed in silver solution without previous chloroform treatment. The wax layer is therefore lost by the time the hypopus case is fully formed. The polyphenol layer remains in the fully formed hypopus case. As in the normal stages, the tips of the tubercles became very dark. The reticulated markings stained slightly darker than the intervening areas.

The /

The examination of serial sections of cuticle treated with silver solution showed that the greater thickness of the cuticle forming the reticulated markings was responsible for the increased staining reaction in these areas.

A cuticulin layer was shown to be present by treatment with 10% caustic potash.

The tests applied to the hypopus case of G. domesticus indicated the presence of similar cuticle layers to those found in G. destructor. The different markings on the case, however, resulted in the appearance of the treated case differing from that of G. destructor. The vermiform markings remained visible when the case was treated for chitin but due to the small size of these markings it was not possible to determine if they stained more deeply than the intervening areas.

In a hypopus case treated for the argentaffin reaction the vermiform markings appeared darker than in an untreated case. In serial sections of the treated case thin dark coloured lines were visible running vertically in the cuticle, most of the lines originating at the ridges in the inner surface of the cuticle. The distance between these lines is the same as the distance between the lines of the vermiform markings. It is probable therefore that these lines correspond to the vermiform markings seen from the surface of the cuticle.

The cuticle of the normal stages of H. polyperi. The component layers of the cuticle of the normal stages of H. polyperi were similar to those of Glycyphagus spp. The chitosan test indicated the presence of chitin in the endocuticle and exocuticle regions and /

and the presence of an epicuticle. The epicuticle was isolated by treatment with concentrated mineral acids.

The deposit of silver produced by the argentaffin reaction on cuticle previously treated with hot or cold chloroform, was uniformly spread over the surface of the cuticle. In serial sections the silver deposit was visible as a thin blackish-grey boundary.

The cuticulin layer was identified by treatment with 10% caustic potash.

The cuticle of the hypopus of H. polyperi. The application of the tests to the cuticle of the hypopus indicated the presence of chitin in the endocuticle and exocuticle regions and an epicuticle consisting of cuticulin, polyphenol and wax layers. The argentaffin reaction was uniformly spread over the cuticle surface. As in the normal stages, no cement layer was present.

The cuticle of Forficula. Chitin was present in the endocuticle and the exocuticle. The epicuticle consisted of cuticulin, polyphenol and wax layers. There was no cement layer present.

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EXPOSURE OF HYPOPUS FORMS TO DIFFERENT RELATIVE HUMIDITIES.

It was well known that Tyroglyphid mites succumbed easily to a harsh, dry environment and that they multiplied most rapidly when the relative humidity was high, the optimum humidity depending on the temperature. Hora (1934) has shown that G. domesticus multiplies rapidly at relative humidities above 70% R.H. while relative humidities below 60% R.H. are fatal. Polezhaev (1940) stated that the conditions for optimum development of G. destructor was 80% R.H. at 23°C. and Ushatinskaia (1945) found that at 50% R.H., when the temperature was 15°C. to 22°C., all normal stages were killed. Hypopi appear to resist adverse conditions of humidity and temperature far more successfully than the normal stages. According to Hora (1934) the hypopus of G. domesticus can survive for about seven days at 10% R.H. Ushatinskaia (1945) found that hypopi of G. destructor survived for several months at 0°C., a temperature which was fatal to the normal stages.

It is legitimate to criticise previous work on the reaction of mites to various humidities on the grounds that there was often a failure to take temperature into account at the same time. It is also somewhat surprising how little work has been done on the effects of humidity relative to work on the effect of other environmental factors. Hence the survival of hypopi and normal stages of Glycyphagus spp. and H. polypori was investigated when exposed to different combinations of humidity and temperature. The importance of the hypopus case (old protonymphal cuticle) of Glycyphagus spp. in helping with respect to resistance of its hypopus against desiccation was also investigated.

In nature the hypopus forms of H. polypori are normally attached /

attached for an indeterminate period, to the cuticle of insects. They appear to show a predilection for earwigs. They may remain attached to the cuticle for several weeks, or months, or until the insect dies. Experiments were, therefore, also devised to discover whether attachment to the cuticle of an earwig afforded some measure of protection against low humidities.

The Humidity Chamber and the Arrangement for Varying
the Relative Humidity.

In order to expose animals to a constant relative humidity they are usually placed in a closed chamber. The humidity of the air is controlled by enclosing within the chamber sulphuric acid or some such agent of the appropriate concentration. This arrangement has one serious disadvantage. Because the volume of air in the chamber is stationary considerable variations in the humidity in different parts of the chamber are likely to occur, the required humidity expected by introducing the acid mixture being obtained only immediately above the surface of the acid. The best arrangement for exposing animals to a constant humidity is one in which a stream of air of the required humidity is passed through a chamber in which the animals are placed. The principle of acquiring air at the desired humidity was similar to the discarded method. A stream of air was bubbled through glycerine of a strength appropriate to give the required humidity before it passed through the chamber. The flow was regulated to change the air in the chamber several times in one hour.

A chamber for use in containing and exposing mites to a stream of air at a constant humidity was made in the following way (Plate IV). A block of perspex 2" x 1 $\frac{1}{8}$ " x 1" was excavated to /

to form a resultant rectangular chamber with side walls and floor $\frac{1}{8}$ " thick. The volume of the chamber was approximately 25 ccs. Two perspex tubes of $\frac{3}{8}$ " diameter and $\frac{1}{8}$ " bore were inserted and sealed into holes made in the walls. They were placed at diagonally opposite corners of the longer sides of the chamber to act as inlet and outlet air tubes. The opening of the tubes within the chamber were covered with muslin. The muslin was stuck to the perspex. This was done by softening the perspex with chloroform and pressing the muslin against it. The mesh of the muslin was sufficiently fine to prevent the escape of the smallest mites used in the tests. The open top of the chamber was covered with a glass plate sealed down with vaseline.

Johnson (1940) described a method for producing constant relative humidities inside a chamber by first bubbling the air through glycerol-water mixtures of known specific gravity. He provided data showing how a given relative humidity corresponded to a given specific gravity of the mixture, and what was more important, that the relative humidity governed by the mixture remains practically constant over a wide range of temperatures. However, he stated that the method is probably not suitable for providing relative humidities of below 70% because of the viscosity of the mixture. But, in the present experiments, it was possible to produce a relative humidity of 50% using a glycerol-water mixture, because the small size of the chamber allowed a much slower rate of airflow than that employed by Johnson. At relative humidities below 50%, the mixture was too viscous to allow bubbles of air of sufficiently small size to travel up through the mixture and acquire appropriate moisture to give the desired humidity.

Mixtures /

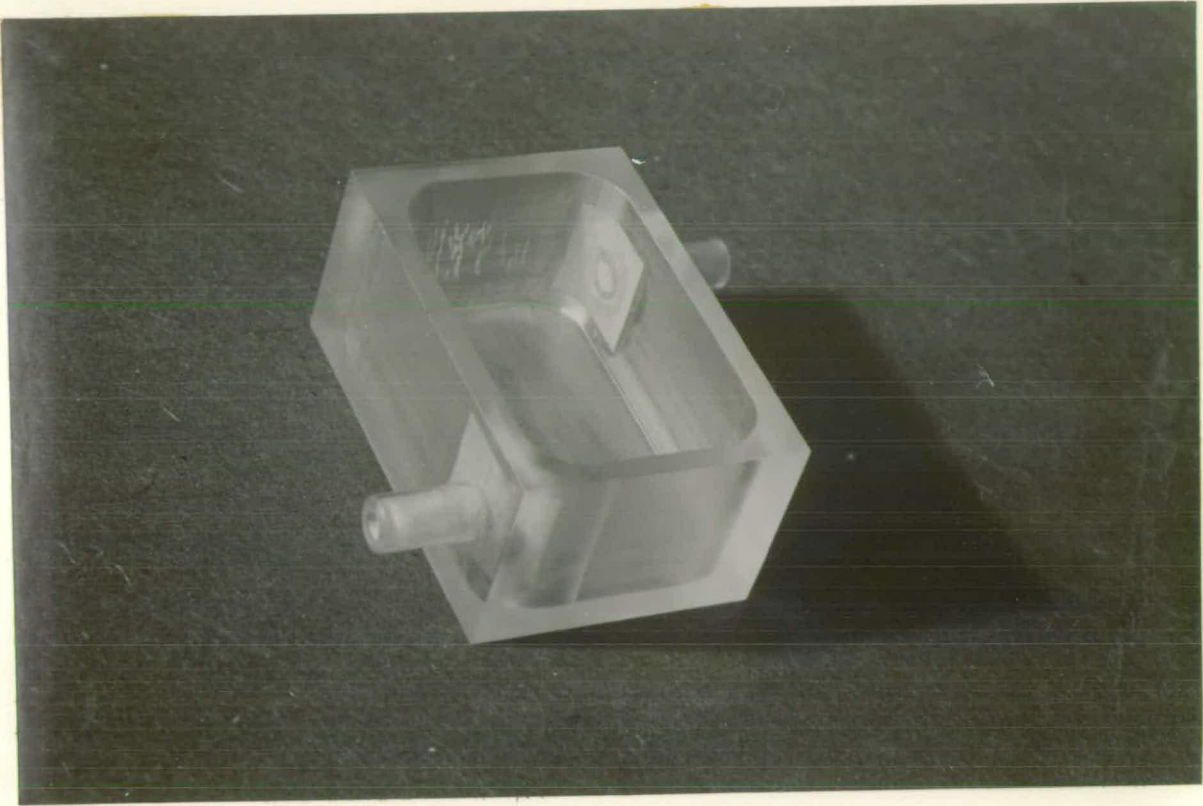
PLATE IV. The humidity chamber (see text for details).

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Mixtures of glycerol and water were made up to give corresponding relative humidities of 50%, 70%, 90% and 100%.

Plate V shows the arrangement of the apparatus used for subjecting mite material to relative humidities ranging from 50% R.H. to 100% R.H. An aspirator containing water was placed about five feet above the bubbler so that sufficient pressure was provided to force the air through the fine jet of a capillary into the glycerol water mixture. By simple displacement, the water from the aspirator forced the air out of the Winchester and through the bubbler containing the glycerol. The bubbler tube (9" high and 1" diameter) was filled with glycerol to about three-quarters capacity. The end of the inner capillary tube of the bubbler, which almost reached the bottom, was drawn out into a fine jet so that the air was released into the mixture as minute bubbles. The air flowed from the bubbler into the experimental chamber. The air flow (about 100 ccs./hr.) was regulated by adjustment of a clip on the rubber tubing between the bubbler and the chamber. Immediately after mites had been placed in the chamber, or after the cover had been removed for some other purpose, the rate of flow of air was increased to about 500 ccs./hr. for the first quarter of an hour in order to bring back the humidity in the chamber to equilibrium as quickly as possible. A check on the specific gravity of the mixture was made at suitable intervals and if any adjustment was needed, either glycerol or water was added.

A relative humidity of near 0% was obtained by substituting a U-tube containing calcium chloride for the bubbler tube of the glycerol-water mixture.

When mite material had to be exposed for periods of a week /

PLATE V. Arrangement for exposing mites to different
relative humidities (see text for details).



week or longer at 0% R.H. it was placed in crystallising dishes containing calcium chloride. Each dish ($3\frac{1}{2}$ " diameter and 2" high) was half filled with a layer of calcium chloride. A circle of black filter paper punctured with small holes was placed over the calcium chloride. The dish was covered by a glass plate sealed down with vaseline, after the perspex cells containing the mites had been placed upon the circle of filter paper inside the dish. When mites had to be exposed in saturated moisture conditions for similar periods, moist cotton wool was substituted for calcium chloride in the dish.

Hypopi of Glycyphagus spp. were removed from the case by means of two small tungsten needles, care being taken to minimise the chance of disturbance to the epicuticular wax layer. A test was carried out on ten hypopi removed from their cases to make certain that the wax layer was not being damaged in the process of removal. The hypopi were immersed in ammoniacal silver nitrate in a cavity slide in the normal manner (see page 37), and kept for seven days. At the end of this period there was no argent-affin reaction from the polyphenol layer. It was therefore concluded that the continuity of the wax layer on the surface of the epicuticle was unbroken.

Twenty to fifty mites were placed in the chamber or dish for each test, and the total number of individuals exposed to a given humidity was not less than one hundred, except in some of the tests on hypopi. The mites were examined at intervals through the glass cover of the chamber or dish, whichever was being used.
The /

The cover was removed only when some of the mites appeared to be dead.

Preliminary tests were carried out to discover the best method of assessing mortality. This was done by simply exposing mites to harsh dry conditions either in the chamber or the dish. Those which had collapsed and were more or less immobile were provisionally regarded as dead or nearly so. Mites in varying degrees of this condition were transferred to perspex cells and kept, at the favourable conditions of 25°C. and an atmosphere saturated with moisture, for a protracted period.

The larvae, nymphs and adults of the different mites tested and the hypopi of H. polypori were considered as dead if at the end of a twenty-four period they were incapable of normal locomotion. It was found that even if the period of twenty-four hours was extended, mites in this condition seldom recovered. Since these stages of different mites were immobile and did not react to the touch of a camel hair brush before they were transferred to the favourable conditions, it was therefore possible to assess the condition beforehand as being one from which the mites would not recover, and therefore a condition which indicated impending mortality.

Hypopus stages of Glycyphagus spp. previously subjected to harsh dry conditions were kept for five days at the favourable conditions. If during this time they collapsed completely, or became discoloured, it was an indication of mortality. Hypopi which shrunk rapidly after they had decreased their bulk to about half the normal size before transference to moist, warm conditions, never recovered. Hypopi whose bulk had decreased to about half the /

the normal size but in which the shrinkage had not yet begun to take place more rapidly, sometimes recovered in the favourable conditions. Hence this mode of shrinking was regarded as an indication of mortality.

In the actual experiments mites conforming to the above descriptions were regarded as dead, removed from the chamber or dish and kept in conditions detailed above for confirmation of mortality.

It was therefore possible in these experiments of testing various humidity conditions on the different stages of mites to assess, by observation, whether or not a mite was either dead or moribund. This assessment of mortality was important as a standard for measuring the biological effect of the various combinations of humidity and temperature.

The time in which 50% mortality of the mites exposed under given conditions was attained, was considered as the average time of survival of the mites under these conditions. This average survival time was used in comparing the survival of the mites under varying conditions.

Resistance of Isolated Individuals.

The normal stages of Glycyphagus spp. were exposed in the humidity chamber to constant relative humidities of 100%, 90%, 70%, 50%, and near 0% at an average room temperature of 17°C. and at 25°C. The results of the separate trials in each of the humidity-temperature conditions were combined and from these combined results the block diagrams in Fig. 16 were constructed. The survival times of the two species were found to be similar so the separate /

FIGURE 16. Histograms showing the survival of batches of a 100 normal stages of Glycyphagus spp. when exposed to different relative humidities at 17°C. and 25°C.

White area - 17°C.; black area - 25°C.

separate species are not distinguished in the results. At 17°C. the average survival time of normal stages was two days in 0% R.H., three and a half days in 50% R.H., four days in 70% R.H., six days in 90% R.H. and seven days in 100% R.H. At 25°C. the survival time was much shorter, being about one-quarter of that at 17°C. Normal stages kept in cells in saturated moisture conditions at 17°C. and at 25°C. survived until they died of shortage of food or natural causes. At 2°C. the normal stages survived for five days in 100% R.H. and for four days in 0% R.H.

Fifty hypopi of G. destructor, removed from the hypopus case, survived for at least six months in room conditions of 50% to 80% R.H. at 12°C. to 18°C. When exposed to 0% R.H., one hundred hypopi, removed from their cases, survived for an average time of nine weeks at a room temperature of 17°C. and for eighteen days at 25°C. (Fig. 17). At 2°C., 0% R.H., the survival time for hypopi was at least twenty weeks, by the end of which time there was a slight shrinkage in about half of the twenty-five hypopi tested.

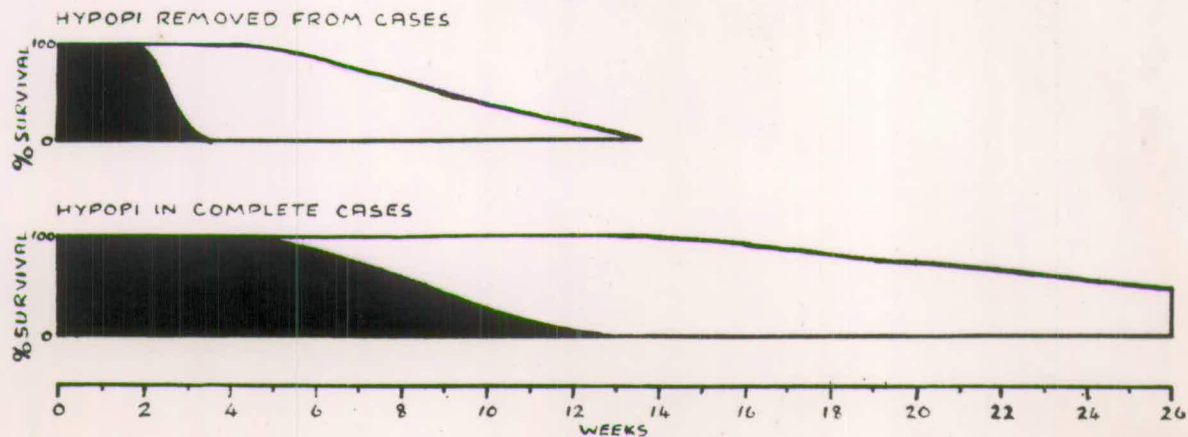
Twenty-five hypopi of G. destructor in hypopus cases which were not split or damaged were able to survive for at least six months in room conditions of 50% to 80% R.H. at 12°C. to 18°C. The hypopi had not shrunk to any visible extent by the end of the six months. In 0% R.H. the average survival time of one hundred hypopi was about twenty-six weeks at a room temperature of 17°C. and eight weeks at 25°C., about three times as long as hypopi removed from their cases, under the same conditions (Fig. 17).

Twenty-five hypopi of G. destructor in cases which were split open at the posterior end were able to survive for about
the /

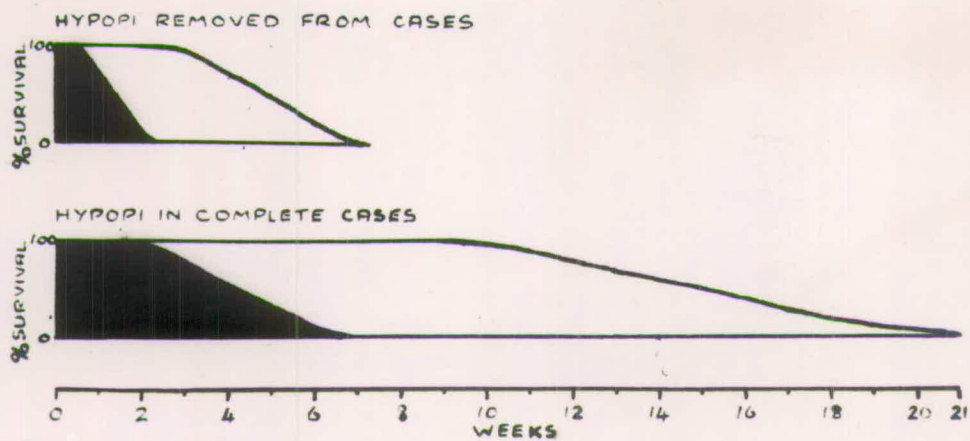
FIGURE 17. Histograms showing the survival of batches of hypopi of Glycyphagus spp. when exposed to 0% R.H. at 17°C. and 25°C.

white area - 17°C.; black area - 25°C.

G. DESTRUCTOR.



G. DOMESTICUS



the same length of time as hypopi which had been removed from their cases, when subjected to 0% R.H. at room temperature and at 25°C.

Fifty hypopi of G. domesticus, removed from the hypopus case were able to survive for at least six months when tested under the same room conditions as hypopi of G. destructor. Five hypopi shrank and died in the six month period of the test. In 0% R.H. at a room temperature of 17°C. and at 25°C., the average survival time of one hundred hypopi was four and a half weeks and ten days respectively, about half that of hypopi of G. destructor under similar conditions (Fig. 17). At 2°C. in 0% R.H. the hypopi survived for at least twenty weeks and by the end of this time all the hypopi had shrunk to varying extents. The results presented in histogram form in Fig. 17 have not taken into consideration the few cases in each test when a hypopus developed into a deutonymph.

Similar batches of hypopi of G. domesticus in undamaged cases tested under the same conditions as the hypopi of G. destructor, survived for about half the length of time as these hypopi. As in G. destructor the presence of an undamaged hypopus case trebled the average survival time of the hypopus (Fig. 17).

Larvae, nymphs and adults of H. polyperi in 100% R.H. at a room temperature of 17°C. survived for about one and a half hours. In lower humidities the time of survival was correspondingly shorter. At 25°C., the survival time of the normal stages was about one-fifth of that at 17°C. ^(Table 1) In 100% R.H. at 2°C. the average survival time was three and a half hours.

Hypopi of H. polyperi were able to survive for longer times /

TABLE 1. Time taken for 50% mortality of normal stages of H. polyperi to be incurred by exposure to different relative humidities at 17°C. and 25°C.

R.H.	Temp.	17°C	25°C
0		5 min.	2 min.
50		10 min.	5 min.
70		30 min.	7 min.
90		55 min.	12 min.
100		90 min.	20 min.

FIGURE 18. Histograms showing the survival of batches of a 100 hypopi of H. polypori when exposed to different relative humidities at 17°C. and 25°C. white area - 17°C.; black area - 25°C.

times than the normal stages in the various humidity conditions. The average survival time of hypopi at 17°C. was five hours in 0% R.H., ten hours in 50% R.H., sixteen hours in 70% R.H., twenty-seven hours in 90% R.H. and thirty-two hours in 100% R.H. At 25°C. the survival time in a given humidity was about one-third of that at 17°C. At 2°C. the survival time of ten hours in 0% R.H. was about twice that at 17°C. (Fig. 18). In 100% R.H. at 2°C. the hypopi survived for about one hundred and thirty hours.

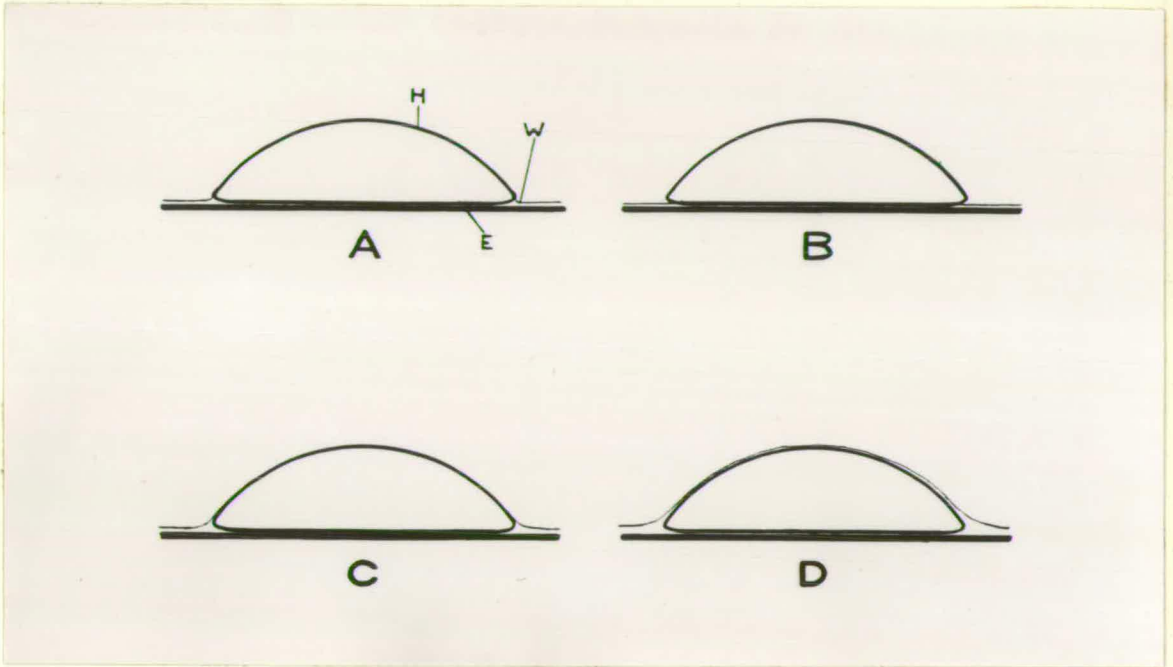
Protection Afforded to Hypopi by the Epicuticular Wax of an Insect to which they are Attached.

Some earwigs supporting hypopus mites which had been attached for at least two days, and others having hypopus mites attached to them for sixteen to twenty-four hours, were examined. Hypopi which had been attached for the longer period appeared to be fused to the insect cuticle by a rim of wax (Fig. 19A). This rim of wax showed up clearly because its concave surface reflected light. Hypopus mites attached for less than twenty four hours were not waxed to the cuticle in the same way (Fig. 19B).

Earwigs with hypopus mites attached for the longer and shorter times were exposed to 0% R.H. at a room temperature of 17°C. Hypopi attached for less than twenty four hours shrunk and fell off the cuticle within seven hours. The wax layer of earwigs became visibly thicker after three days exposure and the cuticle acquired a distinctly glossy appearance. Moreover, the wax around the hypopus mites also thickened (Fig. 19C). The wax of the cuticle of the earwig, in fact, appeared to gradually increase in thickness up to about the sixth day of exposure to the low humidity. The conditions also caused a considerable shrinkage of the abdomen of the /

FIGURE 19. Diagrams showing the relationship at different times between the epicuticular wax of an earwig and an attached hypopus mite, H. polypori (see text for details).

E, cuticle of earwig; H, cuticle of hypopus;
W, epicuticular wax of earwig.



the earwig. Hypopi frequently brushed their legs over the surface of the wax, and in doing so, they covered themselves to such an extent that they practically became embedded in the wax of the earwig (Fig. 19D).

The earwigs themselves succumbed to exposure at 0% R.H., 17°C. after nine or ten days. On the death of the earwig the hypopi normally left the cuticle within a few hours. The hypopi, once they became detached from the earwig cuticle, shrunk and died within a few hours. In one case, a hypopus remained attached to the earwig cuticle for some days after the death of the earwig. Two days after the death of the earwig the hypopus was still alive. By the third day, however, the hypopus had shrunk and was dead. The wax of the earwig appeared to be drying and forming solid lumps.

An attempt was made to obtain serial sections of hypopi attached to the cuticle of an earwig, to examine more closely this waxing down of the mite. Normal methods of fixing and embedding were unsuitable because the wax would be affected by the reagents, and it would be exceedingly difficult to keep the hypopi in situ. Embedding in polyvinyl alcohol was therefore tried, because both the insect and attached mites could be placed directly in the polyvinyl alcohol medium.

An earwig with hypopi attached was exposed to 0% R.H. at a room temperature of 17°C. for seven days to induce a thickening of the layer of wax. A suitable piece of cuticle with hypopi attached was removed from the earwig and kept at -2°C. for half an hour to immobilise the hypopi, before killing them in ammonia vapour.

The /



The polyvinyl medium, into which the cuticle was embedded, was prepared according to the method recommended by Lubkin and Carsten (1942). The polyvinyl block was trimmed to a suitable size and embedded in paraffin wax. However, the method was unfortunately not satisfactory because, when cutting the block, the hypopi became detached from the cuticle of the earwig. But, nevertheless, although it was not possible to obtain sections showing the waxing down of the mite to the insect cuticle, direct observation of whole mites attached to the cuticle clearly showed how the mites take advantage of the insect's thick wax layer to protect themselves.

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HYPOPIUS CUTICLES AND TRANSPIRATION.

The ability of an insect to resist desiccation in a dry environment depends on the rate of transpiration of water through the cuticle. The wax layer of the epicuticle is mainly responsible for the prevention of water loss. The exocuticle, however, also plays a part in controlling the rate of transpiration of water; this depends upon the structure of the sclerotin (Pryor 1940). The transpiration rate is low in the normal range of environmental temperatures. Ramsay (1935) and Wigglesworth (1945) have shown that if insects with their spiracles covered are exposed for short periods to different constant temperatures, transpiration at first increases slowly as the temperature is raised, then much more rapidly when a certain temperature is reached. At this critical temperature which varies considerably in different insects the wax layer becomes more permeable to water (Beament 1945). Lees (1947) states that ticks having higher critical temperatures are more resistant to desiccation at temperatures in the normal environmental range.

A method was devised of assessing transpiration through the cuticle of hypopi and normal stages of mites to gain further information on their resistance to desiccation. Transpiration in hypopi of Glycyphagus spp. enclosed in the hypopus case was also investigated.

The histochemical investigation of the component layers of the hypopus case indicated that the wax layer of the epicuticle was absent. The presence of the hypopus case enclosing the hypopus caused a decrease in the rate of transpiration of the enclosed hypopus. In order, therefore, to discover the role of the epicuticle of the hypopus case in reducing transpiration, early developing /

developing hypopi were also tested. In the developing hypopi the hypopus case was still transparent, pliable, and with the wax layer intact. At the same time the space between the hypopus and the hypopus case was filled with fluid.

Methods for Assessing Water Loss through the Cuticle.

The methods used by previous workers for exposing material to constant temperatures and for measuring the transpiration rate were unsuitable for the present work owing to the small size of the mites.

Glycyphagus spp. were placed in a perspex cell, having a glass plate substituting the filter paper floor. A small quantity of phosphorus pentoxide was placed in the cell and the glass cover was sealed down with vaseline to make the cell airtight. The cell was placed on the floor of a metal box floating in a water bath kept at a constant temperature. A glass cover was placed on the box (Plate VI). Hypopus mites and adult mites were tested in batches of ten. Three batches of hypopus mites and adult mites were exposed at constant temperatures, at 5°C. intervals, within the range of 20°C. to 55°C. The mites before testing were first killed by ammonia vapour. Hypopi of Glycyphagus spp. were left for forty eight hours on filter paper moistened with ammonia to make certain of killing them.

It was noteworthy that the time taken by a mite to obtain a state when no further shrinkage could be detected, was relative to the rate of transpiration. A mite under the conditions of the test usually shrank rapidly to about 1/10th of its original size; /

PLATE VI. Arrangement of apparatus for estimating the rate
of water loss from the hypopi and the adults of
Glycyphagus spp. (see text for details).



size; further shrinkage took place extremely gradually. During this second stage of gradual shrinkage the glossy appearance of the surface of the cuticle of the adults tended to change and become opaque. Hypopi which had reached a similar state of shrunkenness became slightly brownish, and the wrinkled cuticle did not yield to pressure from a tungsten needle. The time taken for fifty per cent of a batch of either hypopi or adults to enter this second stage was taken as the measure of the effect of transpiration at that particular temperature. The reciprocal of this time, expressed in minutes, was used as an indication of the transpiration rate in constructing the graphs relating transpiration rate and temperature. The method does not give the exact relationship between the transpiration rates at different temperatures of hypopi and adults because it takes no account of volume, surface area, percentage water content and other factors, but it does indicate when there is a significant difference between them.

Hypopi and adults of H. polypori were tested in a cavity slide with the coverslip not quite covering the cavity. Phosphorus pentoxide was not used since it resulted in too rapid a shrinkage of the mite. Apart from this slight modification, the procedure for estimating transpiration was the same as that employed for Glycyphagus spp. In H. polypori the brown colour of the shrunken hypopus was much more definite than in Glycyphagus spp.

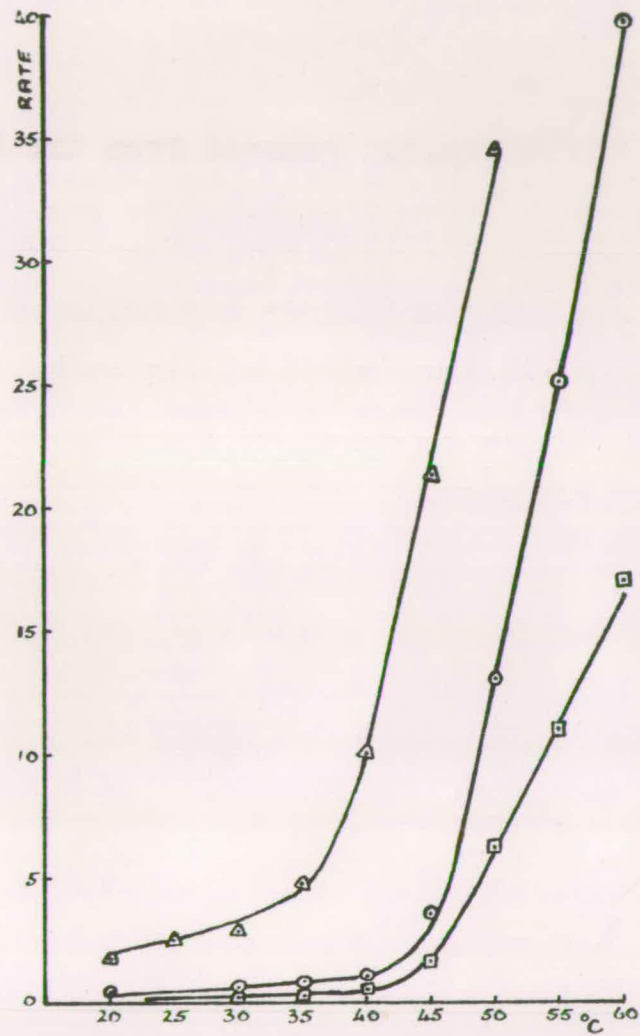
Water Loss by Adult and Hypopus Mites.

The rates of transpiration in the adults of G. destructor and G. domesticus were similar. The results have therefore been collated and they have been presented in the form of a graph (Fig. 20). The critical temperature for adults was approximately 35°C. /

FIGURE 20. Rates of water loss in adults and hypopi of Glycyphagus spp. in 0% R.H. at various temperatures.

- Δ - adults.
- - hypopi removed from the hypopus case.
- - hypopi enclosed in hypopus case.

The unit used to measure rate of water loss is the reciprocal of the time, in minutes, required for the collapse of the mite to a predetermined condition.



35°C.

The hypopi of both species transpired at a much lower rate than the adults. The critical temperature for the hypopus mite was about 43°C., indicating the presence of a layer of wax of a much harder consistency than that of the adult. Observations on the transpiration rate of hypopi of both species in complete cases showed that the rate was about half that of hypopi unprotected by the hypopus case (Fig. 20).

When early developing hypopi were tested, the hypopus case collapsed as the fluid between the case and the hypopus mite evaporated. However, when this occurred, the cuticle of the hypopus was capable itself of preventing further collapse. The hypopus, when removed from the collapsed case, was intact and had not shrunk. The rate of transpiration through the hypopus case, which in fact is an exuvium, was the same as that through the cuticle of an adult. During the early hypopus development of the mite, the hypopus case did not therefore afford protection to the hypopus mite against water loss.

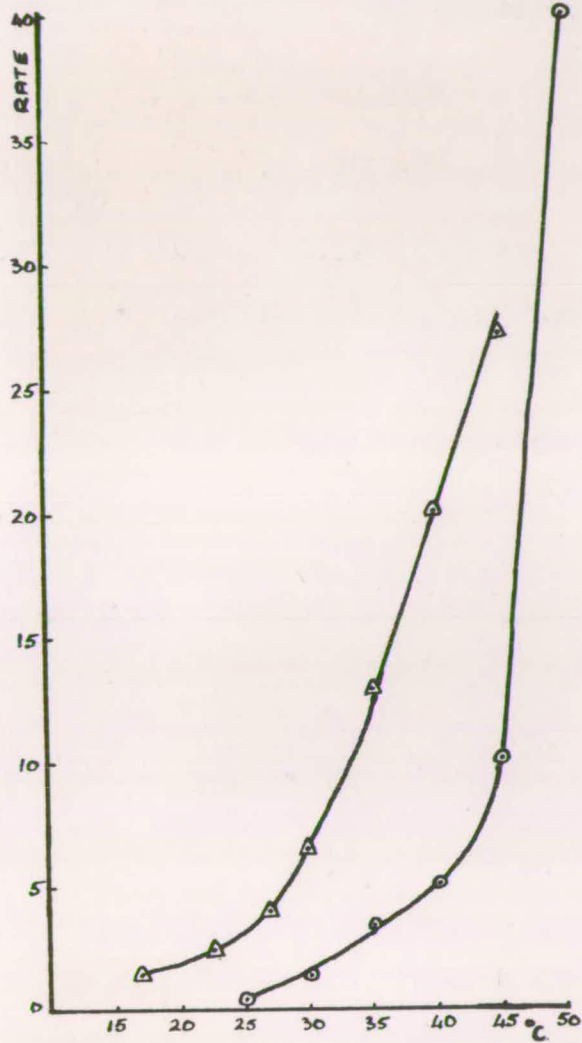
Water passed through the cuticle of adults of H. polyperi much more rapidly than it did through the cuticle of its hypopus forms (Fig. 21). The critical temperature of the adults was about 25°C., and that of the hypopi, about 43°C. The critical temperature of the hypopus mite was much better defined on the curve of the graph than it was in the case of the adult; its higher value indicates that the hypopus has a much harder epicuticular wax than that of the adult.

FIGURE 21. Rates of water loss in adults and hypopi of H. polyperi in 70% R.H. at various temperatures.

Δ - adults.

○ - hypopi.

The unit used to measure rate of water loss is the reciprocal of the time, in minutes, required for the collapse of the mite to a predetermined condition.



THE EFFECT OF ENVIRONMENTAL FACTORS.

Various workers have investigated the effect of different external factors which appeared to modulate the production of hypopus forms in Tyroglyphid mites. Much attention has also been paid to conditions which appeared to convert the hypopus condition back into the normal stage. Schulze (1924b) maintained that in a culture of Caloglyphus rodionovi a proportion of the protonymphs were morphologically distinct from the rest, and that they changed into hypopi irrespective of the environmental conditions. Of the remaining protonymphs a number could be converted into hypopi by depriving them of a suitable food supply. Polezhaev (1938, 1940) found that hypopus forms of G. destructor increased in number as the temperature and the humidity deviated from the optimum conditions which favoured a thriving multiplication of the normal stages. The same worker also observed that, unlike the case of C. rodionovi, a lack of food did not promote hypopus production in G. destructor. Hora (1934) was of the opinion that the production of hypopus stages of G. domesticus was not dependent upon a deterioration of the external environment.

In C. rodionovi hypopi changed into deutonymphs when there was an increase in the environmental moisture above that which obtained when the hypopi were formed (Schulze 1924b). The mobile hypopus of Tyroglyphus farinae changed to the deutonymph within forty eight hours of being placed in saturated moisture conditions, but the immobile hypopus of the same species changed to the deutonymph irrespective of the humidity (Schulze 1924a). Polezhaev /

Polezhaev (1940) found that hypopi of G. destructor changed into deutonymphs at 20°C. when the relative humidity was above 40%, but at higher temperatures correspondingly higher relative humidities were required to induce the reversion of the hypopus to the normal state. At temperatures below 12°C. hypopi would not change into deutonymphs irrespective of humidity.

It was Polezhaev (1940) who stated that fewer deutonymphs of G. destructor appeared in winter and, consciously or unconsciously, he gave a valuable clue to the nature of hypopus production when he also stated that the hypopi at this time of the year appeared to be in a state of diapause.

Although the past findings on the effect of external stimuli upon hypopus production left much to be desired, and left room for further controlled experiments, they seemed to indicate that some hypopi are genetically determined, while others would appear to be produced in large numbers only if the environmental conditions were harsh. But, in this work, although carefully controlled experiments have been designed to discover to what extent the environment governs hypopus production and the conversion of hypopi back to the normal state in certain species of mites, the main aim has been to investigate the nature of the hypopus condition. Whereas this has been the aim of the histological examinations of mites developing into hypopi and of hypopi developing back to the normal state, it has also been the aim of the experiments designed to test the effect of different environmental factors upon this two-way development. It was legitimate to ask the question "Does the hypopus condition simulate a true diapause state?" The investigation on the morphogenetic movements /

movements that take place during the development to and from the hypopus condition suggested that at least in G. domesticus the hypopus condition represented a diapause state. The influence of environmental factors upon hypopus production and upon the altering of the hypopus condition to the normal state have therefore been examined to discover whether or not hypopus development resembled a true diapause state.

The possible influence of light as a modulating factor was also considered in view of recent work showing that photoperiod was causally connected with diapause development. But entry into this field, in the present work, to discover if exposure of hypopi to light could possibly induce a change to the normal condition has only been slight. Hence experiments, of a cursory nature only, were carried out.

The Influence of Humidity and Temperature on Production
of the Hypopus Condition.

Cultures of H. polypori, kept in Petri dishes, were examined daily over a period of ten days at different humidities, temperatures, and light conditions. Hypopus production was estimated by counting the numbers of developing hypopi and developing deutonymphs visible inside immobile protonymphs, within a restricted area. This was accomplished by placing a square-shaped wire boundary with $\frac{1}{2}$ " sides upon the substrate. Discretion was called for in the use of this method, and, for example, when individuals of a sparse colony were scattered, the wire square was placed upon several spots in order to obtain a sufficient total of /

TABLE 2. The influence of moisture content and food supply upon hypopus production in H. polyperi.

Conditions		Ratio of Hypopus Forms to Deutonymphs		
State of Food Supply	Moisture Content	No. of Developing Hypopi observed over a 10-day period.	No. of Developing Deutonymphs observed over a 10-day period.	
17°C.	Adequate	High	6	300
	Adequate	Low	270	35
	Inadequate	Low	0	70
25°C.	Adequate	High	1	277
	Adequate	Low	193	36
	Inadequate	Low	1	83

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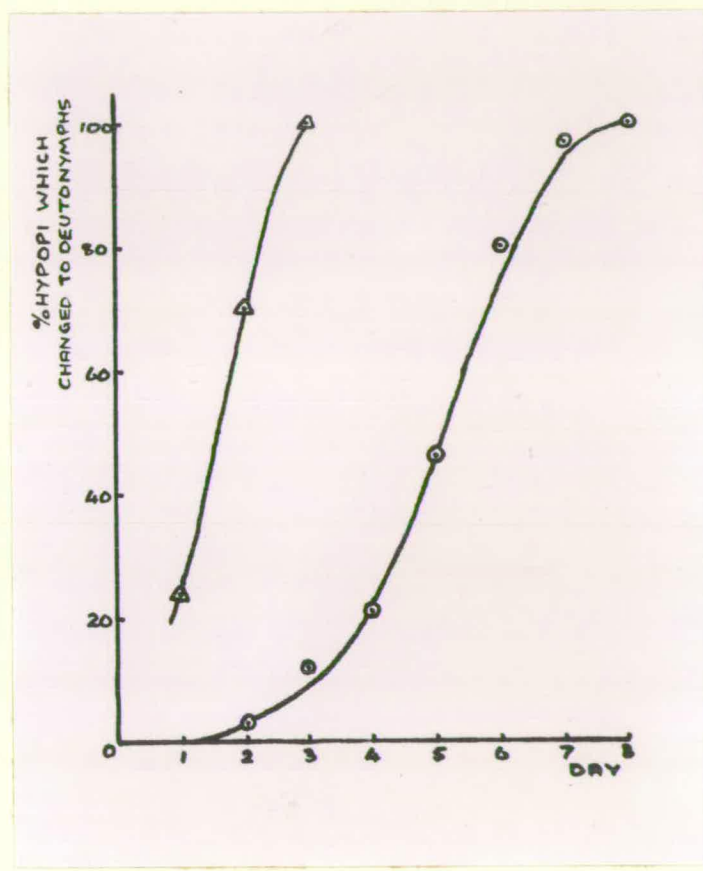
of counted individuals. Since, however, during the early part of the developmental phase which succeeds the protonymph stage, it was not possible to distinguish developing hypopi from developing deutonymphs, a certain proportion of the developing stages remained unidentified. But the method otherwise served the purpose of making a fairly accurate comparison.

When stock cultures were kept at 25°C., and in saturated moisture conditions together with a plentiful supply of earwig remains as food, the numbers of hypopi and developing hypopi were relatively few (Table 2). Identical results were obtained when such cultures were exposed to either daylight conditions or total darkness. Neither was there any change in hypopus production when the temperature was further raised from 25°C. to 30°C. But, when a thriving culture, with an appropriate supply of food, was allowed to dry up slowly, hypopus production increased considerably irrespective of the temperature (Table 2). Even so, a proportion of the hypopi formed were inclined to change back to the normal state where the conditions were relatively moist at the beginning. The keeping of the drying culture in either daylight, or total darkness, made no difference to the production of hypopi. It was noteworthy that when the number of mites in a culture was small owing principally to exhaustion of the food supply, the production of developing hypopi was negligible (Table 2). Even when the mites were kept at 25°C. and in very moist conditions, but without food, hypopus production did not occur. It was therefore legitimate to proceed with the assumption that the lack of moisture and not food influenced the formation of hypopi.

FIGURE 22. The change of hypopi of H. polyperi to deutonymphs induced by exposure to saturated moisture at 17°C. and 25°C.

Δ - 17°C.

○ - 25°C.



61.

The Influence of Humidity and Temperature Changes
upon the Hypopus State.

When hypopi of G. destructor were kept at 25°C., 100% R.H., a single deutonymph emerged after three days, thirty per cent of the hypopi had changed into deutonymphs after five days, and forty per cent after seven days (Fig. 23). Hypopi kept for twenty one days at 17°C., 70% R.H. underwent no change. Only a few hypopi of G. domesticus changed into deutonymphs when they were kept under these same conditions (Fig. 23).

Hypopi of H. polyperi, kept in saturated moisture conditions, reverted to the deutonymphal state in five days at a room temperature of 17°C. and one and a half days at 25°C. (Fig. 22). Moist conditions at either 7°C. or 30°C. also induced the hypopi to develop to deutonymphs. This effect of moisture upon hypopi of Glycyphagus spp. and H. polyperi also took place irrespective of the presence or absence of a food supply or exposure to either daylight or darkness.

The Effect of Alternating Temperatures and
Humidities upon the Hypopus State.

Of one hundred hypopi of G. destructor, kept for twenty four hours at 2°C., 100% R.H. prior to exposure at 25°C., 100% R.H., twenty five per cent developed into deutonymphs within three days of their being transferred to 25°C. and fifty per cent within five days (Fig. 24). When hypopi were kept at 25°C., 0% R.H. for twenty four hours before exposure to 25°C., 100% R.H., it was found that these alternating conditions had no marked influence upon the development of hypopi into deutonymphs. Prolonging the period of subjection to 0% R.H. to seven days also had no effect. /

FIGURE 23. The change of hypopi of Glycyphagus spp. into deutonymphs induced by exposure to 100% R.H., 25°C.

○ - G. destructor.

△ - G. domesticus.

FIGURE 24. The influence of alternating temperatures of 2°C. for 24 hours and 25°C. for 7 days at 100% R.H. upon the change of the hypopi of G. destructor to deutonymphs.

Fig. 23

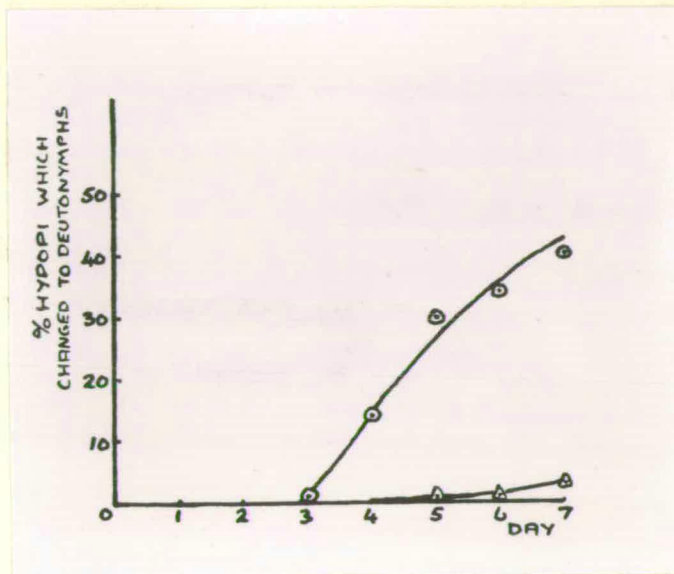
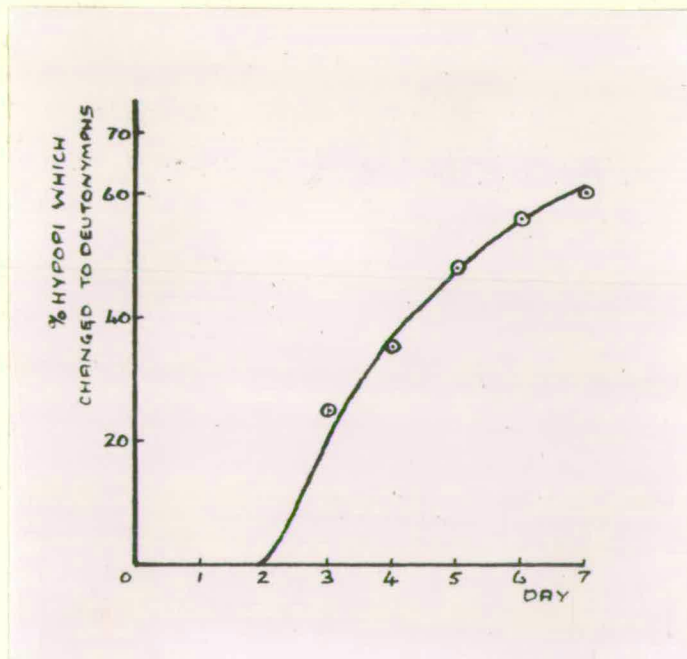


Fig. 24



effect.

Negative results were also obtained when hypopi of G. domesticus were subjected to the above low temperature or low humidity conditions for twenty four hours, or seven days, prior to being kept at 25°C., 100% R.H.

With hypopi of H. polyperi, pretreatment at 2°C. in saturated moisture conditions followed by subjection to 25°C. in saturated moisture did not affect the emergence of deutonymphs. The emergence of deutonymphs was similar to that represented by Fig. 23.

The Influence of Light upon the Hypopus Condition.

It has already been shown that the change of the hypopus condition of G. domesticus to the deutonymphal state could not be induced by changing the moisture content of the environment. Neither were any other of the various factors tested capable of effecting such a change. It was therefore reasonable to suppose that the hypopus condition of G. domesticus occurred spontaneously like a true diapause condition which persisted despite the subjection of the mite to rigorous changes in its environment.

It has been suggested that hypopus production matches the seasons and that peak numbers of mites in this condition are reached during late autumn. Hence, the possibility that photoperiod exerted an influence on the inception of this hypopus condition could not be ruled out.

Although light experiments need careful arranging and planning, if carried out extensively, it was felt that some tests of a cursory and preliminary nature would perhaps prove a pointer to the value of further research in this field. There was some evidence /

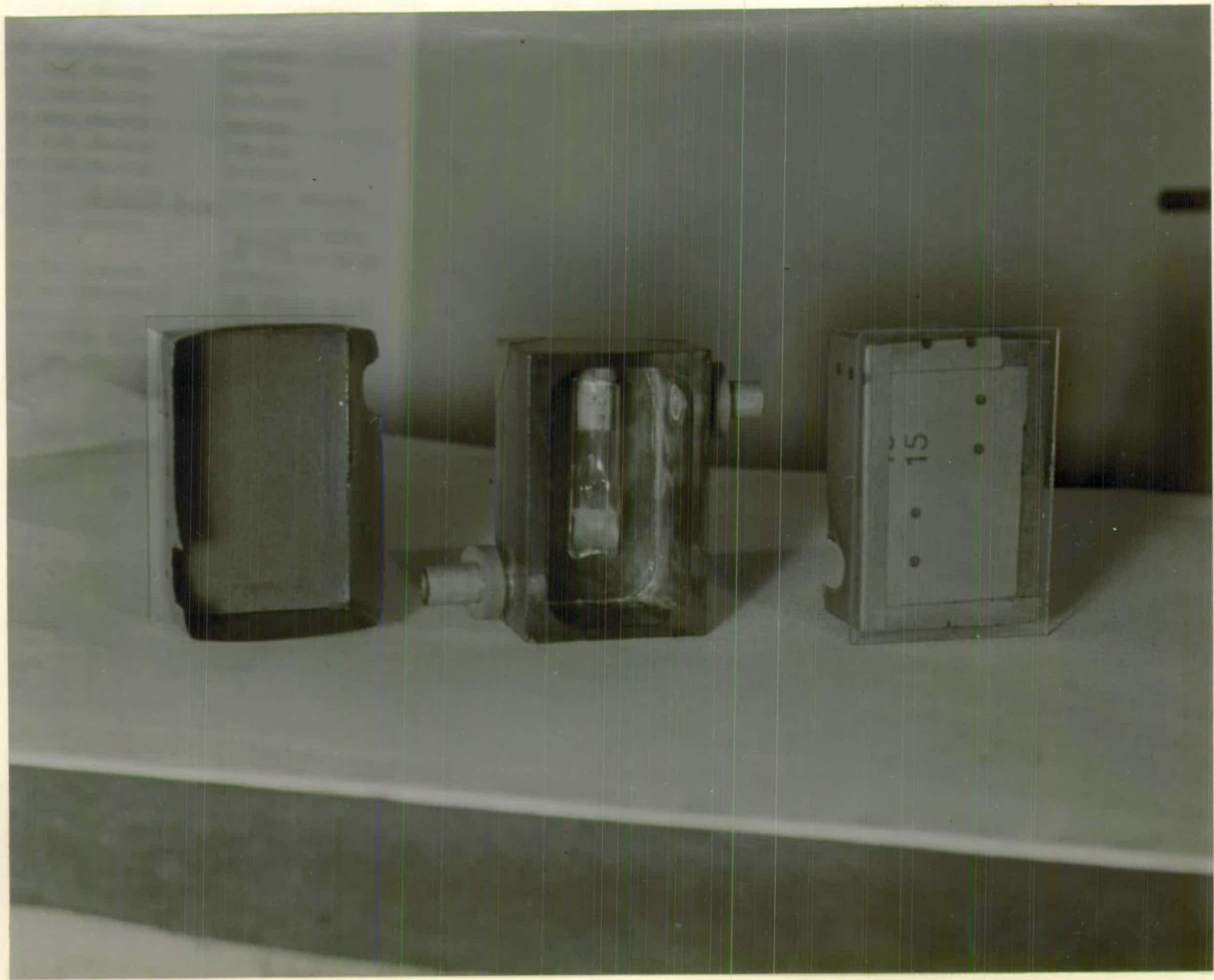
evidence that at least the hypopus form of H. polypori was sensitive to light intensity, hence, it was not unreasonable to expect that the hypopus condition of G. domesticus was also sensitive, in some degree, to light.

Since it was unlikely that a prolonged photoperiod only would induce the hypopus to develop further, it was exposed to a more drastic treatment. This consisted of exposing the hypopi of G. destructor and G. domesticus to alternating bright light and darkness.

The arrangement of the apparatus for exposing the mites to a high light intensity while at the same time preventing any change in temperature by the radiant heat, is shown in Plate VII. It has been designed on the principle of a water condenser, the outer jacket being made of perspex with a glass window, and the inserted inner tube of glass. The glass tube inside the perspex jacket, which was blacked out except for the window, was shaped at the bottom into a rectangular chamber. Its floor was the surface of a plug of paraffin wax, which closed up the end of the tube. The mites were placed upon the plug of wax inside the tube, which was placed against the perspex wall of the jacket opposite the glass window. A $\frac{3}{4}$ " layer of water separated the glass wall of the tube from the window of the jacket. Water kept at 25°C. in an aspirator flowed downwards and through the jacket at a controlled rate so that the water in the jacket was completely changed every five minutes.

The light source was a 100 watt pearl tungsten lamp. It was suspended in a metal box with a hole $1\frac{3}{4}$ " in diameter which was in line with the filament of the lamp. The centre of the hole was placed opposite the surface of the wax plug. A Weston photometer /

PLATE VII. The testing chamber and light-proof cover
used for testing the effect of different light
intensities on hypopus mites of Glycyphagus spp.
(see text for details).



photometer was used to measure the light intensity and the lamp was placed a certain distance away to give a light intensity of 4000 m.c. at the surface of the test chamber. Allowance was made for the absorption of light by the glass plate of the jacket and the glass window of the test chamber. The difference in light intensity at the near and far regions of the wax surface was less than 100 m.c. The jacket was appropriately covered over with black lightproof paper when the mites were kept in the dark (Plate VII).

Twenty five hypopi of G. destructor and twenty five hypopi of G. domesticus, stripped of their cases, were exposed to the alternating periods of light and total darkness. A light period of eighteen hours was alternated with a six hour period of total darkness. The hypopi were exposed to these alternating light and dark periods for seven days. A relative humidity of approximately 65% was obtained inside the test chamber; this humidity corresponded to that of the warm room.

A single deutonymph of G. destructor emerged inside the chamber after five days' exposure to these conditions of varying light intensity. After seven days' exposure, the hypopi were removed from the test chamber and kept for a further seven days at 25°C., 100% R.H., under normal laboratory light conditions. Seven hypopi of G. destructor changed into deutonymphs during the seven days following the test. But those of G. domesticus remained unaffected by this light treatment.

No conclusions can rightly be drawn from limited tests of this kind, but they appear to suggest that the modulating of the environmental factors does not influence the hypopus condition of G. domesticus. The implication here is that the hypopus condition /

condition of this species is spontaneously induced, and that it appears to persist for a prolonged period, despite changes in the environment. In the case of G. destructor, it would be rash to assume that the light treatment was responsible for inducing the change to the normal deutonymphal state because, about the same proportion of hypopi of this species will naturally change into deutonymphs within the length of time the mites were kept at 25°C., 100% R.H. after the test (Fig. 22). But, it will be appreciated, that whereas the conditions were tested on G. destructor, the real purpose of the tests was directed towards the possibility of their breaking down the persistency of the hypopus condition of G. domesticus. However, as already mentioned, they failed to do this, and it is doubtful if exposure to different light intensities would be capable of effecting such a breakdown of this arrest in the development of the mite.

DISCUSSION.

It will be clear from the investigations on the morphogenesis of the three types of hypopus conditions selected for study, that the form expressed by the mite while in this condition appears to depend on (a) the time when development is arrested and (b) the progress of development of the epidermis and its associated muscles.

The cyst-like form of G. domesticus is more or less equivalent to the condition the mite obtains during an early step in the normal developmental phase of the deutonymph. The systems of organs and tissues of the old protonymph at this time are being replaced by new ones. But the potential gut, muscles and salivary glands of the deutonymph are still in an embryonic state. The connective tissue cells are numerous, but they have, as yet, not converted themselves into a new parenchyma network. The new phagocytes have not appeared. In G. domesticus it is reasonable to suggest that, at this step, there is a halt in development, and when it does occur, the gut is a shrunken strand of newly-proliferated gut-wall cells; most of the muscles are represented as groups of myoblasts; the salivary glands are likewise about to develop fully; and the epidermis is ready to deposit cuticle. The central nervous system undergoes little change. The reproductive system, however, has undergone the first stage of its development to the condition in which it is present in the deutonymph. The mite is therefore, at this time, enclosed in the old cuticle of the protonymph, but is not yet invested with a new cuticle. When this arrest in development is accompanied by a deposition of cuticle, and the development of two new systems of muscles, /

muscles, the hypopus form, typical of G. domesticus, is assumed.

The hypopus form of H. polyperi is more difficult to interpret, but nevertheless, the results of the foregoing investigations indicate that the complexity of the hypopus structure is principally due to the activities of the epidermis and its associated muscles. That the epidermis of its own accord, can mould the organism into a given shape independent of the development of the internal systems is well shown in the development of the mobile hypopus of H. polyperi. The shape of the hypopus and its possession of specialised structures are promoted principally by the epidermis.

The hind gut of the hypopus form is present as a fully developed structure. The remaining regions of the gut, however, remain as a strand of regenerated cells which have been halted in development shortly after their proliferation. The salivary glands are composed of new cells not yet fully developed. A large part of the parenchyma network of the protonymph remains. The new parenchyma forming cells have appeared, but have not begun to form the new network. The reproductive system has undergone a slight development as in G. domesticus. The central nervous system has undergone little or no change and has become efficiently geared to the new form, and to its capacity to move at a much quicker pace than the other normal stages in the life-cycle. But, whereas some of the systems of organs and tissues have halted in their development, the epidermis has gone ahead, and has moulded itself into a shape having different spatial relations to that of the normal mite. Furthermore, the cuticle has hardened to a chestnut colour, the nature of the sensory end organs have been changed, /

changed, and, what is even more spectacular, a complex disc of suckers has appeared on the ventral surface of the opisthosoma. As one would expect, muscle development has kept pace with the activities of the epidermis and the muscle systems are appropriately arranged to suit the new form.

The form that the hypopus of H. polypori assumes is therefore primarily due to the differential rates of development of the various systems of organs and tissues. The epidermis and muscles only, reorganise themselves, whereas the central nervous system maintains its normal structural and functional character. The remaining systems, however, which reside within the hypopus mite have developed further than they do in G. domesticus, but they still remain in a more or less incomplete state. One may legitimately suggest that the epidermis and muscles have developed along a side chain, whereas the remaining systems remain checked in their development until final causes intervene to unfold them into the definitive systems of the deutonymph stage. When this change from the hypopus form to the deutonymph takes place, the epidermis sheds the hypopus cuticle and re-deposits that of the deutonymph and the muscles de-differentiate, in some cases only partly, and new muscles are regenerated. The systems of muscles which first appear as the hypopus develops degenerate completely. The epidermis and muscles are therefore brought back into equilibrium with the other systems before harmonious development of the whole organism towards the deutonymphal stage begins.

The hypopus form of G. destructor is attained by the epidermis and the muscles only partially moulding the mite into the shape achieved by the hypopus of H. polypori. The complex sucker disc is not developed. The systems of organs and tissues, other than the epidermis and muscles, assume a condition intermediate /

mediate between that of the hypopus of G. domesticus and that of H. polyperi.

In the light of these observations it is difficult to avoid comparison of this hypopus condition in these mites with the diapause state which occurs in some insects.

The ^{term} diapause was first coined by Wheeler (1893) to describe a step in the morphogenesis of the embryo of the grasshopper Xiphidium. It represents a stationary condition of the embryo during blastokinesis. The term, however, has never gained currency in embryology, and it was Henneguy (1903) who lifted it from its embryological setting and re-defined it to apply to the physiological state of rest or dormancy, which was known to occur in insects. Following Henneguy, ecologists used it loosely to include almost any sort of arrested development. It was Shelford (1929), who suggested that the term diapause be restricted to cases where development is arrested 'spontaneously', and does not respond immediately to any ordinary amelioration of the external environment. It was also recognised that there will be cases, where the first cause of diapause may lie in the external environment but the final cause of arrested development may nevertheless be 'spontaneous' or internal to the organism.

The experiments in the present work on the influence of environmental factors upon hypopus production have shown conclusively that in the case of mites, harsh conditions appear to be a first cause of arrested development. But in cultures with an abundant supply of food and a suitably high moisture content the arrest in development is also liable to occur. In this case it is undoubtedly spontaneous.

A very low moisture content most certainly induces a high /

high rate of production of hypopus forms of H. polypori. A high moisture content will equally induce the hypopus form of H. polypori to develop further, within one and a half days at 25°C., to the deutonymph.

The arrest of development in G. domesticus most closely resembles the diapause state obtained in insects. The halt in development is spontaneous. Amelioration of the environment or subjection to alternating high and low temperatures or humidities or drastic treatment by exposure to bright light alternating with darkness produces no response from the mite while in this hypopus condition. The condition, once established, appears to persist for a protracted period, irrespective of external influences. The re-commencement of development aimed towards the realisation of the deutonymphal state is therefore also spontaneous. The physiological condition of the hypopus form of G. destructor is less responsive to the environment than that of H. polypori, and more so than that of G. domesticus.

It is therefore reasonable to conclude that the hypopus condition of G. domesticus, in which the development of the mite as a whole is arrested, most certainly resembles the diapause state in insects. But, in H. polypori, where the epidermis and muscles deviate and become responsible for producing a specialised hypopus form, the arrested condition becomes less stable and it will respond readily to a change in the moisture content of the external environment. This response of a hypopus form to external influences grows less as it approaches the condition that obtains in G. domesticus, in which the arrest in development of the organism as a whole occurs early in the developmental phase of the /

the deutonymph.

The hypopus form of H. polypori is probably unique because of the extraordinary capacity of the epidermis and muscles of the mite to develop out of gear with the other systems of organs and tissues, and produce a shape so utterly unlike the normal mite. The locomotory behaviour is also attuned to climbing on to a passing animal. The definitive shape and behaviour ascribed to the hypopus form of H. polypori give it, perhaps, legitimate claim to be called a stage. But there is certainly no reason why the hypopus form of G. domesticus should be regarded as anything but a case of arrested development or diapause.

The functions ascribed to the hypopi of mites have been reviewed elsewhere (Solomon 1943). This present work has shown that the mite on assuming the hypopus form is able to resist harsher conditions than it could normally. Hence the value of the hypopus condition for tiding the mite over unfavourable periods. Water loss through the cuticle of hypopi is more controlled, thus mites in the hypopus state resist drought more easily. It is noteworthy that, although the hypopus of H. polypori is appreciably more resistant than the normal form of this mite, the hypopus of G. domesticus, on the other hand, is exceptionally resistant to drought compared with the normal forms of this mite. The mobile hypopus of H. polypori casts the old protonymphal cuticle, but the cyst-like hypopus of G. domesticus retains the old protonymphal cuticle as an outer covering which is generally called the hypopus case. Now it so happens that this hypopus case has an important role in helping the mite to resist desiccation. When the atmosphere is moist the hypopus case, now, to all intents and purposes, dead /

dead material, plays little part in regulating water passage into and from the mite. But, as the environment dries up, so does the hypopus case, and, when the hypopus case assumes this shrunken, taut condition, it helps to prevent water loss. Wigglesworth (1945) observed that the larva of the wireworm, Agriotes, when first removed from the soil, loses water very rapidly, but, if it is exposed in a dry atmosphere, the rate of water loss quickly diminishes. It was suggested that this phenomenon occurred owing to the fact that sclerotin, the protein of the exocuticle, when dry, will prevent the transpiration of water. It therefore seems probable that the reduced rate of water loss from a mite in a hypopus case is also due to this property of the sclerotin component of the hypopus case.

When the hypopus case dries, it becomes extra rigid and it does not distort under pressure as easily as it would when moistened. This ability of the hypopus case to acquire extra rigidity when dry may be of importance in preventing mechanical damage to the enclosed mite.

It is interesting to note that hypopi of H. polyperi, when attached to the cuticle of an earwig, are able to resist desiccation, when exposed to 0% R.H. for a very much longer period than isolated hypopi. This increased resistance, however, is apparent only if the hypopus has been attached to the insect cuticle for at least twenty four hours prior to exposure to these hard dry conditions. On the other hand, if the hypopus has been attached to the insect for less than twenty four hours previous to exposure, it shrivels up and falls off the insect in a very short time. Evidence suggests that the hypopus gains protection from the soft epicuticular wax of the earwig which tends to flow around the attached /

attached hypopus and so prevents excess water loss from the mite. Hypopi, when attached to the wax layer, appear to be capable of sweeping out a depression for themselves and also of pushing the soft wax around their bodies.

The reasons for hypopus development in mites are clear, but the important conclusion resulting from the present work is that hypopus development, especially in G. domesticus, is not unlike diapause development in insects. The hypopus form in these mites represents a true spontaneous arrest of development, the recommencement of development being equally spontaneous. In G. domesticus, therefore, there is a continuity in development as the mite changes from the protonymph to the deutonymph, although it may pass through the hypopus condition expressed more or less as a cyst-like form. But, in H. polypori, the role of the integument and its associated muscles in going ahead to fashion a shape so utterly unlike that of the normal mite, somewhat complicates the condition. This further development of the integument in H. polypori also appears to produce a type of hypopus development, which, perhaps owing to the unstable, disharmonious state of the systems of organs and tissues at the time, is readily influenced by changes in the external environment.

In G. destructor and H. polypori, hypopus development can be induced by exposing the mites to harsh conditions but, nevertheless, hypopus development is also spontaneous in all the three mites studied, and therefore independent of the external environment. The change, on the other hand, from the hypopus condition to the normal deutonymph, can be induced by an amelioration of the environment only in H. polypori, and this is true to a lesser extent in G. destructor. A modulation of the external environment has /

has no effect on the hypopus condition of G. domesticus, hence only final causes appear to promote further development. In H. polyperi and G. destructor first causes, for example moisture content and temperature, appear to act as a trigger which releases the intrinsic, or final cause mechanism responsible for breaking the hypopus condition, and promoting further development to the normal morphological pattern.

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SUMMARY.

1. It has been suggested that the hypopus condition met with in some mites closely resembles the diapause state which occurs in the Insecta.
2. In G. domesticus hypopus development is practically a true diapause. The development of all the systems of organs and tissues in this mite is arrested at an early step in the transition from the protonymph to the deutonymph. During this arrested state of development the mite assumes a cyst-like form. In G. destructor the arrest in development occurs a little later, with the result that small leg buds make their appearance, but, fundamentally, the condition simulates a diapause state.
3. In H. polypori, whereas the internal systems, except the muscles, are arrested in their development, the integument develops out of gear with the rest of the organism and moulds a mobile form utterly unlike that of the normal individual. When the mite develops further to the deutonymph, the epidermis sloughs off the hypopus cuticle and remoulds the mite to its normal form. These muscles, associated with the integument, which have been specially developed in the hypopus mite, de-differentiate, and the new definitive muscles of the deutonymph take their place.
4. The three forms of hypopus development studied are variations on a single theme. But, in H. polypori, a peculiar condition arises.
5. /

5. Hypopus development in G. domesticus is spontaneous and any amelioration of the environment does not induce the hypopus condition to change to the normal form of the mite. G. destructor is, on the other hand, influenced by environmental changes.
6. Hypopus development in H. polyperi is also spontaneous, but, equally well, it can be induced by exposing the mites to a harsh, dry environment. The change from the hypopus condition to the deutonymph can be induced by exposing the hypopus mites to a very moist atmosphere.
7. It is suggested that final causes only induce hypopus development in G. domesticus, but, first causes, for example, a very moist atmosphere, influence hypopus development in G. destructor and H. polyperi. These findings are in line with current interpretations of diapause development in the Insects.

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