An inherited abnormality affecting the development of the yolk plasmodium and endoderm in *Dermestes maculatus* (Coleoptera)

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WITH TWO PLATES

**INTRODUCTION**

In a previous paper (Ede & Rogers, 1964) a method was described for obtaining lines of the beetle *Dermestes maculatus* in which a large proportion of eggs produced specific abnormalities which could be used in investigations into its embryology. One of these abnormalities, type G, occurred in high frequency (up to 70 per cent) in a selected line, and was therefore particularly suitable for further study. Not enough genetic information was obtained to show how it was inherited, but it was sufficient for the purpose of an embryological investigation that it should persist through the three generations over which the stock was maintained.

Though developing so abnormally that they never emerge from the egg, these embryos live up to and slightly beyond the time at which normals hatch. At this time they are generally extremely contracted, and the endoderm, which forms the wall of the midgut in normals, is completely absent. The origin of the abnormality can be traced back to an early stage of embryogenesis when the superficial cellular blastoderm is becoming separated from the 'yolk plasmodium' beneath it. These features give it a particular interest, for the origin of the midgut has always presented problems for insect embryologists (Henson, 1946), and the yolk plasmodium plays an active and important part in a number of developmental processes (Counce, 1961; Krause & Sander, 1962). The present study throws light on both these topics.

**MATERIALS AND METHODS**

The methods of maintaining the beetles and of collecting and treating eggs have been described (Ede & Rogers, 1964). The developmental studies reported here were based upon:

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(1) Observations on living embryos followed through the course of their development. Eggs were collected from females which had been laying for a period of 9 hr., so that the eggs were aged 0-9 hr. at the beginning of the observations. They were then immersed in distilled water in a cavity slide and examined at frequent intervals under a low magnification (70 x) until development was completed. Camera lucida drawings were made at critical periods. Twenty-nine eggs were set up in this way, of which sixteen produced normal embryos and thirteen abnormals.

(2) Observations on fifty-one normal and seventy-eight abnormal embryos sectioned longitudinally at stages ranging from blastoderm formation to the completion of development.

Diagrams illustrating the development of the normal embryo (Text-figs. 2 and 3A) were constructed from camera lucida drawings selected from several normal series. Diagrams f-i (Text-fig. 3B) form a series from a representative abnormal embryo, and diagram j (Text-fig. 3B) is the terminal stage of another abnormal embryo, representing a further development which not all the abnormals undergo. The camera lucida gave an outline picture of the yolk and embryonic tissue, and internal details were reconstructed from corresponding stages seen in sectioned embryos.

RESULTS

In order to make the development of the abnormality clear it will be necessary to outline the main embryonic events which are affected as they occur in normal embryos. The normal development of Dermestes is essentially similar to that of Leptinotarsa as described by Haget (1953).

Development of the normal embryo

Blastoderm formation

The characteristic structure of the insect egg at the beginning of its development has been most recently described by Krause & Sander (1962). Covering the egg is a membrane laid down by the follicle cells of the ovary, the chorion. Within this is the tough vitelline membrane of the egg. The vitelline membrane encloses the egg plasmodium, i.e. the continuous reticulum of cytoplasm which connects the cleavage nuclei and their surrounding islands of cytoplasm (cleavage energids) and contains the yolk granules in its interstices. There is no yolk in the layer of cytoplasm immediately beneath the vitelline membrane, and this clear layer is called the periplasm. The cleavage energids become arranged in an ellipsoid in the middle of the egg. Most of them multiply and migrate to the surface layer, where their cytoplasm fuses with the periplasm and their nuclei form a single layer over the surface of the embryo. There follows a number of tangential nuclear divisions within the periplasm, without formation of cell walls, giving rise to a syncytial blastoderm. Some cleavage energids lag
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behind in the yolk while the remainder migrate towards the periplasm, and these multiply within the yolk to give the vitellophages.

The syncytial blastoderm (Text-fig. 1a) becomes transformed into a cellular blastoderm by a process similar to that observed in *Drosophila* by Ede & Counce (1956). Furrows grow in from the external surface of the periplasm between the nuclei (Text-fig. 1b). They cease their inward growth before quite penetrating the periplasm (Plate 1, Fig. A), and the base of the furrows expands tangentially (Text-fig. 1c) so that each nucleus becomes isolated in its own cytoplasm to give a columnar blastodermal cell (Text-fig. 1d). The innermost layer of periplasm is cut off and remains connected with the cytoplasmic reticulum of the yolk, around which it forms a distinct membrane. The reticulum, together with its vitellophages and enclosed yolk granules, forms the yolk plasmodium (Krause & Sander, 1962).

**Germ band formation and gastrulation**

The blastoderm cells covering the sides of the egg move ventrally and form a thickened germ band, while the blastoderm covering the dorsal side becomes stretched and flattened (Text-fig. 2b). The cells of the germ band along the mid-ventral line become invaginated to form the mesoderm, while the remaining cells close below it to form the ectoderm. The mesodermal cells form a tightly packed block of cells, clearly separated from the yolk plasmodium (Plate 1, Fig. B).

**Extension of the germ band and formation of extra-embryonic membranes**

As gastrulation nears completion the germ band begins to extend backward, curving dorsally and then forward over the yolk (Text-fig. 2c–e). As it does so it

**Explanation of Plates**

*Abbreviations:* AM, amnion; BL, blastoderm; ECT, ectoderm; EN, endoderm; GB, germ band; HC, haemocoele; MES, mesoderm; NT, nervous tissue; PER, posterior endodermal rudiment; PR, proctodaeum; PRI, proctodaeal invagination; SE, serosa; ST, stomodaeum; STI, stomodaeal invagination; VM, vitelline membrane; YP, yolk plasmodium.

**Plate 1**

*Fig. A.* Normal embryo. Longitudinal section showing formation of cellular blastoderm.

*Fig. B.* Normal embryo. Horizontal section showing appearance of mesoderm after gastrulation.

*Fig. C.* Normal embryo. Longitudinal section showing appearance of posterior end of the germ band at its maximum extension.

*Fig. D.* Normal embryo. Horizontal section showing invagination of stomodaeum and formation of endoderm.

*Fig. E.* Abnormal embryo. Longitudinal section showing irregular formation of the cellular blastoderm.

*Fig. F.* Abnormal embryo. Horizontal section showing appearance of mesoderm after gastrulation.

*Fig. G.* Abnormal embryo. Longitudinal section showing appearance of posterior end of the germ band at its maximum extension.

*Fig. H.* Abnormal embryo. Horizontal section showing invagination of stomodaeum.
TEXT-FIG. 1.  

Stages in the formation of the cellular blastoderm in a normal embryo.  
e. Appearance of the cellular blastoderm in an abnormal embryo.  

BL, blastoderm; NU, nuclei; PE, periplasm; VIN, vitellophage nucleus; VM, vitelline membrane;  
YG, yolk granule; YP, yolk plasmodium.
FIG. I. Longitudinal section of an abnormal embryo with minimal development.
FIG. J. Longitudinal section of an abnormal embryo with moderate development.
FIG. K. Longitudinal section of an abnormal embryo with the most characteristic degree of development.
FIG. L. Longitudinal section of an abnormal embryo with maximal development.
FIG. M. Normal embryo. Longitudinal section showing maximum extension of the germ band.
FIG. N. Normal embryo. Longitudinal section showing beginning of shortening of the germ band.
FIG. O. Normal embryo. Longitudinal section showing completed development.
FIG. P. Abnormal embryo. Longitudinal section showing maximum extension of the germ band.
FIG. Q. Abnormal embryo. Longitudinal section showing beginning of shortening of the germ band.
FIG. R. Abnormal embryo. Longitudinal section showing completed development.
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An inherited abnormality in Dermestes pushes a pocket into the blastoderm, and the blastodermal fold so formed grows forward under the germ band. Shortly afterwards a corresponding pocket is formed at the anterior end of the germ band, and the edges of the two folds unite, giving two very fine extra-embryonic membranes which enclose the embryo, an outer serosa and an inner amnion (Plate 1, Fig. B).

At the conclusion of gastrulation there is a cellular mass at the posterior end of the germ band (Plate 1, Fig. C), and another less obvious one at the anterior end. These masses represent the endodermal rudiments which will give rise to the mid-gut wall. The germ band at this time is seen to be clearly separated from the yolk plasmodium, enclosed in its yolk membrane (Text-fig. 3A,f; Plate 1, Fig. C).

Shortening of the germ band and organ formation

After remaining for a short period in its extended state, the germ band reverses its movement and shortens. As it does so, organ formation begins. Segmental divisions begin to appear in the epidermis (referred to as the hypodermis in insects). The nervous system is formed by the proliferation of neuroblasts arising in the ectoderm of each segment. The mesoderm divides into a larger part, the somatic mesoderm against the hypodermis, which will give rise chiefly to muscles of the body wall, and a smaller part, the splanchnic mesoderm. The splanchnic mesoderm gives rise to the very fine musculature of the mid-gut. A distinct haemocoele or body cavity appears, associated with a general contraction of the yolk plasmodium away from the body wall, within which the organs of the body develop.

The ectoderm covering the anterior endodermal mass invaginates to form the stomodaeal rudiment, a blindly ending tube which crosses the body cavity underneath the brain and terminates against the yolk membrane (Plate 1, Fig. D). A corresponding proctodaeal rudiment invaginates as it is carried backward by the shortening of the germ band, and likewise forms a tube ending against the yolk membrane. The endodermal wall of the mid-gut develops from the endodermal rudiments which are carried up against the yolk by the stomodaeal and proctodaeal invaginations. In each case a band of endodermal cells proliferates and migrates along each side of the yolk (Text-fig. 3A,h; Plate 1, Fig. D). The bands eventually meet and fuse with each other ventrally, and with the corresponding bands from the opposite end of the gut, so that the yolk is enclosed ventrally and laterally (Text-fig. 3A,i).

Dorsal closure

As shortening of the germ band is nearing completion, the lateral body walls begin to grow up and eventually fuse in the mid-dorsal line. At the same time the endodermal wall of the mid-gut completes its enclosure of the yolk (Text-fig. 3A,j). Further embryonic development consists of the completion of tissue differentiation, e.g. the formation of functional muscles in the mesoderm and the
production of bristles from the trichogen cells in the hypodermis. The appendages are developed, and a cuticle is laid down over all external parts of the embryo.


Development of the abnormal embryo

All of the abnormal embryos of this type become contracted, but there is some variability in the extent of their development. In a few cases (12.1 per cent.) only nerve cells and fibres are distinguishable, with a serosa around the yolk (Plate 2,
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TEXT-FIG. 3. A. Development of the normal embryo (continued). f. Formation of endodermal rudiments. g. Beginning of shortening of the germ band, and invagination of the stomodaeum and proctodaeum. h. Formation of the endodermal layer. i. Dorsal closure. j. Completed development. B. Development of the abnormal embryo, showing corresponding stages f–j. AER, anterior endodermal rudiment; EN, endoderm; PER, posterior endodermal rudiment; PR, proctodaeum; PRI, proctodaeal invagination; ST, stomodaeum; STI, stomodaeal invagination; YM, yolk membrane.

Fig. I). At the other extreme there are other cases (11·0 per cent.) in which dorsal closure has occurred and in which the embryo, apart from its being contracted, appears to be relatively normal (Plate 2, Fig. L). The majority of embryos (76·9 per cent.) lie between these extremes. They are not closed dorsally, the
embryonic material forms a boat-shaped structure beneath the yolk, and there is a greater (Plate 2, Fig. K) or lesser (Plate 2, Fig. J) degree of tissue differentiation and organ formation.

Living abnormal embryos as seen under low magnification are indistinguishable, except in those cases mentioned above which have the minimum of development, from normals until the germ band begins to shorten. The processes of blastoderm formation, gastrulation and extension of the germ band appear superficially to be normal. Thereafter, however, the embryo begins to contract and the deficiencies in differentiation become apparent (compare Text-figs. 3A and 3B; Plate 2, Figs. M-O and P-R). There is usually no trace of segmentation, bristles are not formed, and the cuticle does not become hardened or melanized. Usually dorsal closure is not completed (Text-fig. 3B, i), but occasionally it does occur (Text-fig. 3B, j).

Examination of sectioned embryos shows that, in fact, development is abnormal almost from the beginning of development, from the time of blastoderm formation. The periplasm is much less regular than in the normal embryo, and over large areas it is considerably thicker. In these areas the formation of the cytoplasmic furrows separating the blastoderm nuclei is irregular, and not all of the cells become completely cut off by tangential cleavages (Text-fig. 1e, Plate 1, Fig. E). Consequently, there is no such clear demarcation of the cellular blastoderm from the yolk plasmodium as occurs in normal embryos, and the two remain connected by strands of cytoplasm.

This connexion persists throughout subsequent embryonic development and must be regarded as the cause of later abnormalities. Gastrulation produces a strip of rather loosely arranged mesodermal cells, whose cytoplasm extends into the yolk and is coextensive with that of the yolk plasmodium (Plate 1, Fig. F). The movement of the germ band in its extension over the dorsal side of the yolk is not impeded, and the extra-embryonic membranes are formed as in the normal embryo. The posterior endodermal rudiment has no clear boundary, since again its cytoplasm merges with that of the yolk plasmodium (Plate 1, Fig. G), and the condition of the anterior endodermal rudiment is presumably similar, though it is more difficult to distinguish in sections. The most dramatic consequence of the faulty separation of the embryonic tissue from the yolk plasmodium appears as the germ band shortens. Simultaneously with this, as in normal embryos, the yolk begins to contract, but since it is connected to it by cytoplasmic strands the embryonic tissue is forced to contract with it (Text-fig. 3Bg–i).

Organ formation begins normally, with the formation of the nervous system by proliferation of neuroblasts in the ectoderm, though its arrangement is not always clearly segmental. Dorsal closure does not generally occur in the abnormal embryos, but in those in which it does (Plate 2, Fig. L) the nerve cord is interrupted and nervous tissue absent in the ventral line where the yolk presses against the body wall. Differentiation of the mesoderm is poor, and since the
contraction of the yolk pulls the body wall up with it rather than contracting away from it the haemocoele space is very poorly developed. Therefore, when the stomodaeal and proctodaeal invaginations are formed they do not cross a haemocoele space but push directly into the yolk (Plate 1, Fig. H). The endodermal rudiments do not develop further; there is no proliferation and migration of cells along the sides of the yolk, and no mid-gut wall is produced. Very small spindle shaped cells representing the splanchnic musculature do however differentiate from the mesoderm lying against the yolk.

The ectoderm of the stomodeum and proctodeum differentiates essentially as in normal embryos, but differentiation of the hypodermis is not completed. There is usually little trace of segmentation and the cuticle remains unhardened and unmelanized. No appendages are developed. Small trichogen cells are found in some cases, but no bristles. The condition of the mesoderm has already been described. The development of the pole cells and gonads has not been followed in these embryos.

**DISCUSSION**

In this abnormality there is a general inhibition of tissue differentiation within the ectoderm. The superior differentiation of the nervous tissue in the abnormal embryos, and its presence in those embryos in which no other embryonic cell type has appeared, is comparable to the situation in a number of embryonic lethal mutants in *Drosophila*. In *Notch* embryos (Poulson, 1945) there is considerable hypertrophy of the nervous system at the expense of other ectodermal derivatives, and also poor differentiation of the mesoderm. In *X10, type 2* *Drosophila* embryos (Ede, 1956b) the embryo is almost identical with the minimally differentiated *Dermestes*, consisting entirely of nerve cells and fibres mingled with yolk. These observations suggest that in insects, where there is a tendency for differentiation of the ectoderm to be inhibited, the differentiation of the nervous tissue will be least affected.

The defective differentiation of the mesoderm would be expected to follow as a consequence of the inhibition of ectodermal differentiation. In many insects (Counce, 1961), and in the beetle *Leptinotarsa* in particular (Haget, 1953), the proper differentiation of the mesoderm has been shown to be dependent upon an inductive stimulation arising from the ectoderm early in development.

The earliest observable defect precedes the formation of the ectoderm as a distinct germ layer, consisting of the defective formation of the cellular blastoderm and its failure to separate clearly from the underlying yolk plasmodium. The most characteristic features of the abnormality follow as consequences of this primary defect. These are:

*The contraction of the embryo as a whole*

It is known that in insect embryos the yolk plasmodium acts as an autonomous dynamic system, and that its controlled contractions play an important part in
several embryonic processes (Counce, 1961; Krause & Sander, 1962). It has been noted in *Drosophila* that the yolk is not enclosed passively by the endoderm, but that the yolk contracts actively during the process (Ede & Counce, 1956). The contraction of the yolk plasmodium at this time, leaving a spacious haemocoele between itself and the body wall, may be a general phenomenon in insects. In this abnormality, because the embryonic tissues are tied to the yolk plasmodium by cytoplasmic strands, the embryonic tissues contract with the yolk, and no haemocoele, or at best a very defective one, is developed.

**The absence of endoderm**

The failure of the endoderm rudiments to produce any trace of an endodermal wall around the mid-gut is not to be accounted for as a failure of the differentiation process. Haget (1953) has shown that in *Leptinotarsa* the differentiation of the endoderm subsequent to its covering the yolk depends upon the presence of an inductive stimulus arising from the cells of the splanchnic mesoderm. If the mesodermal cells were eliminated, the endoderm cells, though present, failed to undergo differentiation as gut cells. On the other hand, if the endodermal rudiments were removed before migration of the cells over the yolk, the splanchnic musculature was developed though no endoderm was present.

The situation in this abnormality corresponds to that in the second experiment. The endoderm rudiment can be clearly seen at the posterior end of the germ band, and though it is not so clear anteriorly it may be presumed that it exists there also. At the conclusion of development there is no endodermal covering over the mid-gut, but scattered splanchnic muscle cells are present. The defect therefore lies in the failure of the cells of the endodermal rudiment to proliferate and migrate. This may be explained by reference to the principle of ‘contact guidance’ developed by Weiss (1961) who has shown that the migration of cells is guided by the microstructure of the substratum, and if this is unsuitable their movement may be altogether inhibited. In normal embryos the substratum in this case is provided by the membrane at the surface of the yolk plasmodium. In the abnormal embryos there is no simple surface, since the yolk plasmodium is merged with the embryonic tissues, and it is reasonable to suppose that it is this circumstance which inhibits the migration of the endodermal cells. Migration from the endodermal rudiment may well be the stimulus for proliferation of the endodermal cells, and it would follow that failure of migration led to failure of proliferation.

This abnormality is particularly interesting in throwing additional emphasis on the function of the yolk plasmodium as an active agent in insect embryogenesis. It is noteworthy that no comparable abnormalities have been found in *Drosophila* embryos. There exist a number of cases in which blastoderm formation is abnormal by reason of defective control of the nuclear divisions (e.g. Ede, 1956; Counce & Ede, 1957), but none in which the cytoplasmic division is
abnormal while nuclear division remains unaffected. Unfortunately, no information was obtained about the condition of the periplasm in the preblastodermal stages of the abnormal *Dermestes* embryo, but its irregularity in the blastodermal stage suggests that the cytoplasm may have been abnormal in the unfertilized egg, following some defect in oogenesis. The primary defect could lie in the submicroscopic structure of the cytoplasm, interfering with cytoplasmic cleavage. If this is the case this abnormality, which appears to be easily obtainable (Ede & Rogers, 1964), could provide excellent material for studies on the ultrastructure of the cytoplasm in the insect egg.

SUMMARY

1. A developmental abnormality characterized most obviously by longitudinal contraction of the embryo occurred in high frequency (up to 70 per cent.) among eggs from females of a selected line of the beetle *Dermestes maculatus*.

2. In the majority of the abnormal embryos dorsal closure does not occur and the embryonic material forms a boat-shaped structure beneath the yolk. Stomodaecal and proctodaecal invaginations are formed, but the endodermal wall of the mid-gut is absent.

3. Development is abnormal from the time of blastoderm formation. The blastoderm is not completely cut off from the underlying yolk plasmodium, to which it remains connected by strands of cytoplasm.

4. It is suggested that the failure of the yolk plasmodium to become clearly separated from the blastoderm accounts for the contraction of the embryo and also for the absence of the endodermal layer.

RÉSUMÉ

*Une anomalie héréditaire affectant le syncytium vitellin et l’endoderme chez Dermestes maculatus* (Coleoptera).

1. Une anomalie héréditaire caractérisée le plus nettement par une contraction longitudinale de l’embryon est survenue avec une fréquence élevée (jusqu’à 70%) parmi les œufs de femelles d’une lignée sélectionnée de Dermestes.

2. Dans la majorité des embryons anormaux, la fermeture dorsale n’a pas lieu et le matériel embryonnaire forme une structure en forme de navire au dessous du vitellus. Les invaginations stomodéale et proctodéale se forment mais la paroi endodermique de l’intestin moyen est absente.

3. Le développement est anormal à partir du moment de la formation du blastoderme. Le blastoderme ne se sépare pas complètement du syncytium vitellin sous-jacent, auquel il demeure rattaché par des travées de cytoplasme.

4. On suggère que l’absence de séparation nette entre le syncytium vitellin et le blastoderme explique la contraction de l’embryon et aussi l’absence d’assise endodermique.
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REFERENCES


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