Downloaded from rstb.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

# The Cell Lineage of Segments and Parasegments in Drosophila

P. A. Lawrence and A. Martinez-Arias

*Phil. Trans. R. Soc. Lond. B* 1985 **312**, 83-90
doi: 10.1098/rstb.1985.0179

**References**

Article cited in:
**http://rstb.royalsocietypublishing.org/content/312/1153/83#related-urls**

**Email alerting service**

Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click **here**

To subscribe to *Phil. Trans. R. Soc. Lond. B* go to: **http://rstb.royalsocietypublishing.org/subscriptions**

This journal is © 1985 The Royal Society

Downloaded from rstb.royalsocietypublishing.org

*Phil. Trans. R. Soc. Lond.* B **312**, 83–90 (1985) [ 83 ]
*Printed in Great Britain*

# The cell lineage of segments and parasegments in *Drosophila*

By P. A. Lawrence, F.R.S., and A. Martinez-Arias
*M.R.C. Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, U.K.*

[Plates 1 and 2]

In *Drosophila*, many good methods of single cell marking are available using mutations that alter the structure of the cuticle or those that alter the activity of an enzyme. *In situ* hybridization of nucleic acids to tissue sections now allows the observer to follow movements of groups of cells in the embryo. These methods challenge the traditional picture of metamerization in the insect embryo: it seems that both in the ectoderm and the mesoderm the embryo is first divided not into segments but into compartments and parasegments. In the ectoderm a parasegment comprises a posterior compartment of one segment and the anterior compartment of the next. In the mesoderm the parasegments are probably not subdivided into compartments.

## Introduction

It could be argued that any objective information about embryonic development is useful: for, in the long history of embryology, what has been described as fact by one author has usually been disputed by others. Accurate confirmable information on the cell lineage of normal wildtype development has only been obtained in a few organisms, particularly in the nematode *C. elegans* (Sulston *et al.* 1984) and to a lesser extent in *Drosophila*. As it happens, even the incomplete information about cell lineage in *Drosophila* has turned out to be valuable, not only for its own sake, but because there have been some surprises that have led to new or sharper ideas about development. These surprises have depended on the precision and clarity of the technique that began with the discovery by Stern (1936) of mitotic crossing over together with the invention of cell marking methods (see, for example, Demerec 1936).

In following the lines of descent of embryonic cells one may simply be describing the indirect outcome of many processes, or one may be close to the mechanisms of development: mechanisms that first select a group of cells in the embryo and then ensure that they generate a specific portion of the adult. One hypothesis is that these mechanisms are essentially genetic, depending on the local deployment of a specific class of genes (Garcia-Bellido 1975) and this can be tested by making clones of cells that lack such a gene and mapping where it is required.

## The methods

One key method is mitotic recombination which is induced by X-irradiation at a time chosen by the investigator. Large numbers of eggs or larvae, that are genetically heterozygous for a marker mutation ($m/m^+$), are irradiated and, rarely, mitotic recombination produces a cell homozygous for the marker ($m/m$). This cell continues to divide and generates a clone of marked cells which shows as a patch in the adult. The marker mutation should have the following characteristics: it should be *autonomous* (so that only and all $m/m$ cells express the phenotype),



The Royal Society is collaborating with JSTOR to digitize, preserve, and extend access to *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences.* www.jstor.org

Downloaded from rstb.royalsocietypublishing.org



it should be *gratuitous* (so that the marked cells develop at the normal rate to give essentially normal structures) and it should have a short *perdurance* (Garcia-Bellido & Merriam 1971). Perdurance describes the persistance of wildtype phenotype after the removal of the $m^+$ allele; if the perdurance is short then even late irradiation produces marked clones and this can be useful. Nearly all markers that have been used affect the cuticle (figure 1, plate 1) or the eye; popular examples include *yellow* (the bristles turn from brown to yellow), *forked*$^{36a}$ (bristles become twisted), *multiple wing hairs* (which increases the number of the little hairs on the wing and elsewhere) and *white* (which removes the red pigment from the eye). Good markers that can be used in the internal organs are few, *sdh* (Lawrence 1981*a*) can be used in almost all tissues; others tend to express their wildtype product only locally: examples are *maroonlike* (lacking aldehyde oxidase activity, see, for example, Janning (1974)) and mutations reducing the level of acid phosphatase (Kankel & Hall 1976).

Morata & Ripoll (1975) improved the standard method of cell lineage analysis by placing the marker *in trans* to a *Minute* mutation. Mitotic recombination produces a $m/m$ cell that is free of the deleterious *Minute* allele. Embryos carrying one *Minute* allele grow slowly but it is found that the clone of marked cells grows at the normal wildtype rate and *competes* with the *Minute* cells around it (Morata & Ripoll 1975) (figure 1). The competition results in the marked clone 'killing' the other cells nearby (Simpson & Morata 1981), filling up defined regions (compartments, Garcia-Bellido *et al.* 1973) and being unable to cross the precisely delineated borders of these regions. This was a surprising result in itself (incredulity was the common reaction of *Drosophila* workers at that time) and it has produced a different and more objective morphology which Jacobson (1978) has heralded as a 'new science of developmental anatomy'.

These methods only describe the lineage of normal flies but they can be simply modified to test local requirements for a gene of interest. For example, a mutation ($p$) of a gene that affects pattern can be placed in cis with the marker ($m$) and in trans with a *Minute*. Embryos of genotype $m\ p\ Minute^+/m^+\ p^+\ Minute$ are then irradiated and large clones of the following genotype are induced: $m\ p\ Minute^+/m\ p\ Minute^+$. These clones grow rapidly and every marked cell must also be homozygous for the mutation $p$. A complete picture of the requirement for the pattern gene ($p^+$) can therefore be built up piecemeal from many clones.

Methods based on mitotic recombination are sometimes inadequate because most of the cell markers can only be seen in the adult fly and much of development therefore remains hidden from analysis. For example, if the marked cell is generated soon after blastoderm it may form a large clone in one adult compartment but we do not know how that clone grew in the embryo or how much of the larva it populated. The pattern of cell lineage during the formation of germ layers and organs is therefore little described. Recently methods of marking have been developed which are beginning to fill this gap. One of these methods, the injection of dyes into groups of adjacent cells in early gastrulation looks very promising because the injected cells can be followed through subsequent morphogenesis (Technau & Campos-Ortega 1985). Another method relies on *in situ* hybridization of molecular probes for selector genes (Akam 1983; Hafen *et al.* 1983). Selector genes are required in defined groups of cells during development (Garcia-Bellido 1975) and with the assumptions that the pattern of transcription does not change and that the transcript itself is stable over the time of observation, the molecular probes hybridized *in situ* can be used to follow groups of cells through morphogenesis (Akam & Martinez-Arias 1985).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org



PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

## Segments and morphology

Traditional morphology suffers from being largely subjective and therefore our names for organs and tissues may have no significance in the way the animal is built. The first compartment boundary was discovered in the *Drosophila* wing (Garcia-Bellido *et al.* 1973) and was a surprise because we thought of the wing as a discrete organ and presumed incorrectly that it was made as a single piece. A better description of the origin of the wing is that it is made by parts of two compartments each of which consists of approximately half of the adult segment including about half of one leg (Steiner 1976). In improving the description we aim to approach what Brenner (1981) has called the 'internal representation' of the fly, that is a more objective picture of the principles, methods and constraints which result in the formation of patterns: we do not mean that in *Drosophila*'s egg there is a minifly!

Another difficulty with traditional morphology is that it is uncritical in the sense that concepts may be vague, for example, a 'segment' means different things to different people. In insects, segments have been best defined from embryos, usually by counting the number of clumps of mesoderm or the number of neuromeres. There is general agreement that there are three segments in the mouth parts, three in the thorax and general confusion about the number of segments in the head and abdomen (Anderson 1972). Another confusion stems from the lack of a definition of a segment at postembryonic stages. Traditionally the intersegmental boundaries in the epidermis are thought to be at the line of longitudinal muscle attachments. In the *Drosophila* larva most of the longitudinal muscles that span the segment attach along a sharply defined line (Crossley 1978) and in *Oncopeltus* along a line coinciding with a groove in the cuticle and a sharp change in the pigment in the epidermis: a groove that turned out, on the evidence of cell marking experiments, to be a compartment border (Lawrence 1973). This suggested that all segments could be defined by cell lineage criteria and that each consists of exactly two compartments, one anterior plus one posterior (Lawrence 1981*b*). In principle this should allow the allocation of each epidermal landmark to a compartment and therefore to a segment, provided that all of the epidermis can be subdivided into anterior and posterior compartments. It does seem that in *Drosophila* much of the epidermis and central nervous system is divided into a chain of 28 alternating posterior and anterior compartments (Martinez-Arias & Lawrence 1985) extending from the posterior mandibular compartment to an anterior compartment belonging to the ninth abdominal segment. At either end of this chain there are poorly mapped regions that form the head and the anal region.

Poulson (1950) had described the 6 h old *Drosophila* embryo as consisting of a head plus 14 segments, which at first sight fits well with the number of 28 compartments. Poulson described the segments as demarcated by grooves which coincided in the ectoderm and mesoderm. The grooves can be seen on the surface of the embryo (Turner & Mahowald 1977; P. Ingham unpublished) in figure 3. Recently we have re-evaluated Poulson's picture and this led to another surprise: we believe that Poulson's 'segments' do not give rise to those regions of the body otherwise defined as segments but instead to a posterior compartment of one segment plus the anterior compartment of the next. We called these units 'parasegments' (Martinez-Arias & Lawrence 1985).

We think that parasegments are earlier and more fundamental units of insect design than segments. Why? Partly because four lines of evidence suggest that the mesoderm appears to be subdivided into parasegments and not into compartments or segments:

Downloaded from rstb.royalsocietypublishing.org



(i) in the embryo the earliest signs of segmentation are surface grooves in the epidermis and, in register with them, deeper grooves in the mesoderm (Poulson 1950). Several arguments, such as the position of the tracheal pits (Keilin 1944) and the size of neuromeres at each end of the central nervous system (Hartenstein & Campos-Ortega 1984; Teugels & Ghysen 1985; C. M. Bate, unpublished) have led us to the hypothesis that the metameres delineated by the grooves are parasegments not segments and include both ectoderm and mesoderm (Martinez-Arias & Lawrence 1985).

(ii) Descriptions of cell lineage of the adult muscles of the thorax and abdomen using the *sdh* marker and the *Minute* technique showed that the muscles of each segment seem to have an origin from separated primordia, there being one not two per segment. The mesoderm is, therefore, divided by cell lineage restrictions into metameric units (Lawrence 1982).

(iii) The *engrailed* mutations, which show phenotypic effects in cells belonging only to posterior compartments (Morata & Lawrence 1975, 1979; Kornberg 1981; Lawrence & Struhl 1982), show no effects in any mesodermal organs (figure 2) (Lawrence 1982; Lawrence & Johnston 1982, 1984*a*). DNA from the *engrailed* region has now been purified and probes used to detect transcripts from the *engrailed* gene and to map their distribution in the embryo (Fjose *et al.* 1985; Kornberg *et al.* 1985). As expected from Poulson's observations, there are 14 stripes of *engrailed* expression (corresponding to the 14 posterior compartments) in the main body of the ectoderm, but in the mesoderm expression is evanescent. It therefore seems that the mesoderm contains no posterior compartments, meaning that there is no subdivision of the metameres as there is in the epidermis (Lawrence & Johnston 1984*a*).

(iv) The *Ubx* gene (Lewis 1978) is active and required only in a subset of all the body compartments and is particularly strongly expressed in one parasegment (Morata & Kerridge 1981; Akam 1983; Struhl 1984; White & Wilcox 1984; Akam & Martinez-Arias 1985; Beachy *et al.* 1985). From *in situ* hybridization it seems that the expression of *Ubx* is compartment-specific in the ectoderm, being strongly expressed in anterior compartments and weakly in posterior ones. In the mesoderm, *Ubx* is strongly expressed in segment-sized units that are first defined in precise register with the parasegments of the overlying epidermis (Martinez-Arias & Lawrence 1985; Akam & Martinez-Arias 1985).

All these arguments lead to a non-traditional description of the body plan of insects. In the mesoderm the units hitherto described as segments are apparently parasegmental in origin, and in the epidermis the unit of design seems to be not the segment but the compartment. It is premature to be confident about this new description as further experiments are required to test it (Martinez-Arias & Lawrence 1985).

## The shift of the mesoderm and some consequences

In his study of *Ubx* expression in third stage larvae, Akam (1983) noted that the *Ubx* probe bound to both ectoderm and mesoderm but not in register: expression of $Ubx^+$ went further anterior in the epidermis than in the muscles. We suggested that during development, there could be a relative movement between the ectoderm and the developing muscles (Lawrence & Johnston 1984*a*, *b*). Direct evidence for a shift has now come from studies of *Ubx* expression during early development; Akam & Martinez-Arias (1985) find that much of the disparity is due to expression in the young embryo being limited to parasegments 6–12 in the mesoderm but extending to parasegments 5–13 in the ectoderm (figure 4, plate 2). In addition, between

Downloaded from rstb.royalsocietypublishing.org



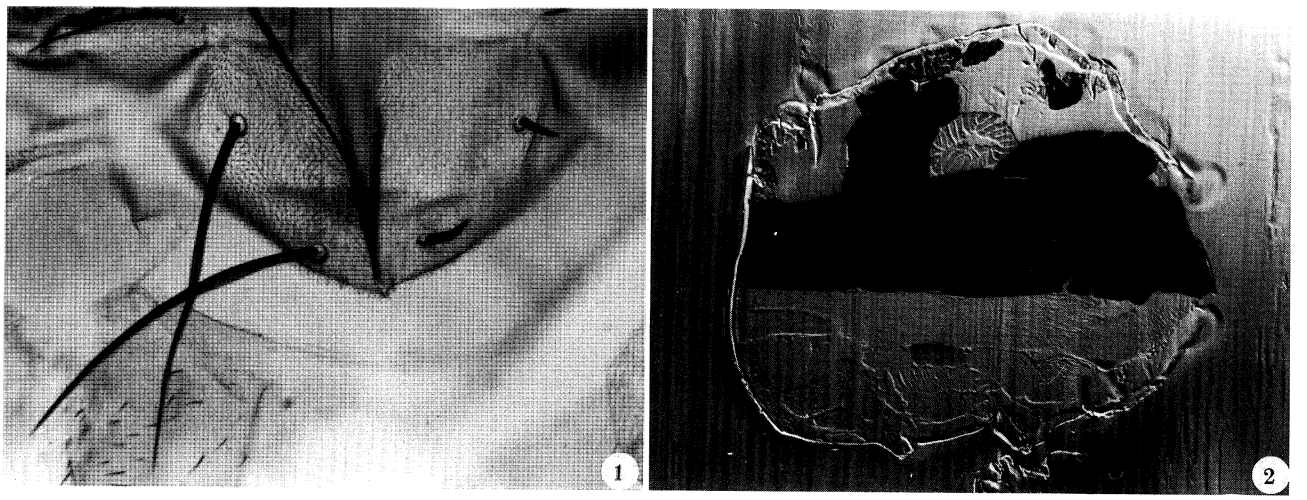



FIGURE 1. A marked clone of cells in the thorax of *Drosophila*. On the right, the genotype is that of the majority of the fly (*Ki Sb*$^{63b}$ *M*(3)*w*$^{124}$/+) and makes the bristles squat and stubby. On the left, wild type bristles (+/+) indicate the presence of a clone induced by mitotic recombination (see Struhl 1982).

FIGURE 2. Horizontal section of the thorax of *Drosophila* in which the unstained parts lack *sdh* activity and are otherwise wild type, while the stained muscles are *sdh*$^{+}$ and also homozygous for a lethal mutation of the *engrailed* gene. The stained muscles develop normally (see Lawrence & Johnston 1984*a*). Anterior to the left. Partial interference contrast.

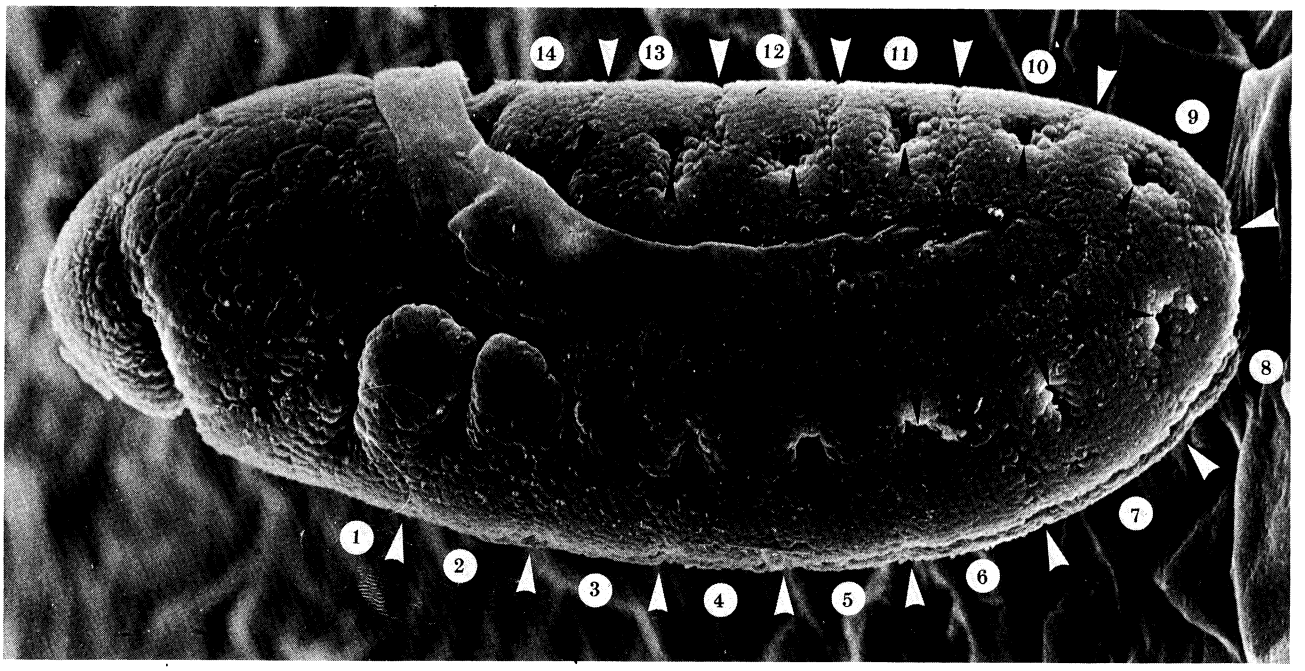


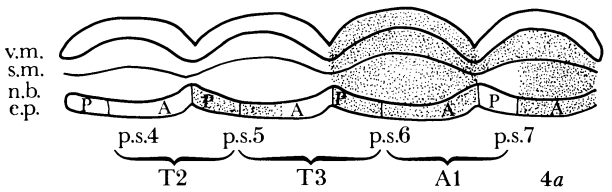

FIGURE 3. Scanning electron micrograph of an embryo at the extended germ band stage showing the first signs of metamerization. According to Martinez-Arias & Lawrence (1985) the ventromedial grooves (white arrowheads) mark the parasegmental borders and the tracheal pits (black arrowheads) the segment boundaries. Notice the eleventh pit (large black arrowhead) located more dorsally than the rest. Numbers indicate parasegments. Photograph kindly provided by Dr P. Ingham.

(*Facing p.* 86)

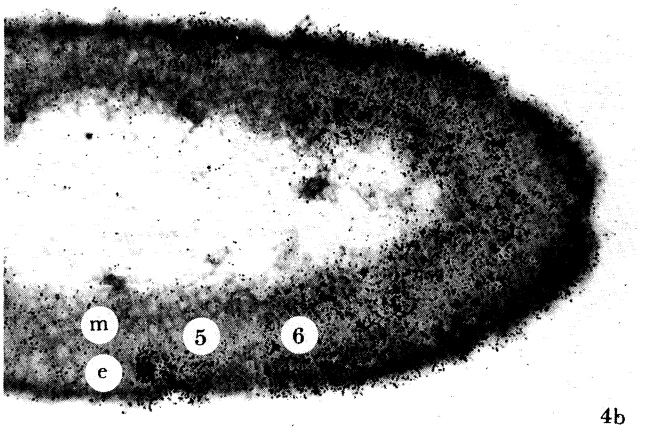
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

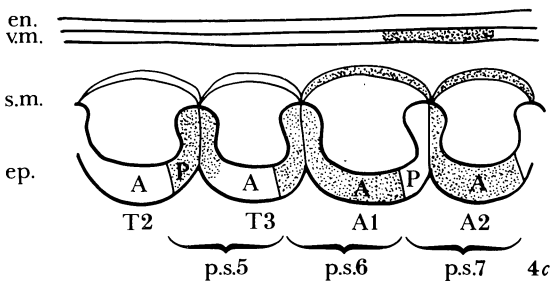




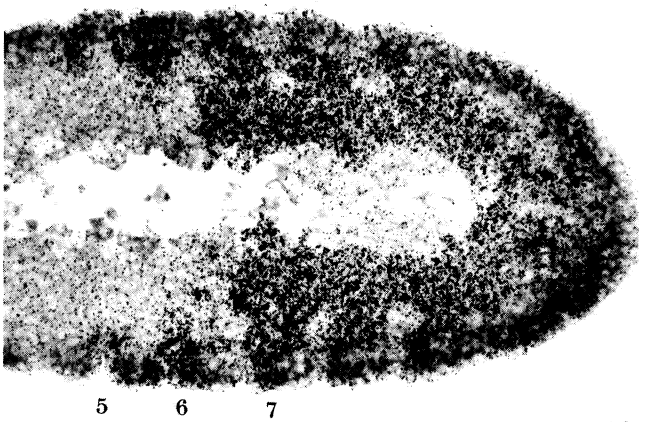

4*a*



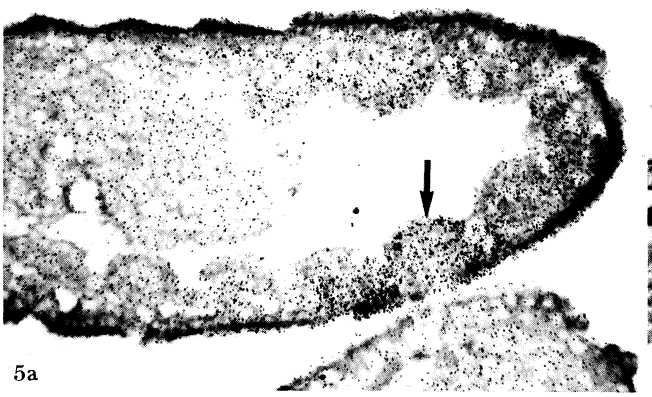

4b



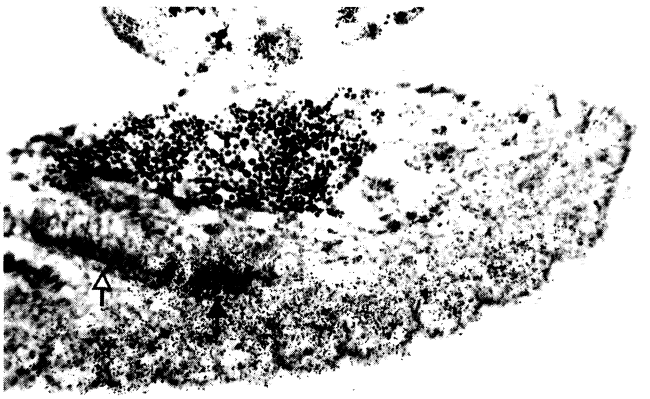

4*c*




4d



5a



5b

FIGURES 4 AND 5. For description see opposite.

Downloaded from rstb.royalsocietypublishing.org



4 and 11 h of development, these authors observe a displacement of most of the mesoderm with respect to the ectoderm of about half a segment, which means that the muscles originating in parasegment 6 (which in the epidermis designates the posterior compartment of T3 and the anterior of A1) shift back and form all the muscles of A1 (figure 4). Taken together with the cell lineage of the muscles (Lawrence 1982; Lawrence & Johnston 1984) discussed above, this means that the segmental muscles of A1 (which are one lineage compartment) originate in parasegment 6, and those of T3 in parasegment 5 (figure 4).

Thus it seems that the *Ubx* gene may be expressed only in abdominal muscles and if so cannot have a direct role in the muscles of the thorax. By contrast, $Ubx^+$ is active and required in much of the thoracic epidermis and central nervous system (reviewed in Lawrence & Morata 1983) and this realization offers a solution to a conundrum: when T3 is transformed to T2 by mutations in the *Ubx* domain an apparently perfect four-winged fly can be made (see cover illustration in *Science, Wash.* 4605, 1 July 1983). Although the transformation is complete exteriorly, inside there are no flight muscles in the ectopic T2 segment and this has been puzzling (Ferrus & Kankel 1981; Lawrence 1984, 1985). However, if $Ubx^+$ is not expressed in the mesoderm of parasegment 4 (muscles of T2) or 5 (muscles of T3), mutations in *Ubx* would not be expected to affect these muscles, as is observed. The four-winged fly demonstrates that transformation of the ectoderm is not sufficient to cause transformation of all associated muscles; even though there is good evidence for some dependence of muscle pattern on the overlying ectoderm (Bock 1942; Williams & Caveney 1980*a*, *b*). This leads inexorably to the hypothesis that muscle pattern is determined autonomously, at least in part. Given that this may be so, how might it be done? We follow the selector gene hypothesis of Garcia-Bellido (1975), according to which sets of epidermal cells act together to construct a particular compartment under the control of a small number of special (selector) genes. In each compartment there is a unique combination of these genes. Applying this logic to the muscles we would expect

## Description of plate 2

Figure 4. Sketches (*a*, *c*) and photomicrographs (*b*, *d*) showing the pattern of *Ubx* transcripts at two developmental stages in *Drosophila* (Akam & Martinez-Arias 1985). (*a*) Sketch of the pattern of *Ubx* expression in the extended germ band; parasegmental folds are shallow in the epidermis (ep.) and the somatic and visceral mesoderm (s.m., v.m.) are divided into metameric units in register with the epidermal folds. The shaded regions indicate *Ubx* expression. The label in the somatic mesodem is continuous in parasegments (p.s.) 6–12 but in the visceral mesoderm only parasegment 6 expresses $Ubx^+$. (*b*) Horizontal section through an embryo 6 h old, hybridized with a probe for $Ubx^+$. The *Ubx* label in the ectoderm (e.) and mesoderm (m.) coincides at the anterior margin of parasegment 6. (*c*) Sketch of the pattern of *Ubx* expression after muscle formation. When the muscles form, they define segments for the first time. The muscles of T3 do not express $Ubx^+$ although those of A1 do (Akam 1983). A region of the visceral mesoderm (v.m.), which is now attached to the gut (en.), also expresses $Ubx^+$ (see figure 5). (*d*) Horizontal section of an embryo after germ band shortening, hybridized with a probe for $Ubx^+$. Notice that the boundary of *Ubx* expression in the mesoderm now lies between parasegments 6 and 7. A, anterior compartment; P, posterior compartment; T2, T3 are the second and third thoracic segments, A1 and A2 the first and second abdominal segments. Results after Akam & Martinez-Arias (1985).

Figure 5. Developmental tracing of the visceral mesoderm of parasegment 6. (*a*) Frozen sagittal section through an extended germ band hybridized with a probe for $Ubx^+$. At about 6 h the visceral mesoderm is visible as a series of ripples in register with the parasegmental grooves. Only one of three units is labelled in the visceral mesoderm (arrow). It is derived from parasegment 6 and by this stage has moved back and is in register with parasegment 7 (M. Akam & A. Martinez-Arias, unpublished). (*b*) Longitudinal wax section through an 11 h old embryo hybridized with a probe for $Ubx^+$. The visceral mesoderm has attached to the gut. Only one part of it is labelled (closed arrow) the rest is not (open arrow) (A. Martinez-Arias & P. Lawrence, unpublished photograph).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org



there to be a gene responsible for the difference between the muscles of T2 and T3 (originating in parasegment 4 and 5, respectively) which would be expressed in one set but not the other. One candidate might be *Antennapedia*$^+$ because it is active (Levine *et al.* 1983) in the thorax, but it is required in all three segments (Struhl 1982) and therefore seems ineligible. A better candidate is *Sex combs reduced*$^+$, which is required in epidermal parts of T1 but not T2 (Wakimoto & Kaufman 1981; Struhl 1982) and so might be expected to be active in parasegment 4 but not 5.

## Segmentation in other tissues

Akam & Martinez-Arias (1985) report that the pattern of *Ubx* expression in the visceral mesoderm is different from that in the somatic mesoderm. In the embryo of 5–12 h, seven parasegments express *Ubx*$^+$ in the somatic mesoderm but in the visceral mesoderm only one parasegment does so. This parasegment is number 6 which is the one where the most expression of *Ubx*$^+$ products is seen (Akam 1983; White & Wilcox 1984; Akam & Martinez-Arias 1985; Beachy *et al.* 1985) and it can be followed as a patch of labelled visceral mesoderm throughout much of embryonic development (figure 5). No other signs of metamerization have yet been seen in the visceral mesoderm; the cell lineage of the muscular lining of the adult gut does not indicate any compartment boundaries, although cells contributing to it come from a large part of the body (Lawrence & Johnston 1985).

The endoderm, which forms the midgut, arises from two primordia situated outside the main chain of 28 compartments in the ectoderm and 14 parasegments in the mesoderm. There are no indications that it is segmented at all. None of the genes involved in segmentation that have been tested so far are expressed (*ftz*; Hafen *et al.* 1984) or required there (*fused* and other segment-polarity genes; Martinez-Arias (1985) and unpublished) and cell lineage studies (Janning 1974; Lawrence & Johnston 1985) do not show any compartment boundaries.

## Conclusions and caveats

Most of our discussion has dealt with the embryo since that is where the origin of the different parts of the body can be traced and where the pattern of gene expression is relatively simple. We find the embryo is built in modules (compartments and parasegments) and that the design and expression of selector genes is different in the four main germ layers: the ectoderm, the somatic mesoderm, the visceral mesoderm and the endoderm. Genetic analysis has already suggested that later stages will be accompanied by increasing complexity – some selector genes may interact so that a region initially under the control of one gene could be taken over by another. For example, it has already been suggested by Struhl (1982) that the early requirement for *Ubx*$^+$ in the posterior compartment of T2 (Morata & Kerridge 1981) is to regulate another selector function, *Scr*$^+$. These complications need not disturb us for they are the stuff of epigenesis, but they do warn that an understanding of selector gene expression in later stages may not be possible without a complete picture of the cell lineage and gene expression in the young embryo.

We thank Michael Akam and Phil Ingham for sharing their results with us and for advice. A. M. A. is supported by an EMBO long-term fellowship.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org



## References


Akam, M. 1983 The location of *Ultrabithorax* transcripts in Drosophila tissue sections. *EMBO J.* **2**, 2075–2084.

Akam, M. & Martinez-Arias, A. 1985 *Ultrabithorax* expression in *Drosophila* embryos. *EMBO J.* **4**, 1689–1700.

Anderson, D. T. 1972 The development of holometabolous insects. In *Developmental systems: insects* (ed. S. J. Counce & C. H. Waddington), pp. 165–292. London: Academic Press.

Beachy, P. A., Helfand, S. L. & Hogness, D. S. 1985 Segmental distribution of bithorax complex proteins during *Drosophila* development. *Nature, Lond.* **313**, 545–551.

Bender, W., Akam, M., Karch, F., Beachy, P., Peifer, M., Spierer, P., Lewis, E. B. & Hogness, D. S. 1983 Molecular genetics of the bithorax complex in *Drosophila melanogaster*. *Science, Wash.* **221**, 23–29.

Bock, E. 1942 Wechselbeziehung zwischen den Keimblattern bei der Organbildung von *Chrysopa perla* (L.). I. Die Entwicklung des Ektoderms in mesodermdefekten Keimteilen. *W. Roux Arch.* **141**, 159–247.

Brenner, S. 1981 Genes and development. In *Cellular controls of differentiation* (ed. C. W. Lloyd & D. A. Rees), pp. 3–7. London: Academic Press.

Crossley, A. C. S. 1978 The morphology and development of the *Drosophila* muscular system. In *The genetics and biology of Drosophila* (ed. M. Ashburner & T. R. F. Wright), pp. 499–560. London: Academic Press.

Demerec, M. 1936 Frequency of 'cell-lethals' among lethals obtained at random in the X-chromosome of *Drosophila melanogaster*. *Proc. natn. Acad. Sci. U.S.A.* **22**, 350–354.

Ferrus, A. & Kankel, D. R. 1981 Cell lineage relationships in *Drosophila melanogaster*: the relationships of cuticular to internal tissues. *Devl. Biol.* **85**, 485–504.

Fjose, A., McGinnis, W. J. & Gehring, W. J. 1985 Isolation of a homoeobox-containing gene from the engrailed region of *Drosophila* and the spatial distribution of its transcripts. *Nature, Lond.* **313**, 284–289.

Garcia-Bellido, A. 1975 Genetic control of wing disc development in *Drosophila*. In *Cell patterning* (ed. S. Brenner), pp. 161–182. Amsterdam, Oxford, New York: Associated Scientific Publishers.

Garcia-Bellido, A. & Merriam, J. R. 1971 Genetic analysis of cell heredity in imaginal discs of *Drosophila melanogaster*. *Proc. natn. Acad. Sci. U.S.A.* **68**, 2222–2226.

Garcia-Bellido, A., Ripoll, P. & Morata, G. 1973 Developmental compartmentalization of the wing disc of *Drosophila*. *Nature, new Biol.* **245**, 251–253.

Hafen, E., Levine, M., Garber, R. L. & Gehring, W. J. 1983 An improved *in situ* hybridization method for the detection of cellular RNAs in *Drosophila* tissue sections and its application for localizing transcripts of the homeotic *Antennapedia* gene complex. *EMBO J.* **4**, 617–623.

Hafen, E., Kuroiwa, A. & Gehring, W. J. 1984 Spatial distribution of transcripts from the segmentation gene *ftz* during *Drosophila* embryonic development. *Cell* **37**, 833–841.

Hartenstein, V. & Campos-Ortega, J. 1984 Early neurogenesis in wild type *Drosophila melanogaster*. *W. Roux Arch. Devl. Biol.* **193**, 308–325.

Jacobson, M. 1978 Clonal origins of the central nervous system: towards a developmental neuroanatomy. *Zoon* **6**, 149–156.

Janning, W. 1974 Entwicklungsgenetische Untersuchungen an Gyandern von *Drosophila melanogaster*. *W. Roux Arch.* **174**, 313–332.

Kankel, D. R. & Hall, J. C. 1976 Fate mapping of nervous system and other internal tissues in genetic mosaics of *Drosophila melanogaster*. *Devl. Biol.* **48**, 1–24.

Keilin, D. 1944 Respiratory systems and respiratory adaptations in larvae and pupae of Diptera. *Parasitology* **36**, 1–66.

Kornberg, T. 1981 *engrailed*: a gene controlling compartment and segment formation in *Drosophila*. *Proc. natn. Acad. Sci. U.S.A.* **78**, 1095–1099.

Kornberg, T., Siden, I., O'Farrell, P. & Simon, M. 1985 The *engrailed* locus of *Drosophila: in situ* localization of transcripts reveals compartment specific expression. *Cell* **40**, 45–53.

Lawrence, P. A. 1973 A clonal analysis of segment development in *Oncopeltus* (Hemiptera). *J. Embryol. exp. Morph.* **30**, 681–699.

Lawrence, P. A. 1981*a* A general cell marker for clonal analysis of *Drosophila* development. *J. Embryol. exp. Morph.* **64**, 321–332.

Lawrence, P. A. 1981*b* The cellular basis of segmentation in insects. *Cell* **26**, 3–10.

Lawrence, P. A. 1982 Cell lineage of the thoracic muscles of *Drosophila*. *Cell* **29**, 493–503.

Lawrence, P. A. 1984 Some observations on the *Drosophila* mesoderm. *Proc. XV Int. Congr. Genet.* p. 215.

Lawrence, P. A. 1985 Notes on the genetics of pattern formation in the internal organs of *Drosophila*. *Trends Neurosci.* **8**, 267–269.

Lawrence, P. A. & Johnston, P. 1982 Cell lineage of the *Drosophila* abdomen: the epidermis, oenocytes and ventral muscles. *J. Embryol. exp. Morph.* **72**, 197–208.

Lawrence, P. A. & Johnston, P. 1984*a* On the role of the *engrailed*$^{+}$ gene in the internal organs of *Drosophila*. *EMBO J.* **3**, 2839–2844.

Lawrence, P. A. & Johnston, P. 1985 Observations on cell lineage of internal organs of *Drosophila*. *J. Embryol. exp. Morph.* (Submitted.)


PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

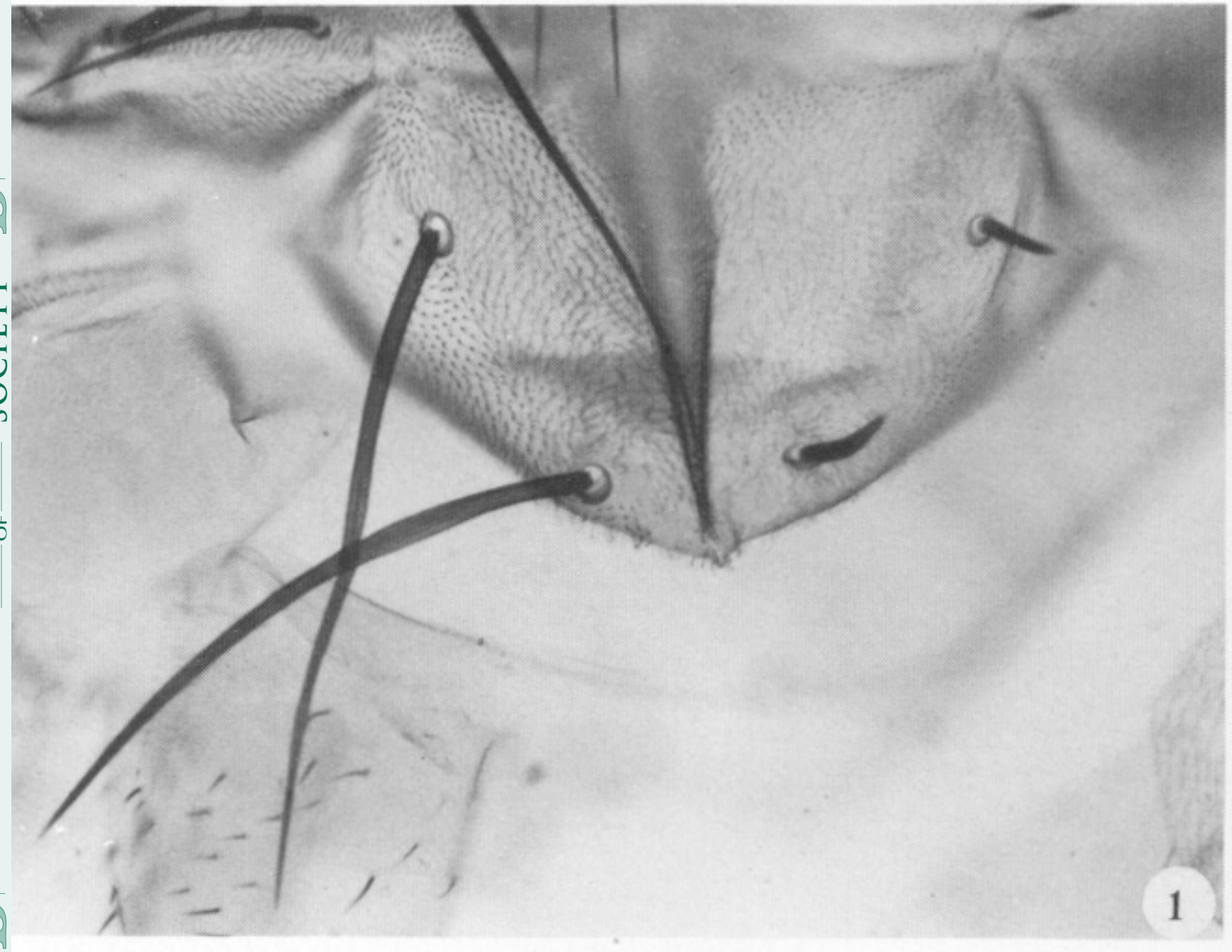
Downloaded from rstb.royalsocietypublishing.org



Lawrence, P. A. & Morata, G. 1983 The elements of the bithorax complex. *Cell* **35**, 595–601.
Lawrence, P. A. & Struhl, G. 1982 Further studies of the *engrailed* phenotype in *Drosophila*. *EMBO J.* **1**, 827–833.
Levine, M., Hafen, E., Garber, R. L. & Gehring, W. J. 1983 Spatial distribution of *Antennapedia* transcripts during *Drosophila* development. *EMBO J.* **2**, 2037–2046.
Lewis, E. B. 1978 A gene complex controlling segmentation in *Drosophila*. *Nature, Lond.* **276**, 565–570.
Martinez-Arias, A. 1985 The development of *fused*$^{-}$ embryos of *Drosophila melanogaster*. *J. Embryol. exp. Morph.* **87**, 99–114.
Martinez-Arias, A. & Lawrence, P. A. 1985 Parasegments and compartments in the *Drosophila* embryo. *Nature, Lond.* **313**, 639–642.
Morata, G. & Kerridge, S. 1981 Sequential functions of the bithorax complex of *Drosophila*. *Nature, Lond.* **290**, 778–781.
Morata, G. & Lawrence, P. A. 1975 Control of compartment development by the *engrailed* gene in *Drosophila*. *Nature, Lond.* **255**, 614–617.
Morata, G. & Lawrence, P. A. 1979 Development of the eye-antenna imaginal disc of *Drosophila*. *Devl. Biol.* **70**, 355–371.
Morata, G. & Ripoll, P. 1975 Minutes: mutants of *Drosophila* autonomously affecting cell division rate. *Devl. Biol.* **42**, 211–221.
Poulson, D. F. 1950 Histogenesis, organogenesis and differentiation in the embryo of *Drosophila melanogaster* Meigen. In *The biology of Drosophila* (ed. M. Demerec), pp. 168–274. New York: Wiley.
Simpson, P. & Morata, G. 1981 Differential mitotic rates and pattern of growth in the compartments of *Drosophila* wing. *Devl. Biol.* **85**, 299–308.
Steiner, E. 1976 Establishment of compartments in the developing leg imaginal discs of *Drosophila melanogaster*. *W. Roux Arch.* **180**, 9–30.
Stern, C. 1936 Somatic crossing over and segregation in *Drosophila melanogaster*. *Genetics* **21**, 625–730.
Struhl, G. 1982 Genes controlling segmental specification in the *Drosophila* thorax. *Proc. natn. Acad. Sci. U.S.A.* **79**, 7380–7384.
Struhl, G. 1984 Splitting the bithorax complex of *Drosophila*. *Nature, Lond.* **308**, 454–457.
Sulston, J., Schierenberg, E., White, J. G. & Thomson, J. N. 1983 The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Devl. Biol.* **100**, 64–119.
Technau, G. & Campos-Ortega, J. 1985 Fate-mapping in wild type *Drosophila melanogaster*. II. Injections of horseradish peroxidase in cells of the early gastrula stage. *W. Roux Arch. Devl. Biol.* **194**, 196–212.
Teugels, E. & Ghysen, A. 1985 Domains of action of *bithorax* genes in Drosophila central nervous system. *Nature, Lond.* **314**, 558–562.
Turner, F. R. & Mahowald, A. P. 1977 Scanning electron microscopy of *Drosophila melanogaster* embryogenesis. II. Gastrulation and segmentation. *Devl. Biol.* **57**, 403–416.
Wakimoto, B. T. & Kaufman, T. 1981 Analysis of larval segmentation in lethal genotypes associated with the Antennapedia gene complex in *Drosophila melanogaster*. *Devl. Biol.* **81**, 51–64.
White, R. A. H. & Wilcox, M. 1984 Protein products of the bithorax complex in *Drosophila*. *Cell* **39**, 163–171.
Williams, G. J. A. & Caveney, S. 1980*a* A gradient of morphogenetic information involved in muscle patterning. *J. Embryol. exp. Morph.* **58**, 35–61.
Williams, G. J. A. & Caveney, S. 1980*b* Changing muscle patterns in a segmental epidermal field. *J. Embryol. exp. Morph.* **58**, 13–33.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES



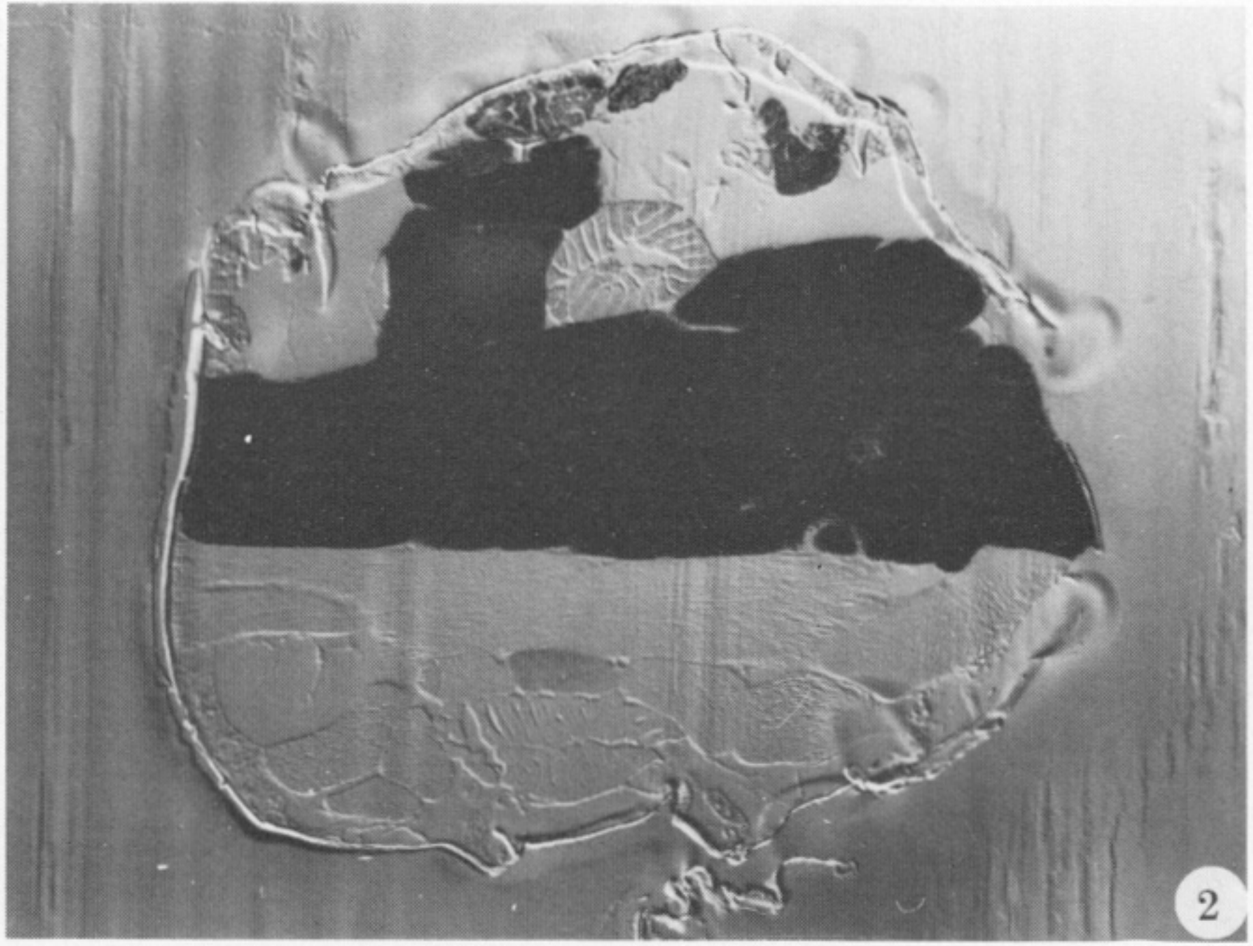




FIGURE 1. A marked clone of cells in the thorax of *Drosophila*. On the right, the genotype is that of the majority of the fly (*Ki Sb*$^{63b}$ *M*(3)*w*$^{124}$/+) and makes the bristles squat and stubby. On the left, wild type bristles (+/+) indicate the presence of a clone induced by mitotic recombination (see Struhl 1982).

FIGURE 2. Horizontal section of the thorax of *Drosophila* in which the unstained parts lack *sdh* activity and are otherwise wild type, while the stained muscles are *sdh*$^{+}$ and also homozygous for a lethal mutation of the *engrailed* gene. The stained muscles develop normally (see Lawrence & Johnston 1984*a*). Anterior to the left. Partial interference contrast.

Downloaded from rstb.royalsocietypublishing.org

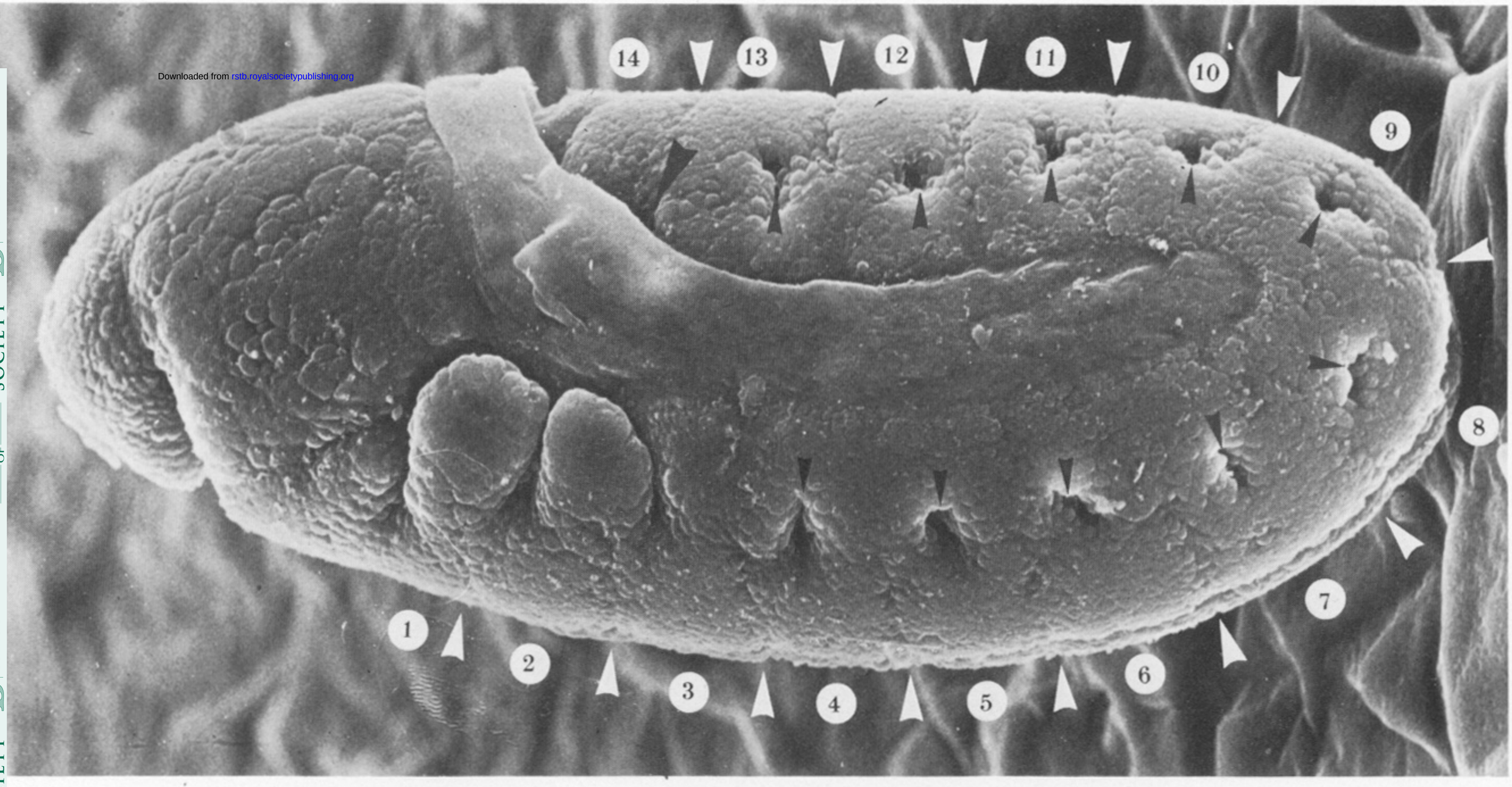
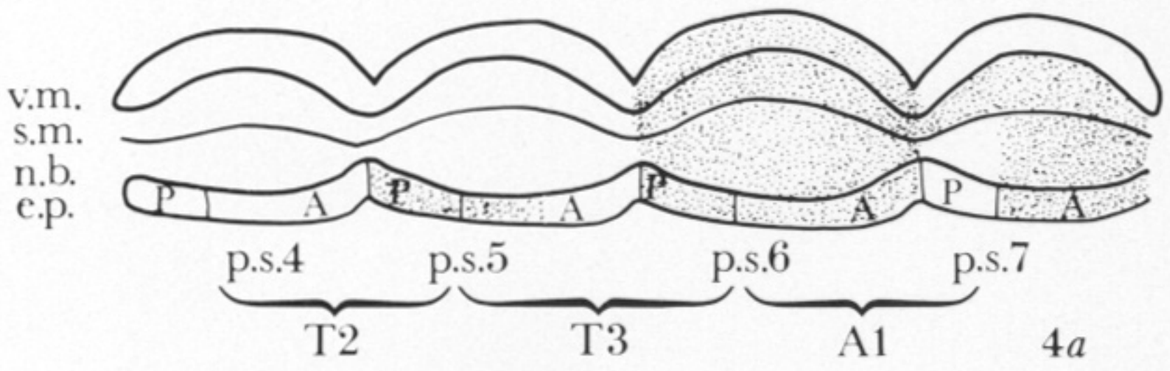
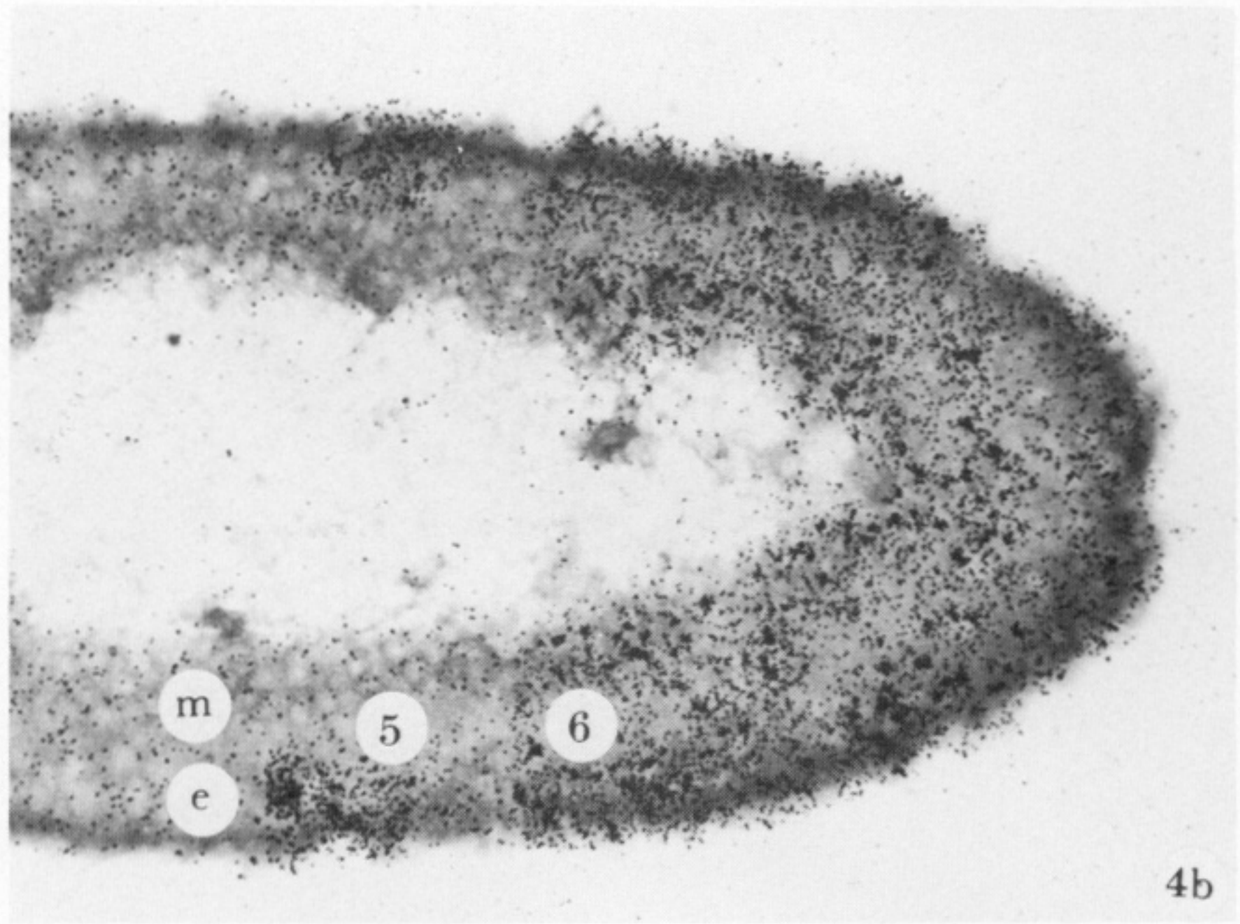



FIGURE 3. Scanning electron micrograph of an embryo at the extended germ band stage showing the first signs of metamerization. According to Martinez-Arias & Lawrence (1985) the ventromedial grooves (white arrowheads) mark the parasegmental borders and the tracheal pits (black arrowheads) the segment boundaries. Notice the eleventh pit (large black arrowhead) located more dorsally than the rest. Numbers indicate parasegments. Photograph kindly provided by Dr P. Ingham.

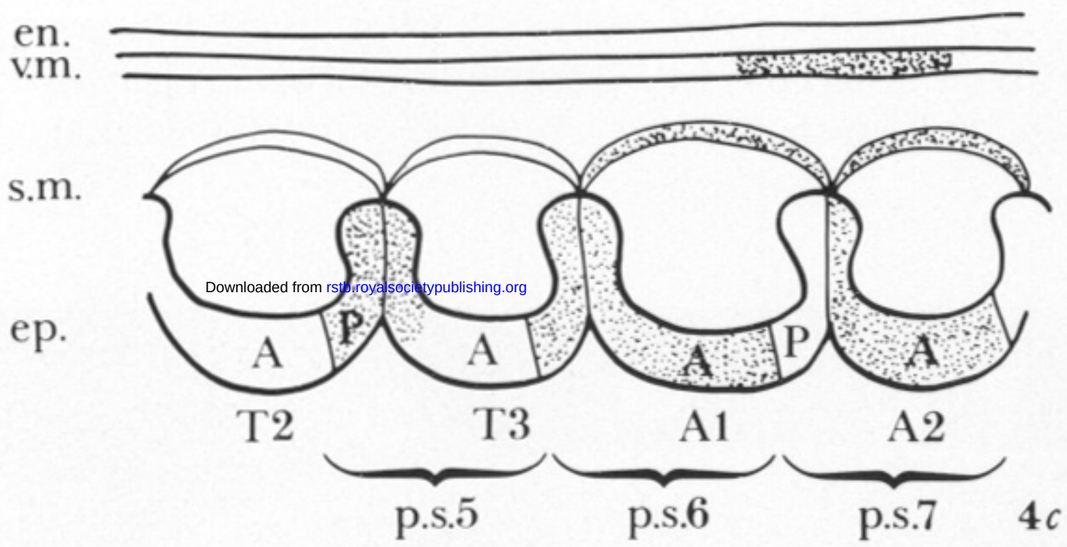
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES




4*a*

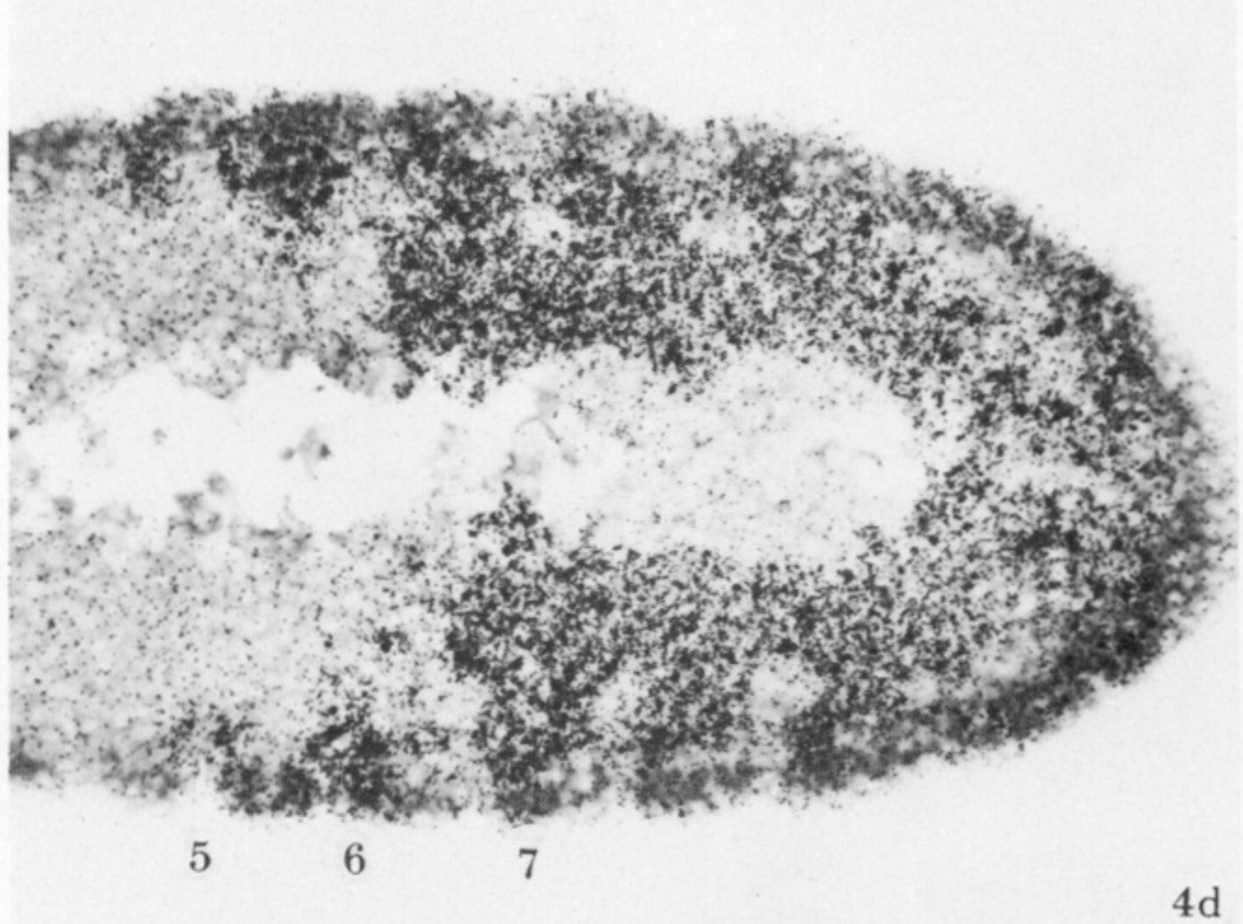



4b

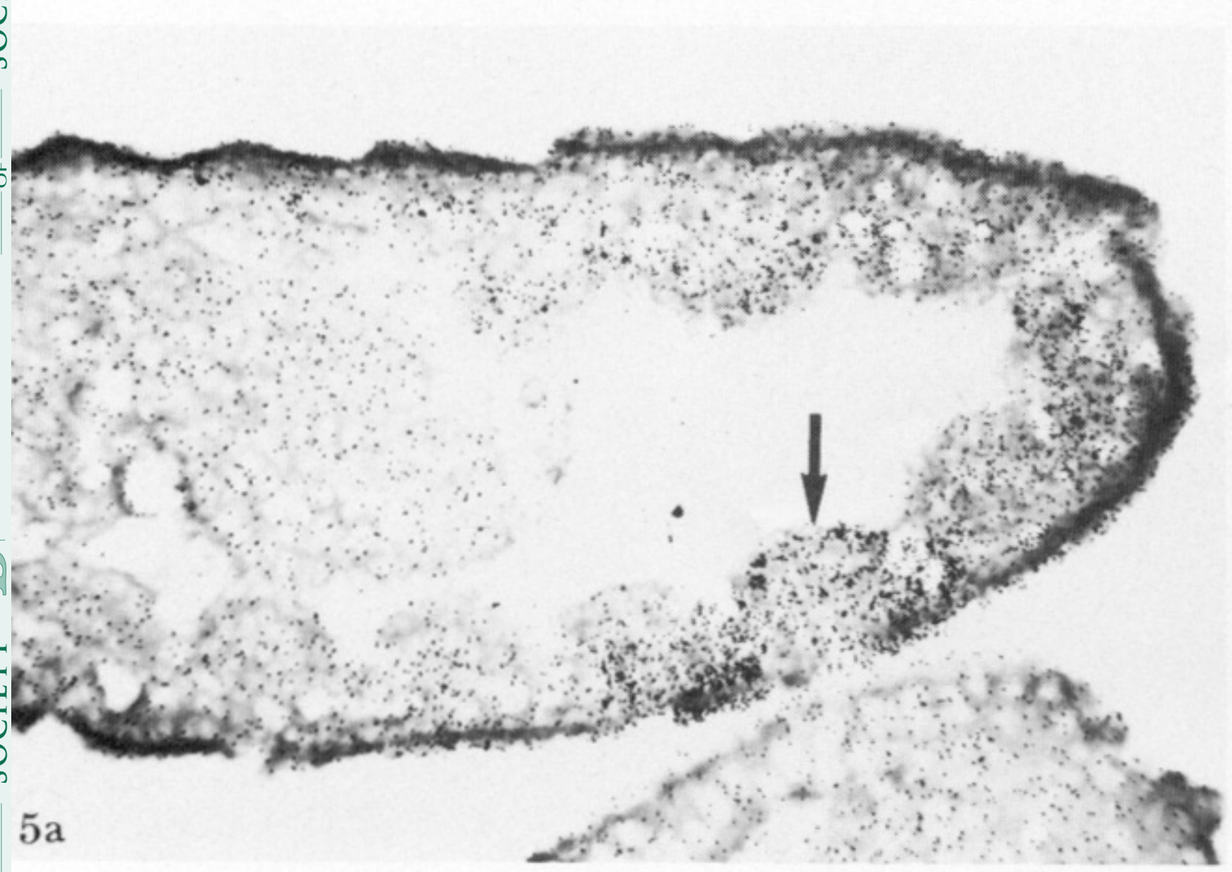



4*c*

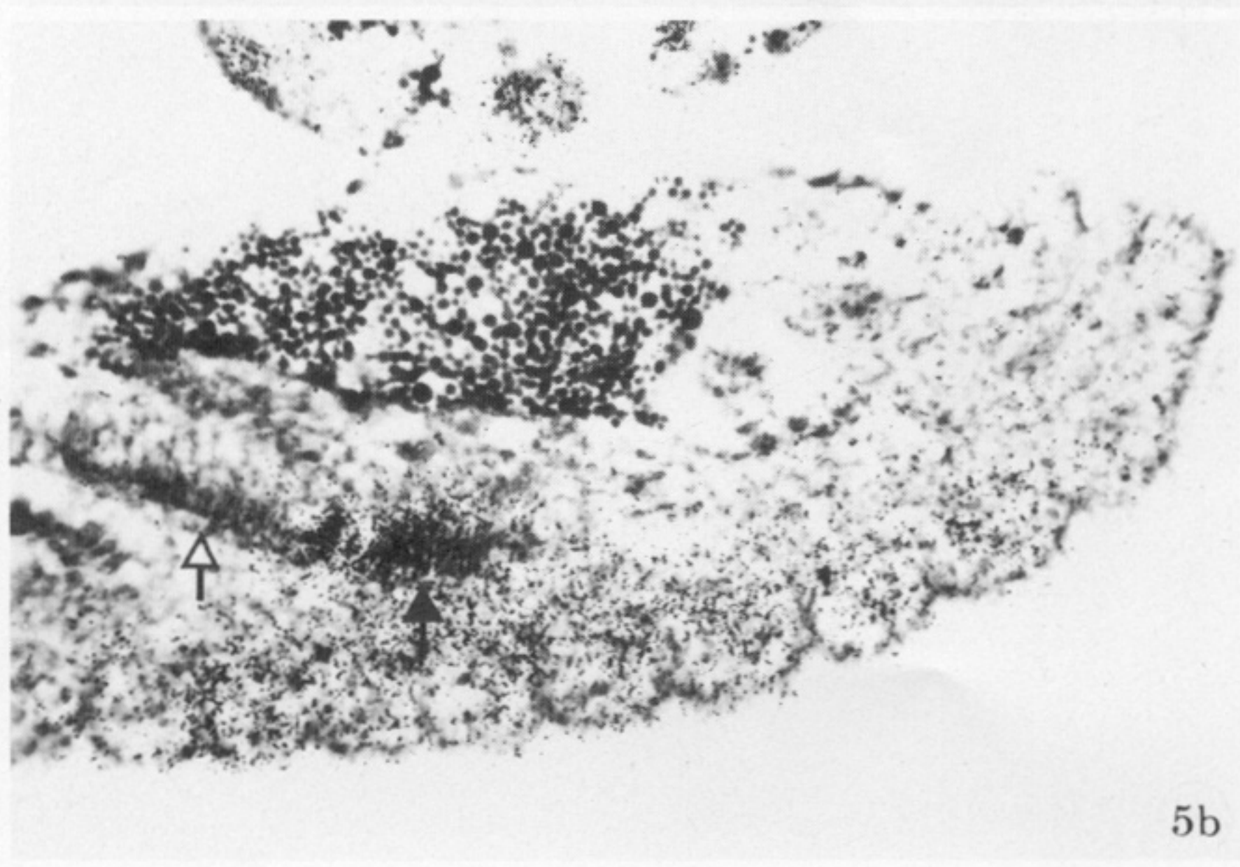
Downloaded from rstb.royalsocietypublishing.org




4d



5a



5b

FIGURES 4 AND 5. For description see opposite.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES