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An Analysis of Abnormal Development in the Cnbw-16 Stock of Drosophila melanogaster

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An Analysis of Abnormal Development in the *Cnbw-16* Stock of *Drosophila melanogaster*[1]

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Abstract. Developmental abnormalities in the embryonic period resulting from cobalt-60 induced mutations in *Drosophila melanogaster* are discussed. Embryos of 16 hours of development were studied. Among 32 abnormal embryos found in 52 eggs in this stock (Cnbw-16), eight having a consistent pattern of abnormality were studied. These embryos, designated as *Cnbw*-16, *type a,* all show a failure of embryonic shortening and midgut development. Associated abnormalities permit interpretation concerning the nature of normal development in three areas: (1) nerve cord condensation, (2) embryonic shortening, and (3) muscle fiber differentiation.

Nerve cord condensation is partial in the absence of embryonic shortening. This indicates that this process is the result of two activities: the mechanical process of embryonic shortening and the process of cell differentiation. Embryonic shortening fails in the presence of partial or complete somatic muscle development. This observation supports the idea that embryonic shortening is independent of the development of the somatic musculature. Muscle differentiation is arrested at several different points. Observations indicate that fusion of myoblasts occurs independently, but that fiber formation depends upon a normal contact with the apodemes.

INTRODUCTION

The study of abnormal development as a means of formulating a comprehensive model of the nature of normal development in a mosaic egg has not been widely exploited. The study of abnormal development in Drosophila has many advantages. A large number of embryonic lethals producing deformed embryos are known and in such stocks numbers of similarly abnormal embryos can be easily obtained. Commonly many variations exist in the expression of the lethal genotype, but these are helpful for comparative purposes. A study of a variety of abnormalities should lead to a fuller understanding of the relationships which may exist between the developing parts of a mosaic egg. Complete descriptions of the normal sequence of events in Drosophila development is available from the work of Poulson (1950), Sonnenblick (1950) and Ede and Counce (1956). Wherever reference is made to normal development in this paper credit should be given to these references. This paper will deal with an interpretation of the abnormalities seen in one type of mutant embryo found among several types in a stock of *Drosophila melanogaster.* These particular embryos were selected

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because the abnormalities observed allow interpretation in three areas: (1) nerve cord condensation, (2) embryonic shortening, and (3) the differentiation of the somatic musculature.

Materials and Methods

Source of the stock. The stock, *Cnbw-16* (cinnibar brown eyed), was obtained from Dr. Frank Seto[1]. Dr. Seto isolated the mutant stock from flies treated with 4,000 r from a cobalt-60 source. The name was assigned by Seto. Hatchability tests showed that 79% of eggs laid by this stock failed to hatch. An examination of late stage embryos indicated that most of the lethality was due to abnormal development.

Techniques. Techniques for the collection and preparation of eggs for serial sections are identical to those reported previously (Reitan, 1964). Eggs were allowed to develop for 16 hours prior to fixation. Fifty-two eggs were collected. Twenty of these showed normal development, 32 were grossly abnormal. The abnormalities fell into four non-overlapping groups. Eight of these, designated as *Cnbw-16, type a,* are described. They were selected because the abnormalities seemed to be well suited for the type of developmental analysis proposed.

Description of the Mutant Embryos

General pattern of abnormal development. In the eight mutant embryos studied a consistent pattern of development is seen. Figure 1 is a sagittal section of a normal 14-hour embryo.

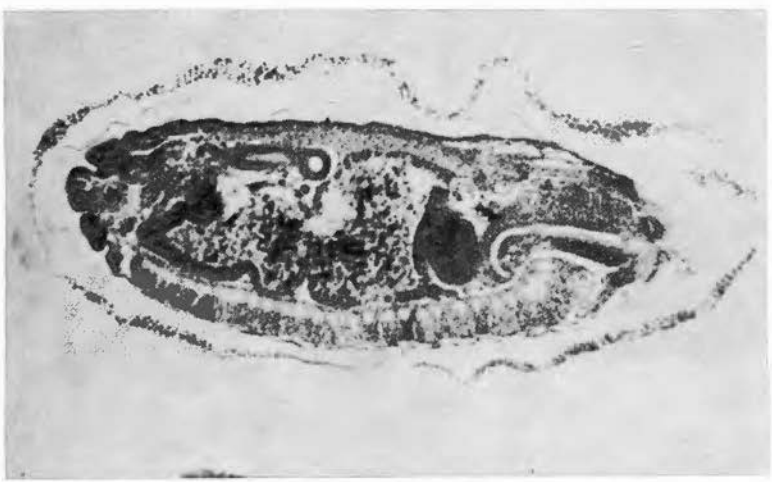


Figure 1. Normal 14-hour embryo

Figures 2, 3 and 4 are sections from three embryos of the *Cnbw-16, type a* type. The major abnormalites noted are the

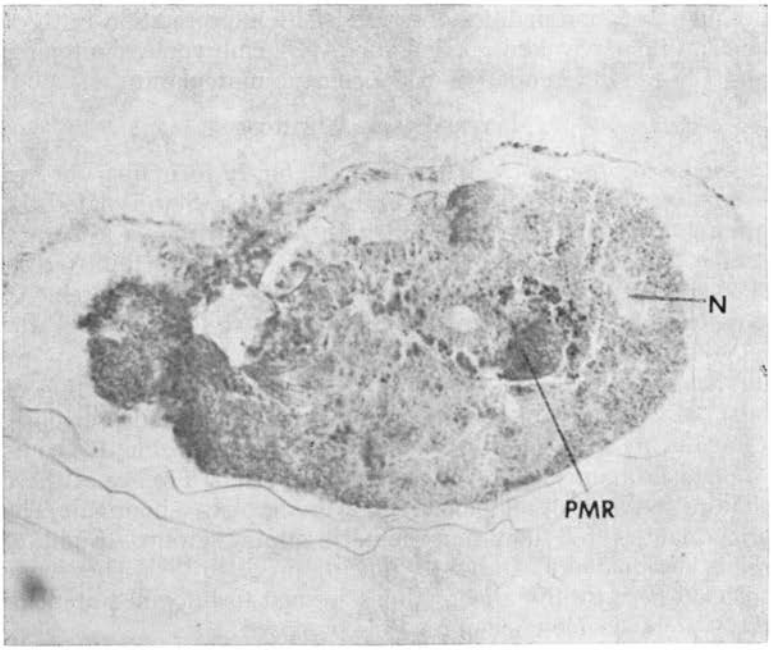


Figure 2. *Cnbw*-16, *type a* mutant

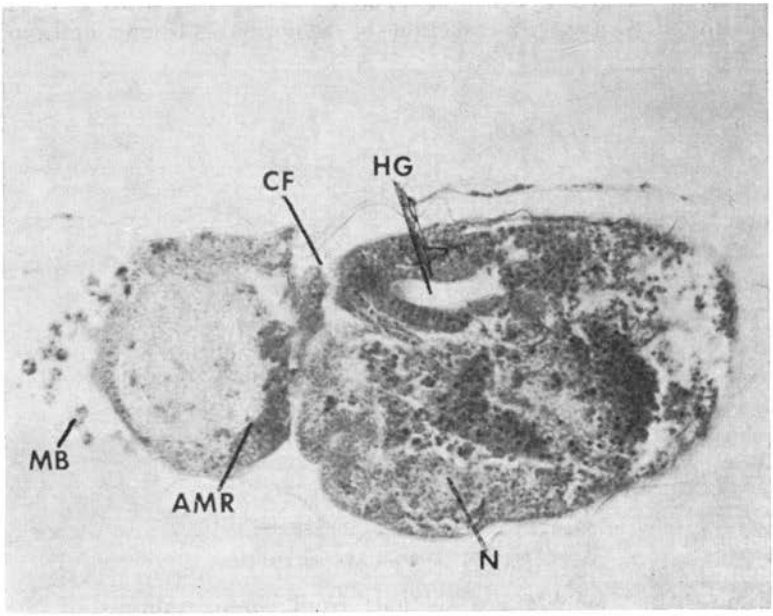


Figure 3. *Cnbw*-16, *type a* mutant

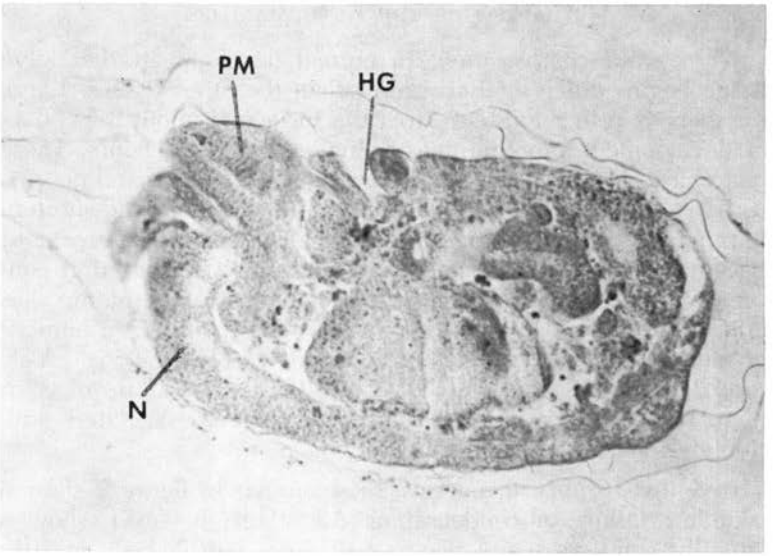


Figure 4. *Cnbw*-16, *type a* mutant

failures of embryonic shortening and midgut development (Figure 5). Cellular differentiation in the hypodermal, nervous and muscular systems in the mutant embryos shows some variation. Two embryos show early differentiation in the nervous and muscle systems along with incomplete hypodermal development. The nervous system is not condensed and is seen as widely dispersed tissue over the ventral and posterior parts of the embryo. Muscle tissue is present as scattered, spherical groups of cells. The hypoderm is incomplete, particularly in the posterior and posterior dorsal parts of the embryo (Figure 2).

Five embryos are like that seen in Figure 3. Here hypodermal differentiation is complete. The nerve cord is partially condensed laterally, but remains as a long, thin, irregular structure stretched around the posterior end to the region of the hindgut invagination. The somatic musculature is partially differentiated. In these embryos the anterior portion of the embryo, including the anterior midgut rudiment and a large mass of yolk, has been nearly severed from the rest of the body. This particular abnormality is presumed to be an exaggeration of a persistent cephalic fold. Such abnormalities have been reported in *Lff 11* mutants by Ede (1956 a) and in *X-23* mutants by Reitan (1964).

One embryo (Figure 4) shows almost completely normal differentiation save for shortening and midgut development. The stomodeum is displaced dorsally; head involution has started but is not completed. The pharyngeal musculature is incompletely differentiated.

Analysis of the Abnormalities

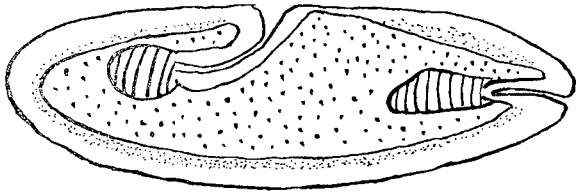
Nerve cord condensation. In normal development the nerve tissue begins differentiation earlier than the other tissues. Large numbers of cells migrate in from the surface at about four hours of development and continue to divide for several hours. These neuroblasts are widely spread throughout the ventral and posterior parts of the embryo. At about the time of embryonic shortening, nerve fiber differentiation becomes evident. The nerve cord becomes shortened, thickened and compact. Condensation continues through 18 hours of development. Condensation has appeared to be closly tied to embryonic shortening. In a number of mutants such as vg^B (Bull, 1956), *Lff 11* (Ede, 1956a), *X-20 type 1,* (Ede, 1956d) and S-9 (Ede, 1956f) the failure of complete or partial embryonic shortening can be correlated with the failure of nerve cord condensation.

In *Cnbw-16, type a* mutants, those similar to figure 2 show a complete failure of condensation. All others, however, show a laterally condensed and compacted nerve cord which remains long and stretched around the posterior end. It is possible to divide the process of nerve cord condensation into two independent activities. The first is the mechanical movement associated with embryonic shorting. This brings all the parts of the developing cord to the ventral side of the embryo. Second is the process of differentiation itself which appears to draw the individual cells of the nerve cord together. This may occur, as it does in these mutants, independently of embryonic shortening.

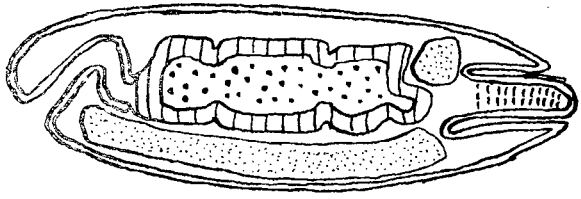
Embryonic shortening. The shortening of the germ band normally takes place as muscle differentiation is getting under way at about 9 hours of development. It had been suggested by Poulson (1950) that the relationship might be a causal one. Evidence has been presented which suggests that embryonic shortening is independent of the differentiation of the somatic musculature. This evidence was based on the appearance of embryos in which muscle was absent, but in which shortening had occurred (Reitan, 1964). Ede (1956f) reported that in S-9 mutants somatic musculature had differentiated but shortening of the germ band did not take place.

Cnbw-16, type a embryos provide similar evidence. Here all but two embryos show the early stages of muscle differentiation including hypodermal attachment. One embryo shows a complete somatic musculature. Nevertheless there is a failure of embryonic shortening. The idea persists that this morphogentic movement is independent of muscle development. In terms of germ band shortening, the timing of these events seems to be coincidental.

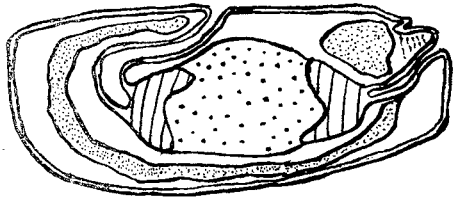
Somatic muscle fiber development. In normal embryonic de-



Normal 7 hours



Normal 14 hours



Mutant Cnbw-16, type a

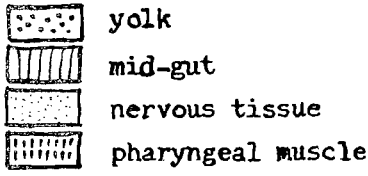


Figure 5. Diagrammatic comparison of normal and *Cnbw*-16, *type a* embryos

velopment muscle differentiation begins at the time of germ band shortening and is completed by 13-14 hours of development. The differentiation of muscle fibers involves two processes: (1) the fusion of presumptive muscle cells to form multinucleate fibers, and (2) the attachment of these fibers to the hypoderm at specific, segmentally arranged sites, the apodemes. Experimental work on *Chysopa* (Bock, 1939; and Seidal, Bock and Krause, 1940) and in *Leptinotarsa* (Haget, 1953) indicated that the hypoderm was a self differentiating system which exerts an inductive influence upon the mesoderm. Ede (1956c) noted that in *X-27* mutants the development of musculature seemed to be dependent for normality upon a normal hypodermal contact.

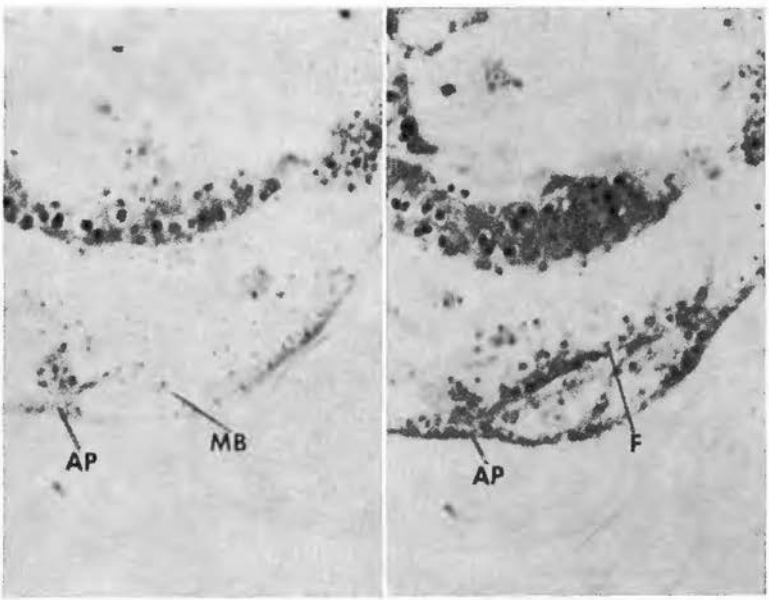


Figure 6. Apodemes and myoblasts in *Cnbw*-16, *type a* mutant
Figure. 7. Muscle fiber, fusion incomplete, in *Cnbw*-16, *type a* mutant.
Abbreviations used: AMR, anterior midgut rudiment; AP, apodeme; CF, cephalic fold; F, muscle fiber; HG, hindgut; MB, myoblast(s); PM, pharyngeal musculature; PMR, posterior midgut rudiment

Cnbw-16, tpye a embryos show considerable variability in the degree of differentiation seen in muscle fiber development. The arrest of development at several different points and the continued differentiation of hypodermal and nerve cord tissues have left several developmental stages exposed in large spaces. These developing fibers are much more easily seen than in the normally developing embryo (Figures 6 and 7). In mutants such as those seen in Figures 2 and 3, fusion occurs without

hypodermal attachment. The result is that groups of myoblasts form spheres of cells floating free in the embryo. Others show myoblasts to be aggregated around the apodemes and to be united into radiating chains of cells (Figure 6). In Figure 7 chains of myoblasts can be seen to have fused with similar chains from adjacent apodemes to form a complete fiber. In this particular fiber the cells have retained their individuality so that final fusion to form a multinucleate fiber has not occurred. Where potential fibers do not make contact with cells from adjacent apodemes they round up to form attached spheres (Figure 6). Similar fan-shaped rosettes of muscle fibers have been described by Counce (1956) in some *fu* mutant embryos.

A model picture of muscle fiber differentiation can be pieced together from these observations. The myoblasts have the independent ability to fuse. Some influence from the apodemes leads the myoblasts to aggregate at these spots. Here fusion occurs along with a normal hypodermal contact and attachment. With normal attachment, chain formation and fusion end-to-end occur. This leads to the formation of a normal fiber if contact is made with the developing fibers of adjacent apodemes. The evidence presented here supports the suggestion by Ede (1956f) that a proper hypodermal contact is essential for normal muscle development.

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