EFFECTS OF HOMOZYGOUS FIRST, SECOND AND THIRD CHROMOSOME MINUTES ON THE DEVELOPMENT OF DROSOPHILA MELANOGASTER[1]

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THE Minutes of *Drosophila melanogaster* include a large group of factors which are dominant over wild type when heterozygous, and lethal when homozygous. All heterozygous Minutes are characterized by the presence of short, slender bristles in the adult and by a retardation in development in larval life. Although the Minutes mimic one another in expression, they have been found to occupy a variety of loci in all four chromosomes and are considered by most authors to be chromosomal deficiencies (BRIDGES and BREHME 1944). The behavior of these factors when combined with modifiers or with other Minutes has indicated that many are qualitatively different (SCHULTZ 1929).

The purpose of the present study is to determine whether or not different Minutes, so similar in heterozygous phenotype, also resemble one another with respect to homozygous phenotype. This investigation is concerned with an analysis of the factors responsible for the death of individuals homozygous for seven different Minutes, one of which, *M(1)o*, is located on the X chromosome; two, *M(2)l* and *M(2)l*^{2}, are overlapping deficiencies of the second chromosome; while the other four, *M(3)w*, *M(3)124*, *M(3)B* and *M(3)B*^{2}, comprise an allelic series of the third chromosome. Some information concerning these different Minutes is available in the literature and will be reviewed briefly in the following section.

Minute (1)o resembles other members of the Minute series in that heterozygous females possess smaller bristles and a longer larval developmental period than the wild type. This mutant is lethal in males. Salivary chromosome studies have shown the locus to be between bands 14B1–2 and 15E7 (BRIDGES and BREHME 1944).

Minute (2)l appeared spontaneously and salivary chromosome analysis by BRIDGES has shown that the chromosome is deficient for bands 58A–F (BRIDGES and BREHME 1944). Studies by LI (1927) have demonstrated that the *M(2)l* homozygote dies in the egg stage. According to LI, heterozygous *M(2)l* females regularly lay eggs which are abnormal in appearance not only in regard to shape but also with respect to the character of the yolk, which he describes as glassy in appearance, rather than opaque white. In the present study, similar abnormalities in egg shape were observed, but differences in the appearance of the yolk were not noticeable in living, developing embryos. It should be noted, however, that unfertilized eggs, as well as partially developed embryos, frequently appear quite transparent 24 or more hours after egg deposition. DUNN and MOSSIGE (1937) have reported that a two-day delay occurs during the larval stage of *M(2)l* heterozygotes although the final body size of the

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Minutes is generally smaller than that of wild type sibs. The presence of this mutant is considered by these authors to result in a retarding rather than an extending effect upon larval life.

Minute (2)l^2 is regarded as a deficiency, although salivary analysis by BRIDGES has shown no detectable abnormalities. The locus is associated with band 58F and is thus included within that of *M(2)l* (BRIDGES and BREHME 1944). A developmental delay in larval life is characteristic of *M(2)l*^{2} as reported by DUNN and MOSSIGE (1937) and by BREHME (1939 and 1941a). According to BREHME, Minute male larvae are about 9.9 and female larvae 13.3 hours behind their wild type sibs in puparium formation. A slight lengthening of the pupal period was recorded by DUNN and MOSSIGE (1937). Body size of the adult is significantly smaller than that of controls, when tibia length is used as an index (BREHME 1939). In addition, the surface area of cells of wing epithelium has been found to be smaller than that of wild type. From this evidence, BREHME (1941a) has suggested that a reduction in cell size may account for the smaller body size of the Minutes. The death of *M(2)l*^{2} homozygotes occurs in the first larval instar (BREHME 1939). Homozygous larvae grow only very slightly and do not molt. These observations are confirmed in the present study.

Of the four allelic third chromosome Minutes, *M(3)w* has been considered the most extreme, while *M(3)124* lies between *M(3)w* and *M(3)B* and *M(3)B*^{2} in degree of expression (BRIDGES and BREHME 1944). The growth of larvae heterozygous for *M(3)w* has been investigated by DUNN and COYNE (1935), DUNN and MOSSIGE (1937) and by BREHME (1939, 1941a and 1941b). BREHME reports a 42-hour delay in puparium formation as compared to the wild type. Although the relative growth curves of heterozygotes are practically identical with controls, adult body size is smaller. The transplantation of eye disks of *M(3)w* heterozygotes to wild type larvae has shown that the more rapid growth rate of the host has no effect on the growth rate of the transplant. Facet number of adult Minutes is not significantly lower than that of controls, but the size of individual facets is smaller (BREHME 1941b). Minute (3)w homozygotes have been found to die in the first larval instar and BREHME (1939) has reported a general necrotic condition of larval tissues in these individuals.

METHODS

All stocks used in the present study were originally obtained from the laboratories of either DR. A. H. STURTEVANT or DR. M. DEMEREC. Heterozygotes on a background of Canton-S wild type were maintained by continuous inbreeding for over twenty generations and were the source of the homozygotes studied.

Eggs were collected at hourly intervals at 25°C and prepared as previously described (FARNSWORTH 1957). The ages of all eggs and larvae were calculated from the midpoint of the laying period. Over 500 embryos were studied for each of the Minutes here considered.

With the exception of *M(2)l*, which is lethal in the egg stage, almost all of the homozygotes of the other six Minutes died in larval life. In each of these stocks, however, there were always a few individuals which failed to hatch. In view of this fact, it was necessary to make certain that only one lethal was being considered and that

the total homozygotes, hatched or unhatched, did not exceed the 25% expected from a cross between heterozygotes. To verify this point, a number of egg collections were initiated and the subsequent history, from hatching through eclosion, of every egg laid within a given period was followed and recorded. All unhatched eggs were dechorionated and examined and, if development had occurred, these individuals were fixed and prepared for histological study. Hatched larvae were isolated, counted and classified as to normal or lethal type and transferred to fresh media. Those placed in the normal group included heterozygotes and the homozygous wild type, a classification confirmed upon eclosion. The individuals designated as lethals were placed in small dishes of fresh media and examined daily for evidence of growth or molting. In all such experiments, the number of lethal larvae plus the individuals which had undergone development, but had not hatched, was very close to the 25% expected. With respect to the adults, approximately two thirds were Minute in phenotype, while one third was wild type. From this evidence, it was concluded that the only lethal factor involved in each instance was the specific Minute being investigated.

RESULTS

Of the seven different Minutes here considered, only *M(2)l* homozygotes die in the egg stage. All other Minutes are lethal in the first larval instar. Since these latter forms are almost identical in phenotype, they will be considered together, while *M(2)l*, which represents a much more extreme departure from the normal condition, will be described separately.

Minute (2)l

The Minute (2)l homozygotes cannot be distinguished from their wild type sibs until after 12 hours of development. Just before and during this stage in ontogeny, a number of rapid changes involving complex cell movements normally occur. Anteriorly and ventrally, the rolling inward of cells contributing to the stomodeum proceeds while, dorsally, involution of the head with frontal sac formation is occurring. The anterior and posterior midgut rudiments have joined and are extending laterally and dorsally to enclose the yolk, a process usually completed by around 12 hours of development. Dorsal closure of the body wall with subsequent formation of the heart and aorta proceeds concurrently. For a complete description of these stages, the reader is referred to POULSON (1950).

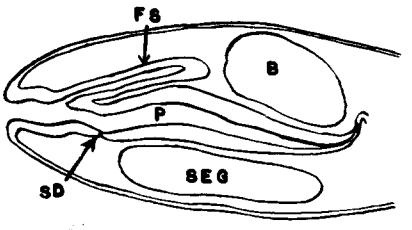
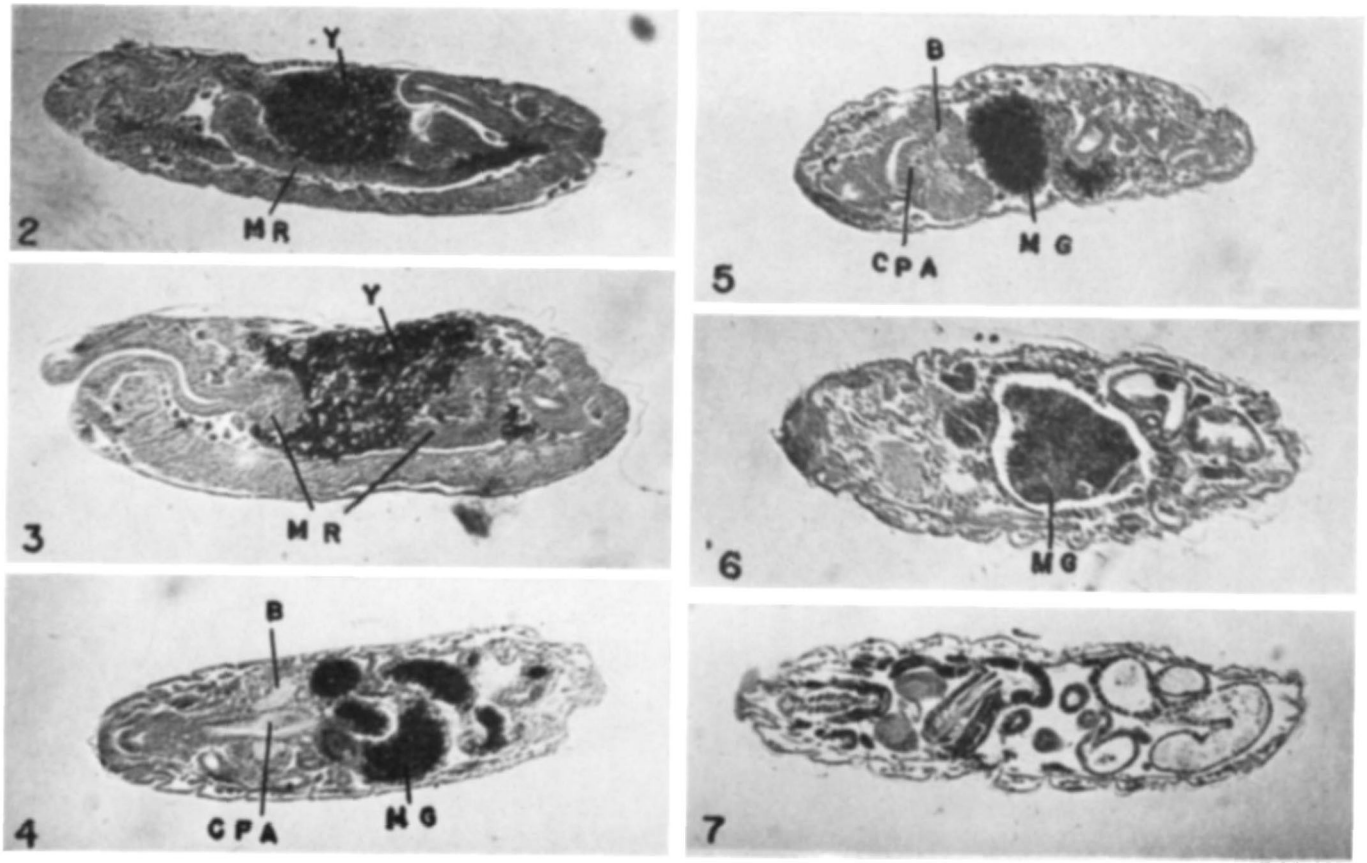


FIGURE 1.—Diagram of a sagittal section of the anterior end of a 16-hour *M(2)l* homozygote showing relationships of frontal sac and pharynx. B, brain; FS, frontal sac; P, pharynx; SD, opening of salivary duct into pharynx; SEG, subesophageal ganglion.



FIGURES 2–7—All figures are oriented with the dorsal surface to the top of the page and the anterior end to the left. B, brain; CPA, cephalopharyngeal apparatus; MG, midgut, MR, midgut rudiment; Y, yolk.

FIGURE 2.—Longitudinal section of 12-hour normal embryo. Midgut rudiments have fused and are enclosing yolk.

FIGURE 3.—Longitudinal section of 12-hour *M(2)l* homozygote illustrating abnormal anterior and posterior midgut rudiments.

FIGURE 4.—Frontal section of 18-hour normal embryo. Midgut is lengthening and cephalopharyngeal apparatus is well formed.

FIGURE 5.—Frontal section of 18-hour *M(2)l* homozygote showing abnormal pharyngeal apparatus and midgut.

FIGURE 6.—Frontal section of 24-hour unhatched $M(2)l^2$ homozygote. Midgut is unsegmented and structures of the anterior end are disorganized.

FIGURE 7.—Frontal section of hatched homozygous $M(2)l^2$ larva.

The first detectable morphological difference between the *M(2)l* homozygotes and the controls is found in the formation of the midgut. The anterior, and to a lesser extent, the posterior endodermal rudiments extend into the yolk as a blunt mass and fail to spread out adequately to enclose this material (figs. 2 and 3). Fusion of these rudiments is thus delayed and, consequently, midgut formation is retarded as compared to the controls. Although the yolk is eventually surrounded, the mid intestine remains abnormal throughout the remainder of development. The walls are thin and in various regions yolk can be found escaping from the gut into the body spaces.

A second major anomaly concerns the foregut. The frontal sac is invaginated only over the anterior end of the pharynx with the result that the pharyngeal dilator muscles are formed in a shortened region. Apparently, invagination of the head is not carried to completion in the *M(2)l* homozygotes for, if the frontal sac were normal in its spread over the dorsal surface of the pharynx a much more extensive cephalopharyngeal apparatus would be formed (figs. 1, 4, 5). The movement of cells into the stomodeum occurs normally, however, and the ducts of the salivary glands reach their definitive location and fuse.

In the Minute homozygote at 16 hours, further abnormalities of the midgut become evident. The cardia differentiates slowly into an organ which is reduced in size and more elongate than that of the controls. Although caecae appear as bud-like evaginations from the mid intestine, they remain very small and fail to achieve normal length. The midgut itself lengthens slowly, and anterior portions in particular remain rounded, sac-like and crowded with yolk (fig. 5). In contrast, the hindgut with appended Malpighian tubules is essentially normal.

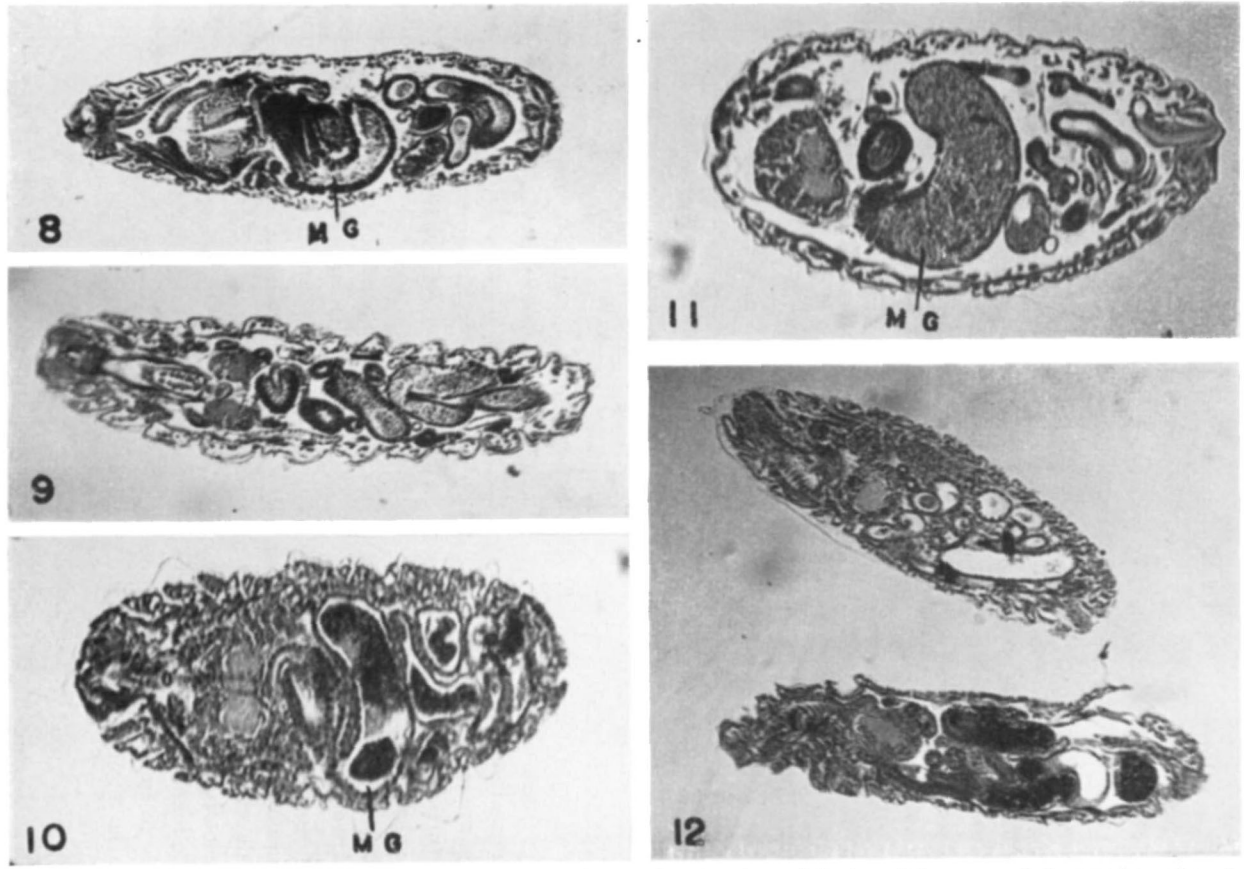
The brain and ventral nerve cord develop in a manner comparable to that found in the controls. Some disorganization in the neuropile is evident in late stages and complete condensation of the ventral ganglia is not usually achieved. However, no ventral ganglia are missing and nerve formation generally proceeds normally.

Other structures, such as the dorsal vessel, gonads, and the very small fat bodies can usually be identified although the presence of the ring gland is not certain. Longitudinal tracheal trunks and their developing side branches are generally normal.

Twenty to twenty-two hour *M(2)l* homozygotes present an appearance similar to that described above. Although the cephalopharyngeal apparatus remains abnormal in position and extent, the various sclerotized plates and hooks of the mouth armature do develop. The midgut does not attain a length comparable to that of controls and not only is yolk withdrawal greatly retarded, but this material frequently can be found scattered through the hemocoel. The *M(2)l* lethal embryo, in general, greatly resembles *M-4* homozygotes with respect to the pattern of abnormalities although the anomalies are not so extensive.

Larval lethals

Almost all homozygotes of *M(1)o*, *M(2)l²*, *M(3)w*, *M(3)124*, *M(3)B* and *M(3)B²* hatch within 22 to 26 hours after egg deposition. Of the few homozygotes which do not escape from the egg, a number are fully developed and are often found to be moving within the vitelline membrane 30 or more hours after normal hatching time. Other unhatched, but developed, embryos present a number of anomalies upon



FIGURES 8–12—Unless otherwise stated, all figures are oriented with the dorsal surface toward the top of the page and the anterior end to the left. MG, midgut.

FIGURE 8.—Frontal section of 24-hour unhatched *M(1)o* homozygote.

FIGURE 9.—Frontal section of hatched homozygous *M(1)o* homozygous larva showing yolk in gut.

FIGURE 10.—Frontal section of unhatched 22-hour *M(3)w* homozygote. Midgut is slow to lengthen and contains much yolk.

FIGURE 11.—Frontal section of 22-hour *M(3)124* homozygote showing large unsegmented midgut.

FIGURE 12.—Top. Sagittal section of 22-hour normal embryo, oriented with anterior end to left and ventral side to top of page. Bottom. Sagittal section of 22-hour unhatched *M(3)B* homozygote showing abnormal midgut. Embryo slightly damaged in preparation.

histological examination. In the majority, in all six stocks, the midgut is usually unsegmented and crowded with yolk, the cephalopharyngeal apparatus may be undeveloped or only partially differentiated, and the nervous system is frequently disorganized or not condensed anteriorly (figs. 6, 8, 10, 11, 12). In some of these individuals, it would appear that coordinated development ceased around 12 to 14 hours of incubation. Although there are never very many of these abnormal types, they are most frequently encountered in *M(3)w* and *M(3)124*, less frequently found in *M(3)B* and *M(2)l*^{2} and rarely found in *M(3)B*^{2} and *M(1)o*. In addition, in *M(3)w* and *M(3)124*, three to five grossly aberrant embryos were found in which dorsal closure was incomplete or in which only two thirds of the embryo had developed with one end consisting of a cap of scattered yolk and a small amount of cytoplasm. Whether or not these rare types can be attributed to the homozygous presence of a Minute deficiency is not known.

Studies of sectioned embryos, fixed at various stages in development, reveal no differences between controls and Minute homozygotes until the time of yolk enclosure. At this stage in the lethal types, the midgut rudiments are slow to enclose the yolk and some embryos resemble the condition found in *M(2)l* (fig. 3). Occasionally, involution of the head and frontal sac formation is atypical and incomplete, but this occurrence is not common. It is probable that such abnormal embryos represent a portion of the unhatched, but developed eggs identified in egg counts.

In later stages, the only abnormality usually present involves the midgut. Although cardia and caecae form normally, the anterior and often the middle regions of the midintestine remain large, rounded and filled with yolk. This entire structure lengthens more slowly than is the case in controls and even in hatching stages, some undigested food is almost always present, a condition rarely encountered in wild type individuals (figs. 7, 9). In the majority of the sectioned homozygotes, the only basis for identification rested on the appearance of the midgut, for all other structures were morphologically normal.

Grossly, the homozygous Minute larvae of all stocks can be distinguished easily from controls. Such individuals are exceedingly small (around 1 mm) and do not increase appreciably in either length or width even though they may live for four or more days. Although normal feeding and crawling motions are carried out, such movements tend to be very sluggish. It has been found that lethal homozygotes survive longer when allowed to remain in the same dish with active growing sibs than when isolated to a separate container. It is thought that this greater longevity may possibly be attributed to the mechanical breaking up of the media by the larger larvae.

DISCUSSION

When the phenotypes of homozygotes of these seven Minutes, along with *M-4*, are compared with one another, certain striking similarities become apparent.

In all Minute types, the metabolism of yolk is impaired and this aberration is accompanied by abnormalities in midgut formation which range from extreme in *M-4*, to less severe in *M(2)l*. In the other Minute homozygotes, the only detectable deviation from the wild type lies in the slowness of midgut differentiation and yolk withdrawal. The cardia and caecae are absent altogether in *M-4* and poorly formed in *M(2)l*, although normal in the other Minutes of the series studied.

In the region of the foregut, head involution ceases at an early stage in *M-4* and proceeds further in *M(2)l*. In both cases, however, an abnormal cephalopharyngeal apparatus results. The foregut is normal in *M(1)o*, *M(2)l²* and in the third chromosome Minutes.

Other structures, such as the nervous system, can be arranged in the same sequence with respect to degree of development in different Minutes, *viz.* grossly abnormal in *M-4*, somewhat disorganized in *M(2)l* and comparable to controls in the case of the other Minutes. It should be noted that of those few homozygotes of the larval lethals which fail to hatch, a number exhibit the same pattern and type of aberrations which have been found in *M-4* and *M(2)l*.

Another similarity between these eight mutants is the time of appearance of the abnormalities. All anomalies can be traced to the same period in development, i.e., around 12 hours of incubation, a period just before and during which rapid changes involving different organ-systems occur. The abnormalities of the various Minutes are invariably found in one or more of the structures intimately concerned in this period of intense morphogenetic activity and can be recognized either in 12-hour stages or shortly thereafter.

The development of the phenotype of homozygous *M-4* has been discussed and interpreted in terms of a severe restriction in growth rate occurring at this critical period in development (FARNSWORTH 1957). The same hypothesis can be applied to the first, second and third chromosomal Minutes studied in the present work, with the modification that such a restriction, even though occurring at a similar time, must be less pronounced. Certainly differences in homozygous phenotype do occur between these eight Minutes, but they seem to be differences in degree, rather than in kind.

The pronounced mimicry in heterozygous phenotype displayed by the Minutes should also be considered. All Minutes are characterized by short bristles, dominance over wild type and by delayed development in larval stages. Even though the larval period is prolonged, heterozygous Minute larvae are smaller than their control sibs. This size difference also extends into the adult stage and has been shown by BREHME to be due to a decrease in cell size, not in cell number. A restriction in growth which affects the size of individual cells of many tissues must be due to an alteration of one or more general physiological processes common to most cells. If the presence of a given heterozygous deficiency results in delayed development and retarded growth, perhaps the homozygous phenotype is due to the same fundamental physiological aberration which becomes a completely limiting factor effective in earlier stages. It should be recalled that failure to grow is one of the outstanding characteristics of the larval lethals studied.

An explanation to account for the similarities in gene action on the part of these different loci can only be speculative at the present time. Further work to clarify the Minute phenotype on physiological grounds is being undertaken.

SUMMARY

1. The effects of seven different homozygous Minutes on the development of *Drosophila melanogaster* are described. The stocks studied include *M(1)o*, *M(2)l*, *M(2)l²*, *M(3)w*, *M(3)124*, *M(3)B* and *M(3)B²*.

2. Minute (2)l homozygotes die in the eggstage. Differences between controls and lethal homozygotes become apparent around twelve hours of development and consist of the following:

Fusion of the midgut rudiments is delayed and the wall of the midgut, when formed, is frequently incomplete, thus releasing yolk into body spaces. Lengthening and coiling of the gut proceeds slowly and even in late stages this structure remains large, sac-like and crowded with yolk, especially in more anterior portions. Cardia and caecae are poorly differentiated.

Abnormal head involution results in a small, aberrant cephalopharyngeal apparatus.

Some disorganization of the nervous system is usually present and condensation of the ventral ganglia is frequently incomplete.

3. Almost all homozygotes of the other six Minute stocks die in the larval stage and are practically indistinguishable from one another. The only morphological anomaly usually present appears around twelve hours of development and involves the midgut which is retarded in formation and differentiates more slowly than is the case in controls. Yolk frequently remains in the lumen of this structure even in hatching stages.

Hatched homozygous Minute larvae are approximately 1 mm in length and often live for several days. During this time, however, no appreciable growth occurs nor has any evidence of molting been observed.

In each of these six stocks, there are always a few homozygotes which fail to hatch. Such individuals are frequently characterized by abnormalities of the foregut, midgut and nervous system.

4. The similarities in the phenotypes of these seven Minute homozygotes are discussed.

LITERATURE CITED

BREHME, K. S., 1939 A study of the effects on development of "Minute" mutations in *Drosophila melanogaster*. Genetics **24:** 131–161.

1941a Development of the Minute phenotype in *Drosophila melanogaster*. A comparative study of the growth of three Minute mutants. J. Exp. Zool. **88:** 135–160.

1941b The growth of transplanted Minute and wild type optic disks in *Drosophila melanogaster* Growth **5:** 183–195.

BRIDGES, C. B., and K. S. BREHME, 1944. *The Mutants of Drosophila melanogaster*. Carnegie Inst. Wash. Publ. **552.** Washington.

DUNN, L. C., and J. COYNE, 1935 The relationship between the effects of certain mutations on the developmental rate and on adult characters. Biol. Zentr. **55:** 385–389.

DUNN, L. C., and J. C. MOSSIGE, 1937 The effects of the Minute mutations of *Drosophila melanogaster* on developmental rate. Hereditas **23:** 70–90.

FARNSWORTH, M. W., 1957 Effects of the homozygous Minute-4 deficiency on the development of *Drosophila melanogaster*. Genetics (in press).

LI, J., 1927 The effect of chromosome aberrations on development in *Drosophila melanogaster*. Genetics **12:** 1–58.

POULSON, D. F., 1950 Histogenesis, organogenesis, and differentiation in the embryo of *Drosophila melanogaster* Meigen. *Biology of Drosophila*, Chap. 3: 168–274. Edited by M. DEMEREC. John Wiley. New York.

SCHULTZ, J., 1929 The Minute reaction in the development of *Drosophila melanogaster*. Genetics **14:** 366–419.