THE GENETICS OF DOPA DECARBOXYLASE IN *DROSOPHILA MELANOGASTER* I. ISOLATION AND CHARACTERIZATION OF DEFICIENCIES THAT DELETE THE DOPA-DECARBOXYLASE-DOSAGE-SENSITIVE REGION AND THE α-METHYL-DOPA-HYPERSENSITIVE LOCUS[1]

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ABSTRACT

A detailed cytogenetic investigation of 16 overlapping deficiencies in the 36C-40A region on the left arm of the second chromosome (2L) in *Drosophila melanogaster* is reported. These deficiencies permit a localization of both the dopa-decarboxylase-dosage-sensitive region and the α-methyl-dopa-hypersensitive locus, *l(2)amd*, to the same region, 37B10–37C7.

A problem of primary interest in biology is the elucidation of the organization in the eukaryote chromosome of genetic elements concerned with the time-specific and tissue-specific control of gene activity during development. The work reported in this paper is part of the continuing effort of both our laboratories to investigate the genetic regulation of dopa decarboxylase in *Drosophila melanogaster* (HODGETTS and KONOPKA 1973; SPARROW and WRIGHT 1974; SHERALD and WRIGHT 1974; HODGETTS 1975; WRIGHT, BEWLEY and SHERALD 1976).

Dopa decarboxylase (DDC) (E.C. 4.1.1.26 3,4-dihydroxyl-L-phenylalanine-carboxy-lyase) catalyzes the decarboxylation of dopa (3,4-dihydroxy-L-phenylalanine) to dopamine (3,4-dihydroxyphenylethylamine). In the Diptera, the enzyme appears to function in embryogenesis (SCHLAEGER and FUCHS 1974), in the production of neurogenic amines (DEWHURST *et al.* 1972) and in the maturation of larval and adult cuticles. This last aspect of function has been most extensively investigated, primarily in the blowfly, *Calliphora erythrocephala* (for reviews, see KARLSON and SEKERIS 1966; SEKERIS and KARLSON 1966). The enzyme is located primarily in the epidermal cells and peaks of activity occur

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as the organism undergoes sclerotization (hardening) of the cuticle, at the larval molts, puparium formation and eclosion of the adult.

SEKERIS and KARLSON (1962) demonstrated that DDC was involved in the production of the sclerotizing agent N-acetyldopamine according to the reaction sequence: tyrosine → dopa → dopamine → N-acetyldopamine. They suggested DDC could be the rate limiting enzyme and presented evidence indicating that the activity which appears at puparium formation is induced by the steroid molting hormone, ecdysone. This suggestion has received additional support from subsequent work with Calliphora (SEKERIS and KARLSON 1964; SHAAYA and SEKERIS 1965; HOFFMEISTER, GRÜTZMACHER and DÜNNEBEIL 1967) and the fleshfly, *Sarcophaga bullata* (CHEN and HODGETTS 1974). The recent experiments of FRAGOULIS and SEKERIS (1975) now provide strong evidence that the hormonal induction of DDC activity results from a stimulation of the transcription of the mRNA for the enzyme.

The general details of the sclerotization of the puparium, as postulated for Calliphora, appear to hold for Drosophila. The enzyme is found in the epidermal cells (LUNAN and MITCHELL 1969) and peaks in activity are correlated with the times during which sclerotization occurs (LUNAN and MITCHELL 1969; MCCAMAN, MCCAMAN and LEES 1972). N-acetyldopamine has been identified as a sclerotizing agent (SEKERIS and HERRLICH 1966; MITCHELL, WEBER-TRACY and SCHAAR 1971), and the enzyme activity responsible for its conversion from dopamine measured (HODGETTS and KONOPKA 1974). Although the description of sclerotization presented here may not hold for all insects (ANDERSON 1971; ANDERSON and BARRETT 1971) and is probably incomplete even for adult *Drosophila melanogaster* (HODGETTS and CHOI 1974), DDC unquestionably plays a key role.

Genetic experimentation has progressed along two different lines of research. WRIGHT and his co-workers have investigated the use of the analog inhibitor α-methyl dopa (αMD) as a selective agent for mutations affecting DDC. Of the resistant strains isolated, three have been extensively analyzed (SHERALD and WRIGHT 1974). Two of them had elevated DDC activities, and for both, resistance was controlled by genes on both the right (2R) and left arm (2L) of the second chromosome, whereas only genes on 2L caused the elevation of DDC activity. No detectable effect either on the level of DDC activity or on the structure of DDC could be found for the third, most highly resistant strain, R_2, in which a mutation located at 54 on 2L is responsible for resistance.

Seven dominant αMD-hypersensitive mutations were isolated (SPARROW and WRIGHT 1974). All are alleles at a single locus designated as *l(2)amd* located immediately to the right of *hook* (53.9) on 2L. As heterozygotes all seven *amd* alleles effect lethality on levels of αMD on which homozygous wild type larvae live, and on standard food all are recessive lethal when homozygous. None of these αMD-hypersensitive alleles has any effect on DDC activity (SPARROW and WRIGHT 1974), on *in vitro* DDC thermolability, or on *in vitro* inhibition of DDC by αMD (WRIGHT, BEWLEY and SHERALD 1976). Except for sensitivity to the dietary administration of αMD, no biochemical phene has been established for mutations at the αMD-hypersensitive locus.

HODGETTS (1975) employed another approach in an attempt to locate the structural gene of the enzyme. Using a methodology developed by LINDSLEY and SANDLER *et al.* (1972), it was possible to test segmental duplications covering virtually the entire genome of Drosophila for their effect on enzyme activity. Dopa decarboxylase activity was affected by hyperploidy of only one region in the genome, 36EF to 37D on chromosome 2L. Since a strict proportionality between the number of copies (1, 2 or 3) of this region and the activity of DDC was observed, the region was postulated to include the structural gene. In this paper, we show that this region also includes the *l(2)amd* locus.

In the papers to follow in this series we shall examine our current working hypothesis, which is that the dosage-sensitive locus is the structural gene for DDC. As a criterion for cytological localization of the putative structural gene, we have used the observation that 50% levels of DDC activity are associated with strains heterozygous for deletions of the dosage-sensitive region. The detailed cytogenetic analysis of the series of deficiencies described in this paper permits a more refined localization of both the DDC-dosage-sensitive region and the *l(2)amd* locus. In the companion paper, we describe the isolation and characterization of point mutations that reduce DDC activity and examine the genetic relationship between these mutations and the *l(2)amd* mutations.

MATERIALS AND METHODS

Culture media: Flies were raised in half-pint bottles on a yeasted standard cornmeal-oats-agar-glucose medium or in 85 × 23 mm shell vials on a medium including yeast, agar, glucose, and salts (CARPENTER 1950).

Genetic variations: All standard genes and balancer chromosomes are described in LINDSLEY and GRELL (1968). The following abbreviations of the designations of complex chromosomes are used throughout these papers.

CyO = *In(2LR)O,dp^{lvl} Cy pr cn^2*
SM1 = *In(2LR)SM1,al^2 Cy cn^2 sp^2*
SM5 = *In(2LR)SM5,al^2 Cy lt^v cn^2 sp^2*
bw^{V1} = *In(2LR)bw^{V1},bw^{V1} ds^{33k}*
T(Y;2)D219 = *Dp(2;Y)D219,y+Y^S·Y^{LP}36EF-21A;Df(2L)D219,B^{S}Y^{LD}36EF-60F/SM1*
T(Y;2)H174 = *Dp(2;Y)H174,B^{S}Y^L·Y^{SP}37D-21A;Df(2L)H174,y+Y^{SD}37D-60F/SM1*

In addition to the listings above, females of both translocation stocks carry the attached-*X* chromosome, *C(1)RM,y*, and males the attached-*XY* chromosome, *Y^{S}X·Y^L,In(1)EN,y*. Further, *T(Y;2)D219* has the inversion *In(2L)35F-36A*; *36E-37A* superimposed on it. The designation for these two translocations are derived from data in LINDSLEY and SANDLER *et al.* (1972).

Segmental aneuploids for the 36EF-37D region: Individuals haploid, diploid, and triploid for the 36EF-37D region on 2L were produced by crosses between flies from stocks T(Y;2)D219 and T(Y;2)H174 (LINDSLEY and SANDLER *et al.* 1972; HODGETTS 1975). Newly eclosed progeny were classified, counted and either immediately frozen or aged for five days prior to freezing and subsequent assay for DDC. Percent survival on αMD food was calculated on the basis of the total number of hatched eggs as described below.

Deficiency screens: Three separate screens were run for deficiencies in the *rdo* (2–53), *hk* (2–53.9), and *pr* (2–54.5) region of 2L by X-raying males with 4000 r. Each of the first two screens was initiated in 100 half-pint cultures by mating X-rayed *CyO/Tft l(2)74i* ♂ ♂ to *rdo hk pr* virgins (20 pairs per bottle). (The presence of *l(2)74i* on 2R was discovered subsequent to both screens.) All non-Cy progeny from this cross were scored for putative deletions by noting the reversion of *Tft* and the appearance of one or more of the *rdo*, *hk*, *pr* phenotypes. The third screen was also set in 100 half-pint cultures by mating X-rayed *cn bw* (lethal-free) homozygous

males to *rdo hk pr* virgins (20 pairs per bottle), and all the progeny were examined for the *rdo, hk,* or *pr,* phenotypes. The total number of progeny examined was not counted, but can be estimated to be approximately 150,000 flies from average half-pint culture yields. All flies carrying a putative deficiency were mated to CyO/bw^{V1}. Some of the *CyO/Df?* progeny were mated to *rdo hk pr* flies to verify the isolation of a new genetic variation; others were used to establish a stock. All isolated chromosome aberrations have been maintained over the *CyO* balancer.

Control strains which were not deficient for the *rdo, Tft, hk* nor *pr* loci were established with mutagenized, lethal 2nd chromosomes isolated from the same screens as the deficiencies, and carried through the same series of crosses. *Control 4/CyO = Tft l(2)74i/CyO* served as the control for deficiencies isolated in Screens 1 and 2 (with numbers $\leq$ 108), *Control 5/CyO = cn bw (l)/CyO* served as the control for deficiencies isolated from Screen 3 (with numbers $\geq$ 109), and *Control 9/CyO = rdo hk pr (l)/CyO* was the control for the EMS-induced deficiencies (WRIGHT, BEWLEY and SHERALD 1976).

Deficiency complementation crosses: Routinely, complementation between two deficiencies was tested by setting 5 vial cultures with 1 virgin female $\times$ 2–3 males in each vial at 25°. This was done to avoid crowding and thus permit weak, complementing deficiency heterozygotes to emerge. Most of the complementation crosses were set at least twice and some involving deficiencies in crucial areas of overlap were set three times. For some of these confirmatory crosses 3 vial cultures were set with 2 virgins $\times$ 2–3 males per vial at 25°.

Since all the deficiencies isolated in the *Tft* chromosome in screens 1 and 2 (those with acquisition numbers $\leq$ 108) carried the recessive lethal *l(2)74i* on 2R, complementation crosses between them would be meaningless. To permit some of these crosses to be made, *l(2)74i* was removed from *Df(2L)50* and *Df(2L)9* by crossing over.

Salivary gland chromosome analyses: The cytological extent of each putative deficiency was determined by an analysis of the salivary gland chromosomes of mature larvae heterozygous for the deficiency and the balancer chromosome *CyO*. Since no breakpoint of this multiply inverted chromosome occurred in the 36–40 region of interest, outcrossing, the balanced deficiency stocks to a stock with normal 2nd chromosomes was unnecessary. A disadvantage in this procedure is that additional aberrations that might have been present in the deficiency-bearing chromosomes outside the 36–40 region went undetected. However, we have used the set of deficiencies described in this study to localize unequivocally the 82 lethals described in the companion paper, as well as a number of visible mutations. Thus we feel that none of the deficiencies is actually an insertional translocation or transposition of any sort.

Glands, dissected in insect Ringers, were washed in 45% acetic acid, stained 10–15 min in 2% orcein in 85% lactic acid:45% acetic acid (1:1) and squashed. Breakpoints were located with reference to the drawings of BRIDGES (1935) and photographs of LEFEVRE (personal communication). Band designations were then assigned with the revised map of chromosome 2L, prepared by the younger BRIDGES (1942). In comparing the original and revised maps, care must be exercised. As we shall discuss below, changes that were made occasionally in the designations of the bands present some problems in correlating the two maps.

DDC assay: Adult flies were collected from half-pint culture bottles started with 7 pairs of parents each to minimize crowding. Usually cultures were cleared in the evening and newly eclosed adults collected in the morning. Flies were sexed, counted, and stored at —67° with either 25 or 50 flies per tube. For the assays of the deficiency heterozygotes, 25 males mixed with 25 females were homogenized in 1 ml of homogenization buffer (SHERALD, SPARROW and WRIGHT 1974) or 12 males and 13 females were homogenized together in 0.5 ml of homogenization buffer. Homogenization and centrifugation were performed as described previously (SHERALD, SPARROW and WRIGHT 1974), and the crude supernatant obtained was either assayed immediately or stored at —67°.

DDC activity was determined spectrophotometrically by the method of SHERALD, SPARROW and WRIGHT (1974) with 0.6 mM pyridoxal-5'-phosphate and an incubation time of 30 min at 42° for the enzyme reaction. Normally, for each assay reported three replicate enzyme activity determinations were made on each of three separate homogenates. Protein was determined by the method of LOWRY *et al.* (1951). DDC activity is expressed as nm dopamine/min/mg protein.

Determination of αMD sensitivity: Eggs of strains to be tested were picked by a method described previously (SPARROW and WRIGHT 1974) and placed in small containers on CARPENTER's (1950) medium containing no inhibitor (control) or increasing concentrations of αMD. Five replicates of 100 eggs per container were done for each concentration except for the segmental aneuploid crosses (Table 1) when 6 to 10 replicates of 200 eggs per container were done. Two days after the eggs were picked, unhatched eggs were counted and this number subtracted from the number picked to determine the number of treated individuals. Adults which eclosed from each container were counted and (except for the segmental aneuploid crosses) an estimate of natural viability made for each strain using the control containers plus those in which the inhibitor caused no additional lethality. The percent affected by αMD was then derived for each effective concentration of the inhibitor by the formula:

$$\left(1 - \frac{\#\text{ adults}}{\#\text{ eggs hatched} \times \%\text{ natural viability}}\right) \times 100$$

A separate batch of culture media containing αMD was made for each experiment and stored at −20° until the day it was used.

RESULTS

Localization of l(2)amd *to the 36EF-37D region*: The strict proportionality between the activity of DDC and the number of copies of the region 36EF-37D has recently been reported by one of us (HODGETTS 1975). These observations have been confirmed in the Charlottesville laboratory using a different assay and have been extended to 5-day-old flies, well after the peak of DDC activity which occurs at eclosion.

In order to determine if the α-methyl-dopa-hypersensitive locus, *l(2)amd*, is located in the DDC-dosage-sensitive region, 36EF–37D, *l(2)amd^{H1}cn bw/SM5* virgins were mated to males haploid for the 36EF–37D region. These *Dp(2;Y)-D219;Df(2L)H174/SM1* males were derived from the cross T(Y;2)D219 ♀♀ × T(Y;2)H174 ♂♂. The absence of *Dp(2;Y)D219;Df(2L)H174/l(2)amd^{H1}cn bw* segregants from the progeny indicated that the *amd* locus is in the 50 band, 36EF–37D, DDC dosage-sensitive region. A similar cross of *rdo hk pr* virgins to the same 36EF–37D haplo males showed that the *rdo* and *pr* loci are not included in the region, but that the *hk* locus is.

The effect of different doses of the 36EF–37D region on αMD hypersensitivity: In order to determine if individuals carrying different doses of the 36EF–37D region are differentially sensitive to the dietary administration of αMD, eggs obtained from reciprocal crosses of *T(Y;2)D219* to *T(Y;2)H174* were placed on culture media containing 0, 0.2, 0.4, and 0.6 mM DL-αMD and the viability of haplo, diplo, and triplo 36EF–37D segregants determined (Table 1). The results from both crosses indicate that haplo 36EF–37D individuals are more sensitive than diplo individuals, and that triplo individuals are more resistant to αMD than diplo individuals.

Deficiency screens: In order to locate the putative DDC structural gene and the *l(2)amd* locus more precisely a series of three separate screens for X-ray-induced overlapping deficiencies in the *rdo* (2–53), *hk* (2–53.9), and *pr* (2–54.5) region were undertaken. From these three screens a total of 164 flies were isolated and matched as putative deficiencies. The genetic variations are numbered by their

TABLE 1

αMD sensitivity of individuals carrying different doses of the DDC-sensitive region 36EF–37D

mM DL-αMD	Eggs picked	Eggs hatched	Survival % of eggs hatched		Ratios 1 dose / 2 dose	Survival % of eggs hatched		Ratios 3 dose / 2 dose
			Haplo	Diplo		Triplo	Diplo	
T(Y;2)D219 ♀ ♀ × *T(Y;2)H174* ♂ ♂								
			Males			Females		
0	1200	443	4.7	8.8	.53	4.7	7.0	.67
0.2	1200	393	0.3	12.2	.02	6.4	8.7	.74
0.4	1200	398	0	4.3	—	4.8	2.5	1.92
0.6	1200	431	0	2.8	—	1.2	0.7	1.71
T(Y;2)H174 ♀ ♀ × *T(Y;2)D219* ♂ ♂								
			Females			Males		
0	2000	766	7.7	9.9	.78	6.3	12.8	.49
0.2	1800	772	1.6	8.4	.19	6.9	9.1	.75
0.4	1600	473	0	0.6	—	10.1	0.4	25.25
0.6	2000	725	0	0.6	—	8.4	0.8	10.50
HC8 cn bw/SM5: Control stock*								
			Diplo ♀ ♀	Diplo ♂ ♂	Total			
0	500	344	19.8	17.4	37.2			
0.2	500	333	29.1	16.8	45.9			
0.4	500	321	9.0	1.6	10.6			
0.6	500	363	4.7	1.1	5.8			

* The αMD-sensitive control stock, *HC8 cn bw/SM5* (SPARROW and WRIGHT 1974) serves as a control for the αMD culture medium.

putative deficiency acquisition number where 1–22 were from the first screen, 23–108 from the second screen, and 109–164 from the third screen. In addition to the aberrations listed in Table 3, the final yield included a viable point revertant of *Tft* (Tft^{Rev54}), two *hk* point mutations (hk^{127} and hk^{131}), two *pr* point mutations (pr^{112} and pr^{156}), three Minutes (*M(2)92*, *M(2)105*, and *M(2)126*), a brown-Dominant mutant (bw^{D114}), a putative black-Dominant deficiency with a severe Minute phenotype (*Df(2L)68*, $b^{D}M$) which was lost prior to confirmation, a Curly revertant ($Cy^{Rev76} = In(2LR)O,dp^{lvl}Cy^{Rev76}pr\ cn^{2}$), and a Y;3 translocation with a strong Minute phenotype (*T(Y;3)136,M*).

Two additional ethyl methanesulfonate-induced (EMS) deficiencies, *Df(2L)-E55* and *Df(2L)E71*, were recovered in a screen for point lethal mutations over *Df(2L)50* (WRIGHT, BEWLEY and SHERALD 1976). Since these deficiencies were induced in a lethal-free *rdo hk pr* chromosome, it has not yet been possible to determine genetically if these loci have been deleted. The cytological limits of *DfE71* indicated that the *hk* locus is deleted, whereas the limits for *DfE55* do not include the *rdo*, *hk*, or *pr* loci.

Deficiency complementation: The results of intercrossing deficiencies to determine if various heterozygous combinations survive are presented in the comple-

mentation matrix in Table 2. Most of the results are also presented diagrammatically in Figure 2, in which noncomplementation between any two deficiencies is indicated by an overlap in the two horizontal lines representing the extent of the regions deleted. These genetic data are completely consistent with the cytological analyses presented below.

Clarification of the salivary gland chromosome band designations in the 36C–40A region of 2L: We would like to preface our analysis of the cytogenetics of this region with several comments. The original salivary chromosome maps drawn by C. B. BRIDGES (1935) have been recently indexed on a set of photographs by LEFEVRE (personal communication) and these reproductions were most useful to us in identifying the breakpoints of the aberrations. However, the numbering of each band along 2L was introduced by P. N. BRIDGES (1942), whose map (shown in Figure 1—bottom) includes more bands than the original and several important changes. These changes can be ascertained from Figure 1. Shown in the middle is a photograph of the 36–40 section of 2L, and the original and revised maps are above and below this, respectively. The vertical lines correlate bands on the three representations.

The major change between 36C–40F is the repositioning of region 39. In the revised map, this region starts with the prominent band formerly designated as 39B. To maintain the standard number (6) of lettered subdivisions in each numbered region, one subdivision was removed from region 38, and one inserted in region 39. In our opinion these changes were made by removing the boundary formerly between 38C and 38D while inserting a boundary between the two heavy bands which were formerly in 39E. BRIDGES (1942) purports to have indi-

TABLE 2

*Complementation matrix indicating survival of deficiency heterozygotes**

	Df119	*Df137,M*	*Df50*	*DfE71*	*Df3*	*Df158*	*Df130*	*DfE55*	*Df2*	*Df9*	*Df12*	*Df84*	*Df150*	*Df65*	*Df1*
Df137,M	0														
Df50	+	0													
DfE71		0	0												
Df3	+	0	0	0											
Df158	+	0	0	0	+										
Df130	+	+	0	0	+	0									
DfE55		+	0		+	0	+								
Df2			0			0	+	0							
Df9			0	+		0	+	0							
Df12			0			+	+	.6%‡							
Df84			0			+	+	+							
Df150			0	+		+	+	+	0	0	0	0			
Df65			0			+	+	+					0		
Df1			+			+	+						0		
Df161,M			+†			+	+		0	0	0	0	0	0	0

* + = complement. 0 = non-complementary. Deficiencies listed in order of distal breakpoints.
† 7.5% of expected (strong Minutes) in one cross at 21–22°. 0 in two sets of crosses at 25°.
‡ % of expected.

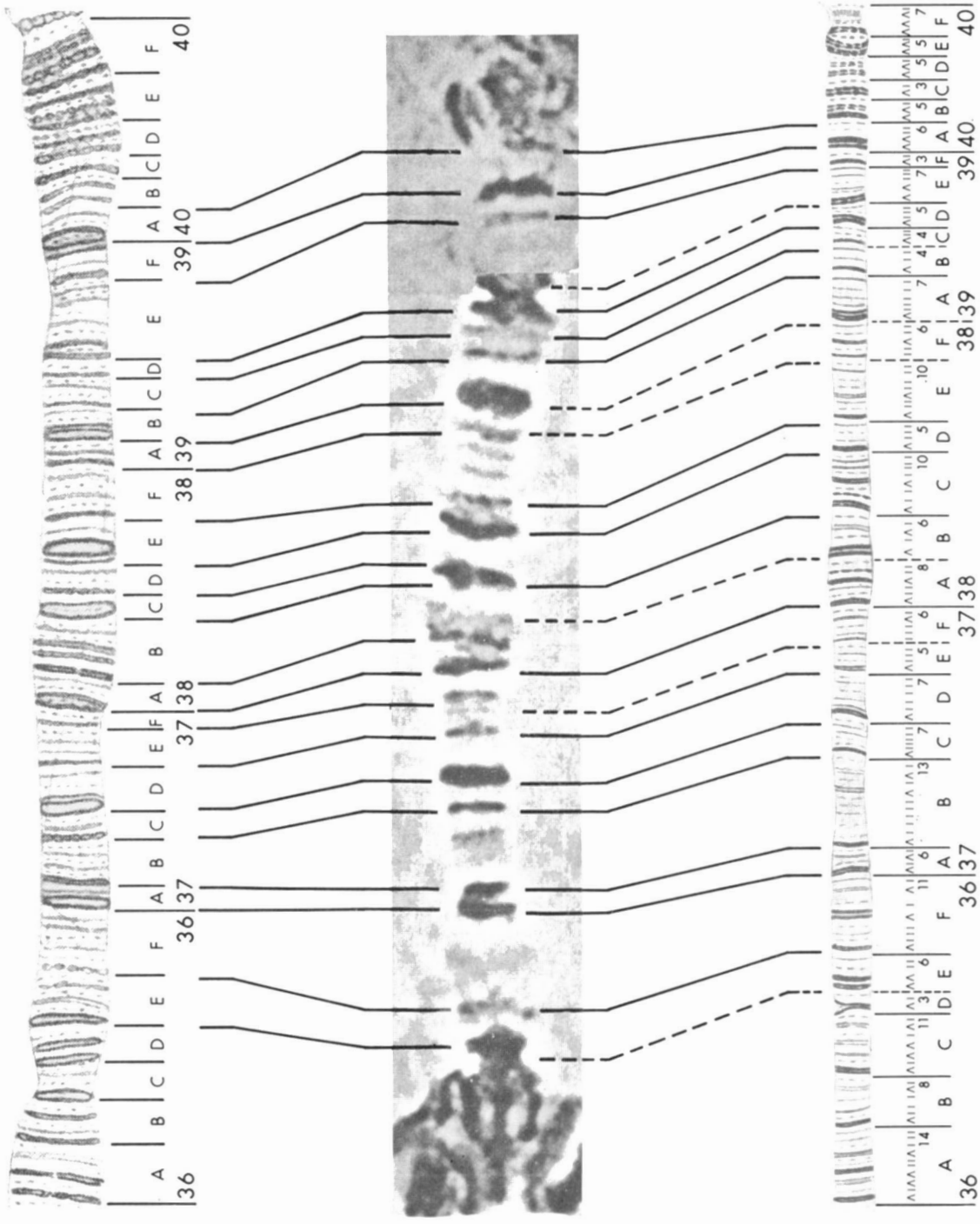

FIGURE 1.—Cytological correlation of the original (BRIDGES 1935, top) and revised (BRIDGES 1942, bottom) salivary chromosome maps with a typical photographic representation of the proximal part of chromosome 2L. The heavy dashed lines indicate our interpretation of the changes made by BRIDGES (1942) in revising the map. Note the lack of correspondence between our interpretation and that of BRIDGES (1942) in 39CE.

cated repositioning of boundaries by dashed lines. Thus, a dashed line marks the boundary between the *numbered regions* 38 and 39 on the revised map, since on the original map this position was the boundary between two *lettered subdivisions* (39A and 39B). Conversely, the 38EF boundary is shown as dashed, since this was the 38–39 boundary on the original map. However, the dashed line which is expected in region 39 as a result of the repositioning is incorrectly shown between 39B and 39C. As Figure 1 demonstrates, a subdivision at this position was also present in the original map, albeit designated differently. The boundary not present in the original is that shown on the revised map between 39D and 39E; it is therefore incorrectly represented by a solid line.

A comment is also necessary on the repositioning of the boundary between 38A and 38B. In the revised map, 38B1,2 is shown as a very pronounced doublet, which in our experience is misleading. As our photograph shows, this region is subject to puffing which normally obscures the banding and makes identification of the 38B1,2 doublet difficult. In some preparations, we have been able to distinguish 38A7, the doublet, 38B1,2 and 38B4,5. However, even under favorable conditions these bands are much less pronounced than the bands 38A5,6 and 38C1,2 which flank this region.

Two final points should be made with reference to the cytology of this region as represented on the revised map. First, the strong doublet shown for 36F6,7 is misleading. The entire 36F region is subject to puffing (see Figure 1) and even when the banding is not obscured by a puff, this particular doublet is always very weak. Second, the repositioning of the subdivision between 37E and F has the effect of making 37F4,5 the most pronounced band in the F subdivision. While this is contrary to the usual criterion for positioning the subdivisions (in that the most pronounced band is generally the first), it was done to effect a more uniform distribution of the bands between 37E and F.

In presenting the cytology of the deficiencies (Figure 2), we have used the revised map without changing the position or designation of any subdivision. However, we did alter the nature (dashed or solid) of two subdivisions in region 39 to conform with the preceding discussion. We feel the map which is displayed in Figure 2 can now be unambiguously correlated with photographs of this region and the original drawing of C. B. BRIDGES (1935). New subdivision boundaries occur in the five locations indicated by dashed lines and the designations of the subdivisions differ from the original between 38D and 39D.

Cytogenetics of the 36C–40A region on 2L: Cytological examination of 15 putative deficiencies induced by X-rays revealed 1 inversion, 13 deficiencies and 1 apparently normal chromosome. These deficiencies, plus 2 more induced by ethyl methanesulfonate as described in the following paper (WRIGHT, BEWLEY and SHERALD 1976), are shown in Figure 2. This set of deletions covers the entire region from 36C to 40B, the 30 end-points being distributed over 16 distinct cytological intervals. These intervals are indicated by the brackets immediately below the chromosome in Figure 2. In the 38A5,6–38C1,2 interval, slightly more refined breakpoint intervals are known for some of the deficiencies, but to preserve clarity these were not shown in the figure. Complete breakpoint data are

TABLE 3

*Cytogenetic data on the 2L aberrations**

Deficiencies	Breakpoints† Distal	Proximal	Genetic loci include‡
Df(2L)119, cn bw	Not visibly deficient		*rdo l(2)Bld*
Df(2L)137,M cn bw	36C2–C4	37B9–C1	*rdo, l(2)Bld, M(2)m^{S6}*
Df(2L)50,cn§	36E4–F1	38A6–A7	*M(2)m^{S6}, Tft, hk, l(2)amd, Ddc*
Df(2L)E71,rdo hk pr	36F2–F6	37C6–D1	*l(2)amd, Ddc, M(2)m^{S6}*
Df(2L)3,l(2)74i	36F7–37A1	37B2–B8	*Tft, M(2)m^{S6}*
Df(2L)158,cn bw	37B2–B8	37E2–F4	*hk, l(2)amd, Ddc, esc*
Df(2L)130,cn bw	37B9–C1	37D1–D2	*hk, l(2)amd, Ddc*
Df(2L)E55,rdo hk pr	37D2–E1	37F5–38A1	
Df(2L)2,Tft l(2)74i	37D2–E1	38E6–E9	*Bl, pr*
Df(2L)9,Tft cn	37E2–F4	38A6–C1	*pr*
Df(2L)12,Tft l(2)74i	37E2–F4	39C2–D1	*pr, l(2)crc*
Df(2L)84,Tft l(2)74i	37F5–38A1	39D3–E1	*pr, l(2)crc*
Df(2L)150,cn bw	37F5–38A1	38B2–C1	*pr*
Df(2L)65,Tft l(2)74i	37F5–38A1	39E2–F1	*pr, Bl, l(2)crc*
Df(2L)1,Tft l(2)74i	38A7–B1	39C2–D1	*pr, Bl*
Df(2L)161,M cn bw	38A6–B1	40A4–B1	*pr, Bl*
Inversions			
In(2L)47,l(2)74i	37A2–B1	38A6–C1	*Tft*
Translocations			
T(Y;2)20,Tft pr⁻ l(2)74i	38A6–C1		
T(Y;2)124,pr^{V} cn bw	45A2–E1		

* Deficiencies listed in order of distal breakpoints.
† Each breakpoint falls between the extremes of the intervals shown.
‡ No attempt to cross every deficiency against a specific marker was made. Where no data are given, inferences about the inclusion of a locus within a particular deficiency can be made from Figure 2.
§ Very weak Minute.

presented in Table 3 which summarizes the cytogenetics of all the arrangements we analyzed in this study.

In Figure 2 the positioning of vertical lines relative to one another can be used to determine the order of breakpoints from distal to proximal of those deficiencies which do overlap and to which identical or very similar breakpoint intervals have been assigned. The relative position of these breakpoints was determined genetically by crossing the deficiencies to a series of EMS-induced lethal mutations located in these regions (see WRIGHT, BEWLEY and SHERALD 1976 for the origin of these lethals). For example, the distal breakpoints for *Df84*, *Df150*, and *Df65* have been assigned to the same cytological interval, 37F5–38A1. However, of four different lethal complementation groups in this region which die in heterozygous combinations with *Df50*, all are lethal over *Df84*, three are lethal over *Df150* and none is lethal over *Df65*. These data indicate that the order of distal breakpoints from distal to proximal is: *Df84, Df150, Df65*.

Brackets along the top of the chromosome in Figure 2 indicate the location of most of the mutants listed in LINDSEY and GRELL (1968), which are proximal to *rdo*. The recessive mutations *rdo*, *hk*, *sple*, *pr*, *rh*, *esc* and *lt* were tested in heterozygous combination with a subset of the deficiencies, which covered the entire

region from 36C to 40B. Mutant phenotypes were observed in at least one combination for all the above mutants except *lt*, *sple* and *rh*. HESSLER (1958) placed *lt* in 40B–40F, which is the region to which we assign the mutant since this is consistent with our data. The map position of *sple* was apparently determined by GOLDSCHMIDT with reference to *b*, and is given as 54 in LINDSLEY and GRELL (1968). Although this would indicate a position for *sple* between *hk* and *pr*, we believe that *sple* is probably distal to *rdo*. The tentative assignment of *rh* to 2L (LINDSLEY and GRELL 1968) was made on the basis of its map position, 54.7. However HILLIKER (1975) describes experiments on the construction of compound second chromosomes which indicate *rh* is on 2R. This is consistent with our observation that *rh* was not included in the region 36C–40A.

The mutant *esc* was uncovered by *Df158*, placing the gene between 37B2 and 37F4. The penetrance of *esc* is often very poor and TOKUNAGA and STERN (1965) report values as low as 10% in homozygous mutant males. We observed only 3 mutant phenotypes among 44 males heterozygous for *Df158* and *esc*. These observations discouraged us from attempting to make a more refined localization until we had obtained a stock with better penetrance.

One of the most useful markers in this region of 2L is *pr*, which has been localized to 38A8–38B6, by virtue of its inclusion in *Df1* (whose distal breakpoint is between 38A7 and 38B1, Table 3), and *Df150* (whose proximal breakpoint is between 38B2 and 38C1, Table 3). ROBERTS (1971) placed *pr* between bands 38B2 and 38C1 but was using the original (1935) map of BRIDGES (personal communication). In terms of the revised map his location (38A6–38C1) is consistent with ours.

The recessive lethals *l(2)B1d* and *l(2)crc* and the dominant mutant *Bl* were located on the basis of the survival of heterozygous combinations of these mutations with the deficiencies. The position of *Tft* has been conservatively placed in 36F8–37B7 from the observation that the induction of *Df3* in a *Tft* chromosome caused a reversion to Tft^{+}. However, the fact that *In(2L)47* with a breakpoint between 37A2 and 37B1 also caused the reversion of *Tft* suggests that *Tft* may be located in the four-band interval 37A3–6 or very close to it.

Two distinct Minutes were uncovered by the set of deficiencies we studied, since two nonoverlapping deficiencies, *Df137* and *Df161*, exhibited moderately strong Minute phenotypes. No other deficiency (with the exception of *Df50*, which was a weak Minute) was Minute. These observations agree with those of LINDSLEY and SANDLER *et al.* (1972), who placed *M(2)m* in the region 36C to 36F and *M(2)H* in 38F to 40B2. On this basis, our observations would suggest a localization of *M(2)m* to that portion of *Df137* which is covered by *Df50* but not *DfE71*; that is, 36E4 to 36F6. Since *M(2)m* has been lost, we examined a putative allele, $M(2)M^{s6}$. This Minute behaves as a genetic deficiency, since it is lethal in heterozygous combination with representatives from four different lethal complementation groups in the vicinity of the distal breakpoint of *Df3*. Cytological analyses confirmed the presence of a small deletion, lying entirely within the interval 36E6 to 36F9. Thus our data permit a localization of *M(2)m* to the region between 36E6 and 36F6.

The other Minute revealed by our deficiencies would appear to be *M(2)H*, and the data suggest a localization to that part of *Df161* not common to *Df65*. However, it was not possible to confirm this directly. Stocks of a purported allele $M(2)H^{S5}$, from either the Pasadena or Bowling Green collections, survive in heterozygous combination with *Df161*. Genetics tests showed that these stocks were lethal in combination with *Df137*, *Df50*, *DfE71*, *Df3* and $M(2)m^{S6}$. Thus, $M(2)H^{S5}$ is not behaving as it did originally for SCHULTZ (see LINDSLEY and GRELL 1968), who found it to survive in combination with $M(2)m^{S6}$. A cytological examination of the stock from Bowling Green revealed a deficiency extending from 36D to 36F, in accord with our genetic data. Since the original data clearly placed *M(2)H* proximal to *M(2)*, we have positioned *M(2)H* in 39E2–40B1, consistent with LINDSLEY and SANDLER *et al.* (1972).

DDC activity of deficiencies: To determine if any of the newly isolated deficiencies had deleted the DDC-dosage-sensitive region, newly eclosed adults heterozygous for the deficiency and the balancer chromosome, *Df/CyO*, were assayed for DDC activity. These data are reported in Table 4. For each assay the activities obtained are compared with the activities measured for the appropriate control strains. It is evident that only four strains, *Df50*, *Df130*, *Df158*, and *DfE71* produce consistently low DDC activities ranging from 49% to 59% of controls. These data are similar to the reduction in DDC activity observed by HODGETTS (1975) in aneuploid flies carrying a single dose of the 36EF–37D region. Therefore, we infer that these deficiencies have deleted the DDC-dosage-sensitive region. The data in Table 2 and Figure 2 show that all four deficiencies overlap. Since only the region 37B10 through 37C7 is deleted by all four deficiencies, the DDC dosage-sensitive region must be located in this region.

Localization of the αMD-hypersensitive locus, l(2)amd, *with overlapping deficiencies*: In order to establish a more precise cytological location for the αMD-hypersensitive locus, *al dp b l(2)amd pr bw/CyO* flies were mated to all the newly isolated deficiencies, *Df/CyO*, and the progeny scored for the presence or absence of *al dp b* $l(2)amd^{H1}$ *pr bw/Df* (non-Cy) progeny. Crosses with all the deficiencies except *Df50*, *Df130*, *Df158*, and *DfE71* produced non-Cy progeny. Crosses with these four produced no heterozygous *l(2)amd/Df* progeny at all. The four deficiencies were then crossed to $l(2)amd^{H45}cn$ *bw/SM5* and $l(2)amd^{H82}cn$ *bw/SM5*, representative mutations in two other complementation groups in the *l(2)amd* locus (SPARROW and WRIGHT 1974), with the same results: the absence of *l(2)amd/Df* segregants among the progeny. Since these four deficiencies, *Df50*, *Df130*, *Df158*, and *DfE71* are identical to the complement of DDC-dosage-sensitive deficiencies, one can conclude that the DDC-sensitive region and the αMD-hypersensitive locus, *l(2)amd*, are both located in the region common to these four deficiencies, 37B10–37C7.

αMD sensitivity of the DDC deficiencies: Since the four deficiencies *Df50*, *Df130*, *Df158*, and *DfE71* both reduced the level of DDC and deleted the αMD-hypersensitive locus, *l(2)amd*, it was of interest to determine the response of these deficiencies to the dietary administration of αMD during larval development (Table 5).

TABLE 4

*Dopa decarboxylase activities of deficiencies and other mutations in the 36C–40A region of chromosome 2L**

Strain	Assay No.	Sp. Act.	%	Assay No.	Sp. Act.	%
Control *4/CyO*	1	11.17	100	2	12.32	100
Df(2L)50/CyO		5.55	50		6.17	50
Control *4/CyO*	3	12.46	100	4	13.62	100
Df(2L)50/CyO		7.41	59		7.40	54
Control *5/CyO*	5	12.64	100	6	11.93	100
Df(2L)130/CyO		6.46	51		6.26	52
Df(2L)158/CyO		6.13	48		5.82	49
Df(2L)150/CyO		14.35	114		12.47	105
Control *5/CyO*	7	13.93	100	8	12.99	100
Df(2L)119/CyO		11.00	79		11.08	85
Df(2L)137/CyO		11.03	79		11.82	91
hk^{127}/CyO		12.34	89		13.65	105
hk^{131}/CyO		12.86	92		12.46	96
Control *4/CyO*	9	13.13	100	10	11.27	100
Df(2L)2/CyO		15.65	109		10.06	89
Df(2L)3/CyO		12.75	97		9.42	84
Df(2L)9/CyO		15.25	116		12.13	108
Df(2L)12/CyO		18.54	141		11.36	101
Control *4/CyO*	11	15.65	100	10	11.27	100
Df(2L)65/CyO		15.24	97		11.18	99
In(2L)47/CyO		16.65	106		10.32	92
Tft^{Rev54}/CyO		12.27	78		8.84	78
Control *4/CyO*	12†	9.06	100	13‡	13.58	100
Df(2L)1/CyO		10.05	111		11.01	81
Df(2L)84/CyO		11.47	127		11.31	83
Control *5/CyO*	14†	10.10	100	15‡	13.50	100
Df(2L)161/CyO		12.60	125		13.73	102
Control *4/CyO*	16	11.46	100	17	11.62	100
$M(2)m^{S6}/SM5$		9.68	84		12.48	107
Control *9/CyO*	18†	13.78	100	19†	12.12	100
Df(2L)E71/CyO		6.89	50		6.06	50
Df(2L)E55/CyO		14.71	107		13.09	108

* Specific activity; n moles dopamine/min/mg protein at 42°. The specific activity reported for each strain for each assay is the overall mean of a minimum of three replicate determinations on each of three homogenates.

Except where noted assays were carried out on mixtures of equal numbers of newly-eclosed males and females.

† Males only.

‡ Females only.

TABLE 5

α-methyl dopa sensitivity of the DDC deficiency strains expressed as percentage of individuals affected by L-αMD

	Percent affected											
	Exp 1 mM αMD			Exp 2 mM αMD			Exp 3 mM αMD			Exp 4 mM αMD		
	0.1	0.2	0.3	0.1	0.2	0.3	0.1	0.2	0.3	0.1	0.2	0.3
*HC8 cn bw/SM5**	0	69	94	0	0	96	0	28	94			
*amd^{H1} cn bw/SM5**	93	100	100	93	100	100	46	100	100	85	100	100
Control *4/CyO*	39	83	100									
Df9/CyO†	0	89	100				5	28	96			
Df50/CyO	88	100	100				11	100	100	56	100	100
Control *5/CyO*				24	24	100	4	13	95	0	77	100
Df130/CyO				83	99	100	13	100	100	74	100	100
Df158/CyO				31	74	100	0	5	82	20	97	100
Control *9/CyO*										17	40	99
DfE71/CyO‡										26	98	100
DfE71/CyO‡										35	100	100

* Original control and αMD hypersensitive strains from SPARROW and WRIGHT (1974).
† Used as a control strain for *Df50/CyO*.
‡ Eggs collected separately for replicate experiments on the same batch of medium.

It is evident that the strains carrying *Df50* and *Df130* are clearly more sensitive to αMD than their respective controls and appear to be almost as sensitive as the original hypersensitive strains, *amd^{H1}cn bw/SM5*. Except in Experiment 3, the *Df158/CyO* and *DfE71/CyO* strains are also more sensitive than their respective controls. However, they appear to be not as sensitive as the *Df50* and *Df130* strains. Unfortunately, to make completely valid comparisons among strains the residual genome (including as much of the X-ray and EMS-treated 2nd chromosome as possible) should be replaced with genetic material from a strain that is not resistant, *e.g.*, the *HC8 cn bw/SM5* strain (Table 5).

Five other deficiency strains, *Df3*, *Df2*, *Df9*, *Df150*, and *Df65*, were tested and found not to be hypersensitive. Two others, *Df137* and *Df161*, both phenotypically Minute, were more sensitive than the control but not as sensitive as the *amd^{H1}*, *Df50*, or *Df130* strains. This sensitivity of the Minute deficiencies might derive from the generally reduced viability of Minutes and the extended exposure to αMD by their prolonged life.

In spite of the reduced sensitivity of the *Df158* and *DfE71* strains, one can conclude that individuals carrying one dose of the DDC dosage-sensitive region are more sensitive to αMD than those with two doses.

DISCUSSION

αMD as a discriminator for mutants affecting DDC: SHERALD and WRIGHT (1974) and SPARROW and WRIGHT (1974) hypothesized that the dietary administration of αMD to growing larvae should serve as a discriminator for individuals with different levels of DDC activity, i.e., individuals with lower levels of DDC activity should die on an αMD concentration on which individuals with higher

levels of DDC activity will live and complete development .Although there is some evidence to support this hypothesis (SHERALD and WRIGHT 1974), it is not completely conclusive. Since HODGETTS (1975) has shown that DDC activity is proportional to gene dosage in the 36EF-37D region of 2L, one might suppose that the response to the dietary administration of αMD of individuals carrying different doses of this region would provide a direct means of testing the hypothesis.

It is evident from the data in Table 1 that individuals carrying different doses of the 36EF-37D region are differentially sensitive to αMD. Those with three doses are more resistant than those with two doses, and those with one dose are more sensitive than those with two doses. Of those deficiencies isolated in the present work, which as heterozygotes effect a 50% reduction in DDC activity, the *Df50* and *Df130* strains are clearly hypersensitive, while the *Df158* and *DfE71* strains appear to be moderately sensitive. These responses of the 36EF-37D aneuploids and DDC deficiencies still do not provide definitive support for the hypothesis, since these genetic variations not only change levels of DDC activity but also change the number of doses of the wild-type allele at the αMD-hypersensitive locus, *amd*. However, strains heterozygous for point-mutations in the putative DDC structural gene are not αMD hypersensitive even though DDC activities are reduced to as little as 30% of controls (WRIGHT, BEWLEY and SHERALD 1976). This indicates that αMD cannot act as a discriminator for strains with reduced DDC activity and suggests that the hypersensitivity of deficiencies for the DDC-dosage-sensitive region is due to dosage effects of the *amd* locus. These data do not eliminate the possibility that αMD can serve as a discriminator for strains with elevated DDC activities as may be the case for the two αMD-resistant strains with increased DDC activities isolated by SHERALD and WRIGHT (1974).

Cytogenetics: Several comments on the cytological data included in Table 3 and Figure 2 are appropriate. First, no cytological abnormality could be identified in the stock Df119, although it behaved as a genetic deletion. However, the region between 35CD and 36D in which *Df119* lies is notoriously difficult to analyze. Commonly a tight loop forms in this region (Figure 1) and the extent of any deficiency within this would be difficult to determine.

Second, haplo flies deficient for virtually the entire region between 36C and the centromere can survive. LINDSLEY and GRELL (1968) report that the deficiency from *T(Y;2)H* (which has now been lost) survived despite the loss of all the material between 37B1,2 and 40B2,3. Likewise, we found that a few *Df50/Df161* heterozygous survived, exhibiting an extreme Minute phenotype. These flies were haploid for some 129–138 bands, or approximately 3% of the entire genome. In this context it is interesting to note that *pr* deficiencies are often very large. ROBERTS (1968) cites this observation in support of BRIDGES' contention that extensive gene duplication has occurred in the proximal part of 2L.

The DDC-dosage-sensitive region and the l(2)amd *locus*: The screen for second-chromosome deficiencies was undertaken to refine further the cytological limits of the DDC-dosage-sensitive region (HODGETTS 1975) and to examine the

relation of this region to a set of mutant alleles which confer dominant sensitivity to αMD, an inhibitor of this enzyme (SPARROW and WRIGHT 1974). A total of 16 deficiencies in or near the dosage-sensitive region (36EF to 37D were isolated, analyzed genetically and cytologically, and assayed for DDC activity. Only four of the deficiencies, *Df50*, *DfE71*, *Df158*, *Df130*, were found to effect a significant decrease in DDC activity, and cytological examination revealed that the region common to all four deletions was 37B10–37C7 on 2L. BRIDGES (1942) identified 11 bands in this region, although our own observations (6–8 bands) are more in line with those of BRIDGES (1935), who identified 6 bands in the region.

The localization of the DDC dosage-sensitive region to 37B10–37C7 by means of those deficiencies is consistent with the observations of HODGETTS (1975), who demonstrated a strict dosage dependency for DDC activity of 1:2:3 in monosomics, disomics, and trisomics for the segment 36EF-37D (which includes the region 37B10–37C7) and found that trisomics for any part of the entire genome other than this segment did not significantly affect activity. Although other possibilities exist (O'BRIEN and GETHMAN 1973; RAWLS and LUCCHESI 1974), these results suggest that the region 37B10–37C7 includes a structural gene for DDC.

It is significant that the same subset of deficiencies, *Df50*, *DfE71*, *Df158*, and *Df130*, are lethal when heterozygous with the αMD-hypersensitive mutants placing the *l(2)amd* locus in the same 11-band segment, 37B10–37C7, as the DDC-dosage-sensitive region. The relation between the *amd* locus and the DDC-dosage-sensitive region remains obscure. αMD is an analog inhibitor of Drosophila DDC *in vitro*, yet none of the alleles hypersensitive to the inhibitor *in vivo* has any demonstrable effect on DDC (SPARROW and WRIGHT 1974; WRIGHT, BEWLEY and SHERALD 1976), and even the most drastic point mutations in the DDC dosage-sensitive region do not increase *in vivo* susceptibility to the inhibitor (WRIGHT, BEWLEY and SHERALD 1976). The functional relationship or possible identity of these loci will be considered further in the following paper (WRIGHT, BEWLEY and SHERALD 1976).

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