HETEROCHROMATIC POSITION EFFECT AT THE ROSY LOCUS OF *DROSOPHILA MELANOGASTER*: CYTOLOGICAL, GENETIC AND BIOCHEMICAL CHARACTERIZATION

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ABSTRACT

This report describes cytological, genetic and biochemical studies designed to characterize two γ-radiation induced, apparent "underproducer" variants of the rosy locus (*ry:3*-52.0), ry^{ts1149} and $ry^{ts11136}$. The following observations provide a compelling basis for their diagnosis as heterochromatic position effect variants. (1) They are associated with rearrangements that place heterochromatin adjacent to the rosy region of chromosome *3* (87D). (2) The effect of these mutations on rosy locus expression is subject to modification by abnormal *Y* chromosome content. (3) The rearrangement alters only the expression of the rosy allele on the same chromosome (*cis*-acting). (4) The *Y* chromosome modification is only on the position-affected allele's expression. (5) The recessive lethality associated with the rearrangements relate to specific rosy region vital loci, and for $ry^{ts11136}$, the lethality is not *Y* chromosome modified. (6) The peptide product of the position-affected allele is qualitatively normal by several criteria. (7) Heterozygous deletion of 87E2-F2 is a suppressor of the rosy position effect. (8) The rosy position effect on XDH production may be assayed in whole larvae and larval fat body tissue as well as in adults.

MOVEMENT of a gene to a different position in the genome may alter the expression of that gene. This phenomenon, termed position effect, has long been recognized as a fundamental problem of genome organization and expression and has been extensively documented in Drosophila (reviewed by LEWIS 1950; BAKER 1968; SPOFFORD 1976). Heterochromatic position effect is the effect upon the expression of a euchromatic gene placed adjacent to or in heterochromatin. In instances of altered gene function involving cell autonomous phenotypes, the position effect is visualized as a variegated phenotype (*e.g.*, white-mottled eyes in the case of heterochromatic position effect alterations of white locus function). In instances of position effect on nonautonomous cell functions (*e.g.*, amylase locus function) position effect is seen as reduced expression per individual.

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The present report describes the results of experiments that identify and characterize heterochromatic position effects at the rosy (*ry:3*-52.0) locus of *Drosophila melanogaster*. The accompanying report (RUSHLOW, BENDER and CHOVNICK 1984) describes experiments that explore the underlying mechanisms. The rosy locus of *D. melanogaster* has been the subject of extensive genetic and biochemical characterization (see reviews in CHOVNICK, GELBART and MCCARRON 1977; CHOVNICK *et al.* 1977). It carries the coding information for xanthine dehydrogenase (XDH) which is a homodimer with a subunit molecular weight of 150,000 daltons. XDH^- flies exhibit a visibly mutant brownish red eye color from which the locus derives its name. Mutants with as little as 5% of normal XDH activity exhibit the wild type, reddish eye color.

The question of tissue specificity of rosy locus function and its relationship to XDH and adult eye pigmentation has been addressed in previous developmental studies. Implants of rosy mutant larval eye discs into wild-type larval hosts demonstrated that rosy locus function is nonautonomous (*i.e.*, that some substance is transmitted from ry^+ tissue that is capable of producing normal red pigment in a mutant eye disc) (HADORN and SCHWINCK 1956). Since hemolymph is high in XDH activity (MUNZ 1964), and since eye discs develop high levels of XDH activity at the time of eye pigment formation, BARRETT and DAVIDSON (1975) suggested that XDH itself is transported to the eye disc via the hemolymph after synthesis in some other organ. The malpighian tubules and the fat body may be the principal sites of synthesis since small pieces of ry^+ tubule or fat body implanted into rosy mutant larvae produce wild-type eyes in the host. Other tissues are not so effective (HADORN 1956; SCHWINCK 1956). Further support for this notion comes from studies of XDH tissue distribution which show XDH activity primarily associated with malpighian tubules and fat body (URSPRUNG and HADORN 1961). XDH activity is found elsewhere in histochemical studies of frozen-sectioned material. However, such activity is probably a secondary manifestation, reflecting the high levels of XDH in the hemolymph (URSPRUNG and HADORN 1961).

MATERIALS AND METHODS

Special chromosomes, rearrangements, balancers and mutants: Throughout the text, we have used abbreviated designations as follows: *MKRS* = *Tp(3)MKRS, M(3)S34 kar* ry^2 *Sb; P18* = *In(3L)P* + *In(3R)P18, Ubx* ry^{41} *kar* e^4; $Y^SXY^L = Y^SXY^L$, *In(EN), y v f B*·y^+. A complete description of all mutants, ry^+ alleles and rearrangements that are used in the present study (and not described herein) may be found in LINDSLEY and GRELL (1968), CHOVNICK *et al.* (1976), MCCARRON *et al.* (1979) and HILLIKER *et al.* (1980).

Cytology: Rearrangement chromosomes were made heterozygous with a normal third chromosome marked with red (*3*-53.6) to facilitate identification of heterozygous larvae. Salivary glands were dissected from late third instar larvae (red^+), fixed in a drop of 45% acetic acid, 1% orcein and squashed. Slides were observed and photographed using a Zeiss Standard Universal photo microscope.

Enzyme preparation and fluorimetric assay: The procedures used were slight modifications of those described elsewhere (MCCARRON *et al.* 1979).

XDH thermolability tests were performed by taking aliquots of enzyme extracts and heating them in an oil bath at 53° for 5 and 10 min. XDH activity was measured as described before.

XDH kinetic measurements were taken by varying the substrate concentration from 0.006 to 0.034

ml of 10^{-3} M 2-amino-4-hydroxypteridine (AHP) for each sample being tested and assaying XDH activity.

Polacrylamide gel electrophoresis procedures are described by McCARRON *et al.* (1979).

Protein concentration in enzyme extracts was determined by the method of WADDELL (1956).

Rocket immunoelectrophoresis procedures are described by McCARRON *et al.* (1979).

RESULTS AND DISCUSSION

Origin of the mutant chromosomes: The mutations used in these experiments, ry^{ps1149} and $ry^{ps11136}$, were recovered from a γ-ray mutagenesis of ry^{+11}-bearing third chromosomes. The specific mutagenesis was part of a pilot study designed to develop a screening protocol for the recovery of "underproducer" control mutants (CLARK, HILLIKER and CHOVNICK 1979). The mutations were considered to be of particular interest in that they exhibited the following features in preliminary studies. (1) Mutant heterozygotes with such noncomplementing rosy mutants as ry^{2} or ry^{41} exhibit the rosy mutant eye color of XDH^{-} alleles when reared on low levels of the XDH inhibitor, 4-hydroxypyrazolo (3,4-*d*) pyrimidine (HPP), but exhibit normal eye color on standard medium. (2) Such flies die on purine-selective medium, hence their designation as purine-sensitive alleles. (3) They are lethal when homozygous or hemizygous with rosy region deletions that extend beyond rosy into adjoining genetic units (HILLIKER *et al.* 1980).

Cytogenetic analysis of ry^{ps1149} *and* $ry^{ps11136}$: Complementation testcrosses were conducted with an array of rosy region deficiencies (Figure 1) that served to localize the vital defect to the *pic* locus for ry^{ps1149} and to the *l(3)S12* locus for $ry^{ps11136}$. This inference was further confirmed by appropriate testcrosses against representative mutant alleles of the vital loci in the immediate region flanking the rosy locus.

Cytological analysis confirmed that these variants are associated with gross chromosomal rearrangements. Examination of salivary gland chromosomes revealed the juxtaposition of the rosy locus and centromeric heterochromatin in both cases (Figure 2). The ry^{ps1149}-bearing chromosome is a translocation with breakpoints in polytene section 87B and fourth chromosome heterochromatin (Figure 2A). From other chromosome preparations, we believe that, in addition to the reciprocal translocation, the 87B-D region is inverted, thus placing 87D into heterochromatin. The $ry^{ps11136}$ chromosome has an inversion with breakpoints in 87D and centrometric heterochromatin (Figure 2B).

These initial cytological observations suggest that the defects in rosy locus function might be due to heterochromatic position effects. A series of experiments were carried out to examine this hypothesis making use of classical criteria or proofs of heterochromatic position effect.

In the following sections that characterize position effect at the rosy locus, the $ry^{ps11136}$ mutation is described in detail. Unfortunately, the semisterility of the ry^{ps1149} chromosome did not encourage elaborate experimental characterization of this mutation. However, small-scale experiments, carried out with this mutant, permit extension of the generalizations drawn from the $ry^{ps11136}$ studies to ry^{ps1149}.

XDH phenotype variation as a function of varying dosage of Y *chromosome heterochromatin*: In our prior experience with phenotypic characterization of wild-type

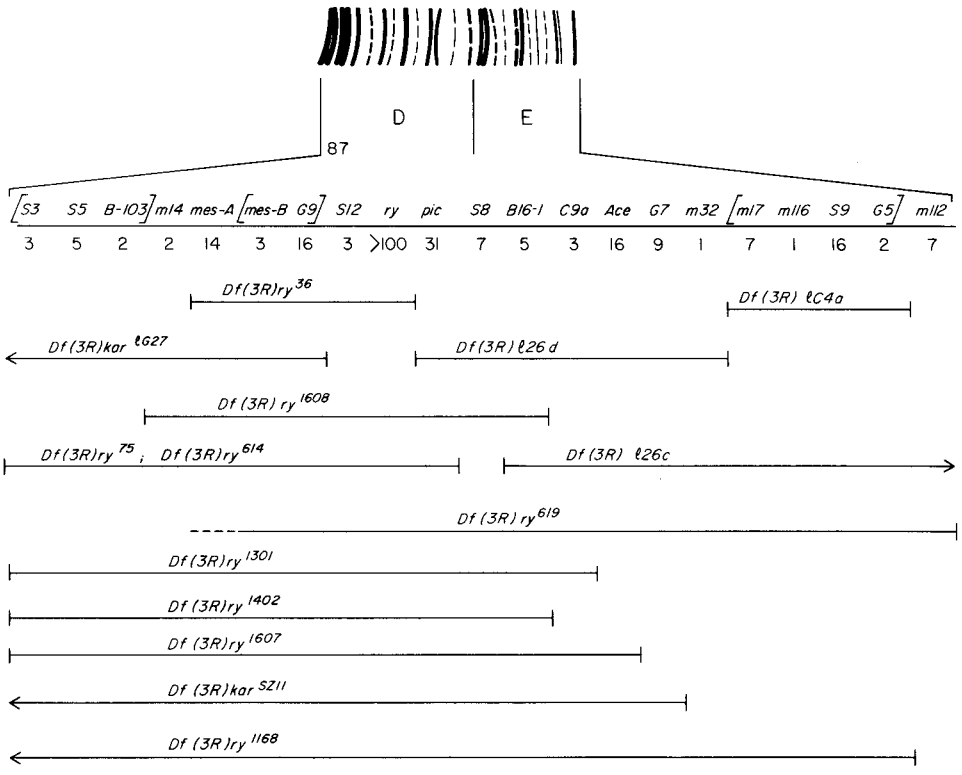

FIGURE 1.—Summary of the cytogenetic analysis of polytene chromosome region 87DE (HILLIKER *et al.* 1980). The order of established complementation groups and the extent of deficiencies are indicated.

and rosy locus variant strains, we have noted a common pattern or developmental profile of enzyme activity and cross-reacting material (CRM) production (CHOVNICK *et al.* 1977). Additionally, there is a quantitative concordance of phenotypic effects whenever we compare enzyme and CRM levels with purine sensitivity (a function of XDH activity in very early larvae) and sensitivity to phenocopy by HPP (a function of XDH activity just prior to eye pigment formation in pupae). In this section, we describe the results of experiments that measure XDH enzyme activity and CRM levels in adults and sensitivity to phenocopy with HPP as a function of variation in amounts of *Y* chromosome heterochromatin. Purine sensitivity is not included in this study because it is the least sensitive of the various phenotypes.

Table 1 summarizes the results of an HPP titration test in which the progeny of the indicated crosses developed on standard Drosophila medium supplemented with HPP, and the emerging adults were scored for rosy mutant or normal eye color. The progeny of all crosses will be $ry^{ps11136}$ or ry^{+11} and heterozygous for the *MKRS* balancer (ry^{2}) or the *P18* balancer (ry^{41}). The putative positive effect variants were recovered originally as rosy eye color mutants on standard medium supplemented with 60 μg/ml of HPP but were normal in eye color on standard Drosophila medium. The results of Table 1 confirm and elaborate on the initial

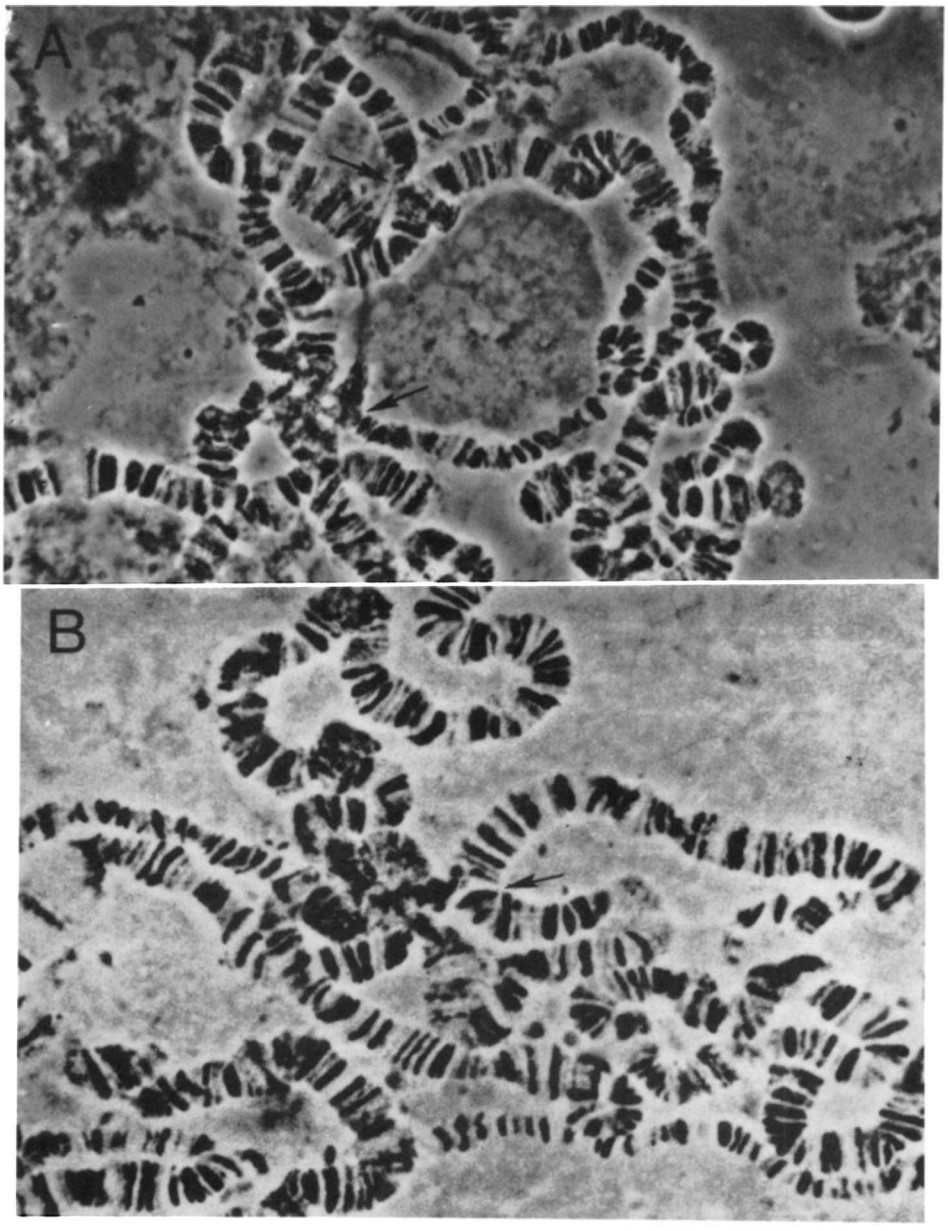

FIGURE 2.—Salivary chromosome squash preparation. A, $ry^{ps11149}$/red. B, $ry^{ps11136}$/red. Breakpoints are indicated by arrows.

observation of HPP sensitivity for $ry^{ps11136}$ compared with ry^{+11} in standard *XX* and *XY* genotypes. In genotypes with abnormal *Y* chromosome constitutions, the $ry^{ps11136}$ flies exhibited a dramatic shift in HPP sensitivity that was not seen with ry^{+11}. Thus, *XO*, $ry^{ps11136}$ males exhibit an increase in HPP sensitivity, whereas *XXY*, $ry^{ps11136}$ females exhibit a decreased sensitivity approaching that of the ry^{+11} flies.

TABLE 1

Percent phenocopy[a] as a function of the HPP concentrations needed to produce rosy mutant eye color in ry^{+11} *and* $ry^{ps11136}$ *genotypes*

HPP (μg/ml)	$ry^{ps11136}$				ry^{+11}			
	XXY[c]	*XX*[b]	*XY*[b]	*XO*[c]	*XXY*[c]	*XX*[b]	*XY*[b]	*XO*[c]
0	0	0	0	0	0	0	0	0
30	0	40	30	94	0	0	0	0
60	10	100	100	100	0	0	0	0
90	80	100	100	100	22	40	50	40
120	100	100	100	100	90	95	95	92

Number of offspring varied among the crosses from a high of 322 to a low of 71. There is a systematic decrease in total progeny among the highest HPP treatments (90 and 120 μg/ml).

[a] Percent phenocopy $= \frac{\text{Number of flies with rosy eye color}}{\text{Total number of flies}} \times 100.$

[b] *XX* and *XY* flies were derived from the cross *XX; ry*$^{+11}$ or $ry^{ps11136}$*/Tp(3)MKRS* × *XY; In(3R)P18/Tp(3)MKRS.*

[c] *XXY* and *XO* flies were derived from the cross *XX; ry*$^{+11}$ or $ry^{ps11136}$*/Tp(3)MKRS* × $Y^{S}X{\cdot}Y^{L}$*/0; In(3R)P18/Tp(3)MKRS.*

Fluorometric assay of XDH activity of matched (wet weight) extracts of whole flies was carried out to confirm that the purine and HPP sensitivity characteristics of the putative position effect variants did, indeed, reflect XDH activity levels. The results of these tests, summarized in Figure 3, confirm that the several phenotypic characteristics associated with $ry^{ps11136}$ are reflections of alteration in XDH activity, and that a concordance of phenotypic effects is seen in response to abnormal *Y* chromosome constitution. In contrast, the ry^{+11}-bearing genotype is unresponsive to abnormal *Y* chromosome constitution.

An immunological study, using the method of "rocket" immunoelectrophoresis, was next carried out on the same matched extracts tested for enzyme activity in the previous section. The results (Figure 4) demonstrate that the reduced enzyme activity associated with $ry^{ps11136}$, and the variation in that activity as a function of abnormal *Y* chromosome constitution, in fact, reflects variation in numbers of molecules of XDH present in the extracts.

Taken together, the cytological observations and the phenotypic effects upon rosy locus expression provide strong support for the notion that the mutational lesion associated with $ry^{ps11136}$ is a heterochromatic position effect.

Effect of extra Y *chromosomes on the recessive lethal phenotype*: Experiments were also carried out to examine the effect of extra *Y* chromosomes on the specific recessive lethality associated with the $ry^{ps11136}$ lesion. All three extant lethal alleles of *l(3)S12* (HILLIKER *et al.* 1980) were tested for lethality with $ry^{ps11136}$ in genotypes carrying extra *Y* chromosomes. Females of the genotype $+/Y^{S}X{\cdot}Y^{L}$; $ry^{ps11136}$*/MKRS* were crossed to *+/Y; l(3)S12/MKRS* males. No Sb^{+} progeny were recovered either among the progeny with normal *Y* chromosome constitutions or among the progeny carrying the marked $Y^{S}X{\cdot}Y^{L}$ chromosome (thereby having an extra *Y* chromosome).

The very specific recessive lethality associated with the $ry^{ps11136}$ and ry^{ps1149} lesions is not consistent with the notion of radiating heterochromatic position

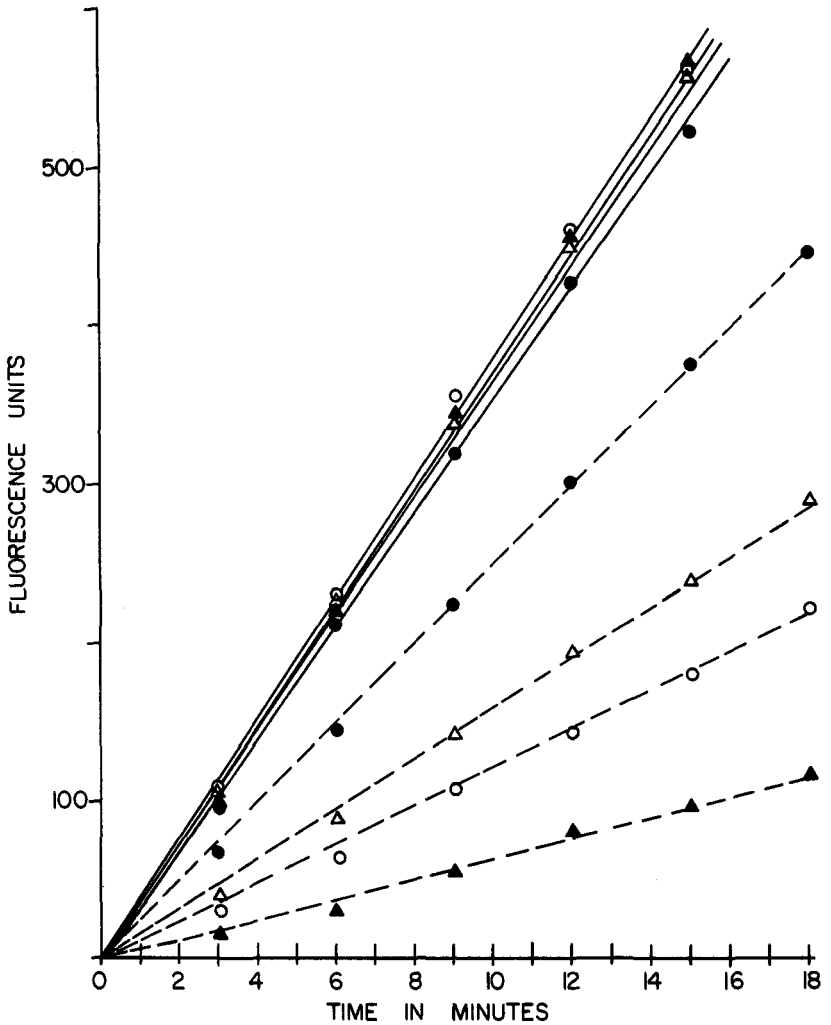

FIGURE 3.—Fluorimetric assay of XDH activities of matched extracts of $ry^{+11}/MKRS$ (——) and $ry^{ps11136}/MKRS$ (– – –) adults with *XXY* (●), *XO* (▲), *XX* (○) *and XY* (△) chromosome constitution.

effects. The failure of extra *Y* chromosomes to modify the $ry^{ps11136}$ lethality provides still additional evidence that the lethality is not a position effect. Rather, these observations favor the viewpoint that the rearrangement break has occurred in the *l(3)S12* gene itself thereby destroying that gene's function. Further evidence bearing on this question is presented in the accompanying report (RUSHLOW, BENDER and CHOVNICK 1984).

Demonstration of the cis*-acting nature of the position effect lesion, and the* Y *chromosome modification*: A classic feature of position effect rearrangement is that only genes located in the rearranged chromosome are affected. Examination of this question was facilitated by the use of XDH electrophoretic variants as illustrated in Figure 5. The ry^{+11} homodimer enzyme, as seen in extracts of ry^{+11} homozygotes (lane A), exhibits a faster mobility (1.02) than the ry^{+14} homodimer

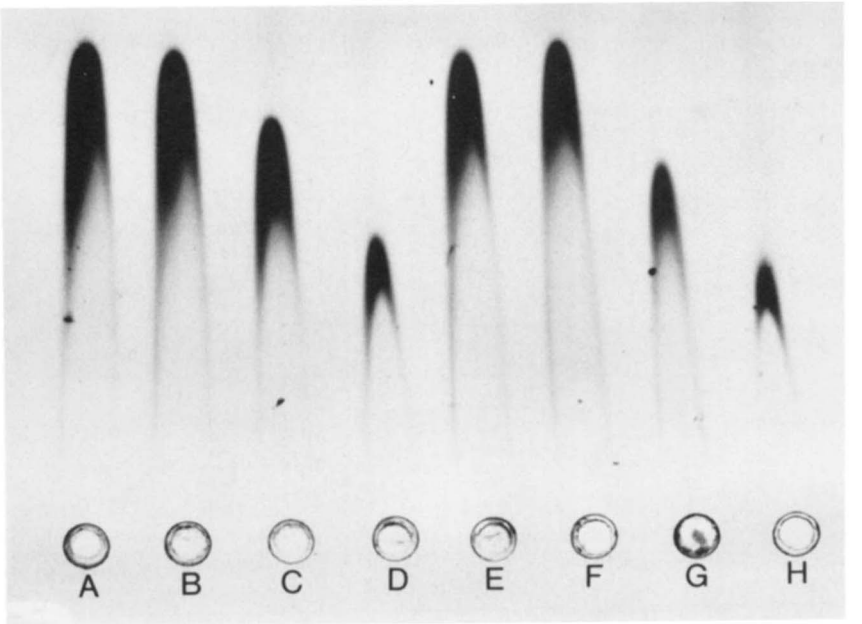

FIGURE 4.—Rocket electropherogram. Matched extracts of adults of the following genotypes run against anti-XDH serum: A, ry^{+11}/*MKRS;XXY*; B, ry^{+11}/*MKRS;XX*; C, $ry^{ps11136}$/*MKRS;XXY*; D, $ry^{ps11136}$/*MKRS;XX*; E, ry^{+11}/*MKRS;XY*; F, ry^{+11}/*MKRS;XO*; G, $ry^{ps11136}$/*MKRS;XY*; H, $ry^{ps11136}$/*MKRS;XO*.

(lane F). The heterozygote, ry^{+11}/ry^{+14}, exhibit a three-banded pattern of dimers which includes the two parental dimers and a hybrid dimer of intermediate mobility (lane C). In contrast, extracts of the heterozygote, $ry^{ps11136}/ry^{+14}$ display a three-banded pattern of dimers (lane E) which differs from that of lane C in that it indicates a very low production of the peptide product of $ry^{ps11136}$. Only traces of the fast (1.02) dimer are visible in lane E of Figure 5, and most of the $ry^{ps11136}$ product forms hybrid dimers of intermediate mobility with the more abundant ry^{+14} monomers. Lane D illustrates the three-banded pattern of XDH dimers produced in *XXY* females of the heterozygote, $ry^{ps11136}/ry^{+14}$. Here, one sees an increase in the amount of fast monomer product of the $ry^{ps11136}$ allele leading to an increase in 1.02 and hybrid dimers at the expense of 0.94 dimer production. Lane B illustrates the lack of a *Y* chromosome effect in *XXY* females of the genotype ry^{+11}/ry^{+14}.

These observations provide further evidence in support of the heterochromatic position effect nature of the rosy locus lesion.

The peptide product of a position-affected gene: A key feature of the classic notion of heterochromatic position effect derives from observations on the variegated expression associated with those cases involving cell autonomous phenotypes. From such cases, the inference is drawn that there is gene inactivation in some cells and not in others. Moreover, whenever the gene is active, the quality of its expression is believed to be normal. A corollary of this observation is that the structure of the position-affected gene is, itself, unaltered. This latter inference is additionally supported by experiments that separate the heterochromatic-

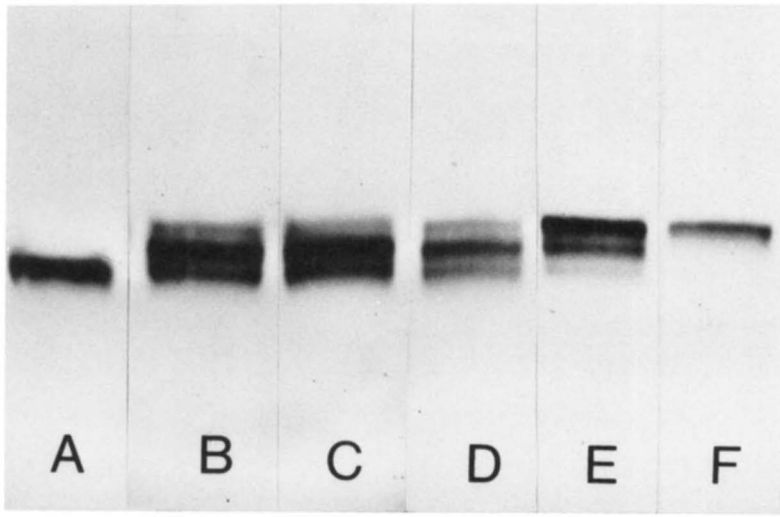

FIGURE 5.—Polyacrylamide gel electropherogram indicating the relative amounts of $XDH^{1.02}$, $XDH^{0.98}$ and $XDH^{0.94}$ present in matched extracts of the following genotypes: B, $ry^{+11}/ry^{+14};XXY$; C, $ry^{+11}/ry^{+14};XX$; D, $ry^{ps11136}/ry^{+14};XXY$; E, $ry^{ps11136}/ry^{+14};XX$. A and F are reference standards of ry^{+11} (1.02) and ry^{+14} (0.94), respectively.

euchromatic rearrangement from the gene under investigation by recombination or further rearrangement and restore the normal phenotype (see review by BAKER 1968).

In the present case of rosy locus position effect, support for these notions may be inferred from the following superficial observations. (1) The peptide produced by the position-affected allele forms homozygous and hybrid dimers identical in mobility to those produced by the parental, ry^{+11}, allele. (2) Response of these molecules to our XDH-specific antibody appeared to be normal. (3) Response of these molecules in their *in vivo* reaction to HPP inhibition appears to be normal.

Two additional tests were carried out to further examine the possibility of structural differences between the peptides produced by the position-affected allele, $ry^{ps11136}$, and its parental wild-type allele, ry^{+11}. One approach considers the possibility that the enzyme product of the position-affected allele is structurally altered, and that this may be reflected in a changed substrate affinity. Figure 6 summarizes the results of experiments designed to examine this question in matched XDH extracts of ry^{+11} and $ry^{ps11136}$. The difference in V_{max} between the preparations is expected on the basis of previous assay results. However, the absence of a K_m difference between the two preparations provides further support for the notion of a quantitative rather than a qualitative difference between the two preparations.

The second test questions the possibility that the decreased level of XDH activity associated with the position effect reflects an alteration in XDH molecular stability. If true, then enzyme thermolability tests might elaborate such differences in XDH preparations of normal and position effect strains.

Table 2 summarizes the results of thermolability studies in extracts of ry^{+11} and $ry^{ps11136}$ preparations. No significant difference in thermolability is seen

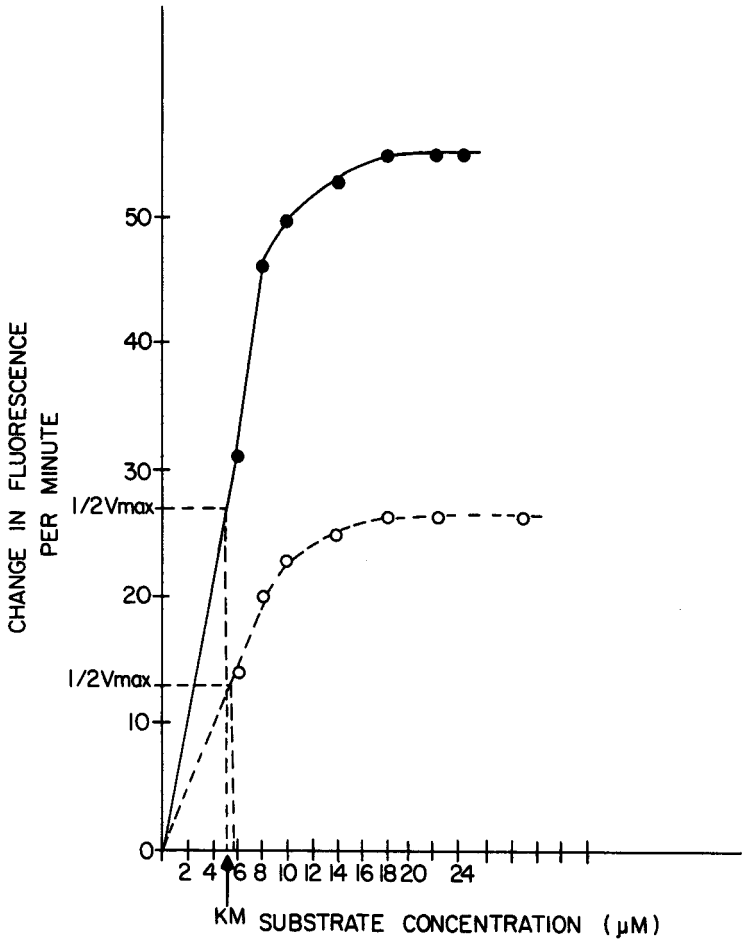

FIGURE 6.—XDH activity as a function of substrate concentration in matched extracts of ry^{+11}/*MKRS* (●——●) and $ry^{ts111136}$/*MKRS* (O– –O) adult females.

TABLE 2

Percent XDH activity[a] remaining following heat treatment (53°) of extracts of the indicated genotypes

Genotype	Time of heat treatment	
	5 min	10 min
ry^{+10}/ry^{+10}	15	6
ry^{+11}/*MKRS*	52	27
$ry^{ts111136}$/*MKRS*	47	33

[a] Percent XDH activity =

$$\frac{\Delta \text{ fluorescence units/time (heated)}}{\Delta \text{ fluorescence units/time (unheated)}} \times 100.$$

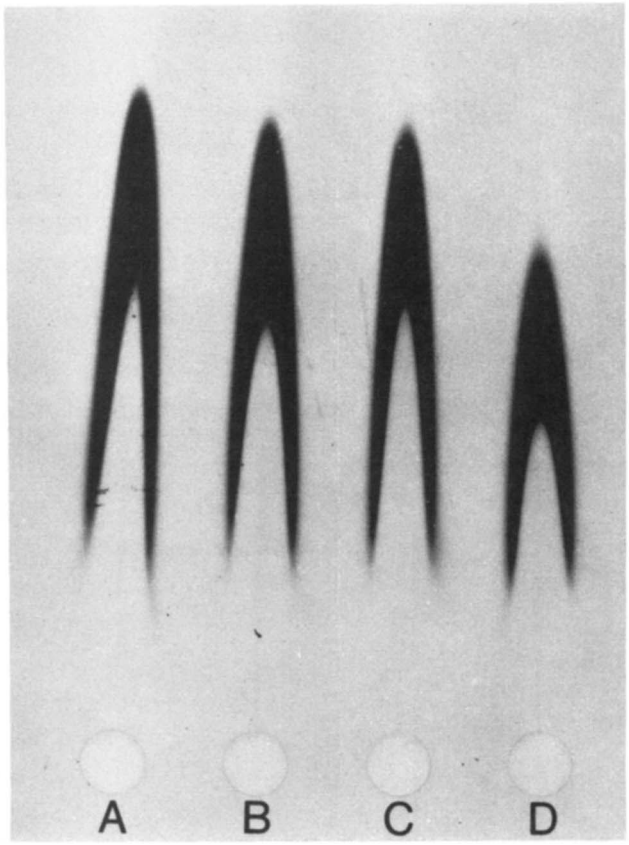

FIGURE 7.—Rocket electropherogram. Matched extracts of adult females of the following genotypes run against anti-XDH serum: A, ry^{+11}/ry^{+11},*Df(3R)l26d*; B, ry^{+11}/ry^{+11}; C, $ry^{ts11136}/ry^{+11}$,*Df(3R)l26d*; D, $ry^{ts11136}/ry^{+11}$.

between these preparations. In contrast, this experiment is capable of demonstrating the XDH thermolability associated with the ry^{+10} structural element as described in a prior report (MCCARRON *et al.* 1979).

Thus, the observations described in this section are entirely consistent with the notion that position-affected gene expression is normal in terms of the quality of the gene product. In the case of a nonautonomous phenotype such as that of XDH production, the position-affected gene expression is seen to be entirely quantitative.

Modification of the rosy position effect by deletion of 87E2-F2: As noted before, the rosy position effect responds in a typical way to abnormal *Y* chromosome constitution. In this section, we question its response to another known modifier of position effect. Heterozygous deletion of polytene region 87E2-F2 has been found to suppress a position effect lethal allele of a vital gene located in 87C and to strongly suppress the white-mottled variegation associated with the w^{m4} position effect (HENIKOFF 1979). Several of the rosy region deletions (Figure 1) are deficient for 87E2-F2. Of these, kar^2ry^{+11} *Df(3R)l26c* and kar^2ry^{+11}*Df(3R)l26d* are

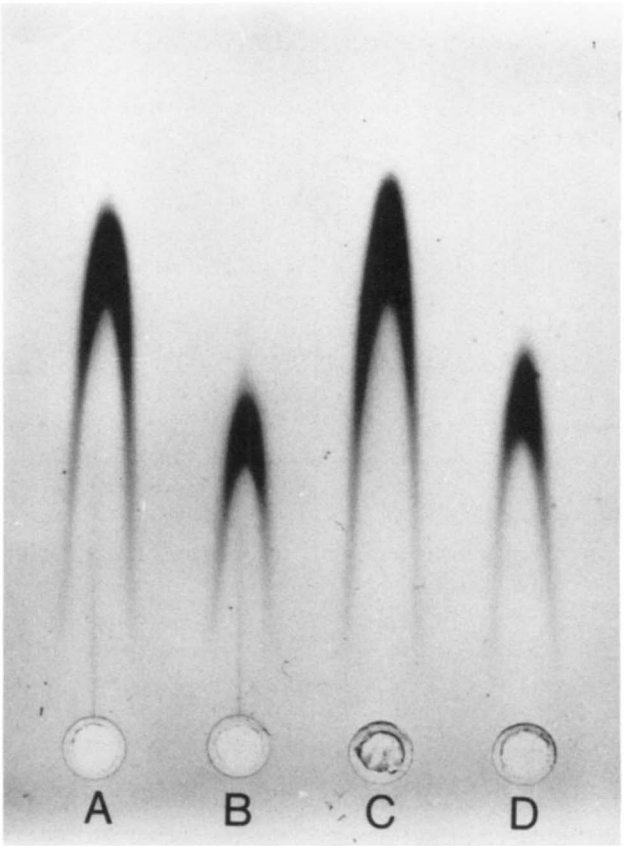

FIGURE 8.—Rocket electropherogram. Extracts of late third instar female larvae and late third instar fat body adjusted to identical protein concentrations and run against anti-XDH serum. A, ry^{+11}/*MKRS* fat body; B, $ry^{ps11136}$/*MKRS* fat body; C, ry^{+11}/*MKRS*, larvae; D, $ry^{ps11136}$/*MKRS* larvae.

l(3)S12^{+}. Consequently, $ry^{ps11136}$/*Df(3R)l26c* and $ry^{ps11136}$/*Df(3R)l26d* survive and may be examined for position effect modification. Figure 7 presents a rocket electropherogram that compares XDH CRM levels in matched extracts of $ry^{ps11136}$/*Df(3R)l26d* and appropriate controls and demonstrates the effect of 87E2-F2 deletion heterozygosity in modifying the rosy locus position effect. Although not shown, *Df(3R)l26c* exhibits the same degree of position effect suppression in parallel experiments.

There are six known lethal complementation groups that are uncovered by both *Df(3R)l26c* and *Df(3R)l26d* (Figure 1). In an effort to localize the 87E modifier to one of these vital genes, one mutant allele of each complementation group was tested as a possible modifier of the $ry^{ps11136}$ position effect phenotype following the experimental protocol described in the previous paragraph. In all instances, these experiments failed. Such a negative result is of little use either in the analysis of the position effect or in the mechanism of position effect modification. As discussed by HILLIKER *et al.* (1980), not all existing genes in the rosy microregion may have been identified by the EMS mutagenesis screens for

vital loci. Since vital loci are not of equal mutability, there may remain one or more unmutated vital loci in the region. Moreover, those vital loci consisting of repeated sequences (either tandem or dispersed) would not have been found by the mutagenesis protocol. Additionally, there remains the possibility that the modifier is encoded by a nonessential gene that does not have an obvious mutant phenotype and would have been missed by the screen for lethals. Finally, there remains the possibility that the position effect modification associated with the 87E deletion heterozygosity does not relate to a specific gene action but rather to a cumulative physiological effect associated with the deficiency of two or more gene functions (*e.g.*, a developmental delay that might effect an apparent increased expression of the position affected gene).

Comparison of XDH CRM levels in ry^{+11}/MKRS *and* $ry^{ps11136}$/MKRS *whole larvae and larval fat bodies*: Characterization of the rosy locus position effect in larvae has utilized indirect phenotypes such as HPP sensitivity and purine sensitivity. In the accompanying paper (RUSHLOW, BENDER and CHOVNICK 1984), experimental tests of hypotheses concerning the mechanism of position effect involve late third instar larvae and larval fat body. Consequently, it is important to establish that the position effect is seen in larvae and larval fat body at the level of the enzyme product of the rosy locus.

XDH CRM levels were measured in samples of mutant and control larvae (third instar) and dissected fat bodies. All samples applied to the gel shown in Figure 8 were equilibrated for total protein. Clearly, the reduction in CRM previously shown for $ry^{ps11136}$ adult fly extracts compared with ry^{+11} extracts is also seen in whole larvae and isolated fat body preparations.

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