REGULATION OF GENE ACTIVITY BY DOSAGE COMPENSATION AT THE CHROMOSOMAL LEVEL IN DROSOPHILA[1]

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ABSTRACT

Two models of dosage compensation have been tested by the measurement of G6PD and 6PGD enzymatic specific activities in flies hyperploid for regions of the *X* chromosome. Females duplicated for the proximal half of the *X* chromosome (2½ *X*'s) have an increased level of G6PD and a normal level of 6PGD. Females duplicated for the distal half of the *X* chromosome (2½ *X*'s) have a normal level of G6PD and an increased level of 6PGD. Males bearing duplications of various segments of the *X* chromosome show control levels of G6PD and 6PGD, except where the duplicated region includes the structural gene for G6PD or 6PGD. These results fail to provide evidence for either the presence of discrete *X*-linked compensator (regulator) genes reducing the activity of other *X*-linked genes, or for a factor in limiting supply necessary for the transcription of all the genes on the *X* chromosome. Superfemales (3 *X* chromosomes) have the same G6PD and 6PGD activity levels as their diploid sisters. It would appear that the regulation of gene activity by dosage compensation is a chromosomal phenomenon in that the level of activity per gene copy for loci on the *X* chromosome is modulated in a stepwise fashion according to the total number of *X* chromosomes present.

DOSAGE compensation, which refers to the phenomenon that both sexes, in species where males have one *X* chromosome and females have two, produce the same amount of *X*-coded gene product despite the differing number of *X* chromosomes, provides a system for the study of gene regulation in a eukaryotic organism. Unlike mammals, where one *X* chromosome appears to be inactivated in the somatic cells of females, both *X* chromosomes are thought to be transcribed in each cell of Drosophila females (KAZAZIAN, YOUNG and CHILDS 1965; SEECOF, KAPLAN and FUTCH 1969). Autoradiographic measurements (MUKHERJEE 1966; KORGE 1970; HOLMQUIST 1972) suggest that the dosage compensation process is active at the level of gene transcription. Insight into how this apparent inequality of expression per gene copy is brought about would add to our understanding of the control of eukaryotic gene activity.

Two models of dosage compensation were outlined in a recent review by LUCCHESI (1973). One is a system of "negative" control, restricting the activity of *X*-linked genes in females; the other is a "positive" control system, in which a special factor in limiting supply is required for the activity of genes on the *X*

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chromosome in both males and females. Here tests are proposed and carried out to distinguish each model by means of *X* chromosome hyperploidy.

In the first case, MULLER (1950) created a model of dosage compensation in which the activity of *X*-linked structural genes depends on the number of copies of some "compensator" gene loci present. The latter were proposed to be genes which are also *X*-linked and act to reduce the output of each copy of the structural genes in females. Thus, the phenotype resulting from an extra dose of the compensator gene would be to depress the activity of each copy of an *X*-linked structural gene. LIEB (1946), then a student of MULLER's, did observe such a "compensating" effect in females homozygous for w^a (white apricot eye color) carrying a duplication (i.e., 3 copies) of the middle segment of the *X* which did not include the w^a locus. If such a result is typical, the change in gene activity expected with an extra copy of the compensator gene should localize to a single site on the *X* chromosome. Differences in activity could be more easily monitored by the measurement of known gene products whose structural genes are on the *X* chromosome. Examples are the enzymes glucose 6-phosphate dehydrogenase (G6PD, coded by the *Zw* gene) and 6-phosphogluconate dehydrogenase (6PGD, coded by the *Pgd* gene). The G6PD and 6PGD phenotypes both exhibit dosage compensation, and the measured activity responds to changes in gene dosage (SEECOF, KAPLAN and FUTCH 1969; STEELE, YOUNG and CHILDS 1969; BOWMAN and SIMMONS 1973; STEWART and MERRIAM 1974).

In the second case, both MARONI and PLAUT (1973) and SCHWARTZ (1973) proposed the presence of a special gene product of autosomal origin which has a positive effect on the transcription of *X*-linked genes. The amount of the substance is limited and all active *X*-linked genes are in competition for it. Hence, one copy of an *X*-linked structural gene has the same competitive ratio in males as do two copies of the structural gene in females. In this case the phenotypic effect of *any* large *X* chromosome duplication would be to diminish the activity from those *X*-linked genes not included in the duplication owing to the further dilution of the special substance among all *X*-linked gene copies. There would be only slight enhancement of the activity from those genes within the duplication.

Flies hyperploid for specific regions of the *X* chromosome may be constructed by means of a series of translocations between the *X* and *Y* chromosomes described by STEWART (1973). The effects of hyperploidy of either the right or left half of one translocated *X* chromosome were examined in females which also carried an attached *X* chromosome. Smaller duplications that resulted from crosses between different translocation stocks, in the manner utilized for the autosomes by LINDSLEY *et al.* (1972), were examined in males. Assays of G6PD and 6PGD activities were made on the various hyperploid flies and their euploid sibs. The activity of the autosomally-specified enzyme, isocitrate dehydrogenase (IDH-NADP) was also measured on the same flies as a control.

A study on *X* chromosome hyperploidy and its relation to dosage compensation was published by FAIZULLIN and GVOZDEV (1973) during the preparation of this manuscript. They found that duplications of the *X* chromosome in females did not give the results expected of a compensator gene as proposed by MULLER for

either G6PD or 6PGD. However, their measurements of G6PD and 6PGD in superfemales, with three *X* chromosomes, showed greater activity for those two enzymes than in diploid females. Neither our results nor those of LUCCHESI, RAWLS and MARONI (1974) show differences in enzyme levels between superfemales and their diploid sisters.

MATERIALS AND METHODS

Enzyme assays

G6PD-, 6PGD-, and IDH-NADP specific activities were measured on separate flies using the fluorometric assay system described by STEWART and MERRIAM (1974). In order to reduce variation between flies of like genotype, newly eclosed flies of the desired genotype(s) and their euploid sibs serving as controls were harvested together from the same bottle and aged one day. Groups of 16 to 24 flies, counting both experimental and control flies, were handled in parallel. Each fly was homogenized separately and its extract assayed for the three enzymes. Standard errors of the means of each group of experimental or control flies ranged from 3% to 8%. We feel this is sufficiently low to reliably distinguish changes in activity of at least 15–20% between groups of that size. Measured enzyme activity shows a linear dependence on amounts of fly extract in the range used here (data in STEWART and MERRIAM 1974, Figure 1). Flies carrying a duplication of the structural gene of one of the enzymes have from 1.4 (females) to 1.8 (males) times the activity of euploid sibs, while females with a heterozygous deficiency of a structural gene have 0.5 times the activity of normal sisters.

Drosophila strains

T(X;Y) B26 has an *X* chromosome breakpoint at 9C. The distal piece of the *X* is marked with *y* and B^S, while the proximal piece is marked with *y+*. A mating of *B26* translocation-bearing males to *y w f* attached-*X* females produces some daughters with the normal two arms of the attached-*X* (*y w f*), other daughters with the two attached arms plus the proximal piece of the *X* (phenotypically *y+ w f+*), and some daughters with the two attached arms plus the distal half of the *X* (phenotypically *y w+ f* B^S), as shown in Figure 1.

The following crosses were made to generate males bearing duplications of varying portions of the *X* chromosome and control brothers.

Salivary chromosome region 1–3A: *y+* males (duplication-bearing) from the F_1 of the the cross *y* females to *y* $dor^1/Dp(1;f)R$ males were compared with euploid *y* brothers.

Region 2C1–3C4: *y sc* w^i; $w^{v\ co}/Sb\ Ser$ females, kindly supplied by DR. W. KAPLAN, were crossed with *w* sn^3 *m* males. Among the progeny were *y sc* w^{var} males with red variegating eyes (duplication-bearing) and *y-sc* w^i; *Sb Ser* males with white eyes (euploid).

Region 3C2–3F1: The cross of *y w spl* females to *T(1;2)* $(w\text{–}ec)^{64d}$ males, kindly supplied by DR. G. LEFEVRE., generated both *y w spl* euploid males and *y w spl+* duplication-bearing males.

Region 3E–20: Duplication-bearing males for portions of the remainder of the *X* chromosome were recovered from crosses of stocks having translocations between a *y*-marked *X* and the B^SYy+. Both duplication-bearing and euploid males are found among the progeny of a cross of two *X;Y* translocations with displaced *X* breakpoints and opposite *Y* arm breaks, as is shown in Figure 2. No males were found carrying a duplication of the region 11D–12E. The stocks used, together with their *X* and *Y* breakpoints, are listed in Table 1.

Superfemales (3 *X* chromosomes and 2 sets of autosomes) were obtained from a stock of *y w f* attached-*X* females and *FM7* males, and were distinguished from their diploid sisters by their y^2 w^a *f+ B* phenotype.

To generate flies duplicated for the *Zw* and *Pgd* loci simultaneously, *T(X:Y) B26/FM7* females were crossed to *T(1;3)*$w^{v\ co}$ males carrying the *X* chromosome region 2C1–3C4 inserted

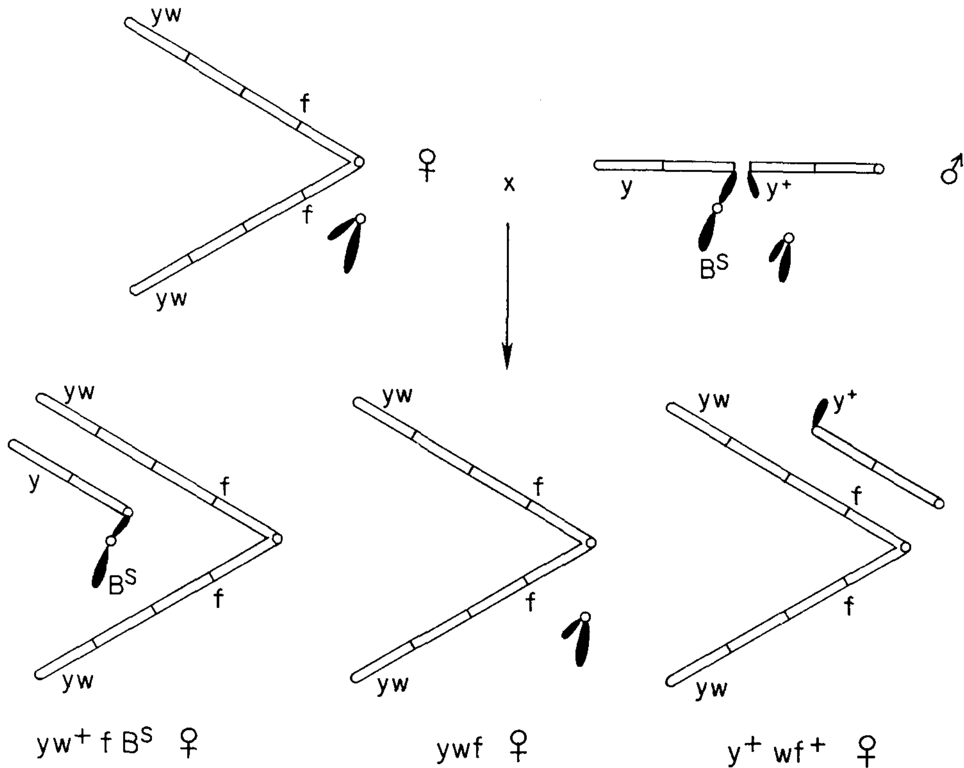

FIGURE 1.—The diagrammatic production of *X* chromosome hyperploids by use of an *X;Y* translocation.

y w f attached-*X* females are crossed to *T(X;Y) B26* males, in which the proximal piece of the *X* is marked with $y+$ and the distal piece with y and B^S. Since *T(X;Y) B26* is male viable and fertile, the two classes of duplication-bearing female progeny represent hyperploidy of the entire *X* chromosome. The attached-*X* chromosome is represented on the upper left and the lower half of the figure by two *X* arms (clear bars) joined to a single centromere. The pieces of the translocated-*X* chromosome are represented in the upper right and lower left half of the figure as half-size lengths of the *X* chromosome joined to different centromeres. The *Y* chromosomes throughout are represented by solid bars. The attached-*X* chromosome is marked with yellow body (y), white eyes (w) and forked bristles (f). The translocated *X* chromosome carries the y marker on the *X* chromosome and the dominant markers $y+$ and B^S (Bar eyes) on the short and long arms of the *Y* chromosome, respectively. The intact *Y* chromosome carried by both male and female parents is unmarked. Phenotypic designations of the three kinds of viable daughters are given below each character.

into the base of the third chromosome. $y+$ B^S translocation-bearing males also carrying the duplication were selected from the F^1 and were crossed to *y w f* attached-*X* females. Five classes of female progeny were obtained: euploid females ($y\ w\ f$), females duplicated for the region 9C–20 ($y+\ w\ f+$), females carrying a duplication of the region 1–9C ($y\ w+\ f\ B^S$), females with a duplication of the region 2C1–3C4 ($y\ w+\ f$), and females carrying duplications of the regions 2C1–3C4 and 9C–20 ($y+\ w+\ f+$).

Mutant markers are described in LINDSLEY and GRELL (1968).

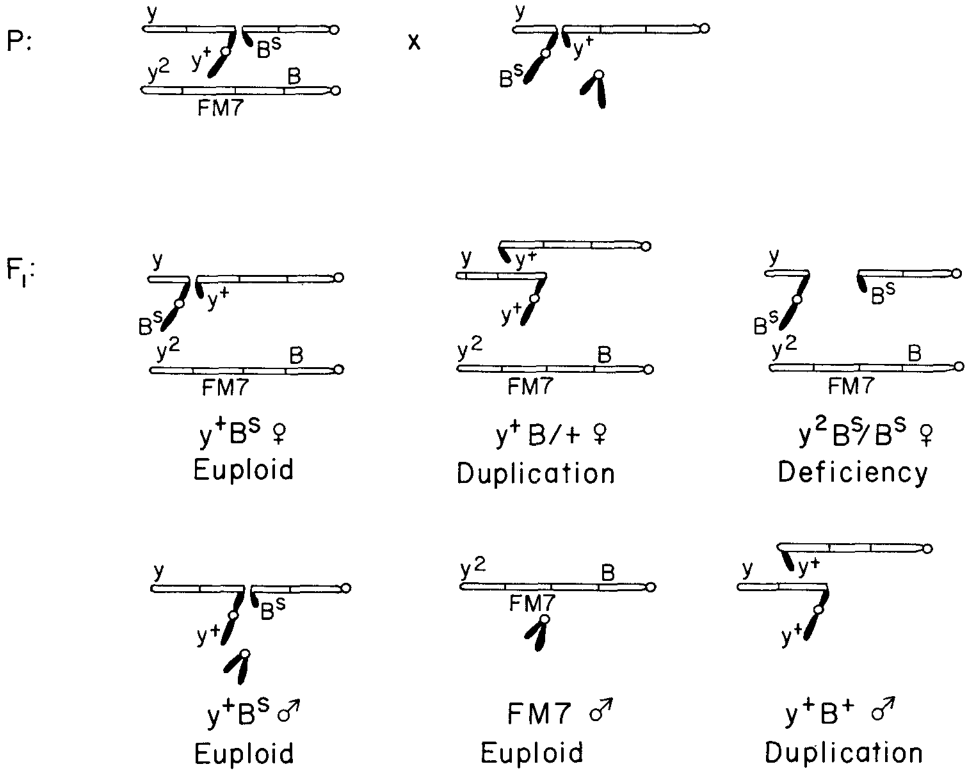

FIGURE 2.—The production of *X* chromosome duplications and deficiencies using two *X;Y* translocations. Crosses between flies bearing translocations with displaced *X* breakpoints and opposite *Y* arm involvement yield both euploid and aneuploid progeny. Phenotypically $y+$ B^S males and females are euploid. In this example the y^2 B^S/B^S daughters are heterozygous for the deficiency while the $y+$ non-*B* sons and daughters carry the corresponding overlap duplication.

TABLE 1

X;Y *translocation stocks used to generate males duplicated for regions of the* X *chromosome*

Stock	*X* breakpoint	*Y* breakpoint
T(X;Y) B29	3E	L
T(X;Y) B36	5C	S
T(X;Y) J8	8C	L
T(X;Y) J2	9A	S
T(X;Y) B44	11A	L
T(X;Y) B39	11D	S
T(X;Y) B24	12E	L
T(X;Y) B28	13F	S
T(X;Y) B35	15B	S
T(X;Y) B10	15E-F	L
T(X;Y) B18	16F-17A	L
T(X;Y) L4	17B-C	L

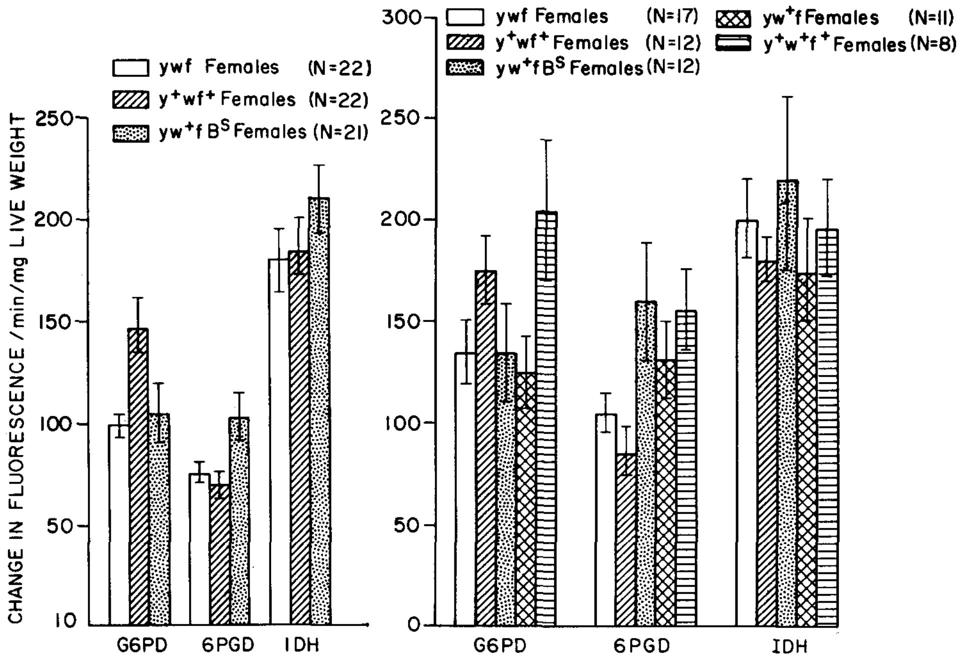

FIGURE 3.—Bar graph comparisons of G6PD, 6PGD, and IDH-NADP enzyme activities between control sibs and females bearing duplications of either the proximal or distal halves of the *X* chromosome. The height of the bars on the ordinate measures the means of specific enzyme activities measured in arbitrary units for the number of flies listed. The vertical lines at the top of each bar indicates the 95% confidence interval calculated from the observed standard deviation and the appropriate *t* value for the number of flies (=*N*) assayed separately in each group.

a. Female progeny from the cross of *y w f* attached-*X* females to *T(X; Y)B26* males. *y w f* attached-*X* females (euploid) are compared with y^+ *w* f^+ females (duplicated for the proximal half of the *X*, 9C–20) and *y* w^+ *f* B^S females (duplicated for the distal half of the *X*, 1–9C).

b. Simultaneous duplication of the *Zw* and *Pgd* loci. The flies are derived from the crosses explained in MATERIALS AND METHODS. *y w f* females (euploid) are compared with y^+ *w* f^+ females (duplicated for 9C–20), with *y* w^+ *f* B^S females (duplicated for 1–9C), with *y* w^+ *f* females (duplicated for 2C1–3C4), and with y^+ w^+ f^+ females (duplicated for 2C1–3C4 and for 9C–20).

RESULTS

Each of the two models of dosage compensation discussed by LUCCHESI (1973) can be tested by the measurement of the activity of *X*-linked enzymes in flies duplicated for regions of the *X* chromosome. By crossing males of the stock *T(X;Y) B26* to marked attached-*X* females, it is possible to recover in the progeny three types of females: euploid females, females duplicated for the distal half of the *X*, and females carrying a duplication of the proximal half of the *X*. These flies were assayed for the *X*-linked enzymes, G6PD and 6PGD, and, as a control, the autosomally-specified enzyme IDH-NADP. The results of these assays normalized to weight are shown in Figure 3a. Results of assaying females of the same genotype as in Figure 3a are also shown in Figure 3b. The females

in the latter figure were derived from a different series of crosses, but those of the phenotypes $y\ w\ f$, $y^+\ w\ f^+$, $y\ w^+\ f\ B^S$ correspond in genotype to the like females in Figure 3a. In both cases, females duplicated for the proximal half of the *X* chromosome, which contains the gene specifying G6PD, have increased G6PD activity compared to their euploid sisters. Flies bearing a duplication of the distal half of the *X*, in which the gene specifying 6PGD is located, show greater 6PGD activity than euploid sibs. Otherwise, G6PD and 6PGD activities, and the autosomal control enzyme, IDH-NADP, are unchanged between controls and either group of experimental flies.

The effects of duplications of smaller regions of the *X* chromosome were also measured in males obtained by using the stocks described in MATERIALS AND METHODS. Duplication-bearing males for all areas of the *X* chromosome, with the exception of the region 11D–12E, were assayed along with their euploid brothers as controls. Results of these assays are recorded in Table 2. The *Pgd* gene speci-

TABLE 2

Results of assaying enzyme activities in males bearing duplications of regions of the X *chromosome*

Duplicated region	N	G6PD (95% limits)	SE	6PGD (95% limits)	SE	IDH (95% limits)	SE
1–3A							
control	11	148 (129–167)	8.7	118 (109–127)	4.4	239 (204–274)	15.5
duplication	11	147 (135–159)	5.4	193 (170–216)	10.3	250 (222–278)	12.4
2C1–3C4							
control	11	184 (161–207)	10.2	158 (138–178)	9.2	207 (176–238)	9.3
duplication	8	160 (127–193)	14.2	230 (187–273)	18.4	221 (172–268)	20.6
3C2–3F1							
control	6	198 (169–227)	11.2	122 (96–149)	10.4	194 (167–221)	10.6
duplication	6	208 (157–249)	16.2	109 (103–115)	2.5	217 (195–239)	8.9
3E–5C							
control	15	167 (145–189)	10.5	132 (104–160)	7.2	341 (302–379)	17.9
duplication	14	171 (151–191)	9.3	140 (123–158)	8.1	288 (251–326)	17.3
5C–8C							
control	14	175 (162–188)	6.0	134 (124–145)	4.9	297 (265–328)	14.7
duplication	12	157 (138–176)	8.8	130 (116–145)	6.6	256 (232–281)	11.3
8C–9A							
control	15	147 (132–162)	7.0	124 (110–137)	6.2	261 (228–295)	15.5
duplication	14	156 (138–175)	8.5	138 (124–153)	6.7	251 (225–277)	12.0

TABLE 2—Continued

Duplicated region	N	G6PD (95% limits)	SE	6PGD (95% limits)	SE	IDH (95% limits)	SE
9A–11A		181		135		305	
control	15	(167–196)	6.8	(127–144)	4.1	(274–337)	14.9
		160		126		300	
duplication	14	(143–178)	8.2	(116–136)	4.5	(271–330)	13.7
11A–11D		163		123		291	
control	15	(149–177)	6.4	(112–134)	5.1	(264–318)	12.6
		167		120		269	
duplication	16	(146–189)	10.0	(110–130)	4.7	(230–307)	12.2
12F–13F		172		133		262	
control	16	(151–193)	9.9	(122–145)	5.4	(227–295)	15.7
		180		146		213	
duplication	15	(155–205)	11.7	(132–162)	6.9	(172–255)	19.4
13F–15E-F		182		147		287	
control	11	(169–194)	6.2	(133–161)	6.5	(261–314)	12.1
		176		129		291	
duplication	11	(157–196)	8.8	(114–144)	6.8	(252–330)	17.5
15B–17B-C		174		135		321	
control	13	(157–191)	7.8	(121–148)	6.2	(282–360)	18.0
		199		146		302	
duplication	14	(179–218)	9.0	(133–157)	5.5	(269–334)	14.9
(16F-17A)-20		198		204		304	
control	14	(176–221)	10.3	(177–230)	12.5	(271–337)	15.1
		334		207		262	
duplication	14	(304–364)	13.8	(198–218)	5.2	(232–293)	14.2

Genotypes of the control and duplication-bearing males are given in MATERIALS AND METHODS. Enzyme measurements are described in arbitrary units corresponding to the units in Figures 3 and 4.

fying the 6PGD activity is located in chromosome region 2D–E and flies bearing an extra dose of that region show elevated 6PGD levels. The *Zw* gene specifying the G6PD activity is located in chromosome region 18D–E and flies bearing an extra dose of that region show elevated G6PD levels. In no other instances were the enzyme levels in duplication-bearing males significantly different from those of their euploid brothers.

Enzyme levels were measured in females carrying an entire third *X* chromosome (superfemales). These flies have the same activity for each of the three enzymes as do their normal diploid sisters, as can be seen from the results in Figure 4.

This last result was initially unexpected by us and at the time we considered that it could be the reflection of some peculiarity of the two *X*-linked enzymes measured. G6PD and 6PGD are responsible for catalyzing sequential steps in the hexose monophosphate shunt, and we wondered if perhaps both levels cannot be raised simultaneously, at least in the same fly. This could possibly account for

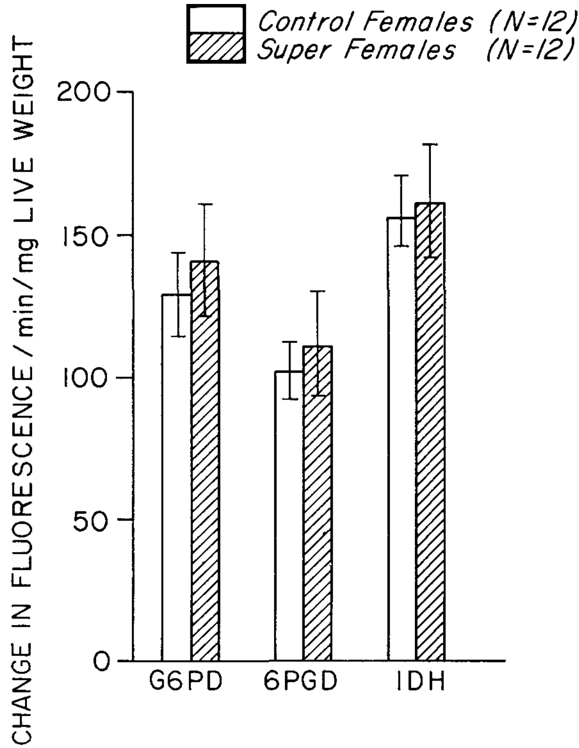

FIGURE 4.—Bar graph comparisons of G6PD, 6PGD, and IDH-NADP enzyme activities as in Figure 3, between diploid females (2*X* 2*A*) and superfemales (3*X* 2*A*).

the discrepancy between our results and those of FAIZULLIN and GVOZDEV (1973), who found elevated levels of both G6PD and 6PGD in extracts from massed homogenates of superfemales. It may be that under some conditions some 3 *X* females have higher levels of one enzyme and some of another. To test among these possibilities by genetic means, flies were constructed in which both the genes specifying G6PD and 6PGD were duplicated, but not as an entire third *X* chromosome. Figure 3b gives the results of assaying these flies. Flies bearing duplications of either proximal or distal halves of the *X* chromosome are the same genotype as those in Figure 3a and also show the expected increases in G6PD or 6PGD activity. Females duplicated for 2C1–3C4 and 9C–20 simultaneously show increased levels of both G6PD and 6PGD compared to the other groups, thus demonstrating that the levels of both activities can be increased at the same time.

DISCUSSION

Changes in gene activity are basic to the study of genomic expression. Dosage compensation in Drosophila is an example of the modulation of genetic activity in a higher organism, albeit specialized in that the activity changes are quantitative in level rather than being a qualitative on-off switch determining when

transcription occurs. What we would like to know is on what basis gene activities are modulated to behave like those characteristic of single *X* chromosome males or of double *X* chromosome females.

LIEB (1946) found that some regions of the *X* chromosome, when present in an extra copy, influenced the phenotypic expression of *X*-linked genes outside of the duplicated region. She studied the forked, scute, white apricot, and Bar phenotypes; all had compensator regions, as well as some augmenter regions which shifted the mutant phenotypes toward normality. In particular, her results indicated the presence of a compensator region in 9B–14C and an augmenter region in 1–9B for the white apricot phenotype. To try and repeat her observations, strains were constructed here such that the following females could be compared: those carrying an attached-*X* homozygous for white apricot, those carrying that attached-*X* and a duplication of the region 1–3E marked with white apricot (3 copies of the white apricot allele), those carrying that attached-*X* and a duplication of the region 1–11A marked with white apricot (3 copies of white apricot and hyperploid for the distal half of the *X*), and those carrying that attached-*X* and a duplication of the proximal half of the *X*, 11A–20 (2 copies of white apricot and hyperploid for the proximal portion of the *X*). As expected from LIEB's results, females with three copies of the white apricot allele were darker in eye color than were females with two copies of the gene. The eye color of females duplicated for 1–3E and for 1–11A could not be distinguished from each other, however, providing no evidence for an augmenter of white apricot in the distal half of the *X* between 3E and 11A. Females with two copies of white apricot and hyperploid for the proximal half of the *X* had a somewhat lighter eye color than did the normal diploid control females, thus indicating that this region may serve as a compensator of white apricot. These flies, however, clearly showed the effects of hyperploidy; they were smaller in size than normal, the wings were held in a different position, and some of the bristles were abnormal. The lighter eye color could also be an effect of hyperploidy in general, rather than being caused by an extra dose of "compensators". If this is so, we could expect flies hyperploid for smaller portions of the proximal half to have the usual w^a eye color.

We find no evidence here for a single locus on the *X* chromosome controlling the modulation of the activity of the structural genes of either the G6PD or 6PGD enzymes. If such a compensator locus were present for either structural gene, we should have detected it by its effect on the enzyme levels in hyperploid flies. We expect males with one copy of the G6PD locus and two copies of the compensator locus to have only half of the G6PD activity of normal brothers. By the same logic, females with two copies of the G6PD locus and three copies of the compensator locus should have two-thirds the normal G6PD level. However, we did not observe such an effect on either G6PD or 6PGD levels measured in hyperploids of the *X* chromosome in either males or females.

It also seems unlikely that a positive activator, such as that in the model proposed by MARONI and PLAUT (1973) and by SCHWARTZ (1973), is sufficient to account for the observations reported in the studies of dosage compensation. If

there were such a substance necessary in a competitive fashion for normal levels of *X*-linked gene activity, we would expect that the duplication of half an *X* chromosome in a female would result in a decrease of activity of 20% for a gene located outside the duplication and an increase of only 20% in the activity of a gene covered by the duplication. In our hands, flies bearing a duplication of the proximal half of the *X* chromosome do not show lower 6PGD levels than control sibs, and flies bearing a duplication of the distal half of the *X* chromosome show the same increase in 6PGD as do flies bearing only a small duplication including the *Pgd* gene. The same observations hold true for G6PD activities.

However, examination of the data in Figures 3a and 3b does show some changes. For example, the ratio of the mean G6PD activity to the mean IDH-NADP activity in flies carrying a duplication of the proximal half of the *X* chromosome is less than that ratio in control flies. Hence, it is possible that another way of analyzing the data may indeed uncover differences between classes of flies which were obscured by our analysis. The original data were, therefore, re-examined by normalizing the G6PD and 6PGD activities in each single fly extract with respect to the IDH-NADP activity for that extract. The means and standard deviations of the ratios for each class of flies are listed in Table 3. Females in part A that bear a duplication of the proximal half of the *X* chromosome ($y^+ \, w \, f^+$) have a slightly lower 6PDG/IDH-NADP ratio than do control females. This difference is not significant. Females in part A bearing a duplication of the distal half of the *X* chromosome ($y \, w^+ \, f \, B^S$) have a somewhat lower G6PD/IDH-NADP ratio than do control females, although again this difference is not significant. Moreover, flies of the corresponding genotypes in part B show little decrease in activity compared to their controls. The 6PGD/IDH-NADP ratios are the same for flies bearing a small duplication containing the *Pgd* gene ($y \, w^+ \, f$) and for those bearing a duplication for the distal half of the *X*, which includes the *Pgd* gene ($y \, w^+ \, f \, B^S$). Although we cannot rule out the possibility of some positive activator substance, the variations in enzyme activity observed in this way, as well as between flies of different genotypes, do not

TABLE 3

The means of relative activities

Phenotype	N	G6PD/IDH-NADP (S.D.)	6PGD/IDH-NADP (S.D.)
A. $y \, w \, f$	22	.57 (.13)	.43 (.07)
$y \, w^+ \, f \, B^S$	21	.48 (.13)	.50 (.14)
$y^+ \, w \, f^+$	22	.78 (.13)	.37 (.07)
B. $y \, w \, f$	17	.69 (.19)	.54 (.07)
$y \, w^+ \, f \, B^S$	12	.66 (.15)	.77 (.24)
$y \, w^+ \, f$	11	.72 (.08)	.76 (.19)
$y^+ \, w \, f^+$	12	.96 (.21)	.48 (.11)
$y^+ \, w^+ \, f^+$	8	1.04 (.24)	.82 (.11)

Results for normalizing G6PD and 6PGD activities with respect to IDH-NADP activity for each single fly extract. The phenotypes in part A of this table correspond to those in Figure 3A; those in part B to Figure 3B.

support a model in which dosage compensation is mediated only by a positive activator.

Given these results, we prefer the conclusion that the regulation resulting in dosage compensation involves elements located throughout the entire chromosome, possibly like those hypothesized to be responsible for sex determination (DOBZHANSKY and SCHULTZ 1934). These elements may conceivably act in one of two ways: a group of genes may be responsible for each structural gene on the *X* chromosome, or the entire series of elements may act coordinately on all the *X*-linked structural genes. In the latter case, the activity of these elements may be envisioned as regulating on a gene by gene basis, or as modulating the chromosome as a whole in some way not requiring the physical continuity of the chromosome. (For another example of chromosomal level regulation see LIFSCHYTZ and LINDSLEY 1972.) Unlike sex determination, however, our results indicate that modulation of gene activities by dosage compensation occurs *in steps*, with each step representing the difference of an entire *X* chromosome.

FAIZULLIN and GVOZDEV (1973) carried out experiments similar to ours in observing the effects of *X* chromosome hyperploidy on G6PD and 6PGD activities in diploid females. They also failed to find evidence for the compensator genes proposed by MULLER (1950). They did additional studies on the enzyme levels in triploid intersexes, finding higher enzyme levels in male-like intersexes than in female-like intersexes. On this basis, they concluded that the setting of dosage compensation level in individual flies is tied to that of the determination of sexual development, a position originally espoused by GOLDSCHMIDT (1954).

If the phenomenon of dosage compensation is a direct outgrowth of the sex determination in each fly, then superfemales would be expected to have higher levels of *X*-linked gene products than diploid females. Since superfemales are generally sterile and of low viability, at least some *X*-specified gene products must be made in enough quantities to be harmful to the fly. FAIZULLIN and GVOZDEV did find higher G6PD and 6PGD levels in superfemales. However, we and others find that superfemales do not have higher G6PD and 6PGD levels than their diploid sisters. The reasons for these differences are not clear. We conclude that the critical variable in dosage compensation must be thought to occur at the level of entire *X* chromosomes and independently of sex.

It is possible that in an evolutionary sense some mechanism may have appeared to distinguish between one and two *X* chromosomes for purposes of sex determination. Subsequently, the mechanism may have also become part of the apparatus regulating dosage compensation. In that case, our results indicate that the mechanism must also distinguish 3 *X* chromosomes and modulate the activity per gene copy accordingly at a level lower than that normally found in diploid females.

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